HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

Introducing ...

This Newsletter on harmful algae responds to the expressed wishes of participants in several IOC workshops on harmful algal blooms, in particular the IOC-SCOR Workshop in Newport, Rhode Island (USA), 2-3 November 1991. Its purpose is to disseminate information on harmful algal events and on research results as well as to announce research and management programmes, conferences, meetings etc.

***Harmful Algae News* is initially published in conjunction with UNESCO's quarterly *IMS Newsletter*. Tim Wyatt has, at IOC's request, agreed to be the initial Editor. Ideas, manuscripts or information appropriate to include in coming issues, should be addressed to him or Yolanda Pazos, Instituto de Investigaciones Marinas, Eduardo Cabello 6, 36208 Vigo, Pontevedra, Spain; tel: (34-86) 23 19 30; fax: (34-86) 29 27 62.**

***Distribution*: Although initially to be mailed together with *IMS Newsletter*, it is envisaged that *Harmful Algae News* will eventually be distributed separately. Participants at the 5th International Conference on Toxic Marine Phytoplankton will automatically be on the address list; others wishing to be included should write to: *Harmful Algae News*, IOC of UNESCO, att.: Henrik Enevoldsen, 1 rue Miollis, 75015 Paris, France.**

Intergovernmental Oceanographic Commission of UNESCO

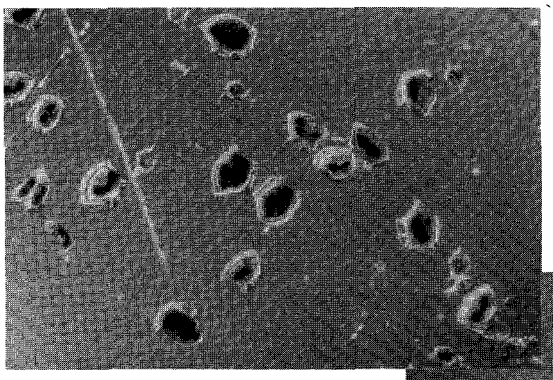
Harmful algal blooms

by Tim Wyatt and Yolanda Pazos

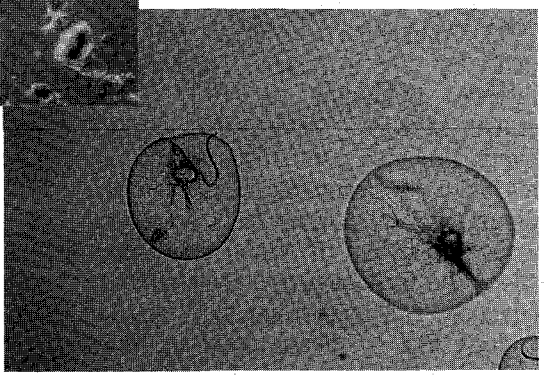
For about a century, studies of phytoplankton and algal blooms have been regarded as fundamental components of fisheries research, since in the last analysis the yields of fisheries must depend on them. The notion that algal blooms can be harmful therefore requires some explanation. The word 'bloom' in the present context refers to discolouration of the water caused by plankton growth, and was used in this sense at least as early as 1838[1]. These blooms are fairly predictable annual events, both in the sea and freshwater, and sampling reveals that diverse mixtures of species are involved. The sometimes harmful algal blooms (HABs) of concern here are, in contrast, not at present predictable, are usually dominated by a single species, and frequently colour the water red or brown rather than green or olive, hence the term "red tide". Just how unpredictable these events can be was dramatized for example by Scott Nixon[2] in his search for the causes of a harmful bloom which occurred nearly a century ago. He wrote: '... it must be remembered that the events of the late summer of 1898 have only been noted once in the entire recorded history of Narragansett Bay'. The *Chrysochromulina polylepsis* bloom which took place in the Skagerrak in 1988 provides a recent example.

Harm is inflicted by these events in a variety of ways. Growth and accumulation, followed by cessation of photosynthesis and subsequent decay of the accumulated plankton can cause deoxygenation of the water, leading to death of fish and invertebrates. Even normally innocuous species can cause harm in this way. Some species when abundant secrete polymers into their surroundings which can render the water so viscous that fish have difficulty in pumping it through their gills. Others, like the diatom *Chaetoceros concavicornis*, are spiny, and can irritate fish gills, leading to mucus production, gill damage, and reduced gaseous exchange. Blooms of these kinds are causing serious losses every year to fish farmers in several countries, and put a serious brake on future investments in this industry.

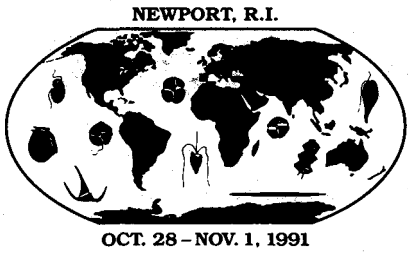
(Cont'd on p. 5)



Left: The potentially toxic dinoflagellate Dinophysis norvegica *(48-67 µm long) is a common species in northern European waters. The toxicity is due to okadoic acid and related substances responsible for diarrhetic shellfish poisoning.*
Photo: Per Andersen, Bioconsult.



Right: Noctiluca scintillans, *a non-photosynthetic dinoflagellate very common world-wide. This species is non-toxic and harmless in low concentrations, but often develops mass occurrences which are subsequently associated with anoxia and mortalities of marine fauna.*
Photo: Courtesy of Instituto Español de Oceanografía, Vigo, Spain.



The Fifth International Conference on Toxic Marine Phytoplankton and the Future

by Ted Smayda

One of the results of our Conference in Newport is the appearance of this newsletter, which I hope will be the first of several new initiatives to network our global activities and concerns about harmful algal blooms. It also gives me this opportunity, as Convener of the Fifth Conference, to thank those of you who attended, gave presentations, chaired sessions, entered into scientific dialogue with your colleagues, and also contributed to its social success. In my judgement, the conference was a great achievement at several levels, and a direct result of your collective participation. I am pleased with the results and promise for the future.

In retrospect, the conference ended one phase in the development of our growing discipline and brought us to the next phase which will probably be characterized by a more integrated, interdisciplinary approach to HAB events. The results of this new approach should already be evident at our 1993 Conference in Nantes, and certainly by 1995 in Sendai. The six conferences (including the 1987 Takamatsu meeting) have now, collectively, provided enough information to allow us to formulate testable paradigms and hypotheses in several key areas. Analyses of these hypotheses should he facilitated by the remarkable advances and application of molecular biology that we heard about in Newport; by the increased sophistication with regard to toxigenesis; and our keener insights into the patterns and varieties of HAB events and organisms. The accumulated data now allowing formulation of a framework of theory and background on bloom patterns and consequences should also allow individual investigators to evaluate bloom events in their local area from regional, global, organismal and toxicological perspectives. This will help to establish the first principles - the commonalities - involved in HAB events. We need such insight to move us from anecdotal or descriptive approaches to such bloom events to quantitative ecological approaches. The diverse expressions, causative organisms and dynamics of HAB events increasingly indicate the need to incorporate regional comparisons into our search (during this next phase) for the causes, mechanisms and underlying principles of such blooms. The number of attendees at the Newport Conference and their global distribution suggest that our regional networks in Asia, Europe and North America are both thriving and increasing, and should facilitate this common need for both regional and interregional perspectives. Unfortunately, we have little information on the status of HAB events in African coastal waters and many coastal regions of South and Central America. We should encourage international bodies such as UNEP, IOC, FAO, WHO, etc., to help nurture such efforts, both in the training of scientists and in sponsoring needed research in these regions.

Another result of the Newport Conference was the very successful post-conference IOC-SCOR meeting, during which plans for a multidisciplinary, multinational integrated programme on HAB events and consequences were drafted. This represents a giant step towards bringing us to a period of more collaborative, interdisciplinary and interregional research - a scientific networking. I believe that this proposed effort is important for several reasons, including its potential for helping to minimize several shortcomings or possible dangers which I am concerned may compromise our collective efforts. For example, our ecological activities directed towards understanding HAB events are woefully inadequate; we have serious problems here, including lack of trained personnel. The collaborative, interdisciplinary research initiatives being proposed should be helpful in this area through the teamwork approach. I am also a little concerned about the seeming proliferation of national, regional and/or agency-sponsored HAB initiatives, carrying with it the risk of duplication or dilution of scientific effort, and possibly fostering a localized focus at the expense of a more holistic, global view. The proposed collaborative, interdisciplinary initiatives should be helpful here, as well as the linking of needed national and regional efforts, as part of an international programme. This might also be more cost effective.

I am mildly concerned about our ability to keep our 'group' together. The considerably increased attendance at Newport and the large number of papers were significant. Is our 'group' becoming too big for its present format? Is it just a matter of time before, say, the toxicologists and taxonomists each decide that they were more interested in hearing scientific reports primarily within their own specialties? That is, will the 'group' begin to splinter into subgroups reformed within their own interests? I believe that this possibility would be a disaster and one we must avoid. The proposed collaborative, interdisciplinary approach encouraged by IOC and SCOR should help, I hope, to reduce this possibility. An additional way to encourage group retention might be for us to establish a society and eventually to publish a journal, which might also be used to publish future conference proceedings.

These various scientific advances, new developments and collective needs of our growing discipline are partly the basis of my view that we are indeed in a new stage in the growth-curve of our discipline. We now need to formulate new research problems, strategies and priorities; to evolve better linkages between our hard science and the concerns of aquaculture, public health and mass media; and to ensure a proper funding base, including both national and international sponsorship for our efforts. I wish to thank all of you who have brought us to this point, and to the many of you who sent me your Season's Greetings and kind expressions on the Newport Conference. To everyone, my best wishes for a happy, fulfilling and scientifically rewarding New Year.

Ted Smayda. Convener.

A Programme on Harmful Algae

The Intergovernmental Oceanographic Commission (IOC) of UNESCO, is responding to an identified need of its Member States to have a program to assist them in dealing with harmful algal events.

There is a wide array of scientific and managerial problems associated with harmful algae. In order to mitigate these problems more knowledge is required about the dynamics of blooms, the succession of species, types of toxins etc,. There are also public health concerns, fisheries are affected, and there are financial impacts on the seafood industry etc.

Furthermore there is a need for management activities to interact with the scientific community in order to function as efficiently as possible. The managers dealing with harmful algal events need an organized scientific community so they can get effective help and advice.

The Harmful Algal Bloom Programme was initiated in 1987 as a subprogramme of the joint IOC-FAO programme on Ocean Science and Living Resources (OSLR). Since 1987 a group of experts has met three times to formulate the programme's goals and to outline its elements. The XVIth Session of the IOC Assembly, Paris, March 1991, adopted a resolution with respect to the formation of an *Ad hoc* Intergovernmental Panel on Harmful Algal Blooms in order to identify adequate resources for a sufficiently broad programme to solve some of the real problems caused by algae.

The Food and Agriculture Organization of the United Nations (FAO) has agreed to cosponsor the *Ad hoc* Intergovernmental Panel, members of which are participating in the capacity of national representatives. The first session of the Panel is planned to take place 23-25 June 1992 in Paris.

There appear to be two major divisions of the problems - the scientific and the operational. It is the aim of the Harmful Algal Bloom Programme to cover both.

The scientific programme can be separated into three branches; ecology and oceanography, taxonomy and genetics, and toxicology and toxin chemistry. The operational problems can be divided into four branches; resource and aquaculture management, information network and training, monitoring, and public health and seafood safety.

The **Overall Goal** of the Harmful Algal Bloom Programme, which should embrace the short, medium and long term aspects of the above-mentioned elements, is to foster and organize the management of and scientific research on, Harmful Algal Blooms in order to understand the causes, predict the occurrences, and mitigate their effects.

To give an idea of the potential of the Programme, the goals for each of the Scientific and Operational Programme Elements are listed below.

Scientific elements:

• *Ecology and oceanography.*
To understand the population dynamics of harmful algae.
• *Taxonomy and genetics.*
To discriminate the causative organisms at the appropriate levels.
• *Toxicology and toxin chemistry.*
To determine the physiological, chemical and toxicological mechanisms underlying toxin production and retention.

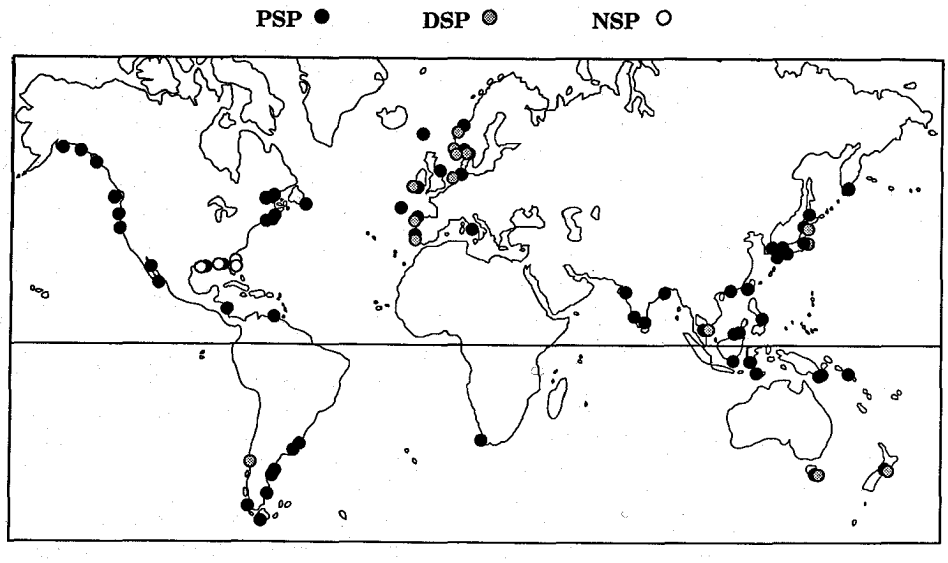
Operational elements:

• *Resource and aquaculture management.*
To develop and improve methods to minimize the environmental and economic consequences of Harmful Algal Blooms.
• *Information, network and training.*
To develop, encourage and maintain the flow of information, technology and expertise to scientists and administrators.
• *Monitoring.*
To assist and facilitate the development and implementation of appropriate monitoring programs.
• *Public health and seafood safety.*
To protect public health and ensure seafood quality.

In November 1991 a preliminary Planning Committee for the Programme was formed at the IOC-SCOR workshop which followed the Fifth International Conference on Toxic Marine Phytoplankton, Newport, USA. The Committee will sustain activities pending complete establishment of the aforementioned programme elements.

In order to ensure as close international collaboration as possible in the development and implementation of the Harmful Algal Bloom Programme, the IOC is cooperating with the Scientific Committee on Oceanic Research (SCOR) and the International Council for the Exploration of the Sea (ICES).

The HAB-Programme has been initiated and will hopefully accelerate during 1992 and 1993. Articles reporting on the development of the Programme will appear in *Harmful Algae News*.



Global distribution (prior to November 1987) of human intoxication by Paralytic Shellfish Poison (PSP), Diarrheic Shellfish Poison (DSP) and Neurological Shellfish Poison (NSP). Fig. 1 in: Toxic Marine Phytoplankton, *1989. Granéli et al. (eds.), p. 538, Elsevier, N.Y.*

WESTPAC Symposium and Harmful Blooms Workshop

From 2-6 December 1991 in Penang Malaysia, the second WESTPAC Symposium was convened, sponsored in part by the IOC. The overall theme for this symposium which attracted scientists and administrators from 25 countries, was the management of the marine environment in the western Pacific. Among the specific topics of interest were sea-level rise, climate change, and other global changes likely to have a significant impact on countries within the western Pacific region.

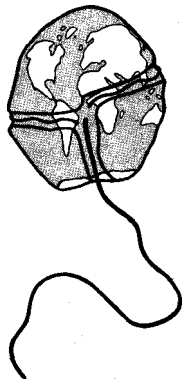
During this symposium, workshops were held in nine different areas represented by task teams established previously by WESTPAC. One of these, the Task Team on 'Toxic and Anoxic Phenomena Associated with Algal Blooms' met for one and a half days to review recent events and to plan strategies for training, research, and networking in the coming years. It soon became clear that in the years since the last meetings of the Task Team in 1984 and 1987, the nature of the red tide or harmful algal bloom problem has changed considerably in the WESTPAC region. Where formerly the predominant problem was paralytic shellfish poisoning (PSP) caused by the dinoflagellate *Pyrodinium bahamense*, countries are now threatened by new species that produce PSP toxins (*Gymnodinium catenatum, Alexandrium tamarense, catenella*) as well as by other species (*Noctiluca, Trichodesmium, Chattonella, Heterosigma*) that cause massive mortalities of fish and shrimp, both farmed and wild. These new problems threaten the rapidly growing aquaculture industry in the region. This regional expansion parallels similar increases in harmful blooms throughout the world, and has led some scientists to conclude that there has been a fundamental change in the structure of marine planktonic ecosystems on a global scale caused by human alterations to the coastal zone.

Red tide workers in the WESTPAC countries are relatively advanced with respect to the formation of a network and regional program, but considerable effort must now be focused on strengthening communication and increasing interactions and collaborations. To this end, three important recommendations from the Task Team were for WESTPAC to support the creation and distribution of: 1) a regional, informal newsletter on harmful blooms; 2) a manual of techniques for field and laboratory studies; and 3) photographs, slides, and preserved samples of harmful algal species. A related recommendation was for a regional symposium on harmful blooms (possibly in conjunction with WESTPAC marine pollution groups).

Considerable discussion was devoted to multi-lateral and bi-lateral programmes planned or underway for harmful bloom studies in the western Pacific region. It became clear that concerted efforts are needed to start new programs or to expand the scope of existing programmes. Task Team members agreed to work closely with national planning and policy agencies to convince them that the bloom problems are severe, growing, and of high priority with respect to competing national needs. It is hoped that effective Task Team 'lobbying' will be directed towards UNDP, where a red tide/prawn recruitment initiative is under consideration, and towards FAO (through the Indo-Pacific Fisheries Commission) which has traditionally not considered harmful blooms to be a priority topic relative to other requests. It cannot be overemphasized that significant progress in funding will come only after those who hold influential national policy positions are convinced by Task Team scientists that it is in their nations' interest to encourage international aid programmes in the area of harmful bloom research and training. An excellent opportunity exists for the IOC and the WESTPAC Subcommission Secretariats to use existing multi- and bi-lateral research and exchange programmes (such as the one between Japan and the Philippines) as leverage to obtain supplemental funds that will permit other countries to participate.

Participants in the Task Team workshop included: T. Okaichi (Chairman), Y. Fukuyo, K. Matsuoka, T. Ogata, M. Komada (Japan); Wu Guiqiu, Zou Jingzhong (China); R. Corrales, C. Gonzales (Philippines); A. Soegiarto, A. Sediaki, Q. Adnan (Indonesia); V.P. Devassy (India); Choo Poh Sze, Wong Tat Meng, Lee Lay Wee, Goh Siew Hoon, Tan Shau Hwau (Malaysia); T. Piykamchana (Thailand); Joo Suck Park (Korea); and D.M. Anderson (USA).

Donald M. Anderson, Woods Hole Oceanographic Institution.



International Directory of Experts in Toxic and Harmful Algal Blooms and their Impact on Fisheries and Public Health

Plans are underway to publish an updated and expanded edition of this directory. The directory is designed to assist countries facing toxic and harmful algal bloom emergencies by facilitating rapid access to scientists, fisheries managers, public health officials and physicians who are experienced in dealing with marine toxins, red tides and harmful algal blooms and their consequences to fisheries, aquaculture and public health.

If you are involved in the basic scientific, applied fisheries, or public health aspects of harmful algal bloom events in your country and would like to be included in this updated directory, please mail or fax your name and address to Alan White (see below) in order to receive a questionnaire. There is no need to send your name and address if you were included in the 1990 directory or if you recently attended the Fifth International Conference on Toxic Marine Phytoplankton held in Rhode Island (USA).

Send your name and address to: Alan W. White, NOAA National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole, MA, USA 02543; fax: (1-508) 548-1158.

NEW - A Seawater Rheology Group

by Ian Jenkinson

Eutrophication in coastal waters seems to be causing more concern every year, with associated problems due to floating slime in the Adriatic, knee-deep *Phaeocystis* foam on North Sea beaches, and the perception that viscous phytoplankton-produced slime kills many millions of dollars worth of fish and shell-fish per year, mostly during blooms.

Waves and ripples are also damped, and whitecap formation affected by organic material absorbed at the sea-air surface. All seafarers have noticed surface slicks in moderately calm weather. This surface material now seems to be similar to that secreted into the bulk seawater by phytoplankton, and its effects on air-sea fluxes of heat, gases and (in rough weather) aerosols of salts are intuitively obvious and scientifically demonstrated.

Are these slimes and surface films, however, just a more obvious manifestation of what phytoplankton organisms do with their secretions wherever they live? Observations by divers and from submarines have now shown that much of this slime coagulates into aggregates, which produce turbulence-free microhavens. Phytoplankton may even have evolved slime production partly to "manage" turbulent and molecular dispersion in their immediate environment. Sinking of these aggregates, along with that of zooplankton faecal pellets, controls vertical flux rates of fixed carbon and other elements, and prevents the return of the carbon to the atmosphere in the short term. These aggregates are mostly sticky and surface active, and their destruction, modulated largely by the interaction between turbulence and the aggregates' rheology, or deformation properties, is likely to slow down this flux. Tougher aggregates should increase flux rates, except that when they trap bubbles they can rise and float, to the annoyance of holidaymakers.

Description of the rheology of these aggregates and, on a larger scale, of the rheology of the water containing them has been slow to develop because of the difficulty of sampling, the lack of suitable rheometers and the problem of describing space-time distributions which include exceptional events. Within the last decade, however, sufficiently sensitive rheometers have become available, better handling techniques have been developed, and space-time distributions can now be described by fractal geometry.

Study of seawater rheological properties has important implications for the statistics of marine turbulence, dispersion and fluxes of all properties and substances, pelagic ecology, mechanisms of toxicology, marine chemistry, particularly near surfaces, and climatology. It is frighteningly interdisciplinary. A Seawater Rheology Group has thus been set up. It is a new, informal interest group, composed of specialists in both *in situ* and theoretical turbulence, wave damping, whitecaps, rheology of bulk and surface phases, surface chemistry, ecological and physical modelling, plankton and fisheries ecology, fluid mud, gas and heat exchange at sea surfaces... Its aim is to promote interdisciplinary research in this field. If this excites you, as a scientist or an administrator, you can write or fax for further information to: Ian Jenkinson, Agency for Consultation and Research in Oceanography, Lavergne, 19320 La Roche Canillac, France; fax: (33) 5529 1982.

(cont'd from p. 1, 'algal blooms')

At the other end of the spectrum are species which are poisonous, and whose toxins can be passed through food chains. Many of these species are flagellates (including dinoflagellates), but a diatom, *Nitzschia pungens*, has recently been added to this group. Paralytic, diarrhetic, and amnesic syndromes resulting from these toxins usually reach man through the consumption of molluscs, and ciguatera via fish.

The word 'bloom' in the HAB context has not yet been effectively quantified. Since these events often take us by surprise, it is frequently only the near maximum cell concentrations which are reported, and the growth periods leading to these maxima are not sampled. These lacunae are now being filled in regions where monitoring programmes for potentially harmful species have been instituted, and studies of the population dynamics of exceptional blooms are becoming possible. When we can estimate the magnitudes of those processes which lead to both increases and decreases in population numbers, we shall be able to see more clearly what changes are needed to transform normal events into abnormal ones.

The virulence of HABs is likely to be exalted as man's demands on coastal zones intensify, and species which at present are harmless may in the future cause problems. This newsletter should provide a communication channel between all people concerned with harmful algal blooms and their effects on coastal zone development and human health. We look forward to your contributions.

Tim Wyatt and Yolanda Pazos,
Instituto de Investigaciones Marinas, Spain.

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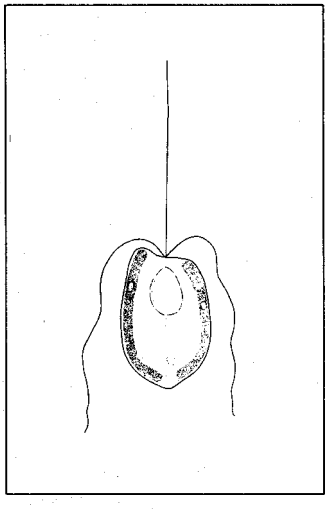
[1]By B. M. Griffiths, in *Proc. Linn. Soc.*, **151**, to describe the greening of Glaslough in Country Monaghan, Ireland.

[2]Scott Nixon's and John Sieburth's papers can both be found in E. M. Cosper *et al* (eds.): *Novel phytoplankton blooms, courses and impacts of recurrent brown tides and other unusual blooms.* Springer Verlag, 1989.



The flagellate Chrysochromulina polylepis *bloomed in Skagerrak in June 1988. Concentrations as high as 100 million cells per litre were registered.* C. polylepis *produces a toxin harmful to fish, invertebrates, and even to other algae. During the massive bloom, some 600 tons of cultured rainbow trout were lost.*
From: Larsen and Moestrup, 1989; Guide to Toxic and Potentially Toxic Marine Algae*; Danish Ministry of Fisheries.*

The Fifth International Conference on Toxic Marine Phytoplankton: A Personal Perspective

by Donald H. Anderson

One thing missing from the Newport Conference (see p. 2), that had been part of the earlier conferences in Lund and Takamatsu, was a retrospective overview, an attempt to place it in perspective relative to past meeting of the same type. Now, as the details of this conference rapidly fade from memory, I find myself charged to attempt just such an overview.

Looking backwards in time for perspective, I can see that each one of our conferences presented one or two topics that, in hindsight, were important steps forward. In Boston in 1974, the main topic was the massive 1972 New England red tide which extended the range of PSP (paralytic shellfish poisoning) in the region. That meeting was also noteworthy because Shantz and co-workers presented the structure of saxitoxin for the first time. In Florida in 1978, the origins of ciguatera poisoning were linked by Yasumoto to the benthic dinoflagellate *Gambierdiscus toxicus*. I also recall a mystified Marie Kat describing illnesses in human consumers who had eaten Dutch mussels that seemed to be free from bacterial contamination, yet which caused severe gastrointestinal distress. The next conference in St. Andrews in 1985 clarified this issue, as many papers discussed diahrretic shellfish poisoning (DSP) and its links to dinoflagellates. It was also the time when Yuzuru Shimizu proposed a biosynthetic pathway for saxitoxin. In Takamatsu in 1987, the extensive work by the Japanese on the toxicology, ecology, taxonomy, and life cycles of several important fish-killing species were presented in great detail. This was also the time of the first public speculations and discussion that harmful algal blooms might be increasing on a global scale. In Lund in 1989, new species such as *Chrysochromulina* and *Aureococcus* took center stage as a result of massive harmful blooms in different parts of the world. In Newport, in 1991, we had a continuation of these themes and issues. New harmful species were identified, such as the fascinating *Gymnodinium* species (described by J.M. Burkholder) that cycles between dormancy and motility in response to the presence of fish, which this species kills in an unknown yet very rapid manner. In his seemingly never-ending series of important discoveries, T. Yasumoto reported the detailed structure of several ciguatoxins and described HPLC (high-pressure liquid chromotography) method for their analysis, findings that should dramatically change the nature of ciguatera research.

These, and other presentations at the Newport meeting continued the general trends established in previous conferences. In my mind at least, three issues set Newport apart.

1) New technologies were presented which will surely have a major impact on our field in coming years. For example, immunological methods are now being employed to develop much needed rapid and sensitive assay methods for toxins. Immunological techniques have also been directed towards the harmful algae themselves and we can now realistically look forward to the availability of a battery of species-specific antibodies to automate the identification and enumeration of harmful species, and perhaps to shed light on important issues in taxonomy and population biology.

In the context of new technology, it is of note that our field, though vibrant and growing in other ways, remains quite backward in the application of the tools of molecular biology to major questions that face us. Of the several hundred papers and posters presented in Newport only a handful involved molecular techniques, whereas in meetings of many other biological, chemical, or ecological disciplines, one would find a far higher percentage of workers using such techniques. One good example of the value of this approach can be found in the presentation by Jovine and co-workers, who have been exploring the molecular biology of pigment/protein complexes in dinoflagellates. Another noteworthy presentation was by C. Scholin who used ribosomal RNA (ribosomal nucleic acid) sequences from many isolates of *Alexandrium* to reveal genetic differences between geographically distant populations. We must now generate a large database containing this type of sequence information for all harmful species. Once we have done so, it will be possible to address issues in species dispersal using the same techniques now common in forensic medicine.

2) Another important characteristic of the Newport conference was the tremendous press coverage that accompanied it. This was in part a function of a well-orchestrated effort by the Department of Communications at the University of Rhode Island, but it was also a reflection of the great public interest in harmful bloom problems these days. This interest has been sparked by several major bloom events that have caused significant damage to coastal resources in different parts of the world. However, it also stems from the fact that more and more scientists are stating that significant changes are occurring in coastal planktonic ecosystems, leading to increased numbers of harmful algal blooms. For some, the publicity surrounding the Newport conference was a concern. Those who have had interviews with journalists are familiar with how difficult it is to control the interpretation of what is said, but somehow we must do more to voice our science rather than our speculations, since it is the speculations that typically get us in trouble. A good example is from an article published in a large Boston newspaper during the conference which indicated that 'a majority of the 325 scientist who met [in Newport] this week said that they have abandoned the previous view of red tide outbreaks as localized, episodic nuisances'. There was no open discussion of this issue, and no vote was taken. The article also claimed that 'the world's oceans are experiencing an explosion of algal blooms such as toxic red tide, and some scientists believe that the phenomenon is an early warning of a massive ecological breakdown'.

C.W. Kao cautioned us to avoid self-serving and unsubstantiated claims of global spreading, arguing

'Whammies'

by Tim Wyatt

It may come as a surprise to readers of this newsletter to find cholera brought to their attention. But patterns and processes recognised in one science (in this case epidemiology) often illuminate problems in another. A recent news item by Christopher Anderson (in *Nature*, 25 Nov. 1991, p. 255) concerning the current cholera epidemic in Peru provides an analogy to the 'chem-tech' hypothesis which John Sieburth proposed (at the 'Novel Phytoplankton Blooms' conference held in Stony Brook, New York, in October 1988) to explain outbreaks of brown tides.

Sieburth's idea was that the fertilizers and pesticides used for shore-side lawn maintenance, when they percolate into coastal embayments, provide the brown tide organisms with two opportunities. The fertilizers raise the potential growth ceiling of the organism (*Aureococcus*) while the pesticides kill its enemies and allow it to reach that ceiling unrestrained by grazing.

Sieburth called this 'a double whammy'. The expression comes from Al Capp's Li'l Abner*, and referred to a hex so powerful he hoped he'd never 'hafta' use it! But the cholera epidemic being witnessed in Peru is worse than Li'l Abner's stare, and has, since January 1991, claimed over 300,000 victims and 3500 lives. The Pan American Health Organization (PAHO) attributes the epidemic to three causes. The inocculum is thought to have reached Peru in the bilge water of a freighter, it is traditional to eat certain seafoods raw in Peru, and a decision was made not to chlorinate drinking water supplies due to the slight risk of cancer which that practice entails.

The factorial combinations of these causes incline PAHO to the view that there would be no cholera epidemic in Peru at present if it were not for the fact that all three coincided.

People involved with harmful algal blooms might recognize, as PAHO has, that the decision on chlorination was a case of poor risk assessment, but without information on the frequency with which bilge waters are contaminated with dangerous pathogens, I doubt that anyone should be blamed for that. The advice on which it was based was sound enough. The delights of eating raw seafoods cannot be denied, and custom is by definition conservative, and in any case above the law. So the epidemic hinges on the inadvertent transport of a microorganism, and that is a kind of event whose threat we are aware of in the context of toxin dinoflagellate blooms.

Gustaaf Hallegraeff has maintained for several years that *Gymnodinium catenatum* which can cause paralytic shellfish poisoning (PSP), was introduced into Tasmanian coastal waters by ships which discharge their ballast water there. This view is strongly supported by detailed studies by Hallegraeff and his colleagues of historical plankton records, sedimentary deposits, *G. catenatum* genetics, and the ballast tank materials themselves of many ships entering Australian ports. Hallegraeff recently reported that twenty different dinoflagellate species have been germinated from cysts collected in these ships. Twelve ships contained cysts of three well known toxic species (two *Alexandrium* species, and *G. catenatum* itself), and one of them (*A. tamarense*) produced toxic cultures. So persistent has the search for the origin of *G. catenatum* in Tasmanian waters been, that it may eventually be possible to indicate with a respectably high probability with which ship the first guilty inoculum arrived.

L'il Abner would call this a single whammy, and it may have been sufficient. Perhaps a double whammy was not needed to introduce PSP to Tasmania. But the possibilities these studies reveal, the numbers of potentially troublesome candidates, and the volume of world shipping during the last century or more, all suggest that one or more additional factors might have been necessary to allow *G. catenatum* to establish itself in Tasmanian waters. Whether other factors were necessary, and of what nature they might have been, remain unknowns.

The more general point is that some algal blooms may be contingent on historical events, hence not predictable from even the most profound knowledge of ecological laws. Accidental changes in coastal waters caused by human activities provide examples of such contingencies, as in the case of Tasmanian PSP. Natural events too may provide those elements of chance which frustrate prediction. Hurricanes and anomalous circulation patterns, just two examples, have both been invoked as preludes to exceptional blooms.

Sieburth's hypothesis, like Hallegraeff's draws attention to the role contingency may play in the creation of algal blooms and has the added virtue of combining 'bottom-up' and 'top-down' arguments. The former emphasize the importance to algal blooms of nutrients and other factors favouring growth, while the latter look to the role of processes damping or preventing population increase, such as grazing or parasitism. These are essential components of the 'hard' ecological theory, and if we can get them right, will improve our predictive powers. But we should not lose sight of those serendipitous events which can enrich nature, confound our predictions, and cause harmful algal blooms as well as cholera.

Donald M. Anderson, Woods Hole Oceanographic Institution

(Cont'd from p. 6)

instead that we should define the problem as best we can, always staying on the side of science and avoiding hyperbole.

3) This brings me to a third important aspect of the Newport conference: the presentation by T. Osborn of the IOC, of the preliminary details of an international programme on Harmful Algal Blooms (HAB). Several small workshops have been held in recent years to develop the concepts of this programme, and another workshop was held immediately after the Newport conference to refine the science plan further (described on p. 3). But for many in our community, the first thing they ever heard of an international HAB programme was Osborn's presentation at Newport. It is a long and difficult road from this conceptual stage to the implementation of a multi-national, multi-disciplinary HAB programme, but the process has begun. I hope that we all can look back in 5-10 years and marvel at how isolated and parochial we all were before international coordination and collaboration became formalized as is now common in other oceanographic disciplines.

Donald M. Anderson, Woods Hole Oceanographic Institution

*A comic strip character (USA).

The First International Red Tide Newsletter

Sherkin Island Marine Station is a small private laboratory off the south-west coast of Ireland, run by Matt Murphy and his family without any State or European community funding. It was founded in 1975 by his late wife Eileen and himself.

In 1978 work began on red tides when the waters around the marine station turned brown. The young graduate volunteer biologists that year began monitoring programmes for plankton which has been carried on each year from April to November ever since.

The monitoring programme at the Station has 11 sites in the area and water samples are taken from the surface to 50 metres. Species are identified. Sherkin's monitoring has been the basis for all programmes in operation in Ireland.

In 1986 the Station organized its first international workshop and conference on the Problems of Toxic Dinoflagellate Blooms in Aquaculture. Those invited were: B. Dale, D.G. Baden, B. Mck. Bary, L. Edler, S. Fraga, I.R. Jenkinson, G.M. Hallegraeff, T. Okaichi, K. Tangen, F.J.R. Taylor, A.W. White, C.M. Yentsh and C.S. Yentsh. Proceedings are available at £12 sterling plus postage.

From that conference the idea of a quarterly *Red Tide Newsletter* evolved and the first issue appeared in January 1987. 1992 sees the publication into its fifth year.

In 1989 a second workshop and conference on Toxic Dinoflagellate blooms was held at Sherkin with E. Balech, D. Dale, M. Elbraechter, K. Tangen, V.L. Trainer, S. Fraga, G. Hallegraeff, K. Steidinger.

Matt Murphy, Director, Sherkin Island Marine Station, Sherkin Island, Co. Cork, Ireland.

Major events in the past

In the last two decades, there have been five international symposia concerning HABs. Their proceedings are listed below. Attention was focussed initially on dinoflagellates, but the scope has gradually expanded, as is reflected in the more recent titles.

1. **Boston, MA, USA, 4-6 November, 1974.** LoCicero, V.R., 1975. *Toxic dinoflagellate blooms* (Massachusetts Science and Technology Foundation), 541 pp., 45 contributions, indexed.

2. **Key Biscayne, Florida, USA, 31 October - 5 November, 1978.** Taylor, Dennis L., and Howard H. Seliger, 1979. *Toxic dinoflagellate blooms* (Elsevier-North Holland), 505 pp., 77 contributions, indexed.

3. **St Andrews, New Brunswick, Canada, 8-12 June, 1985.** Anderson, Donald M., Alan W. White, Daniel G. Baden, 1985. *Toxic dinoflagellates* (Elsevier), 561 pp., 88 contributions, indexed.

4. **Lund, Sweden, 26-30 June, 1989.** Granéli, Edna, Bo Sundström, Lars Edler, Donald M. Anderson, 1990. *Toxic marine phytoplankton* (Elsevier). 554 pp., 89 contributions, indexed.

5. **Newport, RI, USA, 28 October - 1 November, 1991.** Not yet published.

Two other related symposia are:

6. **Takamatsu, Kagawa Prefecture, Japan, 10-14 November, 1987.** Okaichi, Tomotoski, Donald M. Anderson, Takahisa Nemoto, 1989. *Red tides. Biology, environmental science, and toxicology.* (Elsevier), 111 contributions, indexed.

7. **Stony Brook, NY, USA, 27-28 October, 1988.** Cosper, E.M., V.M. Bricely, E.J. Carpenter, 1989. *Novel Phytoplankton blooms. Causes and impacts of recurrent brown tides and other unusual blooms* (Springer-Verlag), 799 pp., 44 contributions, indexed.

Future events

APRIL 92

ICES Study Group on the Dynamics of Algal Blooms, 7-9 April, Vigo, Spain. *Contact*: B. Reguera, Centro Oceanográfico de Vigo, Punta del Apio, San Miguel de Oya, Apdo. 1552, 36280 Vigo, Spain; fax: (34-86) 49 23 51.

ICES Symposium on Measurement of Primary Production from the Molecular to the Global Scale, 21-24 April, La Rochelle, France. *Contact*: ICES, Palægade 2-4, DK-1261 Copenhagen K, Denmark; fax: (45-33) 93 42 15.

ICES Working Group on Phytoplankton and Management of their effects, 27-29 April, La Rochelle, France.

MAY 92

Fourth International Conference on Ciguatera Fish Poisoning, 4-8 May, Tahiti, French Polynesia. *Contact*: P.O. Box 30, Papeete,Tahiti; tel.: (689) 41 64 64; fax: (689) 43 15 90.

Third Canadian Workshop on Harmful Algal Blooms, 12-14 May, Mont Joli, Quebec, Canada. *Contact*: P.O. Box 1000, Mont Joli, Quebec, Canada, G5H 3Z4; tel.: (1-418) 775-0596; fax.: (1-418) 775 0542.

JUNE 92

The IOC-FAO Ad hoc Intergovernmental Panel on Harmful Algal Blooms, 1st Session, 23-25 June, Paris, France.

OCTOBER 92

International Workshop on Marine Environmental Protection and Coastal Living Resources, 29 September - 3 October, Bremen Maritime Training Center, Bremerhaven, Germany. Main topics: eutrophication in semi-enclosed seas, and harmful algal blooms. *Contact*: Polarmar GmbH, Burger 20, 2850 Bremerhaven, Germany; tel.: (49-471) 973 2191; fax: (49-471) 973 2215.

LATE 93

VIth International Conference on Toxic Marine Phytoplankton, Nantes, France.

HARMFUL ALGAE NEWS

This issue was compiled and edited by Tim Wyatt and Yolanda Pazos, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Eduardo Cabello 6, 36208 Vigo, Spain. Tel.: (34-86) 23 19 30 / 23 19 73; fax: (34 86) 29 27 62

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HARMFUL ALGAE NEWS

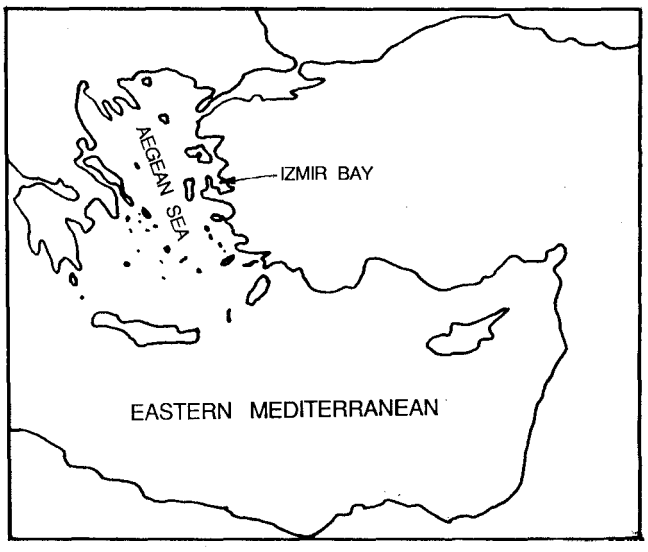
An IOC Newsletter on toxic algae and algal blooms *No. 2*

Noxious Blooms in the Bay of Izmir, Aegean Sea

Fish mortalities associated with red-tides were first reported in the Bay of Izmir in 1955. Since then, red-tides and other noxious algal blooms thought to be due to progressive eutrophication have been observed almost every year by reporters and Turkish scientists.

In January 1990, Ege University and FAO launched the first extensive research programme on these problems in Izmir Bay under the leadership of Dr. T. Koray. Information from this programme is now being applied to questions posed by abnormal fish mortalities during spring.

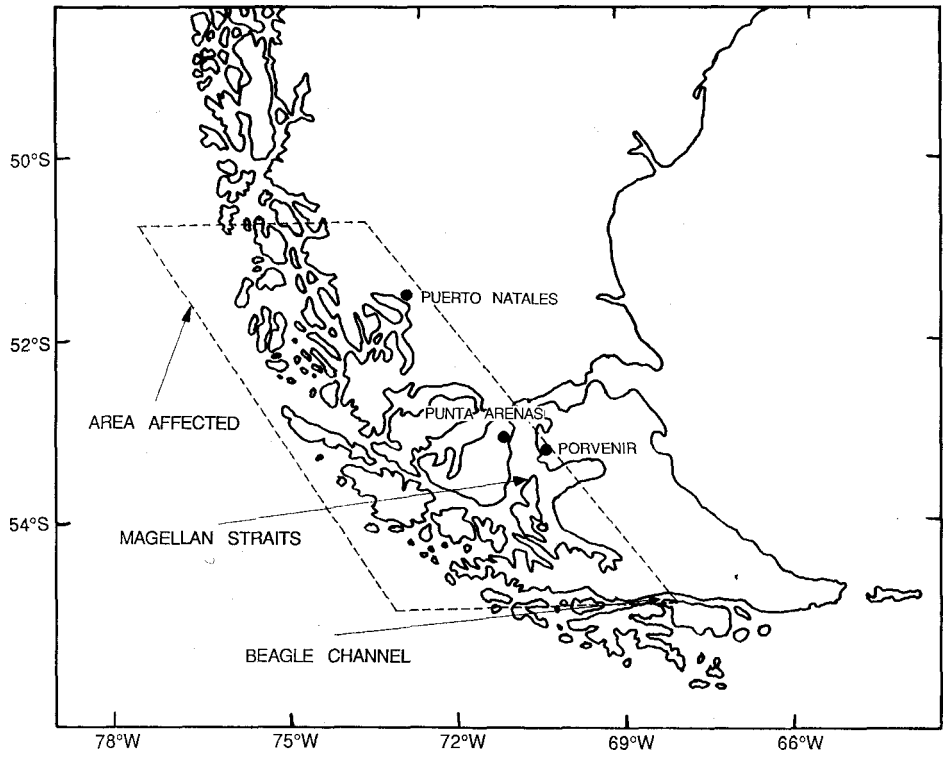
The most common species causing red-tides in this bay since 1980 are *Prorocentrum micans*, *Alexandrium minutum*, *Gymnodinium simplex*, *Noctiluca scintillans*, *Scrippsiella trochoidea*, *Mesodinium rubrum*, *Gonyaulax polyedra* and *Ceratium furca*. Among these, only *A. minutum* is known to be toxic and to be associated with bivalve and fish mortality. So far there is no clear evidence of PSP in the Bay of Izmir. Death of fishes due to this species is characterized by yellowish colouration of the bodies and gills. Levels of *A. minutum* exceed 6-10 million cells per litre when mortalities occur. During blooms in the Bay of Izmir, demersal and pelagic fishes also exhibit symptoms of anoxia. These symptoms are often seen during non-toxic blooms of the diatoms *Thalassiosira anguste-lineata* and *T. allenii* and the euglenoid *Eutreptiella gymnastica* at night. Thousands of the crab *Carcinus mediterraneus* migrate onto land at night when oxygen deficiency occurs. On the other hand, air bubbles are formed by the high rates of photosynthesis during the day in the blooms, and appear at the surface.



Although the impact of algal blooms on some fishes (mullets, sardines, anchovies, gobiids) is frequently

(Cont'd on p. 2)

Major PSP Outbreak in Chile, 1991-1992



Red tides and the accompanying threat of PSP have been a constant worry to the shellfish industry in southern Chile for at least twenty years. The mussels *Mytilus chilensis* (chorito), *Choromytilus chorus* (choro) and *Aulacomya magellanica* (cholga) and the clam *Chlamys patagonicus* (ostión) are the main risk species.

Paralytic toxins were detected in southern Chilean waters during routine monitoring in October 1991. Corresponding emergency measures were taken immediately. These include a ban on harvesting and an extension of the monitoring area. The phenomenon extended from approximately 50°45'S to the Beagle Channel in about 54°55'S latitude (see map) and was detected for the first time in the Straits of Magellan, close to the town of Punta Arenas, and in the eastern mouth of the Strait (e.g. Faro Felix).

Despite the warnings, and due

(Cont'd on p. 2)

(Cont'd from p. 1, 'Noxious blooms')
observed, little is known about which factors are responsible for the mortalities. The adjacent simple speculative model synthesizes the results of our work and offers a coherent explanation of the mixed effects of anoxia - hyperoxia - *A. minutum* and possibly PSP in the Bay of Izmir. The model illustrates how strong stratification and cell accumulation in sub-surface waters affect fish. Both anoxic and hyperoxic layers are formed respectively by the decay of sedimented cells and high photosynthetic rates and are unfavourable environments for many pelagic organisms. Consequently, they are forced to migrate into the narrow layer in which *A. minutum* dominates. Although toxins are produced in amounts too low to cause PSP, fish are stunned by exotoxins and may also feed on zooplankton which has itself concentrated the toxins (the cells stick on gills) in this narrow layer during the day. Mortality of these paralysed fish is caused by maximum oxygen deficiency at dawn.

Harvesting of mussels has been forbidden in the eutrophic inner Bay of Izmir since 1980.

Tufan Koray, Ege University, PK 24, 35102 Izmir, Turkey.

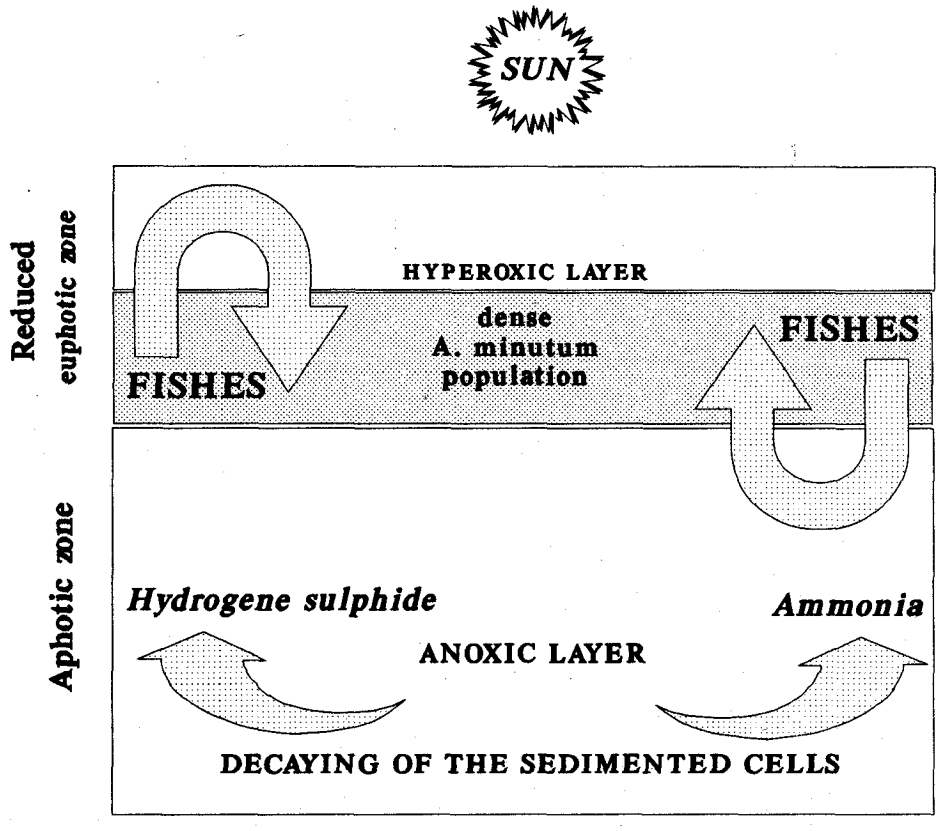


Fig. 1: A speculative model for A. minutum *fish mortality in the bay of Izmir.*

A new *Chrysochromulina* outbreak and fish kills in Danish coastal waters

Several species of the flagellate *Chrysochromulina* (Prymnesiophyceae) are now (April 1992) blooming in Danish coastal waters. The first observations were made mid April in connection with fish kills in fish farms in the south-western Little Belt. *Chrysochromulina* spp. were found in concentrations between 10 and 15 million cells per litre.

In the routine monitoring carried out 24-27 April, *Chrysochromulina* spp. were found in concentrations between 5 and 15 million cells per litre at all sampling sites in the coastal waters of Funen Island, and at several localities along the east coast of Jutland.

Several tons of young Rainbow Trout (*Salmo gairdneri*) have died in aquaculture sites, and fishermen have reported dead fish in their nets. So far, no damage to benthic fauna or vegetation has been reported.

At most of the sampling sites, the vertical distribution of the cells is uniform. Temperature in the affected areas has been 6-8°C, and salinity between 14-20 ‰. Where a halocline has built up, *Chrysochromulina* spp. was only found in low concentrations in the bottom water.

The *Chrysochromulina* community is composed of at least five species: *Chrysochromulina hirta, C. spinifera, C. brevifilum, C. ericina* and a species not yet described (det. Ø. Moestrup, Univ. of Copenhagen). No single species is dominant and the relative dominance varies in time.

Frode Knipschildt, Funen County, Department of Technology and Environment, Ørbækvej 100, DK 4000 Odense SØ, Denmark

(Cont'd from p. 1, 'PSP outbreak')
probably to the unusual duration and extension to new areas, numerous cases of intoxication have occurred (about 300) and eleven people have died. The first cases were reported in November 1991. High levels of toxicity have been detected, of the order of 10,000 microgr/100 g and more (J.C. Uribe), higher than any values previously detected in Chile. Species like the carnivorous gatropod known as 'loco' (*Concholepas concholepas*) gave values of 400 to 500 microgr/100 g in the foot muscle. The muscle of the 'ostión' (*Chlamys patagonicus*) also gave high values.

The Chilean Undersecretary of Fisheries, Andrés Couve, has initiated an educational campaign amongst the local communities, and also relaxed some restrictions on other fisheries to encourage the fishermen to diversify.

We hope to be able to provide further information on this outbreak in *Harmful Algae News* no. 3.

Georgina Lembeye, Laboratório de Microalgas, Freire 385, Casilla 221, Castro, Chile.

ETI - a computer approach to taxonomy and identification of organisms

Taxonomy and systematics are the sciences concerned with the identification, description and classification of organisms. Exact organism identifications are of the utmost importance, not only in building knowledge of the (history of) biodiversity and to preserve wildlife, but also for monitoring changes in the biological environment, identifying causes of pests, diseases, and toxic algal blooms in fisheries, agriculture and mariculture management, etc.

When environmental problems arise, or pests or diseases occur, quick answers are required, but identifying organisms is a time-consuming job requiring expert knowledge. Though taxonomy and systematics form the actual basis for all biological sciences, taxonomy is on the wane and skilled taxonomists are rapidly decreasing in number, leading to serious questions in the scientific community concerning the preservation of this knowledge (*Nature*, August 1990). In developing countries there is a need for directly accessible taxonomic information and expertise, for example, to tackle agricultural problems (pests, diseases) or for management purposes (wildlife, aquaculture, fisheries).

The Expert-center for Taxonomic Identification

The Expert-center for Taxonomic Identification (ETI) will concentrate, preserve and promote taxonomic knowledge and provide the possibilities to answer taxonomic, biogeographic, and ecological questions quickly and efficiently. Present-day computer equipment offers the possibility to modernize taxonomy as it allows efficient handling of very large multimedia databases. ETI is developing a database system that stores taxonomic descriptions, drawings, photographs, video and sounds, as well as species-related information such as distribution maps, ecological characteristics, information on pests, diseases etc. The system makes fast retrieval of the data possible on a 'need-to-know' basis. Identification will be guided by an 'expert system', using both text and images as tools, thereby allowing efficient use by experts as well as laymen. This system makes use of multiple entry keys. This is a great advantage to the traditional dichotomous keys, as the (inexperienced) user can start with any character available on the specimen, instead of being forced to use characters that may be difficult to interpret or may be damaged. Moreover, the system will offer help at every step in the identification process, and therefore provides a maximum of taxonomic expertise to the user.

The ETI taxonomic databases will be stored in the main frame computer of the University of Amsterdam, while Topic Oriented Subsections (TOS) will be made available on CD-ROM or other media which can be run any time any place on a simple microcomputer (Macintosh and IBM compatibles). A TOS may cover various taxonomic groups, areas, or applications of taxonomy. In future, on-line connection to the ETI databases will be possible.

ETI will be developed in three phases: (i) a pilot phase during which the system will be designed and tested using two relatively small marine groups – namely pteropods and chaetognaths, and a larger group – birds; (ii) a building phase during which more data on various groups will be entered into the system and teaching programmes will be developed; and (iii) a consolidation phase during which the database will be expanded further and expertise made available to users. From the start the ETI organization will set up an international network of taxonomic experts, which will serve as the supplier of taxonomic data and can make free use of the ETI system.

With UNESCO support, an international 'protist network' started in December 1991, aiming for an ETI programme covering all protists.

Government funding allows us to bring in necessary expertise and equipment to expand and cover more taxonomic groups as well as related information. It is the intention of ETI to cooperate with other data centers in the field of taxonomy by promoting an exchange of know-how and databases.

In order to build up taxonomic databases we call upon experts all over the world. We intend to have a data input programme available mid-1992 and invite all taxonomists to input data of their specific groups. Inputted data will be accompanied by a full reference to the author. Optimization and organization of the data will be done by the ETI staff. Contributors to ETI will receive a free copy (on CD-ROM) of their taxonomic group in return for their much appreciated services. Also, they will receive a reduction in price on other ETI taxonomic programmes. ETI taxonomic programmes will be made available to users for the nominal fee of the costs of production.

Location and organizational structure

ETI is based at the Institute of Taxonomic Zoology of the University of Amsterdam (The Netherlands). It is financially supported by the Dutch Government (Ministry for Science and Education, Science Policy Department), the University of Amsterdam and UNESCO, through its Promotion of the Marine Sciences Programme (PROMAR).

ETI is a non-profit organization committed to the preservation, concentration and distribution of taxonomic knowledge.

We hope that taxonomists will be interested in helping to build up an ETI programme on their precious groups! Please write to us for a questionnaire.

Further information can be obtained from an article appearing in *BINARY 3/92* entitled 'Computer Aided Taxonomy'.

Peter H. Schalk, ETI, PO Box 4766, N–1009 AT Amsterdam, The Netherlands

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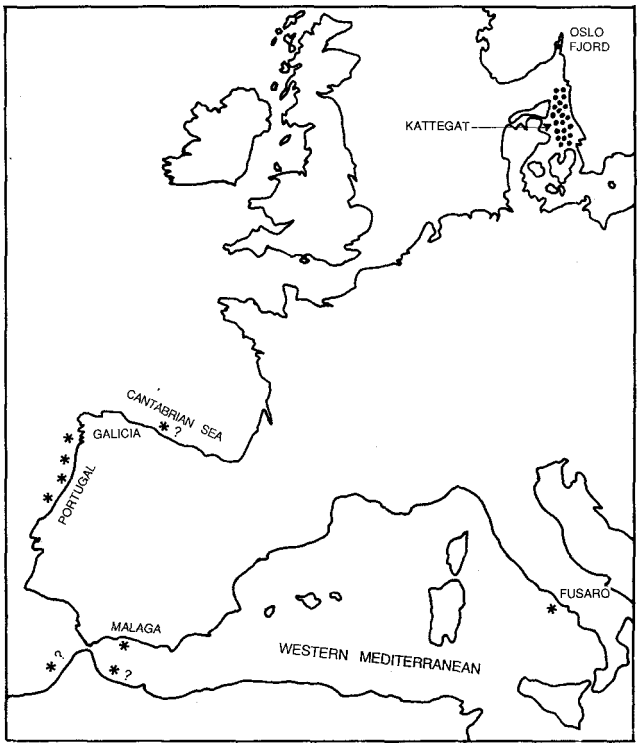
Gymnodinium catenatum in Europe

by Tim Wyatt

Early cases of mussel poisoning in Europe, going back to 1689 AD, were reviewed by Chevalier and Duchesne[1] in 1851. This may be the earliest publication in which a causal link between mussel toxicity and algal blooms was proposed. In some of the cases summarized there, it is reasonably clear that the patients suffered the kind of paraesthesia characteristic of paralytic shellfish poisoning (PSP). The only phytoplankton species in northern European waters today which produce paralytic toxins are members of the *Alexandrium* group (*tamarensis* and *minutum*). But there is at least a possibility that PSP in this region prior to 1700 AD was due to *Gymnodinium catenatum*. This suggestion stems from the fact that *G. catenatum* resting cysts were deposited in large numbers in Kattegat sediments (between Denmark and Sweden), over an area of about 20,000 km^2, from about 2000 until about 300 years ago[2], indicating that the species was then a regular, even prominent component of the phytoplankton there. Apart from a record in the Oslo Fjord, the further distribution of *G. catenatum* in that time interval is unknown.

The present range of *G. catenatum* in European waters extends only as far as northern Spain, where it was first recorded in 1976. Its cysts were not found in sediment cores covering the last 9000 years in Galicia by Margalef[3]. The species has been known in Portugal (between Espinho and Obidos) since 1986[4], but was not detected in Obidos between 1958 and 1962[5]. There are two published Mediterranean records. It has been present in Fusaro Lagoon in Southern Italy since 1985 when sampling began there[6] and has been reported once from Málaga waters in Southern Spain[7]. There are unconfirmed reports that it occurs in the Cantabrian Sea, and in Moroccan inshore waters too.

G. catenatum was first described in 1943 from material collected in the Gulf of California (western Mexico), and has since been found on both sides of the Pacific and Atlantic Oceans and in both hemispheres[8]. As pointed out in *Harmful Algae News* no. 1, the case for its introduction into Tasmanian waters is strong. But Gaines[9] has argued that the distribution pattern is natural. He suggests that 'synergism between ecological variables' has permitted the species to bloom, with the implication that it is autochthonous to all these regions but was formerly very rare.



Outline of Western Europe and Maghreb coast showing:

* *Distribution of Gymnodinium catenatum*

*? *Unconfirmed reports*

▦ *Distribution of subfossil cysts in sediments*

Nordberg's study[2] provides some indications which may help to resolve the issue. Sediments deposited in the Kattegat since the *G. catenatum* extinction there are coarser than before, and the mean grain size now exceeds that of *G. catenatum* cysts. Its sudden decrease and disappearance about 300 years ago might therefore have been due to the cyst being transported elsewhere (and deposited in the Norwegian Deep or beyond) by a change in mixing and transport patterns. Hydrographic conditions in the Kattegat are still seasonally suitable for *G. catenatum*, but deprived of an inoculum or seed bed, the species cannot appear there. Nor, if the sedimentation regime

(Cont'd on p. 5)

Graduate and postdoctoral course

by Stefano Guerzoni

This course, entitled 'The impact of algal growth on coastal waters and lagoons', will be held at the Centro Marino Internazionale, Lungomare Eleonora D'Arborea 22, 09072 Torregrande, Oristano, Italy, from 29 June to 19 July 1992. The course Director is Professor Jean Michel Robert (Marine Biology Laboratory, University of Nantes, France).

Briefly, S. Giusta is a lagoon in the western part of Sardinia Island, where recently (in the past 2-3 years) several episodes of anoxia with massive fish-kills were experienced. Very few (if any) studies were done up to now, so there is no information about the causes. Lagoonal fish-production was reduced drastically recently and, even if no harmful algal blooms were recorded up to now, we are interested in following the future development of the summer blooms. The S. Siusta area will be used as an 'open laboratory' during the course for practical and field work.

For further information, contact: Rosalba Murgia, Istituto di Geologia Marina, Via Zamboni 65, 40127 Bologna, Italy. Tel: (39-783) 22032; fax: (39-783) 22002. The registration fee is 150,000 liras (US$ 120) for school and 500,000 liras (US$ 400) for food and lodging (full board for 20 days).

(Cont'd from p. 4)

continues to prevent local accumulation of its cyst, would its accidental reintroduction be likely to succeed. Thus the absence of *G. catenatum* cysts in Spanish and Tasmanian sediments until very recently suggests the species was introduced in both cases.

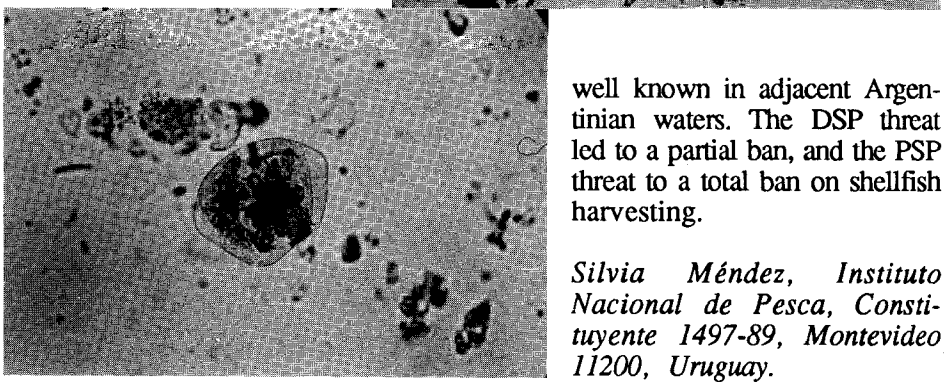
The issue is not exactly between introduction and autochthony, but between 'hierarchical' (long distance) and 'neighbourhood' or local diffusion. A species range can normally be viewed as the balance between local extinctions and recolonizations from local populations. If we accept that available evidence indicates no European source of recolonization for *G. catenatum* has existed for three centuries, then its appearance in Europe in 1976 might be attributable to transoceanic transport, possibly from the western Atlantic. A potential vector would have been the Spanish distant water fishing fleet, based in Galicia, which began operating in Argentinian waters precisely in 1976. *G. catenatum* was first recorded in Mar de Plata in 1962[10].

But another possibility exists. A recent attempt to find *G. catenatum* only a few kilometres from the Galician coast was unsuccessful[11]. Most phytoplankton sampling in the Moroccan sector of the Atlantic has been some distance from the coast, and close inshore waters remain unexplored. If Moroccan inshore waters have provided a refuge for *G. catenatum* since its extinction in the Kattegat, it is not necessary to postulate trans-Atlantic transport. A search for cysts in suitable sedimentary horizons might support a more parsimonious hypothesis.

(1) *Ann. d'Hyg. Publ. et de Méd. Lég.*, 45, Paris (1851).
(2) *Geol. Inst. Publ.*, A65, Göteborg (1989).
(3) *Invest. Pesq.*, 5 (1956).
(4) Franca and Almeida, in: Okaichi et al., *Red tides. Biology, environmental science, and toxicology*, Elsevier (1989).
(5) *Notas e Estudos Inst. Biol. Mar.*, 34, Lisboa (1968).
(6) *Rapp. Comm. Int. Mer Médit.*, 31 (1988).
(7) Bravo et al. in: Granéli et al., *Toxic marine phytoplankton*, Elsevier (1990).
(8) *J. Plankton Res.*, 10 (1988).
(9) *J. Shellfish Res.*, 8 (1990).
(10) *Rev. Mus. Arg. C. Nat. "B. Rivadavia" C. Zool.*, 7 (1962).
(11) Fraga et al, *Rhode Island Conf.* (1991), in press.

Update from Uruguay

At the time of the Rhode Island Conference, the entire coast of Uruguay was closed to shellfish harvesting. The ban was lifted in November 1991. Since then, both DSP and PSP have been detected. The former occurred in January 1992 and was associated with *Dinophysis acuminata* concentrations up to 6000 cells l^{-1} in some zones like La Paloma (about 200 km east of Montevideo). An outbreak of PSP occurred in February 1992, associated with *Alexandrium catenella* and *Gymnodinium catenatum*, with maximum concentrations respectively of 40 x 10^3 and 90 x 10^3 cells l^{-1}. This is the first time these two species have been recorded in Uruguayan waters, though both are well known in adjacent Argentinian waters. The DSP threat led to a partial ban, and the PSP threat to a total ban on shellfish harvesting.



Top: Dinophysis acuta, *vegetative cell.*
Bottom: Dinophysis acuta, *cyst. Photos: Y. Pazos.*

Silvia Méndez, Instituto Nacional de Pesca, Constituyente 1497-89, Montevideo 11200, Uruguay.

PSP in Morocco

Algal blooms commonly cause problems in Moroccan waters, both on the Atlantic and Mediterranean coasts. Red tides are observed several times a year in various localities, and during these periods the consumption of mussels, oysters and other molluscs is forbidden. These problems may increase in the future since there is now a rapid development of aquaculture taking place involving both national and international investors.

There is a large modern aquaculture complex in Nador lagoon, and another covering 2000 hectares began in February this year in the Mouloaya estuary near Oujda.

Another is planned for Larache on the Atlantic Coast South of Tanger. In addition, there are important resources in the Wadi Bou Regreg near Rabat.

Scientific studies of the taxonomy and ecology of potentially harmful algae in Moroccan waters are therefore urgently needed.

M. Baddyr, Institut Agronomique et Vétérinaire Hassan II, CHA, B.P. 121 Aiz Melloul, Agadir, Morocco

Note from the Editor: Dr. Baddyr has just sent us a copy of an article by A. Tber published in Maghreb Vétérinaire, *vol. 2, p. 15-19 (1983), which reports PSP toxicity levels in mussels sampled in 1982 at various Moroccan localities. The highest levels, up to 4880 M.U./100g, were recorded at sites near Agadir and Essaouira in samples collected in October.*

So far as we are aware, Moroccan waters are not marked positive in any chart showing the worldwide distribution of PSP toxins.

Red Tide Research Programme in China

China now experiences a broad range of harmful algal blooms including those that endanger humans (e.g. causing paralytic shellfish poisoning), others that kill farmed fish and shrimp, and still others that alter the general aesthetics of coastal waters due to the noxious character of the blooms. In the past, Chinese red tide activities have generally been restricted to monitoring and descriptive field investigations. A newly established, high priority national red tide research programme includes plans to characterize the taxonomy, physiology, ecology and toxicology of species indigenous to Chinese coastal waters.

Chinese research emphasis on red tides has grown dramatically in recent years, in parallel with the perceived expansion of the phenomenon and its impacts. Nationally coordinated projects were initiated in 1987, and again in 1988 to investigate the East and South China Seas. A significant development occured in 1990 when red tides were given 'major' project status – the highest possible research ranking obtained by only a few projects throughout all scientific disciplines. The resulting four-year project is headed by Dr. Qi Yu-zao, President of the Institute of Hydrobiology, Jinan University and collaborating investigator on the NSF of China proposal. The national red tide programme involves eight institutions and universities, and over 100 scientists. The institutions include: the Institute of Hydrobiology, Jinan University; the South China Sea Branch, State Oceanic Administration of China (SOA); East China Sea Branch, SOA; Third Institute of Oceanography, SOA; the Institute of Oceanology, Academica Sinica; the South China Sea Institute of Oceanology, Academica Sinica; the Institute of Environmental Science, Zhongsan University; and the South China Sea Institute of Fisheries of the Chinese Academy of Fisheries.

There are four research priorities in this national programme: (i) biological basis of red tides (taxonomy, cultivation, life cycles, toxicology); (ii) experimental ecology studies (physiological requirements, growth characteristics, enclosed marine ecosystem experiment); (iii) monitoring (three-year monitoring programme at two sites in the South and East China Sea, plus major coastal cruises); (iv) mathematical modelling.

Qi Yu-Zao and Hong Ying, Institute of Hydrobiology, Jinan University, Guangzhou, China; fax: (86-20) 5516941.

What the haptonema is for

by Ralph A. Lewin

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M. Kawachi and colleagues (*Phycologia* 30, p. 563-573, 1991) have neatly solved (or partly solved) a problem that has worried phycologists for years: why certain chrysophytes have a haptonema.

This organelle is just visible by light microscopy. It is a filament something like a flagellum extending between the paired cilia of certain unicellular algae, but is clearly not involved in swimming.

Short haptonemata are little more than spurs: long ones may exceed the cell in length, and are normally coiled. People had postulated that a haptonema might be involved in feeding, but many of us discounted this idea because haptophytes are generally photosynthetic. Little did we suspect what dark deeds could be done by such little plants.

Kawachi *et al.* (*op. cit.*) show one prymnesiophyte (the class name now preferred) uses the haptonema to catch and eat bacteria and other little morsels. The species they looked at was *Chrysochromulina hirta*. As the cell swims, the organelle is extended straight ahead. Small particles stick to it, and are caused to slither down its surface towards a site some micrometres from the base. After a few have accumulated in a kind of bacterial bolus, apparently held together by slime, the lump is sent back up to the haptonemal tip. Seconds later, the whole filament curves, bending back on itself, and conveys its meatball to a buccal area. There it is ingested into the cell. The haptonema then straightens out again and recommences fishing.

Algae can no longer be regarded simply as primary producers, because even among the apparently vegetative components of phytoplankton pastures there are many facultative predators. Some, such as the colourless dinoflagellates (for instance the giant cells of *Noctiluca*), are obligate predators, but in the past few years we have learned that many smaller dinoflagellates, evidently with good functional photosynthetic apparatus, can also use solid food by extracellular or intracellular digestion of prey cells.

Among predatory algae we now have to include prymnestiophytes such as *Chrysochromulina* which are as small as 5 micrometres in diameter. But what happens in nature? Or does it merely constitute a source of midnight snacks or supplementary food, tiding the algae over periods when photosynthesis is precluded at night, or when the cells sink below the euphotic zone?

(The original article has been slightly shortened for this newsletter.)

ICES-IOC Study group on the dynamics of harmful algal blooms

The ICES-IOC Study Group on the Dynamics of Harmful Algal Blooms met in Vigo, Spain, 7-9 April 1992, to design and propose a programme for studying the dynamics of harmful algal blooms. This multinational, multidisciplinary research programme is intended to include modelling, experimental and field components. The research programme requires investigators from several countries to work simultaneously in the same localities, in order to have the necessary variety of expertise. As well, sequential field studies in different localities will help to resolve the worldwide variation in the population dynamics of harmful algae.

Several localities were suggested as appropriate for a comprehensive study. Among the proposed localities the following three were chosen; Iberia (Spanish-Portuguese Atlantic Coast); Gulf of Maine (USA); Skagerrak/Kattegat (Scandinavia). Furthermore the research programme includes a comparative *Heterosigma* study and mesocosms experiments. The latter will be independent as well as supportive to the field experiments.

This research programme on the dynamics of harmful algal blooms will be a major component of the "Ecology and Oceanography" element of the IOC-FAO Harmful Algal Bloom Programme, outlined in *Harmful Algae News* no. 1. The proposed research programme will be presented to the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms in June 1992 (see below), and published as an *ICES Research Report* later this year.

The development of the IOC-FAO Harmful Algal Bloom Programme continues

The objective of a planning meeting 10-11 April in Vigo, Spain, was to continue the planning process of an international programme to deal with the problems of harmful algae. The broad outline of the programme was completed at a workshop in Newport, Rhode Island, USA, 2-3 November 1991.

The main objectives were to review the broad outline of the programme, to identify staff and resource requirements of a secretariat for the programme, and to prepare recommendations for presentation to the first session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms. Furthermore the first issue of IOC's *Harmful Algae News* was reviewed and the strategy for the development of an international society on harmful algae was discussed as mentioned elsewhere in this issue.

With respect to the present newsletter, it was decided that an editorial support group composed of contributing editors, representing different geographical regions of the world, should be formed as soon as possible, and at the latest in the fall of 1992. One of the group's tasks will be to provide the Editor with material from their region. It was suggested that each 'sub-editor' in turn could be responsible for an issue in order to give a comprehensive overview of the situation in the different regions of the world.

The first session of the Intergovernmental Panel on Harmful Algal Blooms will take place 23-25 June 1992 in Paris, and the ad hoc planning committee will present the following recommendations to the Panel: (i) to accept the programme plan outlined in the *IOC Workshop Report* No. 80; (ii) to establish a secretariat to support coordination and implementation of the programme; (iii) to provide support for continued planning and implementation of the programme; (iv) with respect to the considerations of the ICES-IOC study group to design and propose a programme for studying the dynamics of harmful algal blooms, Member States should consider the ICES-IOC study group report, review it at a national level, and provide the sustained resources to implement the activities; (v) the concerns expressed in past programme documents about long-term trends in harmful algal blooms occurrences and data sets should be brought to the attention of the IOC GOOS Support Office for inclusion in the development and implementation plans for GOOS (Global Ocean Observing System).

Those who would like to receive the outline of the Harmful Algal Bloom Programme (*IOC Workshop Report* No. 80) can send a request to IOC.

INTERNATIONAL SOCIETY

At the IOC-SCOR Workshop on Programme Development for Harmful Algal Blooms held in Newport (USA) following the Vth Conference on Toxic Marine Phytoplankton (November 1991), it was recommended that 'it would be appropriate to form an international (harmful algal bloom) society that would be independent of any one country, but attached to an international body such as the International Council of Scientific Unions (ICSU)'. This recommendation was supported at the recent IOC ad hoc planning programme meeting held in Vigo, Spain. We hereby solicit suggestions, ideas, proposals and assistance from those of you interested in helping to prepare the terms and bylaws of this society for presentation to attendees at the VIth Conference on Toxic Marine Phytoplankton (1993) to be convened in Nantes, France. Your input will help to establish the strategy and working group assignments that we will follow in developing the proposal for this society. Please send your comments etc. to:

Ted Smayda
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882-1197
USA
Tel: (1-401) 792 6171
Fax: (1-401) 792 6682

Early red tides

Many travellers through the centuries have left accounts of red tides from all parts of the world. Here are two extracts from a book by E. Olafsson and B. Pálsson (*'Reise igennem Island foranstaltet...* ') published in 1772.

'This phenomenon is known in other countries but is rare in Iceland. In 1712 it was observed in Reykjaströnd. The sea was blood-coloured off the coast quite far out, so that the oars of fishing boats became bloodstained, and that "blóðkifrar" (coagulated blood), to use the word of the writer of the annales, indicating his belief that it was blood, settled on the seaweed and stones on the beach. Much earlier, or in 1649, the same was observed two miles offshore in Northwest Iceland and now again in Seydisfjördur and western Alftafjördur. In the early morning that day the sea in these fjords was blood-red but the night before it had been fiery red so there must have been some kind of phosphorescence occurring. It cannot be verified whether this was caused by some marine insects or plants...' (para. 84).

'Some time in 1638 fishermen observed "blood in the sea" off the east coast of Iceland. It drifted towards the shore in wide stretches or current bands. This is only mentioned here in reference to what has previously been reported (on para. 84). It is however possible that when whales get into a fight in the sea, especially when killer whales by the thousands attack the huge toothless (sei) whales and tear them to pieces, that the sea will become bloodstained for miles. This might even be one other way to explain the red colour of the sea' (para. 846).

It was common amongst seamen at that time to attribute red water to the menstrua of whales. This is the only account I know in which a fight between whales is suggested.

Gunnar Stein Jónsson, Hollustuvernd Ríkisins, Box 5285, IS-125 Reykjavik, Iceland.

Future events

JUNE 92

The IOC-FAO Ad hoc Intergovernmental Panel on Harmful Algal Blooms, 1st Session, 23-25 June, UNESCO, Paris, France.

AUGUST 92

Environmental Assessment in Developing Countries, 19-22 August, World Bank HQ, Washington DC, USA. *Contact*: M. Voland, Box 70, Belhaven, NC 27810, USA.

12th International Diatom Symposium, 30 August - 5 September, Renesse, Netherlands. Monitoring, eutrophication, acidification and ecotoxicology are expected to be among the issues discussed. *Contact*: AquaSense, PO Box 41125, 1009 EC Amsterdam, The Netherlands. Tel.: (31-20) 592 2244; fax: (31-20) 592 2249.

SEPTEMBER 92

40th Annual Congress of the Society for Medicinal Plant Research, 1-5 September, Trieste, Italy. This congress will include a one-day session devoted to algal toxins. *Contact*: The Congress Secretariat, Via S. Nicolò 14, 34121 Trieste, Italy. Tel.: (39-40) 368343; fax: (39-40) 368808.

Nutrient regeneration and primary production - closing the loop (Society of General Microbiology), 2-4 September, Trinity College, Dublin, Ireland. *Contact*: M. A. Lock, School of Biological Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, UK.

Workshop on red tides and mortality of marine organisms in the Caribbean, 17-19 September, Instituto Oceanográfico de Venezuela, Universidad de Oriente, Cumaná, Venezuela. *Contact:* Elvira Ferraz-Reyes, President of the Organizing Committee, CONCIT, Apartado 70617, Los Ruices, Caracas, Venezuela. Tel.: (58–2) 2390433; fax: (58-2) 2398677.

OCTOBER 92

International Workshop on Marine Environmental Protection and Coastal Living Resources, 29 September - 3 October, Bremen Maritime Training Center, Bremerhaven, Germany. Main topics: eutrophication in semi-enclosed seas, and harmful algal blooms. *Contact*: Polarmar GmbH, Burger 20, 2850 Bremerhaven, Germany. Tel.: (49-471) 973 2191; fax: (49-471) 973 2215.

NOVEMBER 92

8th International IUPAC Symposium on Mycotoxins and Phycotoxins, 8-12 November, Stouffer Presidente Hotel, Mexico City, Mexico. *Contact*: Symposium Secretariat, VIII International IUPAC, Insurgentes Sur 3700 - C, Apartado Postal 101-63, 04530 Mexico DF, Mexico. Tel: (525) 550 58 83; fax: (525) 548 82 07.

LATE 93

VIth International Conference on Toxic Marine Phytoplankton, 18-22 October, Nantes, France. *Contact:* Cité des Congrès, 5 rue Valmy, 44041 Nantes Cedex 10, France. Tel: (33) 40 37 41 30; fax: (33) 40 37 40 73.

HARMFUL ALGAE NEWS

This issue was compiled and edited by Tim Wyatt and Yolanda Pazos, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Eduardo Cabello 6, 36208 Vigo, Spain. Tel.: (34-86) 23 19 30 / 23 19 73; fax: (34 86) 29 27 62

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 3

Eight die in Philippines from red-tide molluscs

A state of emergency has been declared by Philippine President Fidel Ramos in Manila and four other provinces, due to the presence of red tides in various coastal regions and as a consequence of eight deaths caused by eating contaminated mussels.

Since June of this year, Philippine health authorities have banned the consumption of mussels, clams, and other bivalves on account of the extensive blooms of *Pyrodinium bahamense* which have coloured coastal waters red. Despite the ban, eight people have died and 150 have been hospitalized after eating products contaminated with this microorganism.

The red tide covered about 90% of Manila Bay along the coasts of Batàn, Papanga, Bulacan and Cavite.

The authorities state that fishermen in the zones where the consumption of mussels is prohibited are undergoing hardship due to a sudden fall in fish sales. The declaration of a state of emergency allows increased government aid to those affected.

Translated from Voz de Galicia *(a Spanish newspaper) of 5 July 1992. Source EFE (news service), Manila.*

Editor's note:
The dinoflagellate *Pyrodinium bahamense* has caused paralytic poisoning in Papua New Guinea, Brunei Darussalam, Sabah, and Guatemala, as well as in the Philippines, most commonly via shellfish but also via plankton-eating fish. Cases of PSP elsewhere in the western Pacific may be due to the same species. Jay Maclean of ICLARM(1), has pointed out 'the exact coincidence of major toxic red tides [in the western Pacific] between 1972 and 1987 with ENSO (El Niño-Southern Oscillation) events'.

Pyrodinium red tides were the topic of a management and training workshop held in Brunei Darussalam in May 1989. The proceedings are published as ICLARM contribution Nº 585(1).

(1) Hallegraeff, G.M., J.L. Maclean (editors), 1989. Biology, epidemiology and management of Pyrodinium red tides. (Fisheries Dept., Brunei Darussalem, and ICLARM, Philippines).

Artificial respiration saves two from fatal PSP in Canada

In April 1992, a party of seven were fishing in Kingcome Inlet, British Columbia, approximately 250 km north of Vancouver. On 19 April butter clams (*Saxidomus giganteus*) and cockle clams (*Clinocardium nuttalli*) were harvested from Moore Bay for an evening feast the following day. On the morning of the 20th a 54 year-old man decided to have some for breakfast. He threw them on hot coals just long enough for them to open slightly. He offered a butter clam to a 31 year-old companion who, after swallowing a "thumbnail-sized" bite (including the siphon tips) of a butter clam, decided that he didn't want the rest of it. The older man ate five in the period that the younger man went to fetch water.

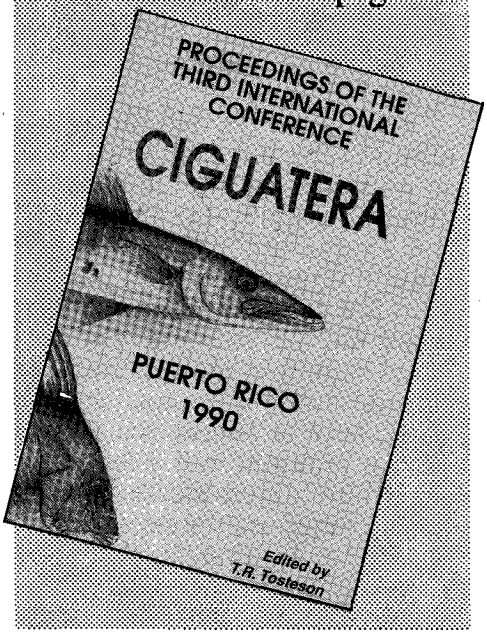
Both began to experience tingling and numbness of the lips and mouth (paresthesia) within several minutes ("like having gone to the dentist") but not on initial contact, the effect spreading to their faces and fingers. Within 10 minutes the older man collapsed on the ground, vomiting, and the younger man was becoming severely paralysed. By 15 minutes the latter was unable to move and by 20 minutes was completely paralysed, unable to breath or see (eyelids closed), total paralysis only coming later for the other (fifteen minutes into the flight). Throughout the whole episode both men were able to hear, including discussions among their friends as to whether there was any point in continuing artificial respiration!

Resuscitation was begun when it was noticed that they were turning blue, although showing no struggle for breath. Fortunately their companions knew industrial first aid and also had a radio. Coast guard helicopters picked them up after 45 minutes and took them first to a nearby town, Port NcNeil, where they were treated for 6 hours, being intubated and given a gastric lavage. Then they were flown to Vancouver where they were connected to ventilators at Vancouver General Hospital. It was found that the older man had aspirated into one lung, a common problem with vomiting while becoming paralysed, the younger one also having done so to a much lesser extent. Atrial fibrillation was also present initially in the older man, probably the results of stress. Both patients were sedated.

(Cont'd on p. 5)

Now available ...

see page 2



Cholera and the environment

For 30 years the search has gone on for an environmental reservoir for cholera to help explain seasonal oscillations. Dr Ventura and colleagues[1] demonstrate that the growth of *Vibrio cholerae* non -O1 on plant samples in sewage lagoons is associated with the seasonality of cholera in Peru. Colwell and her colleagues[2] have also detected *V. cholerae* O1 on marine life, in non-culturable, viable states, and have demonstrated that this reservoir is sensitive to environmental changes.

Under unfavourable conditions, cholera bacteria become dormant, re-emerging to an infectious state with warming and nutrient availability. 'Hibernating' forms of *V. cholerae* co-exist with a wide variety of algae and plankton[3][4], which are also responsive to changes in temperature, pH, salinity, and nutrition. Climatologists report unusually large algae and plankton blooms at sea, and plankton sampled in the harbour near Lima, Peru, are contaminated with *V. cholerae*[5].

Phytoplankton and algae help modulate our climate[6]. They absorb atmospheric carbon (2 of the 7 billion metric tons released annually) and give off oxygen through photosynthesis; they scatter heat and light; and, through emission of volatile dimethylsulphide, help seed rain clouds. Marine plants are thus 'fertilized' by atmospheric CO_2 and by warming, and atmospheric CO_2 is increasing (now about 350 parts per million), mainly from fossil fuel combustion. Pacific waters began warming in late 1990[7].

V. cholerae O1, biotype El Tor, serotype Inaba, may have arrived in the Americas with the bilge or ballast of a Chinese freighter[8], and the studies of Drs. Faruque and Alberts with DNA probes[9] suggest "genetic identity" for the strains causing epidemics in Bangladesh and the Americas. The unexpected intensity of the outbreak, which penetrated cities and towns along the Pacific coast in January and February 1991, is consistent with multiple entry points from marine life blooms, with fish, molluscs, and crustacea as vectors. The internal dissemination throughout South and Central America reflects the vulnerability of host defenses, inadequacies in sanitation, clean water, and fuel supply, as well as the uncontrolled urbanization (eg, Lima's shanty-towns) generated by the economic collapse of the 1980s.

There is now convincing evidence for an environmental reservoir for cholera. Alterations, due to climatic change, in the distribution of water-based and vector-borne diseases have been predicted[10][11], and the pattern of cholera in the Americas may represent the first detectable impact. Of the greenhouse gases, carbon emissions constitute 50%; and an international treaty to limit worldwide release was on the agenda for the UN Conference on the Environment and Development in Rio de Janeiro in June 1992. The changing distribution of old diseases (and the emergence of new ones) makes it essential that public health professionals and scientists participate centrally in planning developments.

By Paul R. Epstein, Cambridge Hospital, Harvard Medical School, Cambridge, Massachussetts 02139, USA.

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(1) Ventura, G. et al. *Lancet* 1992; 339: 937-938.
(2) Huq, A., Colwell, R.R. et al. *Appl. Environ. Microbiol.* 1990; 56: 2370-73.
(3) Islam, M.S. et al. *J. Trop. Med. and Hyg.* 1990; 193: 133-39.
(4) Tampin, M.L. et al. *Appl. Environ. Microbiol.* 1990; 56: 1977-80.
(5) Tampin, M.L.; Parodi, C.C. *Lancet* 1991; 338: 1216-17.
(6) Williamson, P.; Gribbin, J. *New Sci.* 1991; March 16: 48-52.
(7) Kerr, R.A. *Science* 1992; 255:402.
(8) Anderson, C. *Nature* 1991; 354:255.
(9) Faruque, S.M.; Albert, J. *Lancet* 1992; 339:740-741.
(10)WHO Task Group. *Potential health effects of climatic change.* Geneva: World Health Organisation, 1990.
(11)Leaf, A. *N. Engl. J. Med.* 1989; 321: 1577-83.

Editor's note:
From The Lancet *(9 May 1992), Vol. 339: 1167-8, with permission. This aspect of algae, i.e. carrying the potential to cause severe and widespread harm to large communities, has not emerged in any of the toxic phytoplankton conferences.*

International Workshop on Ciguatera Management

This workshop will be held 13-16 April 1993 on Bribie Island near Brisbane, Australia. The event, sponsored by the Australian Fisheries Research and Development Corporation and the Queensland Department of Primary Industries, will focus on current research that has implications on the management of ciguatera. It will include sessions on: *(i)* chemical and immunological aspects of the detection of ciguatera toxins, *(ii)* pharmacology and treatment, *(iii)* origin, and *(iv)* clinical aspects and epidemiology.

The workshop will include talks by invited speakers, discussion sessions and poster presentations. Further information can be obtained from: Workshop Secretariat, Southern Fisheries Centre, PO Box 76, Deception Bay, Queensland 4508, Australia. Tel: (61-7) 203 1444; fax: (61-7) 203 3517.

New publication

Now available are the *Proceedings of the Third International Conference on Ciguatera, Puerto Rico, 1990*, edited by T.R. Tosteson and published by Polyscience Inc.; ISBN 0-921313-35-2; US $60 plus shipping and handling charges. Those interested may send a request to: Polyscience Publications, 44 Seize Arpents, PO Box 148, Morin Heights, Quebec, Canada J0R 1H0. A limited number of copies are available for free to students, scientists and managers in developing countries, from: IOC Secretariat, att. Henrik Enevoldsen, UNESCO, 1 rue Miollis, 75732 Paris Cedex 15, France.

Monitoring in Belfast Lough

Belfast Lough in Northern Ireland is a semi-enclosed, sheltered tidal inlet. The River Lagan, which enters the upper lough is heavily polluted with urban, industrial and agricultural waste. There are power stations on the south shore of the upper lough and on the north shore of the lower lough. The south side is a favoured residential area and has a high BOD (biological oxygen demand) requirement caused by accumulation of domestic wastes and the north has an industrial and residential mix (Carter, 1988). There are commercially harvested mussel beds along parts of both shores (Fig 1). No other shellfish are taken from the waters within the lough.

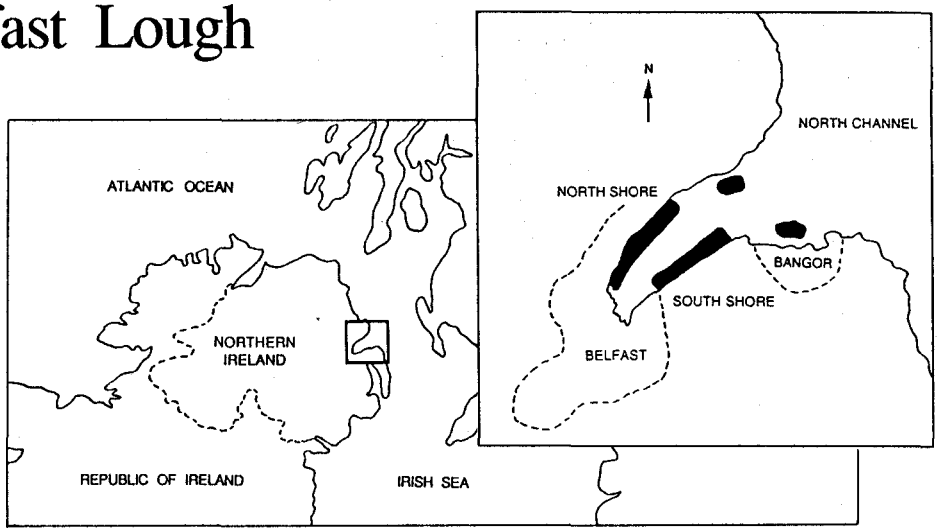

Fig. 1: Outline of mussel beds in Belfast Lough.

During the 1970's and early 1980's commercial shellfish beds in Northern Ireland coastal waters were regularly monitored for paralytic shellfish toxins by the mouse bioassay (Anon, 1984). A small number of these samples, mainly from within the lough, exceeded the accepted hazardous concentration of 400 mouse units (m.u.) per 100 g. After a series of negative results, routine testing ceased in the mid 1980's. A new programme, again based on the mouse bioassay, started in June 1990 following reports of toxic concentrations in mussels taken off the NE coast of England.

The first round of sampling revealed a maximum concentration of 480 m.u. in one sample of mussels collected from the north shore of the lough. Contemporaneous samples from other areas in the lough and more widely around the Northern Ireland coast were negative. Following these results a selective health warning was issued and commercial harvesting ceased.

A more extensive survey, based on weekly sampling of recognised coastal shellfish beds, was instituted and continued until the concentration in all samples fell below 400 m.u. During the survey detectable toxin concentrations occurred only within the upper lough, first off the north and subsequently off the south shore. The elevated concentrations peaked at 5496 m.u. and remained above the hazardous level until early August (Fig 2). The toxin peaks of the north and south shores appear to be similar but the latter increased later and decreased more rapidly. Samples of winkles, crabs, limpets and oysters collected from harvesting areas around the coast were consistently negative for PSP toxin. Additional samples of scallops were taken when concentrations above 400 m.u. were confirmed in scallops from the coast of Scotland. There was no detectable toxin in these samples.

A similar collection and testing programme was maintained in 1991. Toxin was detected only from within the lough and the maximum was 257 m.u. per 100 g in one sample collected from the north shore of the lough. Monitoring in 1992 to date has also failed to detect PSP toxin.

Information collected on the local climatic conditions in 1990 and 1991 show that there were less hours of sunlight, air temperatures were lower, and there was less rainfall in May and June in 1991. The wind speed was higher from May to July in 1991. Within the lough the available data suggest that the water temperature off the north shore is consistently lower (difference: 1 to 3 degrees Centigrade). There were no reports of visible blooms in the lough during either year. If such had occurred it is probable that the event would have been recorded. The area is regularly overflown by commercial aircraft arriving at the Harbour airport, ships frequently cross the lough to enter the harbour and there are several yacht clubs and marinas using the inshore areas.

These results suggest that, in the coastal waters of Northern Ireland, PSP toxins occur only within Belfast Lough. If this is confirmed by further studies over a more extensive period, then selective sampling from this area may be an effective means of regularly monitoring to detect PSP risk at an early stage.

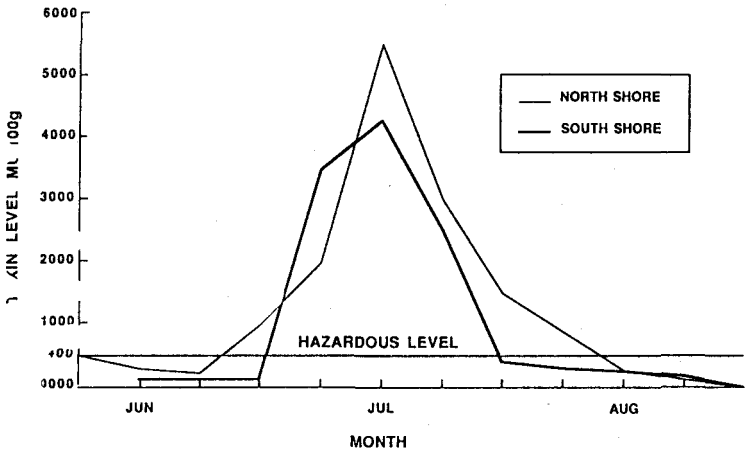

Fig. 2: Paralytic shellfish poison survey, Belfast Lough, 1990.

By W.J. McCaughey and J.N. Campbell, Veterinary Sciences Division, Department of Agriculture, N. I., Stoney Road, Belfast, BT4 3SD, Northern Ireland, U.K.

References:
• Anonymous (1984). *Official Methods of Analysis*. 14th Edition. AOAC. Section 18-086-18-092. Arlington, VA, USA.
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Intergovernmental panel recommends concerted HAB action

At its first session (Paris, 23-25 June 1992) a joint IOC-FAO Intergovernmental Panel on Harmful Algal Blooms (HABs) reviewed IOC's activities on harmful algae and the Programme Plan for HABs as it was prepared at the IOC-SCOR Workshop in Newport last year. Fourteen countries were represented. The Panel serves as an intergovernmental mechanism guiding the international HAB programme and dealing with resource gaps.

Based on its review, the Panel concluded that the information network and training elements of the programme need more emphasis and visibility. The revised Programme Plan consists of educational, scientific and operational elements.

The Panel agreed on a set of recommendations for the further development and implementation of the HAB Programme, which are summarized as follows:

Structural

- The modified Programme Plan on Harmful Algal Blooms suggested by the IOC-SCOR Workshop on Programme Development on HABs should be adopted by the IOC Assembly (Paris, March 1993).
- An IOC-FAO Programme Office for HABs should be established in accordance with the revised Terms of Reference.
- An IOC-FAO group of experts is needed to guide and advise IOC and FAO on scientific and managerial problems relevant to the HAB Programme. Membership and terms of reference should be elaborated by the two sponsors in consultation with the Panel.
- The joint IOC-FAO ad hoc Intergovernmental Panel on HABs should continue its work until otherwise decided by IOC and FAO, the terms of reference remaining unchanged.

Action by Member States

- The promotion of the HAB Programme by:

a) identifying *(i)* national resources and resource personnel to support the programme planning process and, if possible, locate resources to support their participation in the necessary meetings; *(ii)* training needs; *(iii)* institutions and organizations willing to operate, co-sponsor, and/or host training activities, both in developed countries with visiting students, and in developing countries with visiting and resident staff; *(iv)* personnel and organizations to interact with, and provide material for this newsletter;

b) obtaining national support for: *(i)* WESTPAC[1] and IOCARIBE[2] programmes, for the States in these regions; *(ii)* the Global Environment Facility and UNDP[3] activities on harmful algae; and

c) encouraging PICES[4] to include HAB activities in its programme formulation.

Intersecretariat action

The IOC Secretariat, in co-operation with the FAO Secretariat and the Chairman of the Panel, should: *(i)* continue the planning process; *(ii)* solicit offers from Member States to provide support for the establishment of the HAB Programme Office; *(iii)* seek expansion of participation in the HAB Programme by other international organizations; *(iv)* increase the interaction and participation of programme participants in other IOC activities, in particular TEMA[5], GIPME[6] and GOOS[7]; *(v)* prepare a training programme based on identified training needs; *(vi)* continue the publication of this newsletter; *(vii)* proceed with the publication of an updated and expanded version of the

(Cont'd on p. 7)

An appeal from Rumania

During the last three decades, very dense algal blooms have been a regular feature in Rumanian coastal waters (Black Sea). As well, some occurrences offshore have been noted.

The most important species involved, and concentrations of cells reached (in 10^6 l^{-1}) are *Skeletonema costatum* (18-97), *Cyclotella caspia* (14-28), *Nitzschia delicatissima* (4-21), *Prorocentrum cordatum* (50-197), *Heterocapsa triquetra* (11-40) and *Apedinella radians* (1-21). Toxicity has not been identified, but increased DOM (dissolved organic matter) and ammonia levels, anoxia, and fish and shellfish mortalities are common events in summer, especially in association with *Prorocentrum cordatum* blooms.

Rumanian scientists involved in scientific and monitoring programmes centred on these problems would welcome international collaboration in the study of: (1) microflagellate taxonomy, (2) development of culture techniques for physiological studies, and (3) analysis of long term data sets of phytoplankton and accompanying physical and chemical information.

Anyone interested should contact: Pia Elena Mihnea, Rumanian Marine Research Institute, Bd. Mamaia Nº 300, 8700 Constanta, Rumania.



Dead fish and crustaceans on strand line (measure marks/m²). Photo: Pia-Elena Mihnea.

(Cont'd from p. 1, 'PSP in Canada')

The younger man was totally paralysed for 24 hours, unconscious for 48 hours and showed neurological symptoms (difficulty with sight co-ordination and walking) for a further 48 hours. He was released after 6 days. The older man was in intensive care for longer, largely as a result of the complications of his condition and was only released on 29 April.

The only after-effect experienced by both men was pneumonia, more severe in the older man, due to the aspiration into their lungs. The vomitus was not retained for testing but the uneaten remaining shellfish was found to contain up to 10,000 µg toxin per 100 g meat (R. Chiang, Fisheries Inspection Branch, Department of Fisheries and Oceans, pers. comm.).

It is difficult to estimate the toxic doses received by the men in view of the vomiting and chewing of siphon tips, partial cooking, as well as the mix of clams with cockles (the latter had lower toxicity) but one can estimate the possible maximum ingested.

The five clams eaten, assuming an average flesh weight of 35 g, probably contained a total of at least 17,500 µg. In the butter clam the siphons contain as much as 77% of the total toxin (Quayle, 1969) and so the part consumed could have contained more than 2,500 µg. Weights of the older and younger man were 87.5 and 73 kg respectively. Based on these considerations the doses for two could have been 200 µg and 35 µg per kg respectively. Similar estimates have been made by Ewan Todd (Health & Welfare Canada, Health Protection Branch). In view of the extremely rapid and severe onset in the younger man it seems likely that this is an underestimate in his case. Alcohol, known to add to the severity, had been freely consumed the previous evening and the older man had some rum in his morning coffee.

Unfortunately no plankton samples were taken prior to, or during this episode, and so the identity of the causative organism is unknown. On the basis of general distributional data (Taylor, 1984) we suspect that it was due to *Alexandrium (Protogonyaulax) catenella*. Samples taken two weeks later, courtesy of R. Forbes, Institute of Ocean Sciences, did not contain *Alexandrium* cells.

Several significant features of this event stand out. Although the area where the toxicity occurred (Fisheries zone 12) has consistently been an early location for toxin increase (Fisheries Inspection data for six years) such high concentrations are approximately a month earlier than might be expected. The west coast is currently experiencing a waning El Niño effect. Based on this and a 6–8 year cycle, 1992 was predicted to be worse than usual for PSP (Taylor, 1992).

The men undoubtedly would have died had it not been for *(a)* the ability to perform resuscitation by their companions, *(b)* possession of a radio while in a remote area, *(c)* knowledgeable and prompt responses by the Coast Guard, *(d)* steps to reduce the effects at Port McNeil, and *(e)* use of modern hospital ventilators. These are the first cases in British Columbia to survive under such circumstances. They show that resuscitation should always be performed as long as there is the slightest hope of reaching a ventilator. They also confirm observations from sublethal exposures that there do not appear to be lasting after-effects.

This episode also stresses the need for greater public education, even on a coast where PSP is endemic (records extend back to 1793, the last fatality being in 1981). None of the fishermen were more than slightly aware of the risks involved, the potential lethality, the lack of antidote, the most toxic parts of the shellfish, etc. The current shellfish monitoring programme can only protect in a general, statistical way. Samples taken by inspectors the same day elsewhere in the Inlet and vicinity had levels reaching 440 µg, illustrating the well-known problem of small scale variability. Closures, other than blanket regional bans (which are often disregarded by locals), are always after the fact and cannot protect individuals collecting shellfish at the same time as Fisheries Inspectors.

By F.J.R. 'Max' Taylor

References

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- Taylor, F.J.R. 1984. In: Ragelis, E.P. (ed.) Seafood Toxins. *Amer. Chem. Soc. Symp. Ser.* 262. pp.77-97.
- Taylor, F.J.R. 1992 (in press). In: Smayda, T. & Shimizu, Y. (eds), *Proc. 5th. Int. Conf. on Toxic Marine Phytoplankton*, Newport, Rhode Island, Nov., 1991.

Editor's note:
Max Taylor pointed out in his letter that 'these may be the first people to survive unquestionably fatal doses'. Does anybody know of others?

Red-Tide Research and Monitoring Programme in South East Asia

EVS Consultants Ltd. is pleased to have been selected by the Canadian International Development Agency (CIDA) to be the Canadian Executing Agency (CEA) for a five-year project in the southeast Asia region, including: Thailand, Brunei Darussalam, Indonesia, Malaysia, Philippines and Singapore. The project is aimed at red-tide response programmes and the establishment of environmental criteria for protection of marine resources. The objectives are to enhance ASEAN (Association of Southeast Asian Nations) marine science capabilities and human resources through the development of technical studies on red tides and other environmental concerns. This will be pursued through an institutional strengthening strategy designed to develop, among ASEAN marine science institutions and researchers, a capacity to pursue regional environmental management objectives on a long-term basis. Technical support by Canadian and ASEAN marine science professionals for the technical studies will be complemented by formal post-graduate training, practical attachments, workshops and seminars.

EVS Consultants is presently developing research priorities for red-tide projects, in conjunction with the ASEAN countries. We would be pleased to receive inputs/comments from other researchers in this area, especially from the six ASEAN countries, with respect to possible cooperative research activities. Please contact: *(a)* Mr Peter Nix, EVS Consultants Ltd., 195 Pemberton Avenue, North Vancouver, B.C., Canada, V7P 2R4. Fax: (1-604) 662 8548; *(b)* Mr Dwight Watson in Kuala Lumpur, Malaysia. Fax: (1-603) 293 6146; or *(c)* Dr Max Taylor, Dept of Oceanography, University of B.C, Vancouver, B.C., Canada V6N 2L7. Fax: (1-604) 822 6091.

By Peter G. Nix, EVS Consultants.

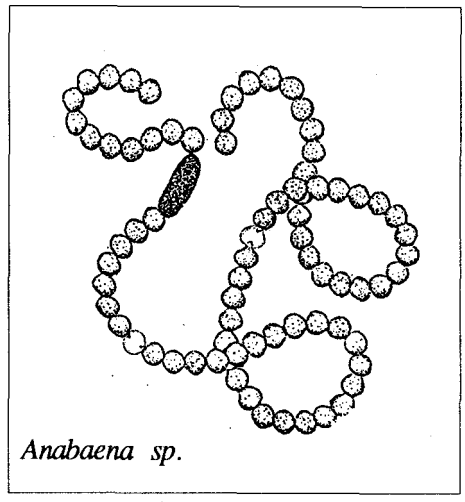
Blue-Green Algae in Australia

In November 1991 what has been described as the world's largest freshwater algae bloom occurred in outback Australia. (Further details can be found in the article by Dr. Gustaaf Hallegraeff on the opposite page). A stretch of the Darling River over one thousand kilometres long experienced a bloom dominated by *Anabaena* species. During the last few years the public in many regions of the country have been sensitized to the issue of cyanobacteria in recreational, irrigation, agricultural and potable waters. The magnitude of the Darling River bloom focussed attention across Australia on the problems of algal blooms and the broader issues of water quality and toxin contamination from cyanobacteria.

Blooms of cyanobacteria, or blue-green algae, are not new to Australia, nor is the recognition of the toxic effects; Francis (1878) reported stock deaths resulting from a cyanobacterial bloom *(Nodularia spumigena)* in Lake Alexandrina (South Australia). More recently the risk to public health has been identified (e.g. Falconer *et al*, 1983 and Hawkins *et al*, 1985). It is only in recent years though that this understanding has been translated into the management of water systems. The summer of 1989-90 saw the closure of several water storages in southeastern Australia due to cyanobacterial blooms. These included storages for irrigation, recreation and potable supplies. Blooms also occur on slow-flowing rivers. The following summers have seen an increase in the number of storages and water supplies closed or affected by cyanobacteria, probably due not to an increase in bloom occurrence but to a greater awareness of the potential risk and recognition of the issues involved.

The closure of water supplies causes significant disruptions to local communities. More often than not the towns affected have had a single source of water. The need to develop contingency plans for dealing with water supplies during algal blooms resulted in the formation of interdepartmental working groups in some States. The leap in the public profile of algal blooms that came from the Darling River incident resulted in a series of Task Forces being established at both the State and Federal levels of government, to bring together the various agencies involved in the water industry in order to minimize the future incidence and impact of algal blooms. Out of these gatherings of expertise it has become apparent that there are many unanswered questions on the causes, management and effects of algal blooms that need to be resolved if the situation is to improve. The Australian Water Resources Council (AWRC), the peak forum for the water industry, established a National Project Manager for Algal Bloom Research to provide a co-ordinated, focussed approach to resolving these issues. At present, the additional demands for further research into algal blooms have to be accommodated in existing budgets. There is some hope that the identification of knowledge gaps on a national scale will promote the allocation of additional resources towards algal bloom issues.

In order to reduce the occurrence of blooms in the longer term, attention is being focussed on nutrient sources and inputs to aquatic systems together with management of flow regimes. This approach is similar to that adopted in other countries, but Australia does have some characteristics that affect the dynamics of aquatic systems and the potential for control of algal blooms. The landscape of Australia is dominated by old erodable soils, and waters are generally turbid due to the suspension of fine clays. These clays provide binding sites for phosphorus, which means that a significant proportion of the total phosphorus load is not readily available to biota. For example, at one site on the Broken River in Victoria, turbidity ranges from 5 to 110 NTU for a suspended solid level of 10 to 55 mg/L and total phosphorus ranges from 0.01 to 0.1 mg/L of which 0.003 to 0.02 mg/L is filtered reactive phosphorus (Harrison *et al* 1990). The suspended clays provide a transport mechanism for phosphorus and an additional sink both within the water column and in areas of deposition. Remobilisation can occur from these sinks. Coupled with these problems are climatic factors. Australia is the driest continent, and even the wetter parts of temperate Australia have high variability in rainfall. The natural pattern of streamflow includes droughts and floods and it is not uncommon for a dry summer to be broken by torrential downpours. This feature encourages the transport of nutrients to aquatic systems. Cullen (pers. comm.) has found in a study of one small catchment that 61% of the phosphorus and 41% of the water moved down a creek in 1% of the time. The variability of runoff and general aridity of Australia have resulted in major constructions to store and manage water so that the flow regimes of rivers now bear little resemblance to natural conditions.



Anabaena sp.

While the Australian cyanobacterial blooms may have local characteristics, the overall mechanisms and impacts are common with eutrophication internationally. The Australian research effort intends to be complimentary to research in other countries. The AWRC National Project Manager for Algal Bloom Research, Dr Phillip Johnstone, would like to hear from any organization or individuals involved in aspects of cyanobacterial bloom research. He can be contacted at: Department of Water Resources, 35 Spring St., Melbourne, Victoria 3000, Australia. Tel: (61-3) 651 3648; fax: (61-3) 651 3989.

By Phillip Johnstone, AWRC National Project Manager, Algal Bloom Research.

References:

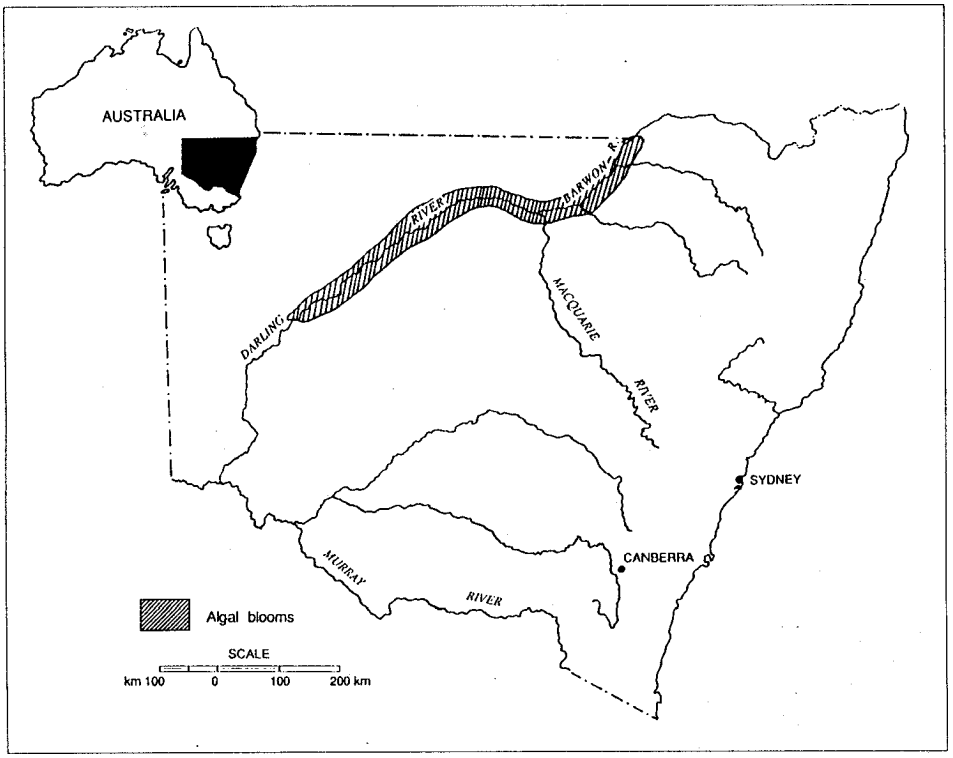
- Francis, G. (1878). *Nature* 18: 11- 12.
- Falconer, I. R., et al. 1983. *Med. J. Aust.* 1: 511-514.
- Hawkins, P.R., et al. 1985. *Appl. Environ. Microbiol.* 50 (5): 1292-1295.
- Harrison, J., et al. Rural Water Commission of Victoria; *Water, Materials and Environmental Science Branch Report* Nº 105.

Massive Bloom of Toxic Blue-Green Algae in Australian Rivers

In mid-November 1991 the largest riverine toxic blue-green algal bloom (mainly *Anabaena circinalis*), ever recorded anywhere in the world, occurred in the Darling/Barwon River system in New South Wales, Australia. Cell counts exceeded 105 cell/ml and most sites tested were found to contain high concentrations of both hepatotoxins and neurotoxins. The bloom contaminated supplies of drinking water for country towns and killed hundreds of sheep and cattle as well as wildlife which had drunk affected water. Farmers in affected areas were forced to drive hundreds of kilometres to satisfy their families requirements for drinking water. Fish kills resulted from the generation of anoxic conditions, and significant economic losses were suffered by tourism and recreation. A state of emergency was declared and this was only lifted in late December after the first heavy rains of the season reduced the algal bloom through flushing and increased turbidity.

Toxic blooms of freshwater and brackish water blue-green algae are not new to Australia. This phenomenon was first recorded from Lake Alexandrina in South Australia in 1878 (caused by *Nodularia spumigena*), and in 1959 one of the worst blooms in Lake Bonney, South Australia (probably a mixture of *Anabaena circinalis* and *Microcystis aeruginosa*) killed 300 sheep, 5 cattle and a horse. What is new, however, is the enormous extent of the 1991 bloom, stretching 1200 km through the Darling River system from Queensland in the north through New South Wales where it joins the Murray River in Victoria and South Australia. The river's catchment area covers 1/7 of the continent of Australia, that is, as large as France and Spain together. The bloom was continuous throughout the length of the river but this was not due to transport because the flow was too low to allow for this.

The latest *Anabaena circinalis* bloom has been blamed on eutrophication by sewage from townships (a population of 600,000 people), run-off of agricultural fertilizers (especially super phosphates) and from wasted cattle feedlot, in combination with high summer temperatures and low river flow rates. Flow rates in the Darling River were only 1/30 of normal due to both drought conditions and significant diversions (up to 60% of total flow) for irrigation of cotton fields. The loss of macrophytes along the river banks in the past 10 years (reduced nutrient removal) and the introduction of European carp (increased sediment nutrient release due to resuspension of sediments) may also have contributed. A Senate Inquiry into the toxic algal bloom was instigated and a National Task Force was created to coordinate the research activities of a large number of overlapping water authorities, and Commonwealth and University scientists. (See accompanying article by Dr Phillip Johnstone.)



By Gustaaf M. Hallegraeff, Department of Plant Science, University of Tasmania, Hobart, Tasmania, Australia.

(Cont'd from p. 4, 'Panel')

International Directory of Experts in Toxic and Harmful Algal Blooms and Their Effects on Fisheries and Public Health; *(viii)* proceed with the publication of a manual which compiles widely different information on the taxonomy, toxicology, and epidemiology of harmful algal blooms, and which should be distributed freely to developing countries; and *(ix)* support national and international conferences related to harmful algae.

It should be the task of the IOC Secretariat, the Intergovernmental HAB Panel, and national research, management and administrative institutions to achieve these goals in concert. Readers may address a request for a copy of the outline of the HAB Programme Plan (*IOC Workshop Report* No. 80) to: IOC Secretariat, attn: Henrik Enevoldsen, UNESCO, 1 rue Miollis, 75732 Paris cedex 15, France.

(1) WESTPAC = IOC's Programme Group for the Western Pacific Region.
(2) IOCARIBE = IOC Sub-commission for the Caribbean and Adjacent Regions.
(3) UNDP = United Nations Development Programme.
(4) PICES = Pacific International Council for the Exploration of the Sea.
(5) TEMA = Training, Education and Mutual Assistance (IOC).
(6) GIPME = Global Investigation of Pollution in the Marine Environment (IOC and UNEP).
(7) GOOS = Global Ocean Observing System (IOC, WMO and UNEP).

2nd Luso-Spanish Meeting on Toxic Phytoplankton, Shellfish Toxicity, and Prevention Measures

Held 10-12 March 1992 in Lisbon, Portugal, this meeting focussed on the management of the marine environment and the seafood industry in the region. Sponsored by INIP (the Portuguese National Fisheries Research Institute), the event aimed in particular to: *(i)* establish research priorities more in line with current knowledge and the concerns of aquaculture, fisheries and public health; *(ii)* develop and improve methods to minimize the environmental and economic consequences of harmful algal blooms (HABs); *(iii)* enhance the flow of information between the two countries (Portugal and Spain); and *(iv)* standardize, as far as possible, the national monitoring programmes both for toxic species and toxin detection in seafoods. Also discussed was the creation of a regional programme to understand the causes, predict the occurrences and mitigate the effects of HABs.

Among the main topics of discussion were:

- Toxic algae occurrences and interaction with physico-chemical parameters in the region.
- DSP[1] and PSP[2] toxins, accumulation by shellfish, detection methods, permitted levels and quarantines.
- The Iberian coast as a pilot region for the study of HABs.

During the meeting, workshops were held on the main topics in order to standardize methodologies and to discuss the preparation of an interdisciplinary international programme to study the dynamics of HABs.

Agreement was reached on methodologies for toxin detection. Protocols for the mouse bioassay for DSP and PSP were written and a resolution passed to present them to the European Economic Community (EEC) Commission. The latter action was in response to an agenda item of an Expert Meeting on 25 March 1992 in Brussels, which called for: "Preparation of a draft for methods, analytical tolerances and sampling plans on mouse bioassay in order to detect biotoxins". Periodical intercalibration exercises were agreed upon between Spain and Portugal. It was agreed that the Iberian coast would serve as a suitable region for an interdisciplinary international pilot study on the dynamics of harmful algal blooms, and that a proposal for such a study should be presented at the forthcoming meeting of the ICES-IOC Study Group on the Dynamics of Harmful Algal Blooms, 7-9 April 1992, Vigo, Spain (see *HAN* No. 2).

Red-tide specialists in Portugal and Spain are relatively advanced in the creation of a regional programme, but considerable effort is still required to strengthen communication and increase interaction and collaboration in order to concert efforts and maximize results.

The need for research funds was emphasized. As well, priority was given to the necessity of closer collaboration with national planning and policy agencies, in particular to convince them that HAB problems are severe, growing and of high priority with respect to other competing national needs.

Maria Antónia de M. Sampayo, Instituto Nacional de Investigação das Pescas (INIP), Avenida de Brasilia, 1400 Lisboa, Portugal.

(1) DSP= Diarrhetic shellfish poisoning.
(2) PSP= Paralytic shellfish poisoning.

Future events

NOVEMBER 92

8th International IUPAC Symposium on Mycotoxins and Phycotoxins, 8-12 November, Stouffer Presidente Hotel, Mexico City, Mexico. *Contact*: Symposium Secretariat, VIII International IUPAC, Insurgentes Sur 3700 - C, Apartado Postal 101-63, 04530 Mexico DF, Mexico. Tel: (52-5) 550 58 83; fax: (52-5) 548 82 07.

APRIL 93

International Workshop on Ciguatera Management, 13-16 April, Bribie Island, Australia. *Contact*: Workshop Secretariat, Southern Fisheries Centre, PO Box 76, Deception Bay, Queensland 4508, Australia. Tel: (61-7) 203 1444; fax: (61-7) 203 3517.

OCTOBER 93

SIXTH INTERNATIONAL CONFERENCE



TOXIC MARINE PHYTOPLANKTON

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44041 Nantes Cedex 10
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HARMFUL ALGAE NEWS

This issue was compiled and edited by Tim Wyatt and Yolanda Pazos, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Eduardo Cabello 6, 36208 Vigo, Spain. Tel.: (34-86) 23 19 30 / 23 19 73; fax: (34 86) 29 27 62

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 4

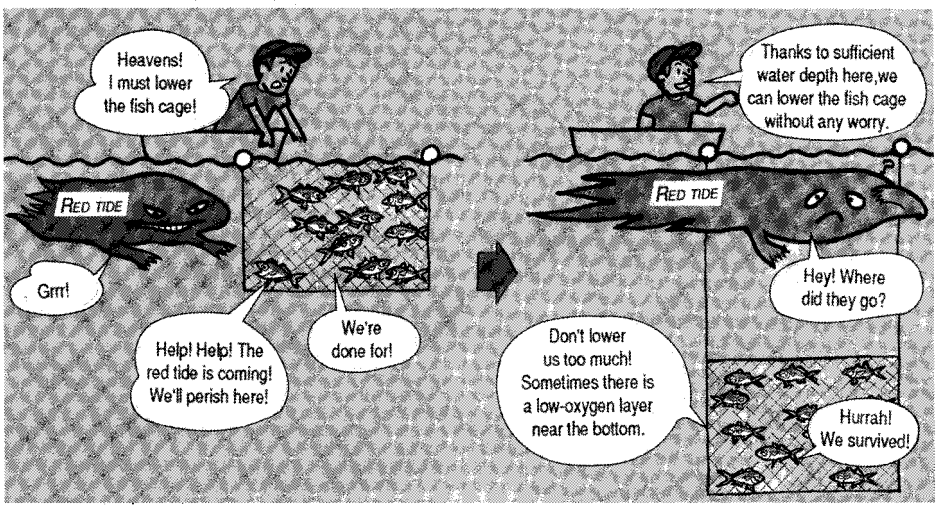
Red tide action in Philippines:
Fishing community involvement sought

Our vision in the Philippines is to have a nationwide, community-based red tide monitoring and management programme. Education of the community through video shows, slide shows and red tide information leaflets has been started. In one area, Bataan (Manila Bay), responsible fishermen and organizations have been mobilized in information dissemination during red tide outbreaks.

The project, entitled "Fisheries Sector Program (FSP) Training Package for Red Tide Monitoring and Data Management", is a collaborative endeavour between the Department of Agriculture of the Bureau of Fisheries and Aquatic Resources (BFAR) and the Marine Science Institute (MSI) of the University of the Philippines Science Research Foundation (UPSRF). Efforts are being made to involve fishermen in certain aspects of monitoring and management. After careful selection and intensive training of qualified fishermen, the latter will be integrated into the community- based red tide monitoring and management programme.

The project aims to: a) train government and interested university personnel in the major aspects of red tide monitoring and data management; b) ensure the use of standard methods in the collection, analysis and interpretation of data by providing trainees with the necessary skills, information and some kits; c) help develop systems of fast and reliable documentation and information exchange between the technical personnel and the general public; d) develop material that could be used beyond the project implementation period for information campaigns on red tides, and e) make recommendations to facilitate the development of a more sustained, permanent and comprehensive red tide monitoring programme to fit efficiently into the overall fisheries management programme of the country.

Involvement of rural inhabitants in coastal zone management is not new in the Philippines. In 1986, the ASEAN*-USA-sponsored Coastal Resource Management Project developed a community-based coastal resource management programme in Lingayen Gulf, in the north of the country. The community-based approach has been summarized as "community-oriented, people-oriented and resource-based" (Ferrer, 1992).



Example of red tide public information material; adapted from Japan's Ministry of Agriculture and Fisheries pamphlet on red tide countermeasures.

For "participatory approaches to development" to succeed, careful planning

(Cont'd on p. 2)

Algal bloom in NW Caribbean

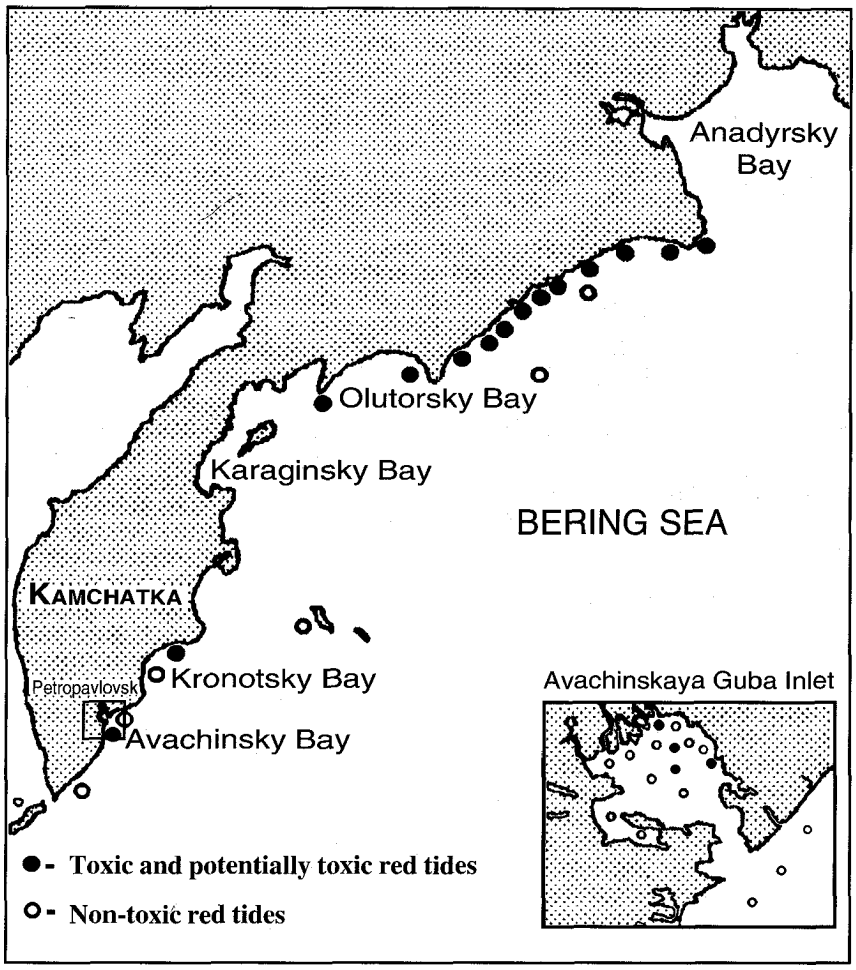
Independent reports from Dr. Carolina Rogers (National Park Service, St. John, Virgin Islands) and Susan White (Saba Marine Park, Netherlands Antilles) indicate that there is an "unprecedented" planktonic algal bloom in progress across the NW Caribbean Sea. Underwater visibility is less than 3 m from an average of more than 20 m. This is the period of the year when the plume of the Orinoco River is known to make incursions into the Caribbean, as evidenced from CZCS(1) imagery, penetrating all the way to Puerto Rico on occasions (F.E. Müller-Karger et al. 1990. *Prog. Oceanog.* 23: 23-64). An attempt is being made to verify the geographic extent, composition and other features of this water mass through coordinated observations by other members of the CARICOMP(2) cooperative network of Caribbean marine laboratories.

The fact that this phenomenon cannot be examined from space through the current lack of an ocean colour scanner argues most strongly for the need for the SeaWIFS(3) sensor and its utility in large geographic scale examination of oceanographic events of potentially critical importance in the structure and function of coastal ecosystems.

John Ogden, Florida Institute of Oceanography, 830 First Street South, St. Petersburg, FL 33701. USA.

(1) CZCS = Coastal Zone Colour Scanner
(2) CARICOMP = Caribbean Coastal Marine Productivity
(3) SeaWIFS = Sea-viewing Wide-field-of-view Sensor

Harmful dinoflagellate blooms along the eastern coast of Kamchatka



The first dangerous red tide event along the coast of Kamchatka occurred in Pavla Bay and adjacent waters of the Bering Sea in the summer of 1945 and led to poisoning and death of some fishermen. The symptoms of poisoning by Pavla Bay shellfish which six men had for breakfast, resembled the effect of paralytic shellfish poisoning (PSP) produced by some species of the dinoflagellate *Alexandrium*. Further investigations proved their occurrence in these areas[1]. In August 1973 a similar tragedy was observed in Petropavlovsk-Kamchatsky on the shore of Avachinskaya Guba Inlet. Twelve people (mainly children) were poisoned after eating toxic shellfish collected in the Inlet and prepared on a fire. Two of them died. Some days before this event, intense water luminescence at night and red water by day were seen in some areas of the inlet. Poisoning of these people was found to be due to saxitoxin contained in mussels, probably derived from *Alexandrium tamarense* or *A. catenella*[2,3]. These species are common in the summer plankton of Avachinskaya Guba Inlet, and are responsible for red tides there.

Since 1983 red tide events have been observed annually in various areas along northeastern and eastern coasts of Kamchatka. Toxic red tides were observed in 1984, 1986, 1987, 1988 and 1990 in Avachinsky, Olutorsky and Kronotsky Bays including the northeastern coast of Kamchatka (see map). These blooms resulted in mortalities of marine mammals, birds and fish. There were no cases of human poisoning, possibly owing to the fact that newspapers and magazines widely disseminated information on the danger of eating mussels, especially during the periods of red tides.

Ten species of toxic and potentially toxic dinoflagellates belonging to the genera *Alexandrium*, *Dinophysis*, *Gymnodinium*, *Gyrodinium* and *Prorocentrum* have been identified in Kamchatka coastal waters. However, only four of them cause visible blooms, i.e. *Alexandrium tamarense*, *A. excavatum*, *A. acatenella* and *Dinophysis acuminata*.

(1) G. V. Konovalova. *Botanical Journal*, **74**, 10 (1989).
(2) G. V. Konovalova. In: *Red Tide. Biology, Environmental Science and Toxicology*. D. M. Anderson, T. Okaichi, T. Nemoto, eds. (Elsevier, New York, 1989).
(3) G. V. Konovalova. *Algologia*, **2**, 4 (Ukraine, 1992).

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(Cont'd from p. 1, "Red tide action")
for intensive education and mobilization of the people is needed. Education, being an important component of the approach, should be given much emphasis, for it will deepen the understanding of people as to the conditions of the coastal environment and its resources, heighten their consciousness and mobilize them for the activities that need to be undertaken.

In the Marine Conservation and Development Project of Silliman University (Dumaguete City, Negros) for example, rural people were mobilized and involved in the monitoring and care of coral reefs. They were given the opportunity to observe plankton samples under the microscope and coral reefs by snorkeling. Dr. Angel Alcala (then Director of Silliman Marine Laboratory, subsequently President of its University, and now the country's Secretary of Natural Resources and Environment) reports that marine reserve areas as in Sumilon Island are now being protected and developed by the community and the fishermen themselves.

* ASEAN = Association of Southeast Asian Nations.

Reference:
Ferrer, E. M. 1992. *Learning and working together: towards a community-based coastal resources management.* 62 pp. College of Social Work and Community Development, University of the Philippines, Diliman, Quezon City, Philippines.

Rhodora A. Corrales, Marine Sciences Institute, University of the Philippines, U.P.P.O. Box 1, Diliman, Quezon City 3004, Philippines.

Joint research programme between Hong Kong and China

A consortium aiming to promote research efforts on harmful red tides in the South China Sea which is a milestone of joint research between Hong Kong and China before 1997, i. e. the date that Hong Kong returns to China after 150 years' colonial administration by the British Government, was signed on July 1992. The institutions (which include Open Learning Institute of Hong Kong, Department of Biology in Hong Kong Baptist College, Department of Applied Science in City Polytechnic of Hong Kong and South China Sea Institute of Oceanology in China) have agreed to conduct a three-year collaborative project in identifying the limiting factors of red tide in the South China Sea, and in developing a computer model to forecast red tides on the basis of ecological data. Studies will concentrate on the harmful red tides, particularly those of *Alexandrium* spp., in the Pearl River Estuary, Hong Kong and Daya Bay. Studies of water quality, phytoplankton diversity and population dynamics, cysts, and limiting factors (by bottle bioassay) began in January 1993, and the institutions are raising grants from governments and private foundations to support this collaborative project.

In April 1992, widespread red tides were observed in the Pearl River estuary near Hong Kong. Characterized by their distinctive orange-juice colour, patches of *Noctiluca scintillans* were carried along the south China coast by the Kuroshio Current and tides. The incident caused temporary closure of 11 bathing beaches in Hong Kong and a severe kill of marine organisms by deoxygenation in aquaculture facilities. The loss in this incident was more than US $100,000.

In the winters of 1989 and 1990, Hong Kong was badly affected by the blooms (and toxicity) of *Alexandrium catenella*. This year, *A. catenella* was also present in the seawater but in low concentrations (<100 cell/ml). A survey suggested that cysts of the species persist in the bottom sediments of the South China Sea. Thus, there is a high possibility of paralytic shellfish poisoning (PSP) recurrence in the coming years.

Dr Ho Kin-chung, Open Learning Institute of Hong Kong, 700 Nathan Road, Mongkok, Kowloon, Hong Kong; and
Wang Zhaoding, South China Sea Institute of Oceanology, Academia Sinica of China, 164 West Xingang Road, Guangzhou, China.

Outbreak of black band disease in Caribbean

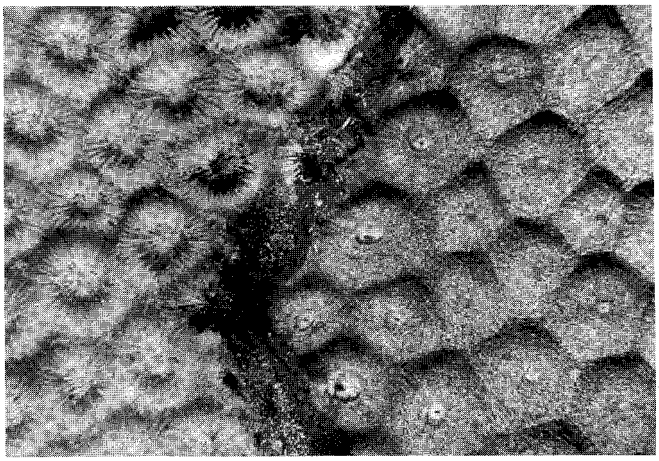
In the summer of 1992, a major outbreak of what is known as black band disease (BBD) was observed in Jamaican waters. This disease, first reported in Belize in 1973 as an algal infection responsible for coral mortality, has subsequently been found throughout the Caribbean and Indo-Pacific on scleractinian corals as well as on milleporinids and gorgonians.

In routine monitoring carried out since December 1991 on the north coast of Jamaica, less than 1% of scleractinian corals have been observed to be infected with BBD in most fore reef and back reef sites. This is comparable with published data reported for other Caribbean localities. However, two of our study sites have shown a rapid rise in BBD cases, with up to 10% of the susceptible corals being infected at any one time.

The primary pathogen of BBD is the cyanophyte *Phormidium corrallyticum*. In well developed cases BBD appears as a ring of finely interwoven black filaments separating living coral tissue from stark white skeleton. Severe infections in our study sites are characterized by a band up to 3 cm wide and a linear spread of 1 cm/day. We have found the disease most frequently attacking *Montastrea cavernosa*, *M. annularis*, *Diploria strigosa*, *D. clivosa* and *Siderastrea siderea*. Few cases have been observed on *Agaricia agaricites*. Three colonies of *Meandrina meandrites* (previously unreported in the literature) have been infected.

Mechanisms responsible for BBD are poorly understood. In our study area the disease spreads most rapidly in the direction of prevailing water currents. The band is easily dislodged, flaking off during rough seas. Previously healthy corals down current of an infected colony most frequently acquire BBD, resulting in a clumped distribution of diseased colonies. Corals adjacent to colonies previously killed by black band disease frequently become infected. The disease can appear on a new victim several months after all signs of BBD have disappeared from the dead colony. Reef temperatures have not significantly increased for the duration of the study, remaining between 26°C and 28°C. The highest incidence of BBD overlaps with an area experiencing high siltation, a seasonal phenomenon which has persisted since March.

Jamaica's coral reefs have been dramatically effected by hurricanes, over fishing, pollution, sedimentation, algal overgrowth and frequent episodes of bleaching. Recent outbreaks of BBD suggest that the corals may be succumbing to environmental stress and the reefs may be rapidly converted to algal gardens, ultimately affecting tourism, shore protection and reef fisheries.



Macrophotograph of Phormidium *band on star coral,* Montastrea cavernosa. *Area represented is 6 cm long by 4 cm high.*

Andrew Bruckner, Hofstra University Marine Lab, St. Ann's Bay, Jamaica.

Canadian workshops on harmful marine algae: *a review*

The domoic acid crisis in eastern Canada in late 1987 was the impetus for a major retargeting and expansion of phycotoxin research and monitoring efforts by several Canadian federal government agencies. A Department of Fisheries and Oceans (DFO) national action plan on harmful algal blooms and their associated effects on human health and marine ecosystems was implemented in 1989, under the direction of an *ad hoc* Phycotoxins Working Group (PWG). The PWG proposed an annual workshop on harmful algal blooms to serve as an informal forum for the exchange of information on phycotoxin research in a Canadian context.

The first DFO workshop, held in Moncton, New Brunswick, in the fall of 1989, was mainly devoted to issues arising from the domoic acid crisis of 1987-88. The second workshop, hosted by the Bedford Institute of Oceanography, Dartmouth, Nova Scotia in October 1990, was somewhat broader in scope, and reflected significant advances made in many areas of phycotoxin research.

The Third Workshop was held at the Maurice Lamontagne Institute, DFO, Mont-Joli, Quebec, from 12-14 May 1992. Nearly 100 people participated. Often at scientific conferences, the more prominent researchers are afforded special priority to give oral review presentations, while newcomers to the field (who may have the freshest insights and most relevant data) are relegated to an obscure poster session. This was avoided by eliminating plenary lectures, by making the poster sessions a focus of discussion, and by featuring a broad spectrum of oral presentations to include graduate students and outsiders.

Third workshop issues

The thematic structure of the third workshop indicated the major issues in phycotoxin research pursued by Canadian scientists, and several new developments in phycotoxin detection methods were reported. Among the most significant were a modified mouse neuroblastoma cell bioassay for PSP, and a comparison of assay methods for ciguatoxins, which included the results of a protein phosphatase inhibition assay and a solid-phase immunobead test. A new competitive indirect enzyme-linked immunosorbent assay (ELISA) for okadaic acid, based on a monoclonal anti-idiotypic antibody, was also described.

Other presentations included: physiological and behavioural bioassays to study the responses of juvenile salmonids to potentially harmful microalgae; recent advances in analytical methods for phycotoxin detection and quantification, including ion-spray mass spectrometry (ISP-MS) coupled with flow injection (FI), high performance liquid chromatography (HPLC), and capillary electrophoresis (CE); the use of an immunofluorescence technique to discriminate closely related morphotypes in the diatom genus *Nitzschia*; proposal to use nucleotide sequences and PSP toxin profiles to characterize variation in PSP-toxin producing *Alexandrium* species.

Focus on *Alexandrium*

The Maurice Lamontagne Institute has played a leading role in conducting studies on the population dynamics of *Alexandrium* and the transfer of PSP toxins within marine food webs. Participants from diverse laboratories gave presentations on the accumulation and metabolism of PSP toxins in blue mussels, lobsters and scallops, as well as on the effects of toxic species on copepod feeding behaviour and larval fish recruitment in the St. Lawrence ecosystem. The hypothesis that resuspended benthic cysts of *Alexandrium* are responsible for wintertime PSP toxicity in mussels was rejuvenated, with a correlation between toxicity and cyst numbers in the digestive tract in the apparent absence of motile *Alexandrium* cells in the water column. Some preliminary evidence tends to indicate that the endogenous clock hypothesis, rather than control by external environmental factors, is a more tenable explanation for *Alexandrium* cyst germination patterns in the lower St. Lawrence estuary.

Since DFO has instituted a national monitoring programme for harmful algae, the efficacy of various regional monitoring strategies was thoroughly addressed. Modelling approaches to toxic bloom description and prediction were also considered. Colleagues from France and Belgium gave perspectives on DSP toxicity which proved useful in interpreting the results of experimental work on bivalve mollusc contamination.

Phycotoxin production and metabolism by various microalgal species grown under controlled conditions is an important component of current research programmes in Canada. The cage-culture turbidostat technique has been successfully applied, to study the effects of growth rate and environmental variables on PSP toxin production rates in *Alexandrium tamarense*. Researchers at the National Research Centre (NRC), Halifax have made great strides in understanding the biosynthesis of domoic acid in *Nitzschia pungens*, using ^{13}C-labelled precursors and nuclear magnetic resonance (NMR) to trace the biosynthetic pathway. The hypothesis that bacteria are ultimately responsible for the production of certain "phycotoxins" continues to attract attention. But it has been demonstrated that domoic acid production by *Nitzschia pungens f. multiseries* can proceed unabated in the absence of associated extracellular bacteria. Arguments for a bacterial role in PSP toxin synthesis in *Alexandrium* are inconclusive and unconvincing, due to evident problems in maintaining good algal growth in control cultures.

In addition to the known phycotoxin-producing species of obvious concern to human health, plankton blooms of nuisance species also plague the Canadian seafood industry. A major project directed by the Maurice Lamontagne Institute on the "blackberry feed" problem, where the flesh of certain commercial fish species is rendered inedible due to a sulphurous odour, has established the link between a pteropod vector and certain prymnesiophyte species known to produce dimethylsulfide. Joint research effectively solved a mystery perplexing mussel culturists in Nova Scotia, which involves an alarming red colouration of the digestive gland. The source is the accumulation of a (non-toxic) ciliate *Mesodinium rubrum*, which harbours a red-pigmented cryptomonad endosymbiont.

Working group assignments

Six highly effective Working Groups (WGs) were established, each containing less than a dozen participants. The major terms of reference were as follows: I. *Origin and propagation of harmful algae*: Group 1 - The American west coast domoic acid crisis; Group 2 - The problem of DSP in Canada; II. *Transfer of phycotoxins in the food web*: Group 3 - The benthic food web; Group 4 - The pelagic food web; III. *Methodology*: Group 5 - Detection and quantification of toxic algae and toxins; and IV. *Monitoring and prediction*: Group 6 - Monitoring toward the year 2000. A particular advantage of this format was that after some exchange of participants among WGs, the groups retained the expertise necessary to formulate realistic goals and recommendations, without becoming unwieldy in size.

Synopses of these discussions will appear in the workshop proceedings. However, a few key observations merit mention here. Phycotoxic events associated with domoic acid, originally believed to be localized in shellfish from eastern Prince Edward Island in Atlantic Canada, have now been reported as seabird mortalities associated with food chain transfer on the Pacific Coast of the United States. The high domoic acid levels in certain shellfish from the northeast Pacific, especially in razor clams and mussels, is particularly disturbing, as no definite causative organism has yet been identified. Canadian regulatory authorities must be vigilant in monitoring shellfish species from adjacent waters, to include non-filter-feeding species (e.g. Dungeness crabs) previously considered exempt from domoic acid accumulation.

DSP in Canada

Even granted that under-reporting may occur, the limited number of cases of DSP in Canada is *a priori* evidence that a crisis does not currently exist. Nevertheless, the discussions revealed poor understanding of this phenomenon in Canada and inadequate knowledge of the taxonomy, ecophysiology and population dynamics of the causative species. At least ten *Dinophysis* species have been identified in Canadian waters, and two species (*D. acuminata* and *D. norvegica*) implicated elsewhere in DSP incidents can form blooms of $> 200{,}000$ cells L^{-1} along the Atlantic coast. Thus the question arises: Why are DSP incidents not more common in Canada than the epidemiological literature indicates? Originally, the confirmed DSP cases in eastern Canada in 1990 were suspected to be due to the presence of *D. norvegica*, based upon circumstantial evidence. However, the lack of DSP toxins in subsequent blooms of this organism and the recent discovery of DSP toxins in *Prorocentrum lima* isolated from a mussel site known to be a source of DSP toxicity has complicated (but not refuted) this interpretation. The WG recommended that a retrospective survey of historical phytoplankton data on the spatial distribution of *Dinophysis* spp. in Canadian waters should be conducted to identify potential sources of DSP toxins in shellfish.

Conclusions

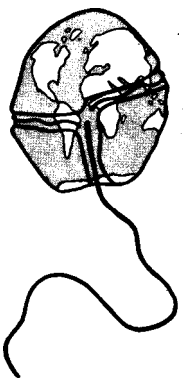
Work is advancing rapidly on the transfer and detoxification kinetics, metabolism, and tissue compartmentalization of various phycotoxins in benthic invertebrates. In contrast, progress in determining the disposition of phycotoxins in the pelagic food web has lagged behind. Such fundamental questions as the acute effects of toxic algae on pelagic grazers (fish larvae, zooplankton) and the resultant deleterious impact on recruitment of commercial fish species must be addressed from an oceanographic perspective. The excellent toxicological studies and bioassay methods emanating from the laboratory should now be extrapolated to the field.

A consensus evolved that the harmful phytoplankton monitoring programmes recently initiated in Canada have been justified, but that they have not yielded much predictive capability. A plea by regulatory authorities and the seafood industry for the development of workable alternative methods for routine phycotoxin monitoring, and for increased availability of analytical standards and reference materials, is being heeded in a major continuing effort by the Halifax NRC laboratory.

The abstracts and proceedings of this workshop will be available in a few months as a Technical Report issued by the Maurice Lamontagne Institute, DFO, Mont-Joli, Quebec. Proceedings of previous workshops may be obtained from the respective DFO host institutions.

Allan Cembella, Institute for Marine Biosciences, 1411 Oxford St., Halifax, N.S., B3H 321 Canada.

SECOND ANNOUNCEMENT



International Directory of Experts in Toxic and Harmful Algae

An updated and expanded edition of this directory will be published in 1993 by IOC-NOAA. If you are involved in the basic scientific, applied fisheries, or public health aspects of harmful algae events in your country and would like to be included in this updated directory, please mail or fax your name and address to Alan White (see below) in order to receive a questionnaire. There is no need to send your name and address if you were included in the 1990 directory or if you attended the Fifth International Conference on Toxic Marine Phytoplankton held in Rhode Island (USA), 1991. We would especially like to include individuals from countries not yet represented at the International Conference on Toxic Marine Phytoplankton.

Send your name and address to: Alan W. White, NOAA National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole, MA 02543, USA; fax: (1-508) 548-5124.

The French national programme on marine phycotoxins

Since 1990 a subprogramme has been implemented which targets phycotoxins produced by different harmful algae from French coastal waters. This subprogramme is part of the National Programme on Toxic Algal Blooms (PNEAT), another subprogramme of which deals with the causes of harmful blooms.

Diarrhetic toxins produced by *Dinophysis* spp.

Every summer several photosynthetic species of *Dinophysis* are associated with positive assays of DSP on French coasts. These species have been shown to synthesize mainly okadaic acid (OA), as checked by HPLC analysis, although DTX1 (dinophysitoxin) is sporadically detected in contaminated shellfish. A monoclonal anti-OA antibody has now been produced, which shows strong affinity for the OA-BSA conjugate, but no affinity for OA alone. Further improvements of this immunological probe are in progress, as well as alternative biossays for DSP, to replace the mouse bioassay. The *Daphnia* test has recently been used, giving 0.16 ± 0.1 µg OA LC 50.

OA extraction and purification procedures have also been investigated using *Prorocentrum lima* cultures as well as mussels contaminated with DSP. The purification procedures are now well defined, and the same approach is to be tried with DTX1. Production of large amounts of these toxins is a major priority of the programme, since they are required for neurophysiological studies. Both OA and DTX1 produce chronic effects on the cardiac activity of ischaemic Guinea pigs.

Finally, preliminary results have been obtained from an investigation of the role of bacteria associated with *P. lima* on OA production in culture. Traces of OA are easily detected by immunofluorescence in *P. lima* cytoplasm, whereas OA and DTX1 can be detected by HPLC not only in the algal fraction but also in the extracellular medium. Further work is in progress.

Paralytic toxins of *Alexandrium* spp.

Experimental bioaccumulation of Paralytic Shellfish Poisons (PSP) by mussels and clams has been investigated using a French strain of *A. minutum*, isolated in 1989. HPLC analysis of toxin profiles in shellfish meat during contamination and depuration phases has revealed the prevalence of GTX2/GTX3 (gonyantoxin), as previously found during similar work on *A. tamarense*. Different algal diets are to be tested during depuration in order to allow better post-contamination siting and management of shellfish by shellfish farmers.

Experimental contamination of scallops with PSP from *A. tamarense* has been carried out to investigate the changes in toxin profiles which take place between algae and different shellfish tissues, during and after bioaccumulation. Toxin biotransfer and hydrolysis were found, with a surprising increase in toxin levels in the gonads during depuration.

In conformity with European concern about the production of standards and the more accurate detection of the toxins prevalent in shellfish, extraction and purification of the GTX2/GTX3 complex has been performed on mass cultures of *A. tamarense*. Production of anti GTX2/GTX3 antibodies is also in progress. The chemical basis behind the studies was recently improved by the adoption of Oshima's HPLC method. Differentiation of C toxins in the toxin profiles of contaminated shellfish should thus be obtained, especially for experimental contamination by the French strain of *A. minutum*.

Ichthyotoxins of *Gyrodinium* cf. *aureolum* (*Gymnodinium nagasakiense*)

Routine tests of both haemolytic activity and algal growth inhibition have been developed for the detection of *G.* cf. *aureolum* extracellular toxins. Fish kills associated with *G.* cf. *aureolum* blooms appear to be caused by the water-soluble part of these toxins. About 3 mg of purified toxins was obtained from mass culture in chemostat and the chemical structure of these toxins, as well as of their precursors, was elucidated by gas-phase chromatography and Iatroscan analysis. Environmental factors involved in release by *G.* cf. *aureolum* have been screened and partly identified.

Ciguatera toxins.

The dinoflagellate, *Gambierdiscus toxicus*, which produces these toxins is tropical in distribution, and does not occur in the waters of mainland France. However, several analytical improvements have been carried out by teams working within the framework of PNEAT, as scientific support for toxicological and environmental studies carried out in French Polynesia and New Caledonia, as well as providing support for the authorities monitoring imported seafood.

Purification procedures for Maitotoxin (MTX) are now developed and verified by cytotoxicity bioassays using sea urchin ovocytes and mammalian neuroblastomas as cellular models. These bioassays appear to be about 100 times as sensitive as the conventional mouse test.

Concerning Ciguatoxin (CTX), trials are in progress using 9 - anthronitrile as the fluorescent reagent for HPLC detection, but further validation of this method with samples from the Caribbean and Polynesia are now needed.

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Domoic acid threat in N. Europe

Reports of extensive blooms of the potentially toxic diatom *Pseudo-nitzschia pseudodelicatissima* in Danish, Norwegian, Swedish and German coastal waters have reached us just as we go to press. Shellfish harvesting was banned in the Danish Isefjord and parts of the Limfjord, where very high cell concentrations were detected. Fuller details will appear in the next issue.

Classification of dinoflagellates using artificial neural networks

It is a generally held belief that the majority of plankton samples will eventually be sorted, counted and taxonomically analysed using automatic techniques. This does not remove the role of experienced taxonomists, it only reinforces their position. No definitive system has yet been established but it is highly likely that pattern recognition and image processing will be the method to carry out the processing of the data. Recognition of biological patterns by neural networks is a new concept and one which we suggest could play a role in achieving these goals.

The current state of the art in automatic plankton classification is to employ standard statistical techniques and classical machine vision techniques[1]. The complex task of automated discrimination of morphologically similar species of phytoplankton has recently been attempted[2,3,4,5]. Although many problems must be solved before an automatic classifier of phytoplankton can exist, the work of a number of our research team members has shown that new computer learning algorithms based on neural network theories can provide a way forward in this difficult area of visual identification.

Our project involves the collaboration of four groups. Two expert groups in aspects of neural network techniques (University of Plymouth, UK and Università di Genova, Italy) possess complimentary skills in neural network research. Plymouth has developed an understanding of the Back Propagation algorithm associated with trainable networks and Genova has developed skills in handling Radial Basis Function (RFB) networks. These two network paradigms have become important research tools in the artificial intelligence community. The other two expert groups possess considerable expertise in marine phytoplankton analysis (Plymouth Marine Laboratory, UK, and Centro Oceanográfico de Vigo, Spain).

Our intention is to develop a computational system capable of automatic taxonomic classification of marine phytoplankton species. We have already shown that for such problems novel neural network (or brain-like) computational techniques appear to offer more robust discriminatory powers than traditional machine vision techniques. We shall build on our previous work to develop a demonstrator device capable of classification of a wide range of dinoflagellates, with special reference to the toxic and nuisance species. An image database of species collected from the EEZ of the European Common Market countries will be created in the course of this project. With approximately 100 examples of each species, the database is expected to hold over 5,000 images of taxonomically labelled photo-micrographs, scanning electron micrographs and camera lucida drawings. Any assistance which can be given to us to achieve this will be greatly appreciated. The demonstrator will consist of a software-based artificial neural network (ANN) classifier that, having been trained on images from the database of dinoflagellate images, reliably classifies unseen examples taken from the same database. The application of the ANN to direct microscopic images of phytoplankton will be studied, and it is hoped that the generality and robustness of our solutions will be extended to operate with relatively unprocessed images and for a wide range of species. The validated phytoplankton image data set will be incorporated in a teaching system to provide marine ecologists with a computer-based teaching package for learning to differentiate between members of the *Dinophyceae*, in particular the toxic and nuisance species. Plymouth University has an up-link to the Olympus satellite and it is intended to use this to provide an alternative means of data dissemination than from computer disk. We hope to offer the teaching tool and validated image database on optical disk, data cartridge and via satellite. The satellite connection will be made via a low cost 100 kbit per second data transfer system (approx. US $650), enabling potential users across Europe to invest in a low cost satellite receiving system connected to their personal computer.

(1) Rolke M., J. Lenz, 1984. *J. Plankton Res.* 6: 637-645.
(2) Graneli, E. et al (eds.) 1990. *Toxic Marine Phytoplankton*. Elsevier Sci. Publ. Co., New York.
(3) IEEE Int. Conf. on Neural Networks in Ocean Engineering, Washington, USA, 1991.
(4) Simpson, R. et al. 1992. Sixth International Conference on Toxic Marine Phytoplankton, Rhode Island, USA, 1992 (in press).
(5) Simpson, R. et al 1992. *Mar. Ecol. Progr. Ser.* 79:303-308.

Bob Williams, Plymouth Marine Laboratory (PML), Prospect Place, The Hoe, Plymouth, Devon PL1 3DH, UK; fax: (0752) 670637.

Alistair Lindley (PML), Phil Culverhouse, Rod Ellis and Rob Simpson (University of Plymouth, UK), Thomas Parasini (Università di Genova, Italy), Beatriz Reguera and Isabel Bravo (Centro Oceanográfico de Vigo, Spain).

Korean-French seminar on red tides

The mariculture industry has grown rapidly in Korea since the 1970s, especially in the Chinhae Bay area on the South coast, and shellfish are exported to North America and Europe as well as supplying the home market. Although red tides have been reported since at least 1961, an unprecedented outbreak of *Gymnodinium mikimotoi* in 1981 and cases of PSP in 1986 have alerted the National Fisheries Research and Development Agency (NFRDA) as to the risks posed by red tides. This agency formed a "sistership" with the French Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) in 1975, and in November 1990, a Korean-French seminar on red tides was held. The proceedings of this meeting are now available as a book entitled *Recent Approaches on Red Tides* edited by Drs. Joo Suck Park and Hak Gyóon Kim.

The book contains eleven chapters, five from Korean authors and five from French authors, and an invited contribution from Prof. T. Okaichi of Kagawa University, Japan. To obtain a copy, write to: Dr. Joo Suck Park, NFRDA, Kijang-up, Yangsan-Gun, Kyongsangnam-do, 626-900 Korea.

Worldwide regulations for marine phycotoxins: current situation

An article under a similar title appeared in the *Journal of Natural Toxins*, Vol 1: 67 - 85, authored by H. P. van Egmond, G. J. A. Speyers, and H. J. van der Top. The abstract follows:

"An international enquiry was undertaken in 1990 within the framework of a project of the International Union of Pure and Applied Chemistry (IUPAC) to obtain up-to-date information about worldwide marine phycotoxin legislation.

Forty-seven countries have responded to the enquiry. The response, supplemented with published information, showed that 21 countries had (proposed) legislation for one or more phycotoxins in fishery products. Details about algal monitoring programmes, tolerances, legal bases, responsible authorities and methods of analysis are given for paralytic shellfish poisons (PSP), diarrheic shellfish poisons (DSP), amnesic shellfish poisons (ASP), neurotoxic shellfish poisons (NSP), and ciguatera toxins. There were some differences noted between nations in tolerance levels established for PSP and for DSP toxins. These differences should be eliminated for consistency of public health protection and for greater harmonization of international trade."

The information summarized is said to reflect the situation as of 1 July 1991. Copies are available from: National Institute of Public Health and Environmental Protection, PO Box 1, 3720 BA Bilthoven, The Netherlands.

IOC Publications

Available free of charge

The publications listed below are available free of charge from the IOC. Please address requests for these publications, as well as for extra copies of issues 1-4 of Harmful Algae News *to: IOC Secretariat, Attn. Henrik Enevoldsen, UNESCO, 1 rue Miollis, 75732 Paris Cedex 15, France.*

Programme on Harmful Algal Blooms. IOC-SCOR Workshop on Programme Development for Harmful Algal Blooms. IOC Workshop Report No. 80, 1991.

Summary Report of the IOC-FAO Ad hoc Intergovernmental Panel on Harmful Algal Blooms, Paris, 23-25 June, 1992. (Includes revised outline of the HAB Programme).

Monitoring the Health of the Ocean: Defining the Role of the Continuous Plankton Recorder in Global Ecosystem Studies. The Intergovernmental Oceanographic Commission and The Sir Alister Hardy Foundation for Ocean Science, 1991. IOC/INF-869.

Also, a few copies are available for scientists in developing countries (priority to libraries) of:

Biotoxines aquatiques (eau de mer et eau douce). Critères d'hygiène de l'environnement 37. International Programme on Chemical safety (IPCS), World Health Organization, Geneva, 1986.

Proceedings of the Third International Conference on Ciguatera, Puerto Rico, 1990. Edited by T.R. Tosteson.

Future events

APRIL 93

International Workshop on Ciguatera Management, 13-16 April, Bribie Island, Australia. *Contact*: Workshop Secretariat, Southern Fisheries Centre, PO Box 76, Deception Bay, Queensland 4508, Australia. Tel: (61-7) 203 1444; fax: (61-7) 203 3517.

AUGUST 93

IOC Training Course on the Taxonomy of Harmful Marine Phytoplankton

This course will be held at the University of Copenhagen, Denmark, 16-28 August 1993, and will focus on identification and preparation techniques supplemented by lectures on different aspects of harmful algae. The teaching staff will be: Dr. Yasuwo Fukuyo (University of Tokyo), Professor Øjvind Moestrup and Dr. Jacob Larsen (University of Copenhagen). The course is organized by the Botanical Institute, Department of Phycology, and co-sponsored by DANIDA (Danish Ministry of Foreign Affairs, Department of International Development Cooperation). Priority will be given to applicants from developing countries.

Application forms can be requested from the IOC Secretariat, Paris.

OCTOBER 93

Sixth International Conference on Toxic Marine Phytoplankton, 18-22 October, Nantes, France. *Contact*: Cité des Congrès, 5 rue Valmy, 44041 Nantes Cedex 10, France. Tel: (33) 40 37 41 30; fax: (33) 40 37 40 73.

Eleventh International Symposium of the World Association of Veterinary Food Hygienists (XI WAVFH), 24-29 October, Bangkok, Thailand. *Contact*: Dr. Vanda Sujarit, Faculty of Veterinary Medicine, Kasetsart University, Bangkhen, Bangkok 10903, Thailand. Tel: (66-2) 5797539; fax: (66-2) 5798781/5790524; telex: 21957 RECOFTC TH.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 5

Unknown poison found in French Atlantic shellfish

In early November 1992, water samples taken in the framework of REPHY (French Phytoplankton Surveillance Network), near mussel ropes in the centre of the Pertuis Breton, showed high numbers of a *Dinophysis* species not normally common on French coasts, particularly at this time of year. Mouse tests for DSP (Diarrheic Shellfish Poison) were immediately carried out, but the rapidity of death and the symptoms observed suggested the presence of PSP (Paralytic Shellfish Poison).

On 13 November, sales of shellfish from the affected area were banned as a precautionary measure. On 16 November, results of chemical analyses by HPLC (high performance liquid chromatography), carried out by the National Centre for Veterinary and Food Research (CNEVA), Paris, appeared to confirm the presence of PSP toxins, but in amounts below the internationally recognized toxic threshold, 80 µg of PSP/100 g meat. The toxic profile moreover, did not correspond to that of cultured and wild stocks of the PSP-producing dinoflagellate, *Alexandrium minutum*.

During this period, no known PSP-producing species of phytoplankton was detected in water samples. Nor did sediment samples, taken on 23 November from under mussel ropes in the Pertuis Breton, show cysts of any known toxic species. As from 10 December, toxic effects could no longer be detected.

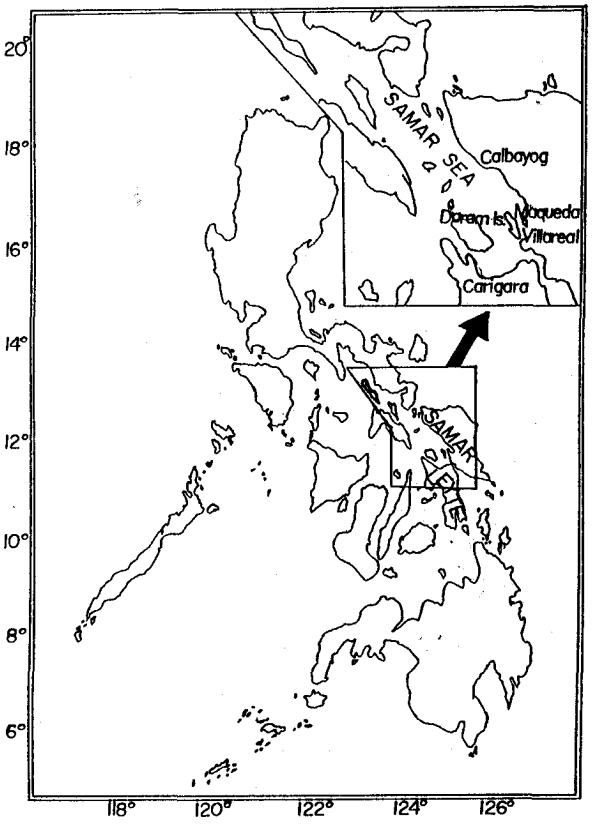
A new toxicity episode began on 26 January 1993. PSP mouse tests carried out on rope-grown mussels from the Pertuis Breton showed intense toxicity (survival time 1 to 3 minutes), even though no species known to be toxic could be detected in the water. Toxicity spread to oysters in the Bassin de Marennes-Oléron and to mussels in the Bassin d'Arcachon, leading to the decision to impose closing orders. Twenty-four hours after obtaining results which were positive for supposed PSP, further tests carried out on the same acid extracts gave negative results, showing that the poison is unstable and that it is not PSP toxin (which is stable).

More complete chemical analyses on the same extracts confirmed the absence of PSP toxin in the samples. Further conclusions as to its origin, however, remain difficult.

Subsequently the contamination rapidly diminished. All areas were reopened on 17 February.

The organism responsible for the production of poison has still not been identified. The molecular structure of the toxin is still unknown.

Claire Marcaillou-Le Baut, Cathérine Belin, IFREMER, Centre de Nantes, rue de l'Ile d'Yeu, B.P. 1049, 44037 Nantes cedex 01, France.



Pyrodinium bloom in Central Philippines

A massive bloom of *Pyrodinium bahamense* var. *compressum* occurred in Carigara Bay* and the Samar Sea in the central Philippines in November 1992, causing illness to 141 persons and death to eight others due to paralytic shellfish poisoning. The marine products implicated in the poisoning cases were pen shell, tongue clam (*Lingula* sp.), green mussel (*Perna viridis*), slipmouth (*Leiognathus* sp.), sardine (*Sardinella longicipeps*), Asian moon scallops (*Amusium pleuronectes*), thorny oyster (*Spondylus* sp.), cuttlefish and blue crab (*Portunus pelagicus*).

The *Pyrodinium* bloom started in Carigara Bay in late October 1992 and spread to the neighbouring Samar Sea in mid-November. On 23 November, a 75-hectare reddish discolouration was detected on the western coast of Daram Island which lies near the entrance of Maqueda and Villareal Bays where the multi-million peso green mussel industry is located. Up to 25 cells per chain were observed from plankton samples collected from the Samar Sea.

The discoloured water subsequently dispersed to neighbouring areas and was seen to have reached as far north as Calbayog City waters in mid-December with the more intense colour observed near Daram Island. A species of blood cockle (*Mimarcaria matsumotoi*

(Cont'd on p. 3)

World record of PSP in Southern Argentina

An unusual PSP toxicity outbreak was recorded in southern Chile and southern Argentina during spring and summer of 1991 and 1992 (G. Lembeye, *HAN* N° 2). Its intensity, duration and extension resulted in a high number of human intoxications (some people died), and in a high mortality of fish, penguins and other marine bird species in the region. All evidence showed that the *Alexandrium catenella* bloom began during November 1991 in the western end of the Magellan Strait, and then spread southwards and eastwards. In the southern Argentine region, the highest toxicity values (127,200 µg STX eq/100 g *Mytilus edulis*) were detected in February, near Ushuaia city (Benavides *et al.*, *CTMFM*, p. 19, 1992). To my knowledge, this is the highest toxicity value recorded for a PSP outbreak, and it is also higher than the saturation value reached in experimental intoxications of *Mytilus edulis* (V.M. Bricelj *et al.*, *Toxic Marine Phytoplankton,* 269-274, 1990). This extreme value seems to be a consequence of the high toxicity of the *Alexandrium catenella* population (325 pg STX eq/cell) as well as of the high cell concentrations detected, which produced discolourations and luminescence.

In the southern Chilean region, the first bloom of this species was detected in 1971. However, P.A. Segers (*La Semana Médica*, Buenos Aires, 1117-1119, 1908) described the intoxication and massive death of native people near Ushuaia, due to bivalve consumption in 1886. It is possible that this species might then have been a common - and occasionally prominent - component of the phytoplankton community from the region.

The scenario of the present outbreak was characterized by periods of unusually calm and high insolation, and the occurrence of near surface pycnoclines. Recent data have indicated that the decrease of the ozone layer is a world phenomenon, and significant in this southern region of South America, near the 'Antarctic ozone hole' (R. Stolarski *et al.*, *Science,* 256, 342, 1992). There is agreement among scientists that the change in the specific composition of phytoplankton communities is one of the expected effects of the increase of the UV radiation (UVR) (R.C. Smith *et al.*, *Science,* 255, 952, 1992). A well-adapted species seems to be *Phaeocystis pouchetii*, which produces surface blooms in the marginal ice-edge zone around Antarctica during spring/summer. As mentioned elsewhere for several dinoflagellates, including *Alexandrium catenella*, *P. pouchetii* produces mycosporine-like amino acids that diminish the damage produced by UVR (Marchand *et al.*, *Mar. Biol.*, 109, 391-395, 1991). On the other hand, small UVR doses produce a significant decrease in the survival of most of the zooplankton species examined and could reduce the fecundity of marine copepods (Karanas *et al.*, *Mar. Biol,* 65, 125-133, 1981).

Li'l Abner might call the UVR increase a 'Whammy' (T. Wyatt, *HAN* N° 1). However, this is not sufficient to explain this massive outbreak detected in the southern region. The UVR doses received by a planktonic population depend on the incident UVR with depth in the water column and on the 'residence time' of a population within the UVR photic zone. Therefore, overall effects, integrated through the water column and over large geographic areas, are difficult to estimate.

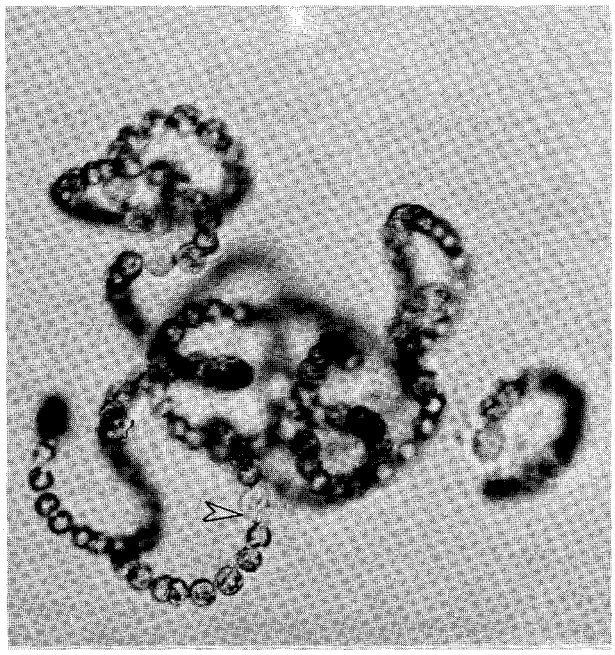
We are ignorant of the ultimate cause which triggered this unusual bloom, although it seems that we are dealing with an event resulting from the hazardous combination of several environmental factors. Is the increase of UVR one of them?

Jose I. Carreto and Hugo R. Benavides, Instituto Nacional de Investigación y Desarrollo Pesquero, P.O. Box 175, Mar del Plata, Argentina.

Nuisance blooms in Argentina reservoirs

The Paso de las Piedras reservoir in Buenos Aires Province, Argentina, is the sole water supply for the two large towns of Bahía Blanca and Punta Alta. Blooms of blue-green algae, *Gloetrichia echimulata*, *Anabaena spiroides* and *Microcystis* spp. are common in summer and autumn, and give the water a bad taste and smell. Some of these blooms are also potentially toxic. Cell counts made on tap water in Bahía Blanca have revealed up to 1.6×10^3 cells ml^{-1} showing that the conventional sand filtration and chlorination procedures are ineffective. The photograph below shows coils of *A. spiroides* (arrow marks a heterocyst).

Ana María Gayaso, Instituto Argentino de Oceanografía, Avda. Alen 53, 8000 Bahía Blanca, Argentina.

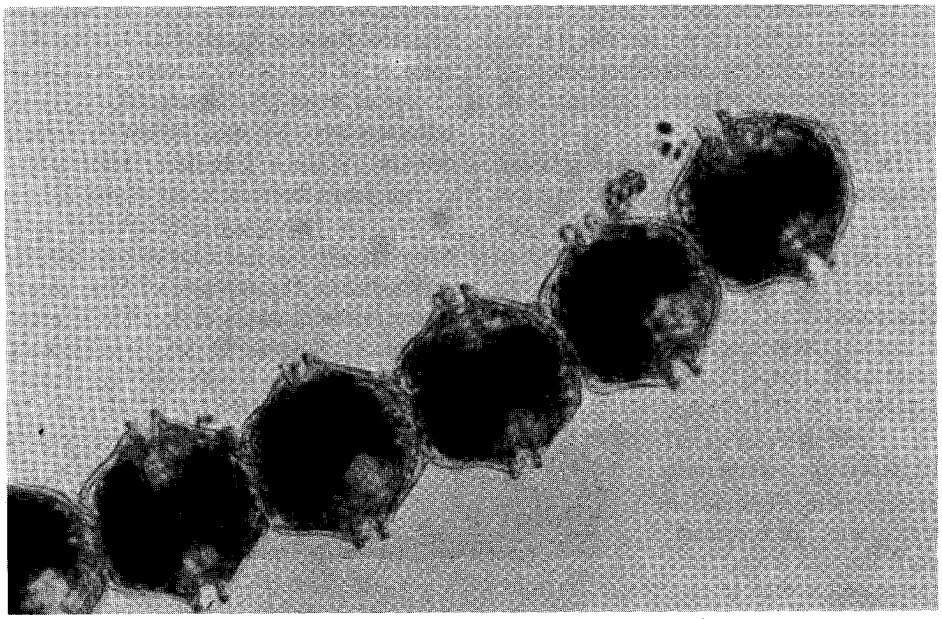


Alexandrium fraterculus in Uruguayan waters

High levels of PSP were recorded in Uruguayan coastal waters in February 1992, as reported in *HAN* N° 2, p. 5. The species responsible was identified at that time as *Alexandrium catenella.* Subsequent consultation with Dr. E. Balech and Dra. R. Akselman of Argentina has led to a re-identification, and it is now believed that we were dealing with *Alexandrium fraterculus.* It is also worth pointing out that the first toxic episode recorded in Uruguayan waters, in 1980, was attributed initially to *Gonyalulax* sp. This is now thought to have been *A. fraterculus* (Balech, pers. comm.).

Thus up until now, three species have been associated with toxic events with PSP in Uruguay: *A. fraterculus*, *A. excavatum*, and *Gymnodinium catenatum.*

Silvia Méndez, Instituto Nacional de Pesca, Constituyente 1497-89, 11200 Montevideo, Uruguay.



Chain of *Alexandrium fraterculus.*

(Cont'd from p. 1, 'Pyrodinium bloom')

Habe) collected from the coastal water of Daram Island reached a maximum toxicity of 3,844 µg/100 g of shellfish meat.

In order to protect public health, the National Inter-Agency Committee on Environmental Health, upon recommendation from its Red Tide Task Force, imposed temporary bans on the harvesting, transporting and marketing of all kinds of shellfish from Carigara, Maqueda and Villareal Bays, and from the Samar Sea, immediately after the bloom was detected.

The issuance of auxiliary invoices, a requirement of the Department of Agriculture for the transportation of fishery products from one place to another, was suspended to prevent the movement of contaminated shellfish to other areas. Other quarantine measures, such as the establishment of checkpoints in strategic places like airports, piers and bus routes, were instituted.

Due to the adverse effect of the phenomenon on the livelihood of marginal fishermen, shellfish farmers, shellfish farm workers and vendors, the Office of the President declared several municipalities of the provinces of Leyte and Samar as disaster areas and released 5 million pesos (approximately US $196,850) for alternative livelihood projects of the affected fishermen.

As of this writing, motile *Pyrodinium* cells are still present in plankton samples collected from the two areas.

Cielito Lindo Gonzales, Bureau of Fisheries and Aquatic Resources, 860 Quezon Ave., Quezon City, Philippines.

*Carigara Bay is about 550 km from Manila Bay, site of the *Pyrodinium* event reported in *HAN* N° 3, p. 1.

Gymnodinium kills farm fish in India

About 5 tons of fish were found dead in a 7-acre brackish water fish farm at Kodi, Karnataka State, on the southwest coast of India during 11-15 December 1989. This fish farm had stocked *Tilapia, Etroplus* and *Chanos* using wild seeds collected during high tide. No information was available on the stocking density in the pond at the time mortality occurred. Mortality was observed in all three varieties of fish stocked. On 11 December 1989 when mortality was first observed, a variant of *Gymnodinium nagasakiense* 22 µm long, 12 µm wide was observed at a concentration of 4×10^8/L. Concentration came down to 7.4×107/L on 13 December and 2.1×10^6/L on 15 December. Inlet water was examined during high tide on 15 December and the same *Gymnodinium* species was observed at a concentration of 2.4×10^5/L. Pond water collected on 11 December was centrifuged and cells deposited from 2 L were extracted with ethanol and dried by flash evaporation. The residues, resuspended in 0.1% Tween 60 in physiological saline, were lethal to mice on intraperitoneal injection. This confirms the toxicity of *G. nagasakiense* which we recently reported in this region (*J. Shellfish Res.*, 11(2): 477-8, 1992).

I. Karunasagar, Department of Fishery Microbiology, College of Fisheries, Mangalore - 575 002 India.

Note: *G. nagasakiense* (also called 'type '65' following an event in Omura Bay, Japan, that year) is morphologically identical to *Gyrodinium aureolum*, responsible for fish kills in Europe. Fukuyo, Y. *et al.* (in: 'Red Tide Organism in Japan', 1990) maintain that it is synonymous with *Gymnodinium mikimotoi* described by Oda from Cohaska Bay, Japan in 1935. Patensky, F. *et al.* (*J. Phycol.*, 24: 408, 1988) provide a recent account of these problems.

Pseudonitzschia pseudodelicatissima ... in Scandinavian coastal waters

From early July to mid-October 1992 massive blooms of the pennate diatom *Pseudonitzschia (Nitzschia) pseudodelicatissima* (Hasle, G.R., 1993, *Nova Hedwigia*, Beiheft 106, in press) were reported in the coastal waters of Denmark.

The first - and the only - time thus far that this species has been associated with the neurotoxin domoic acid was in the Bay of Fundy, Canada, in 1988. In late September, the species here comprised 99% of the total number of phytoplankton cells and the concentration reached 1.2×10^6 cells per litre. Domoic acid was detected in both soft shell clams, *Mya arenaria* and blue mussels, *Mytilus edulis*. It was necessary to close some areas of the bay to mussel harvesting, since the concentration of domoic acid reached levels above 20 µg per g of whole mussel tissue. *Pseudonitzschia pseudodelicatissima* was taken into culture and shown to produce domoic acid (Martin, J. *et al.*, 1990, *Mar. Ecol. Prog. Ser.*, Vol. 67, pp. 127-182).

There are now three species in the genus *Pseudonitzschia* (Hasle, *op. cit.*). Known to produce domoic acid are: *Pseudonitzschia australis* Frenguelli, *P. pseudodelicatissima* and *P. pungens f. multiseries* (Hasle, *op. cit.*).

Under the light microscope *Pseudonitzschia pseudodelicatissima* may be identified by the width of the cell (1.5-2.5 µm) and the presence of a central interspace. The cells are linear and symmetrical along the longitudinal axis when seen in valve view. Electron microscopy is necessary for a critical identification. It reveals that the cell has 30-46 interstriae in 10 µm, 16-26 fibulas in 10 µm, and very characteristic poroids, which are covered by a distinctly perforated membrane (Fig. 1) (Hasle, G.R., 1965, *Skr. Utgitt Norske Vidensk. Akad. i Oslo I.*, Ser. nº. 18, pp. 1-29; Lundholm, N. and Skov, J., 1992, unpubl.).

The blooms in the Danish coastal waters were rather extensive (Fig. 2). The first reports of high cell numbers were from the Limfjord (280.000 cells per litre, 13 July) and the Isefjord (2.2×10^6 cells per litre, 14 August), where it comprised 40% of the total number of cells in the plankton. During August the cell numbers increased to $2\text{-}8 \times 10^6$ cells per litre in many areas, often representing 80-90% of the total cell number. In some areas the cell numbers decreased during September, while in others they continued to increase. In certain areas high cell concentrations seemed to occur in deeper water (7-18 m). Thus in the Lillebælt, on 8 September, 0.7×10^6 cells per litre were found at 1 m, and a maximum of 14×10^6 cells per litre at 14 m. During October the bloom decreased, although some areas still had high concentrations (e.g. 1.6×10^6 cells per litre in Aarhus Bay, 7 October).

During the bloom, *Pseudonitzschia pseudodelicatissima* dominated in most areas, while the bluegreen alga *Nodularia spumigera* was reported to dominate in certain areas of Lillebælt. The summer of 1992 in Denmark was warm with practically no precipitation.

When cell numbers in an area reached 1×10^6 cells per litre, the Ministry of Fisheries closed the area to harvesting of shellfish and it was not reopened until the level was low and stable.

At the end of July the first area (the Isefjord) was closed. Soon after (2 August) certain localities of the Limfjord were closed as well. By 29 August the whole Limfjord was closed. On the east coast of Jutland in Lillebælt, the bloom of *Pseudonitzschia pseudodelicatissima* coincided with DSP in mussels, and this area was closed from 9 August. On 23 August the southern part of the Isefjord was reopened, and later the northern part. Most localities of the Limfjord were reopened 20 October, and all were open by 22 October. In the first two weeks of November the last areas in the Lillebælt were reopened to shellfish harvesting.

Domoic acid was found neither in water samples (Helle Ravn, National Environmental Research Institute, pers. comm.) nor in blue mussels (*Mytilus edulis*) or oysters (*Crassostrea gigas*) (Ministry of Fisheries, Denmark and Matts Hageltorn, Dept. of Food Hygiene, Swedish University of Agricultural Sciences, Uppsala, Sweden). Cultures of *Pseudonitzschia pseudodelicatissima* have now been established, and this facilitates further investigations on this species.

Apart from Danish waters, high cell numbers were reported from Norwegian coastal waters (Hasle, G.R., pers. comm.) and the west coast of Sweden.

Nina Lundholm and Jette Skov, Botanical Institute, Department of Phycology, Oster Farimagsgade 2 D, 1353 Copenhagen K, Denmark; fax: (45-35) 32 2321.

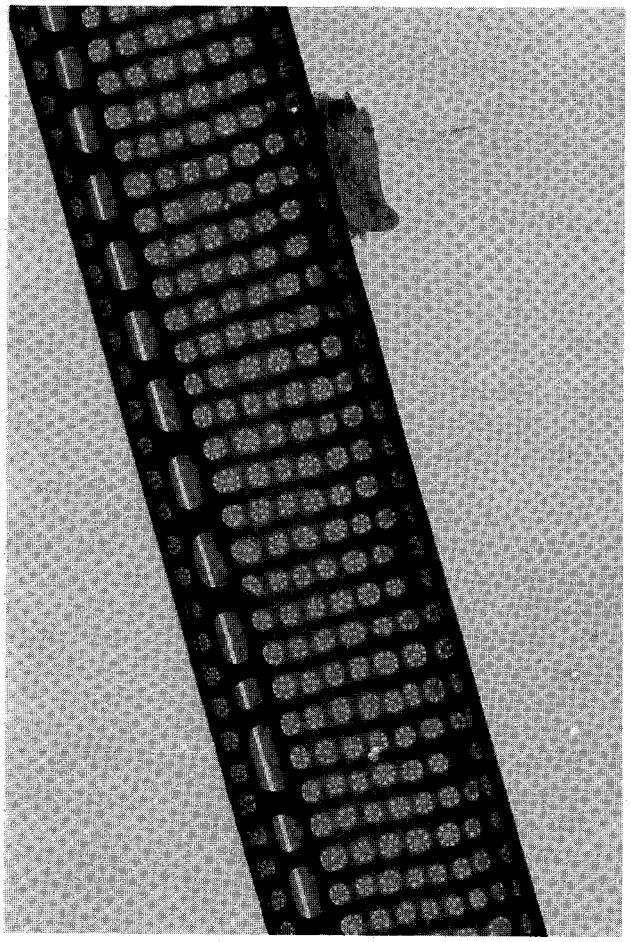


Fig. 1: Pseudonitzschia pseudodelicatissima. *Fibulae, interstriae and striae with poroids covered by a perforated membrane are visible, ×24000, electron microscopical wholemount, material from Øresund, Denmark.*

North Pacific coast of USA:
Toxic diatom blooms and domoic acid

Domoic acid (DA)-producing blooms of *Pseudonitzschia australis* were first recognized on the Pacific coast of the United States at Monterey Bay, California in the late summer of 1991. This toxic event was signalled by the death of several brown pelicans and Brandt's cormorants in mid-September of 1991. DA was found in bird tissues and linked to feeding on northern anchovy, and ultimately to *P. australis* cells upon which the anchovy had been feeding[1]. At the time of these seabird deaths, no DA measurements were being made, but plankton samples indicated that *P. australis* abundances were approximately 6.5 to 20 x 10^4 cells/L[2]. Measurements of DA levels at the University of California at Santa Cruz (UCSC) showed that toxin was present throughout November. Plankton analysis showed a peak of *P. australis* abundance in late November, with cell counts reaching > 1 x 10^6/L (Fig. 1a). Cells from the November bloom were isolated and shown to produce DA in culture[3]. No seabird deaths were associated with this November peak, probably because the anchovy were no longer congregated near the shore.

However, there was also an occurrence of DA poisoning to the north in Washington State during November, when fishermen had consumed DA-contaminated razor clams[4]. In addition to the razor clams, DA was also detected in the viscera of Dungeness crabs from the north Pacific coast[5]. Although no plankton samples were collected in Oregon and Washington during this event, it seems likely that the events in California and those further north were linked.

A toxic bloom of *P. australis* reappeared in 1992 during our monitoring program. In net-collected plankton samples, DA was detected from 10 to 20 November 1992. *P. australis* densities reached a maximum of approximately 4 x 10^5 cells/L during this bloom[6]. Although DA was measured in top smelt collected during the November bloom, and cormorants were observed diving and feeding on these fish, no seabird deaths were reported during this period. Interestingly, and unlike the 1991 event, DA was not detected in California mussels, which are sampled by the California Department of Health Services as part of a PSP and DA monitoring program[7]. Therefore, questions remain about whether this species is an appropriate 'sentinel' organism to detect DA toxicity on the Pacific coast.

In our preliminary examination of archived plankton samples at UCSC, we confirmed that there were massive blooms of *P. australis* in the winter of 1977, but the species had been misidentified as *P. seriata*[3]. Thus, although blooms of *P. australis* have previously been present, there is no evidence of toxicity in Monterey Bay prior to 1991. During our monitoring of the plankton in February 1992, we also found *P. pungens* f. *multiseries*[8], but no toxin was detected in net-concentrated plankton samples during this period.

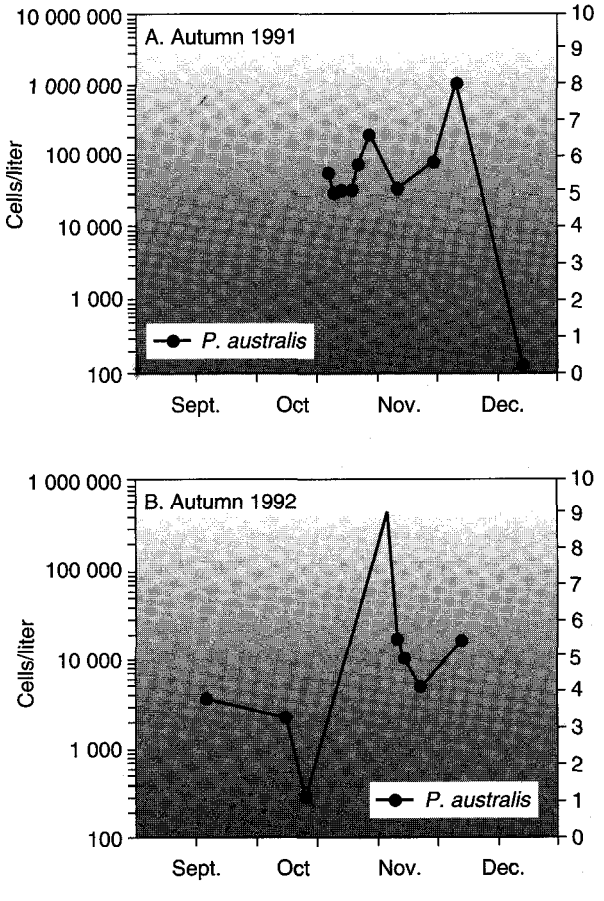


Fig. 1a,b: Pseudonitzschia australis *abundances during the autumns of 1991 and 1992.*

David Garrison and Peter Walz, Institute of Marine Sciences, University of California, Santa Cruz, California, 95064 USA.

New publication

The Proceedings of the Third Canadian Workshop on Harmful Marine Algae, held at the Maurice Lamontagne Institute, Mont-Joli (May 12-14, 1992), are now available as a published Technical Report. Many issues related to domoic acid problems in a Canadian context were addressed at this Workshop. Address reprint requests to:

Dr. Maurice Levasseur,
Biological Oceanography Division,
Maurice Lamontagne Institute,
Department of Fisheries and Oceans,
P.O. Box 1000,
850 route de la mer, Mont-Joli,
Quebec, Canada G5H 3Z4.

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Monitoring Galician coastal waters

In March 1992, the autonomous government of Galicia, northwest Spain, in collaboration with the Spanish Institute of Oceanography (IEO) began a new monitoring system to:

- determine the presence and abundance of organisms already known to be toxic;
- determine the composition and biomass of phytoplankton populations with the aim of forecasting the appearance of future toxicity;
- determine the atmospheric and hydrographic conditions associated with the appearance of particular species of phytoplankton, with a view to developing predictive indices;
- determine the nutrient concentrations associated with phytoplankton populations, and to relate these and their delivery to the rías with the appearance or increase in frequency or biomass of taxonomic groups which may cause problems as a result of their toxicity or abundance; and
- create a data base on phytoplankton populations and environmental conditions which can be used by any marine research team, especially those interested in toxic phytoplankton and marine biotoxins.

Two kinds of stations have been established: (i) primary stations are situated in the rías where aquaculture is most intensive, and (ii) secondary stations lie along the coast not covered by the primary ones. Sampling at the former is done by laboratory personnel, and at the latter by collaborating organizations and aquaculture companies operating in the region. Samples are taken weekly at all stations throughout the year.

Oceanographic conditions are determined, at primary stations only, by profiling the water column with a CTD (conductivity, temperature, depth) system provided with sensors for pressure, temperature, salinity, pH, dissolved oxygen, and optical transmission.

Integrated water samples are taken using Lindahl's (1986) technique*, and net samples of phytoplankton with half-sized bongo nets with 10 µm mesh. Phytoplankton biomass is quantified by vertical profiles of *in vivo* fluorescence using a fluorometer integrated with the CTD system. Pigments are also determined in the integrated samples by the spectrofluorometric method of Neveux *et al.* (1987). An autoanalyser is used to measure nitrate, nitrites, phosphates, silica, and ammonia levels in the integrated samples.

Data management and the issuance of reports are handled by a development of the MS-DOS system (for the local network *Novell Netware*) in *Clipper* and *C* languages (compatible with dBASE). It has a modular structure with the following features:

- general management and issuance of reports, charged with processing and synthesizing information arriving from other units;
- collection and processing of CTD data and associated sensors;
- reading and processing spectrofluorometric data;
- management of taxonomic list and cell counts;
- management of photomicrograph data;
- internal transfer of data from the different units to the central computer;
- transfer of weekly reports by fax and modem;
- management of historical data base stored on optical disc, and selective extraction of information required by other research groups.

Normally, the establishment of monitoring programmes must be justified in economic terms. Justification here is the enormous socio-economic importance of marine resources in Galicia and the requirement for systematic and detailed attention to the phytoplankton community in order to forecast toxic and noxious episodes.

Monitoring of the marine environment is always costly, but its profitability varies in an important way as a function of the planning and objectives. Thus it can be very expensive if it is only aimed at keeping alert to toxic phytoplankton and some parameters which reflect environmental conditions, but very profitable if the data obtained are of high quality, and are immediately available to research groups as backup information to their programmes, and if a good data base is created.

An important factor to bear in mind, based on the experience acquired during the seventeen years throughout which the IEO was responsible for the control of red tides, is that it is difficult to combine the requirements of a monitoring programme of this kind with the simultaneous development of research programmes by the same team. For this reason, it was decided that the basic monitoring work would be done by a team of technical staff, and that the role of the biologists would be limited to exhaustive quality control of the determinations carried out. Thus our team consists of two biologists and five technicians. The biologists are responsible for the taxonomic lists and supervision of the techniques and equipment used, the preparation of reports, and the interchange of information with research centres. The technicians undertake the sampling, cell counting and processing, and analysis of the samples.

Since research is essential if the quality of monitoring is to be maintained, we have established collaboration with the four marine research institutes and the three universities which exist in Galicia. These institutions assist us by training personnel, updating techniques and procedures, and by intercalibration; in exchange, they receive the information we obtain from the monitoring as well as samples or data which aid their research projects.

The viability and profitability of this monitoring programme is confirmed after almost one year of operation by the quantity of important information which has been acquired, the prompt transmission by fax or modem of the weekly reports of oceanographic conditions and phytoplankton in Galician coastal waters, and by the enthusiasm and collaboration of the research centres.

Joaquín Mariño Cadarso and Juan Carlos Maneiro Cadillo, Centro Galego para o Control da Calidade do Medio Mariño, Conselleria de Pesca, Marisqueo e Acuicultura, Xunta de Galicia, Peirao de Vilaxóan, s/n, Vilagarcia de Arousa, Pontevedra, Spain.

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* We have added the Sutherland *et al.* reference below, which is a more accessible and updated description of a pipe sampler.

Lindahl, O., 1986. A dividable hose for phytoplankton sampling. ICES, C.M., L: 26.

Sutherland, T.F. *et al.*, 1992. A segmented pipe sampler for integrated profiling of the upper water column. *J. Plankton Res.*, 14: 915-923.

Neveux, J. and M. Panoune, 1987. Spectrofluorometric determination of chlorophylls and pheophytins. *Archiv. Hydrobiol.*, 109: 567-581.

Mediterranean invasion by the toxic seaweed *Caulerpa taxifolia*

The green, toxic macroalga *Caulerpa taxifolia* (Vahl) C. Agardh is native to the shores of all tropical oceans. Recently it has been used in tropical seawater aquaria, in part because its toxins keep them free from both epibionts and the attention of herbivores. The Oceanographic Museum at Monaco made extensive use of it in its public displays, and in 1984, the alga was found growing on the shore just below the Museum. This did not engender concern, as it was assumed that *C. taxifolia* would be unable to survive the Mediterranean winter.

The discovery, in 1989, of *C. taxifolia* growing in a much used anchorage for pleasure boats, Cap Martin, 1.5 km to the east, took algologists by surprise. Further spreading has now occurred, probably by both drifting of fronds and/or swarmers over distances up to 500 m, while larger scale spreading, over hundreds of km, may be due to the movement of boats with the alga entangled in their anchors and fishing nets. Dumping from other aquaria is also suspected in at least one case. The alga survives local winter water temperatures of 11-13°C, suffering slight depigmentation and die-back in the colder, near-surface waters. Growth and spreading take place from June to December, when temperatures exceed 18°C.

Over 26% of the 16 km stretch of coast west of the Franco-Italian border (including Monaco) is colonized. Further isolated centres are present between the Italian port of Imperia and the French port of Toulon, but a 3-5 m^2 patch at Saint-Cyprien was cleared, with apparent success, by the divers who discovered it. So far, new centres have been reported at the Spanish port of Palma de Mallorca, where 1.5 ha are covered, and at the Italian port of Livorno.

At least eight seaweeds have already been accidentally introduced into the Mediterranean. They include the Chlorophyceae *Codium fragile* (Suringer) Hariot, the Fucophyceae *Colpomenia peregrina* (Sauvageau) Hamel, *Undaria pinnatifica* (Harvey) Suringar, *Laminaria japonica* Areschoug and *Sargassum muticum* (Yendo) Fensholt, and the Rhodophyceae *Asparagopsis armata* Harvey, *Acrothamnion preisii* (Sonder) Wollaston and *Chrysymenia wrightii* (Harvey) Yamada. Unlike any of them, however, *C. taxifolia* colonizes all types of substrate, from rock to mud, and all types of coast except for the most exposed, whether polluted or not. The depths affected are from the surface to 52 m, but between 5 and 15 m, colonization is most vigorous. Both from observations of the patch below the Oceanographic Museum and from work on a much larger scale since 1989, Boudouresque, Meinesz and co-workers estimate that *C. taxifolia* is increasing exponentially by a factor of 6 per year, in biomass of 2-3.5 kg/m^2 (wet weight), representing 3.5-10 g/m^2 of caulerpenyne toxin.

In the Mediterranean, *C. taxifolia* contains larger amounts than in its original habitat of both oxytoxin and the sesquiterpenic toxin, caulepenyne. In addition, four new sesquiterpenes, termed Taxifolial A, B, C and D, have been isolated and characterized from Mediterranean material. Early fears, by some Côte d'Azur fishermen's organizations, that herbivorous fish and urchins could be rendered toxic to humans through incorporation of these toxins from *C. taxifolia*, now seem unfounded, as the herbivores have not switched to eating the invader, even when their original food is eliminated. Vigilance is required, however.

As well as tolerating temperatures lower than in its original range, and producing more toxin, Mediterranean *C. taxifolia* grows fronds 20 to 65 cm long, compared with only 2 to 15 cm in its original habitats. This has led to suggestions that the invader may have evolved new genotypic traits (cold tolerance, larger size, more abundant and diverse toxins) which are helping it to outcompete existing species and resist grazing. *C. taxifolia* has already invaded meadows of the seagrass, *Posidonia oceanica*, already declining under pressure from coastal pollution and reclamation. In the Monaco-Cap Martin area, sparser *Posidonia* beds have succumbed to *C. taxifolia*, but denser beds appear to be resisting it, at least for the present. *Posidonia* meadows represent a key biotope in the Mediterranean littoral. The *Posidonia* and its attached animals and plants provide food, shelter and important nursery grounds for commercial fish, shellfish and urchins. As *C. taxifolia* bears no epibionts and does not represent food for existing animals, the effect of their stands on coastal fisheries is likely to be severe. Nevertheless, it remains to be seen to what extent decomposition of old, toxic fronds might support detritivore communities, and to what extent these fronds may pose a problem when they eventually wash up on tourist beaches.

Even in France, some aquarium shops are still supplying *C. taxifolia* to decorate the increasingly fashionable tropical marine aquaria. To reduce the rate at which distant centres of spreading are established, it would seem essential to ban this practice immediately in all Mediterranean countries, and as rapidly as possible to target information at seawater-aquarium dealers and owners about the danger of discharging *C. taxifolia* (and water with which it had been in contact) into the sea or into coastal rivers and drains.

Even with these precautions, it would seem possible that much of the Mediterranean coast (and perhaps nearby Atlantic ones) will be colonized in the next 10 to 20 years, and much of the existing biota replaced. Although some invading species lose their dominance with time, not all do so, and there is no evidence yet to suggest that *C. taxifolia* will. No time should be lost establishing an infrastructure to help inhabitants of the Mediterranean littoral, particularly those dependent on inshore fishing, prepare for the likely ecological, economic and social changes.

Ian Jenkinson, Agence de Conseil et de Recherche Océanographiques, 19320 La Roche Canillac, France; fax: (33) 55 29 19 82.

Selected references:

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Boudouresque, C.F., Meinesz, A., Verlaque, M. and Knoepffler-Peguy, M., 1992. *Cryptogamie Algologie*, 13, 144-145.

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ICES - IOC Study Group meeting on harmful algae

The ICES-IOC Study Group (SG) on the dynamics of harmful algal blooms (HABs) met in Charleston, North Carolina (USA), 8-11 February 1993, to continue discussions and programme development begun in Vigo (Spain) in 1992 (see *HAN* N° 2, p. 7). On this occasion, two days of the meeting were held jointly with the ICES Shelf Seas Oceanography Working Group.

Drs. Henrik Enevoldsen, HAB Programme Coordinator, IOC, and Bernt I. Dybern, Chairman of the IOC-FAO *ad hoc* Intergovernmental Panel on HABs gave accounts on the development and organization of the HAB Programme.

The SG participants agreed that it would be advantageous for both IOC and ICES if cooperation on HABs could be increased between the two organizations. This would require a more coherent organization of the question within ICES. Until now, problems related to HABs have been dealt with by several groups. However, HABs are special and, in many parts of the world (including the ICES area), they are of immense importance, causing damage to ecosystems, fisheries, aquaculture and human health.

In view of the importance of HABs from the scientific and the management viewpoints and in order to facilitate contacts with other organizations, it was proposed that there be a special body within ICES to take care of HAB problems.

In accordance with this proposal it was decided to recommend to ICES that the Study Group on Dynamics of Algal Blooms be transformed into a working group on harmful algal blooms, and some preliminary terms of reference were drafted.

Other plans discussed for the future were the holding of two workshops, one in which modelling approaches to HABs would be developed, and another in which different techniques for the *in situ* estimation of algal division rates would be compared.

Perhaps the most important result of this meeting was the fruitful interaction that took place between the 'subcultures' of biology and physics with their distinctive approaches to problem solving. It was agreed that this interaction should become an integral part of the proposed new ICES working group on HABs.

The Editor

This is your newsletter!

It is sometimes felt that this newsletter may not be able to effectively respond to specific regional issues caused by harmful algae. This feeling is especially prevalent in remote regions which are not yet in easy contact with the 'global village'. We feel however that almost all harmful algal news from whatever region will be of interest to *HAN* readers in general. The extent to which any international newsletter reflects regional needs is in the hands of its contributors!

To strengthen *HAN*, and to make sure that it is sensitive to the needs of all regions, a team of co-editors is being established. They will be announced in the next issue of *HAN*. We suggest that you keep in touch with them, and make sure that *HAN* serves your needs.

Future events

AUGUST 93

IOC Training Course on the Taxonomy of Harmful Marine Phytoplankton, 16-28 August, Copenhagen, Denmark.

OCTOBER 93

Sixth International Conference on Toxic Marine Phytoplankton, 18-22 October, Nantes, France. *Contact*: Cité des Congrès, 5 rue Valmy, 44041 Nantes Cedex 10, France; tel: (33) 4037 4130; fax: (33) 4037 4073.

Eleventh International Symposium of the World Association of Veterinary Food Hygienists (XI WAVFH), 24-29 October, Bangkok, Thailand. *Contact*: Dr. Vanda Sujarit, Faculty of Veterinary Medicine, Kasetsart University, Bangkhen, Bangkok 10903, Thailand; tel: (66-2) 5797539; fax: (66-2) 5798781/5790524; telex: 21957 RECOFTC TH.

The IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, 2nd session, 14-16 October, Paris, France. *Contact*: IOC Secretariat, Paris (see address below).

NOVEMBER 93

Environmental Management of Enclosed Coastal Seas (EMECS) - Change of dates from 19-21 July to **10-13 November**, Baltimore, Maryland, USA. *Contact:* Helene Tenner, EMECS Secretariat, Tawes State Office Building, Taylor Avenue, Annapolis, Maryland 21401, USA; tel: (1-410) 974 5047; or Dorothy Peterson, Sea Grant College, University of Maryland, USA; fax: (1-301) 314 9581.

12th Biennial International Estuarine Research Conference, 14-18 November, Hilton Head, SC, USA. A session will focus on all aspects of phytoplankton blooms. Individuals wishing to present papers or posters should send their abstract and fee of US $20 (made out to ERF'93 Conference) to: Kevin Sellner, Benedict Estuarine Research Laboratory, ANS, Benedict, MD 20612, USA; tel: (1-301) 274 3134; fax: (1-301) 274 3137; omnet: benedict.lab.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 6

This issue is devoted to domoic acid in North America

Guest editor's notes

As one steeped in the conventional wisdom that only phytoflagellates (excepting cyanobacteria) were phycotoxin producers, it came as a surprise to me that the capacity to synthesize potent mammalian toxins is shared with diatoms. Under-flagellated - thus comparatively boring to watch under the microscope (Victorian diatom gardens notwithstanding), and of limited apparent use other than in such utilitarian products as polishing compounds, diatoms always seemed to lack a certain 'je ne sais quoi' compared to flagellates. Yes, as first among equals at the base of marine food chains, diatoms are certainly important, but roughage for copepods is hardly a sexy role. Frankly, I'm a bit disappointed to admit diatoms into the first ranks of marine 'killer' organisms! Perhaps *Pseudonitzschia* really produce pseudo-domoic acid.

On a more serious note, the recent National Shellfisheries Association Annual Meeting in Portland, Oregon, USA (31 May–3 June, 1993) hosted a special mini-symposium, sponsored by SEAGRANT, on 'Harmful Phytoplankton and Shellfish Interactions'. It was noteworthy that the risk of ASP is of particular concern to the Pacific shellfisheries industry and regulatory agencies, as reflected in the fact that more than half of the invited oral presentations were focused on domoic acid (DA) distribution and effects. Local reports from the Pacific northwest have indicated that > 250,000 people may be involved in the recreational razor clam fishery – obviously a nightmare to monitor adequately! Re-

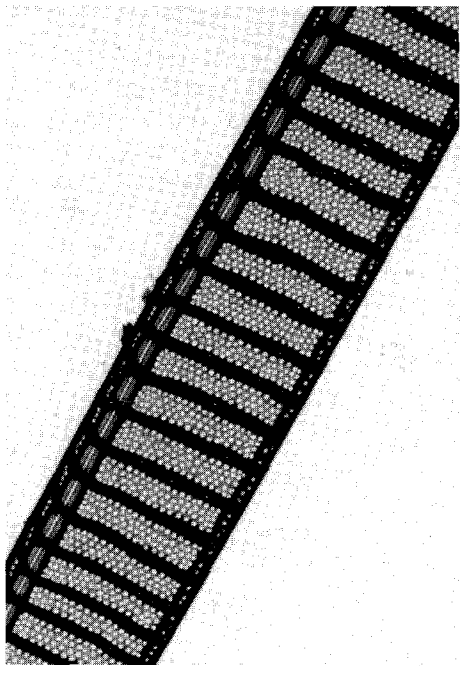
(Cont'd on p. 2)

Toxicity and *Pseudonitzschia pungens* in Prince Edward Island, 1987-1992.

In late 1987 an unusual series of human illnesses and deaths was determined to be caused by consumption of mussels contaminated by domoic acid (DA)[1]. This potent neurotoxin, produced by the pennate diatom *Pseudonitzschia* (formerly *Nitzschia*) *pungens* f. *multiseries*[2], was found during late autumnal blooms in estuaries of eastern Prince Edward Island (PEI), in Atlantic Canada. The chronology of these events[3] and many other aspects of these phenomena have been reviewed recently[4].

Prior to 1987, it was believed that PEI and nearby regions of the Gulf of St. Lawrence were free from harmful algal problems and no regular monitoring program for phycotoxin-producing phytoplankton was in place. Following the tragic events of 1987, projects were undertaken to determine the spatial and temporal patterns of abundance of *P. pungens* and occurrences of DA toxicity in phytoplankton and shellfish. A further aim was to acquire a predictive capability by relating the physical, chemical, biological and hydrographic parameters governing the timing and intensity of these blooms.

Large scale meteorological events appear to be important in determining the magnitude of *P. pungens* blooms. The 1987 bloom was likely to have been immense, although we can only speculate. The summer of 1987 was the driest in many years, resulting in a drop in the water table and low river runoff. Rain in September (while significant) served only to raise water tables, not to increase runoff. Heavy rains followed in November, leading to a sudden increase in runoff, which presumably delivered a large input of nutrients to the Cardigan River estuary within a short period, hence provoking a bloom of *P. pungens*. The heavy rainfall was followed by an extended period of calm weather characterized by light southerly winds,



Pseudonitzschia pungens *f.* multiseries *(x6000) from Danish waters. Photo: Ø. Moestrup.*

which may have been important to the retention of the phytoplankton bloom in the estuary. It is not known where the seed population for the bloom originated, however back calculations were used to estimate the *P. pungens* bloom maximum at 3 to 4 x 10^6 cells/L.

The meteorological events of 1988 were similar to those of 1987, but were less severe. The summer was dry, yet not as dry as in 1987, and the water tables were not as low. In 1988, the autumn rainfall started earlier and the mid-November weather was not as calm. A very strong southeasterly gale in early November injected large amounts of nutrients into the water column, probably including major fluxes from benthic sources. The toxic *P. pungens* bloom in 1988 reached 1.2 x 10^6 cells/L. The morphologically similar (but non-toxic) f. *pungens* formed two blooms which peaked earlier than the toxigenic f. *multiseries* bloom.

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searchers at Battelle Marine Sciences Laboratory divided toxic razor clams from Washington State into edible and non-edible fractions, then determined DA levels in each tissue compartment. For edible tissues the hierarchy was as follows: foot > adductor > siphon > mantle, whereas for non-edible components the toxin content in decreasing order of importance was gonads > siphon tip > digestive gland/gill. This is markedly different from the distribution expected for PSP toxins. Also, surprisingly, measurable DA was retained in tissues for more than four months, and archived samples indicated that DA was present even after eight years in storage. Short-term feeding experiments using *P. pungens* f. *multiseries* from Galveston Bay to toxify oysters, *Crassostrea virginica*, conducted at Texas A&M University, indicated that most DA was concentrated in digestive tissues, with virtually no detoxification occurring in < 72 hours after cessation of feeding.

A review of DA in western Washington State waters, from the University of Washington, noted that at least three known DA-producing *Pseudonitzschia* species (*pungens* f. *multiseries*, *pseudodelicatissima*, and *aus-tralis*) are present in this area. However, *Pseudonitzschia* was apparently absent from the surf zone during the most prominent DA incident. A detailed survey by Texas A&M researchers and their collaborators on the Pacific coast has attempted to determine the taxonomic affinities and geographical range of suspected DA-producing diatoms from southern California northward to the Columbia River. A number of clonal isolates are being screened for their capacity to produce DA in culture. Good luck to all concerned.

Guest editor for this issue:
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British Columbia: Implications of the North American west coast experience

Until 1991, the only region known for domoic acid (DA) produced by diatoms was the Atlantic coast of Canada, particularly the east coast of Prince Edward Island. This was peculiar because the diatoms implicated in that region are not confined to those waters, being common in many temperate coastal regions in both the northern and southern hemispheres. For example, *Pseudonitzschia pungens* is particularly common off the west coast of Vancouver Island, British Columbia, with highest numbers found in July and August, although not in great abundance (max. 10^6 cells/L). Both *P. pungens* forms are present in Sechelt Inlet, a classic mainland fjordal inlet on Vancouver Island, where they occur primarily in late summer, with f. *pungens* predominating. Another species known to produce DA, *P. pseudodelicatissima,* is also present in British Columbia, although its distribution, abundance, and toxicity is not well known since it is difficult to distinguish under routine analytical conditions.

The deaths of seabirds in Monterey Bay, California in September 1991, following the consumption of anchovies, was traced to another related diatom, *P. australis* (see article by Garrison and Walz, p. 5). Thus, the presence of DA on the west coast was demonstrated in dramatic fashion, yet the incident seemed very unusual. Seabird mortalities suspected to be due to phycotoxins have occasionally happened on the west coast of North America and in other parts of the world, but these were attributed to PSP. In fact, the knowledge that anchovies can accumulate enough DA in their guts to harm birds is quite alarming since anchovies are in many foodchains, including our own. This event caused quite a stir at the Fifth International Conference on Toxic Marine Phytoplankton, Rhode Island (Oct.–Nov. 1991), which followed soon after; it was another example of a newly implicated species doing a new diabolical thing with a recently discovered toxin. (This was eclipsed in dramatic impact by Joanne Burkholder's fish-killing dinoflagellate from North Carolina – which even made *Time Magazine* and *National Geographic*).

The illness of some Washington State residents drew attention to the presence of DA in razor clams (confirmed by the Institute for Marine Biosciences, NRC, Halifax) from open coast localities in Washington and northern Oregon. The fact that west coast mussels were effectively free of DA was puzzling at first, but this was interpreted subsequently to be due to rapid detoxification by mussels and remarkably prolonged retention by razor clams. The causative bloom had vanished by the time the toxicity was noted and the clam guts were free of *Pseudonitzschia*, a fact consistent with the above interpretation. However, this also opened the possibility that another source might have been the cause. Shortly thereafter DA was detected at low levels in southern Alaska. Was it conceivable that a bloom of the same species could have extended along practically the entire west coast (not necessarily coincidentally)? The puzzling absence of DA-positive analyses from the coast of British Columbia is at least partially attributable to the fact that razor clams were not part of the phycotoxin sampling program at the time. Nevertheless, the presence of DA was confirmed subsequently on the west coast of Vancouver Island in 1992.

Another new dimension was added in December 1991 when it was discovered that Dungeness crabs from Washington and Oregon, including some shipped unknowingly to Japan, had DA in their viscera. This was the first definite implication of a phycotoxin in these widely eaten crabs (PSP has been subsequently detected in west coast crab viscera, but that is another story). Its presence was restricted to the viscera, but unfortunately the crabs are often cooked whole, at which time the DA enters the meat. Also causing concern is the fact that the viscera are sometimes eaten separately as 'crab butter'. The concern of Canadian regulatory authorities regarding the occurrences of DA along the Pacific coast of the USA has led to an expanded shellfish monitoring program for DA in shellfish species, including crabs, at selected sites in British Columbia. It is not known how the crabs acquire the toxin: are they able to ingest a planktonic bloom that has

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Neurotoxicity by domoic acid and okadaic acid

In 1987, cultured Canadian mussels produced an intoxication characterized by neurological problems, such as headache, confusion, and loss of memory, particularly severe at times[9]. The syndrome was named amnesic shellfish poisoning (ASP). Neuronal damage was found in the hippocampus and amygdala of four patients who did die later on[11]. The toxin responsible for such intoxication was identified as a rare tricarboxylic amino acid, named domoic acid (DA), possibly produced by the unicellular alga *Pseudonitzchia pungens*, and accumulated by the cultured mussels[12]. Since 1987, DA has been found in a variety of seafood in the USA and Canada. In November 1991, on the west coast of the USA, a large number of cormorants died as a consequence of ingesting anchovies contaminated with DA. DA has been classified as an excitatory amino acid (EAA) which action occurs through the ionotropic NON-NMDA (N-methyl aspartic acid) subtype of EAA receptors[6,7,8]. In mammalian central nervous systems, EAA receptors (of the NMDA and NON-NMDA type) are activated mainly by the dicarboxylic aminoacids, aspartic acid and glutamic acid, which seem to be the major excitatory neurotransmitters, and appear to be also responsible for neuronal death following ischemic accidents such as strokes[1,5]. We have reported on the biochemical and toxicological effects of a DA-containing toxic mussel extract, using primary cultures of cerebellar neurons[6,7,8].

Using this experimental system we have also investigated the neurotoxic properties of okadaic acid (OKA), another toxin of algal origin which may be present in seafood, and known to produce diarrhetic shellfish poisoning (DSP). The biochemical properties of OKA have been recently reviewed[2,4]. Thus, this toxin has been found to be a potent tumor promotor. In intact cells, OKA produces a great increase in phosphorylation of several proteins, and modulates a variety of cellular functions such as smooth muscle contraction, fatty acid biosynthesis, protein synthesis, and cathecholamine synthesis and secretion. Calcium influx through voltage-sensitive calcium channels (VSCC) may be increased by OKA treatment, although opposite effects of OKA have been reported on calcium-dependent phenomena such as neurotransmitter release. OKA does not appear to alter only VSCC. Recent data indicated that OKA may increase the ionic influx through the ionotropic NON-NMDA type of EAA receptors. EAA receptors and VSCC may be responsible for neuronal degeneration[1,3,4]. We have recently described the neurotoxic effects of OKA considering the possible involvement of VSCC and EAA receptor in the neurotoxic effects of OKA in neuronal cultures[3,4].

Domoic Acid

Cultured cerebellar neurons were exposed to several dilutions of either mussel extract from amnesic shellfish poison-containing mussels (AEX), or purified DA, the alleged amnesic poison in the mussels. Surprisingly, when the neurotoxicity curve produced by AEX was compared to the concentration-dependent neurotoxicity curve produced by DA, 50% reduction in neuronal survival was achieved at a dilution of AEX containing 1~μM DA as compared with 8~μM for purified DA alone, indicating that subtoxic concentrations of DA may become neurotoxic when in AEX. In order to verify that DA was the only substance responsible for the neurotoxicity of AEX, several concentrations of purified DA were added to a non-toxic mussel extract, used as a control (CEX). We found that the addition of non-toxic concentrations of DA to CEX, produced neurotoxicity similar to AEX. By using specific antagonists for NMDA and NON-NMDA receptors, and chromatographic techniques, we have found that such potentiation of DA neurotoxicity in mussel extract is due to mussels' high content of aspartic and glutamic acid. Thus, DA, by acting at the NON-NMDA receptor, is able to enhance the action of aspartic and glutamic acid at the NMDA receptor, beyond physiological control. The result is an excessive excitation which may lead to neuronal death. In mussels, the combined concentration of glutamic and aspartic acid reported in the literature[7]

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(Cont'd from p. 2, 'British Columbia')

'sunk out', or are they getting DA from a benthic source either directly or indirectly?

As yet unpublished results of a field study we conducted in Barkley Sound on the west coast of Vancouver Island in the summer of 1992 (first of a two-year study) revealed that both *P. pungens* and *P. australis* bloomed within the bay, but in sequence. The former species was not associated with DA in shellfish, yet there was a rise in DA levels (according to the Department of Fisheries and Oceans, Inspection Branch) immediately following the collapse of the latter bloom. These data suggest that, in general, *P. australis* is the primary source of DA on the west coast. This, of course, requires confirmation by testing *P. australis* cultures from the northwest coast for DA production, and the determination of what role, if any, *P. pungens* plays in DA toxicity on the Pacific coast.

Since *P. australis* has not been recorded previously from the west coast, or even from the northern hemisphere, there is a natural tendency to wonder if it represents a recent introduction via ship's ballast water or other means. Several points can be noted in this regard. First, this species greatly resembles *P. seriata* (in fact, its synonym is *Nitzschia pseudoseriata*), differing primarily in having a more symmetrical valve shape. Therefore, it could easily have been misidentified (as *N. seriata*) in earlier west coast collections. This species was not even included in Esther Cupp's much-used taxonomic reference on west coast diatoms, but only its look-alike, *P. seriata*. Second, its known distribution is practically the same as *P. pungens* f. *multiseries* (Argentina, British Columbia), although the latter also has east coast records. The matter should be resolvable by looking at archived samples from which *P. seriata* was previously recorded (as *N. seriata*). Records of the *N. seriata* from California are particularly suspect since Grethe Hasle believes that this is a very cold northern species (Cupp found it in Alaska).

F.J.R. 'Max' Taylor, Dept. of Oceanography, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4.

averages at 0.74 mmol/100 g fresh weight, although it may vary from 0.3 to 1.62 mmol/100 g fresh weight. Nevertheless, human cases of intoxication attributable to mussels' high EAA content have never been reported. These considerations reinforce the idea that DA may have been necessary for the occurrence of neurological problems. Moreover, our data suggest that concentrations of DA that per se would not have been sufficient to cause neurotoxicity, could have induced neurotoxicity in the presence of adequate concentrations of NMDA receptor agonists. The relevance of our results in the explanation of the neurological problems reported during the Canadian episode of intoxication will need further investigation using different experimental systems. Recent data from behavioural studies in rodents did show that low doses of DA in extracts of contaminated mussels were significantly more potent than the same doses of purified DA[10]. Thus, this observation may be explained by our finding of a synergistic interaction between EAAs, assuming a significant passage of alimentary-related EAAs through the blood brain barrier.

Okadaic Acid

Okadaic acid (OKA), at concentrations as low as 0.5 - 1 nM may produce neurotoxicity[3]. OKA action is characterized by a slow disintegration of neurites, followed by visible swelling of cell bodies, and later by cellular death. Neurotoxicity by OKA was not characterized by any fast darkening/swelling of the neurons (characteristic of EAAs), which remained unaltered for up to 16 hours, when degeneration of the neuronal network started to become evident[3,4]. After 24 hours exposure, OKA led to a complete degeneration of the neuronal network, while around 70% of the neuronal cell bodies were still able to stain for the vital probe fluorescein diacetate, and therefore they were considered alive by this criterion[3]. After 48 hours exposure to OKA most of the neuronal bodies had degenerated. The concentration producing 50% neurotoxicity shifted from around 7.5 nM at 24 h, to around 1.8 nM at 48 h. The majority of cerebellar neurons in culture are glutamatergic granule cells, which may release EAAs, such as glutamic acid and aspartic acid, upon depolarization-induced calcium entry via VSCC. Such endogenous EAA release may produce neurotoxicity[3]. Thus, we have explored the possibility that neurotoxicity by OKA may involve endogenous EAA release. Blockers of VSCC, as well as EAA receptor antagonists, completely prevented neurotoxicity by endogenous EAA release, but failed to protect from OKA neurotoxicity. The reported increase in ionic permeability of the NON-NMDA channel subsequent to exposure to OKA may not be relevant for the neurotoxic effect of OKA. In fact, OKA failed to potentiate DA toxicity[4].

It is well documentated that OKA is a potent inhibitor of protein phosphatases PP1 and PP2A, and therefore leads to a great increase in protein phosphorylation when added to intact cells[2]. The inhibition of PP1 and PP2A activity *in vitro* occurs at concentrations of OKA comparable to those we found to be neurotoxic[2,3]. Some of the proteins affected could be particularly important for the neuronal functioning, as recently discussed[3]. OKA is capable of altering the activity of Ca^2+/calmodulin-dependent protein kinase II (CaM-K), which participate in the storage of synaptic weight. Thus, OKA-mediated disruption of the neuronal network might be reflecting changes in the phosphorylating equilibrium determined by CaM-K activity. Other phosphorylated proteins may also be considered to play a role in OKA neurotoxicity, such as synapsin I, which controls vesicular neurotransmitter release. On the other hand, the microtubule-associated protein 2 (MAP2), which is increasingly phosphorylated in the presence of OKA, has been suggested to be necessary for neuronal cells to undergo normal morphological differentiation including neurite extension and cessation of cell division. Another microtubule-associated protein, tau, appears to be abnormally phosphorylated in some neurodegenerative disorders such as Alzheimer's disease, and the appearance of hyperphosphorylated tau and neurodegeneration following microinjections of OKA in rodent brain has been observed. Moreover, OKA has recently been shown to modulate the processing of Alzheimer B/A4 amyloid precursor protein.

Conclusions

The neurotoxic properties of DA and OKA deserve further studies in order to fully evaluate the risk represented by ASP and DSP for human health. Caution must be exercized when considering the toxicological properties of each toxin, since they may produce unexpected synergisms. For ASP, the contemporary presence in the brain of concentrations of DA insufficient alone to be toxic, together with excitatory amino acids of diet-related origin, may have been relevant in the occurrence of the neurological problems reported. On the other hand, the slow neurotoxic effect of OKA may be important when considering its lipophilicity and its possible acumulation in liphilic structures such as the brain.

This investigation has also allowed the preparation of a new bioassay based on the utilization of cultures of cerebellar neurons for the determination of the presence of ASP and DSP in shellfish (as well as in other types of seafood). It should be noted that this assay, based on live cells, allows us to determine the presence of toxicity not only by DA or OKA, but by any DA-like or OKA-like toxin. In this respect it may be an interesting alternative to the mouse assay, in which respect it may offer higher sensitivity.

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North Pacific coast of USA:
Toxic diatom blooms and domoic acid

Domoic acid (DA)-producing blooms of *Pseudonitzschia australis* were first recognized on the Pacific coast of the United States at Monterey Bay, California in the late summer of 1991. This toxic event was signalled by the death of several brown pelicans and Brandt's cormorants in mid-September of 1991. DA was found in bird tissues and linked to feeding on northern anchovy, and ultimately to *P. australis* cells upon which the anchovy had been feeding[1]. At the time of these seabird deaths, no DA measurements were being made, but plankton samples indicated that *P. australis* abundances were approximately 6.5 to 20 x 10^4 cells/L[2]. Measurements of DA levels at the University of California at Santa Cruz (UCSC) showed that toxin was present throughout November. Plankton analysis showed a peak of *P. australis* abundance in late November, with cell counts reaching > 1 x 10^6/L (Fig. 1a). Cells from the November bloom were isolated and shown to produce DA in culture[3]. No seabird deaths were associated with this November peak, probably because the anchovy were no longer congregated near the shore.

However, there was also an occurrence of DA poisoning to the north in Washington State during November, when fishermen had consumed DA-contaminated razor clams[4]. In addition to the razor clams, DA was also detected in the viscera of Dungeness crabs from the north Pacific coast[5]. Although no plankton samples were collected in Oregon and Washington during this event, it seems likely that the events in California and those further north were linked.

A toxic bloom of *P. australis* reappeared in 1992 during our monitoring program. In net-collected plankton samples, DA was detected from 10 to 20 November 1992. *P. australis* densities reached a maximum of approximately 4 x 10^5 cells/L during this bloom[6]. Although DA was measured in top smelt collected during the November bloom, and cormorants were observed diving and feeding on these fish, no seabird deaths were reported during this period. Interestingly, and unlike the 1991 event, DA was not detected in California mussels, which are sampled by the California Department of Health Services as part of a PSP and DA monitoring program[7]. Therefore, questions remain about whether this species is an appropriate 'sentinel' organism to detect DA toxicity on the Pacific coast.

In our preliminary examination of archived plankton samples at UCSC, we confirmed that there were massive blooms of *P. australis* in the winter of 1977, but the species had been misidentified as *P. seriata*[3]. Thus, although blooms of *P. australis* have previously been present, there is no evidence of toxicity in Monterey Bay prior to 1991. During our monitoring of the plankton in February 1992, we also found *P. pungens* f. *multiseries*[8], but no toxin was detected in net-concentrated plankton samples during this period.

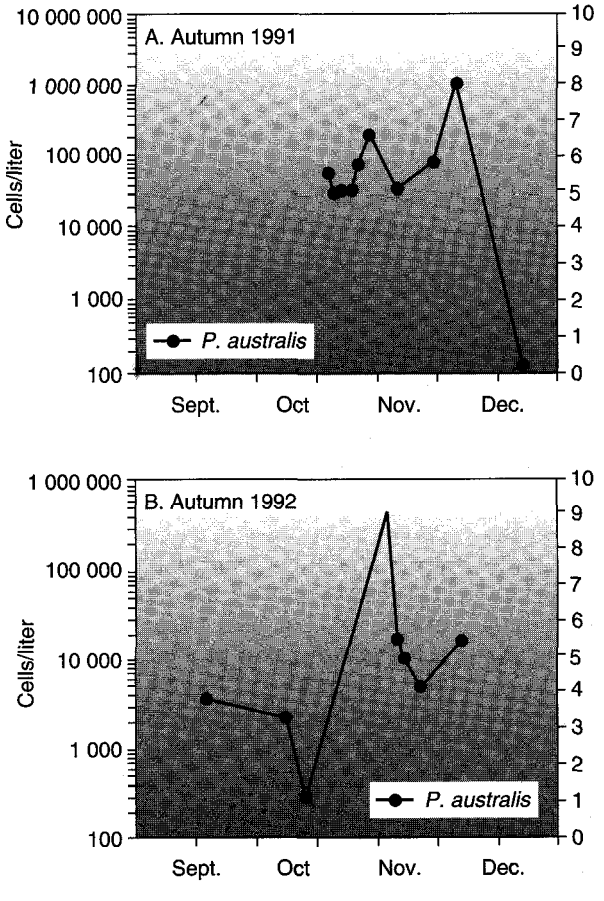

Fig. 1a,b: Pseudonitzschia australis *abundances during the autumns of 1991 and 1992.*

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New publication

The Proceedings of the Third Canadian Workshop on Harmful Marine Algae, held at the Maurice Lamontagne Institute, Mont-Joli (May 12-14, 1992), are now available as a published Technical Report. Many issues related to domoic acid problems in a Canadian context were addressed at this Workshop. Address reprint requests to:

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Maurice Lamontagne Institute,
Department of Fisheries and Oceans,
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850 route de la mer, Mont-Joli,
Quebec, Canada G5H 3Z4.

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Pseudonitzschia/Nitzschia: What's in a name and its consequences?

I thought it might be helpful to explain, as simply as possible, the basis for a recent series of name changes, since some authors may be confused about these toxic and non-toxic diatom species. At the risk of over-simplification, here goes.

In terms of number of species, *Nitzschia* is one of the largest genera of the elongate, boat-shaped pennate diatoms. It has numerous delicate ridges that run across the wall, with rows of pores between them. A tubular canal runs down one edge, and a thin slot is cut along it. To hold it together there are cross bridges that create the illusion of a string of beads, under the light microscope. Species are identified (in part) by the number of these ridges and bridges. The vast majority of species are single and benthic, i.e. they live on surfaces.

However, there are some '*Nitzschia*' that look like the above description but the cells are planktonic, more sharply pointed, and occur in chains caused by the fusion of their overlapping ends. At the turn of the century, the French Peragallo brothers thought that they were sufficiently different to warrant their own distinct genus, *Pseudonitzschia* - the name implied that the resemblance was misleading. Their proposal was generally looked at unfavourably, since the basic structure of the walls turned out to be very similar between the two, and, with a notable exception, future planktonic species were assigned to *Nitzschia*. The exception was the action of J. Frenguelli, who named a locally common species from Argentine waters as *Pseudonitzschia australis* in 1939.

In 1988, Grethe Hasle produced a very useful revision of these planktonic species which she referred to as the '*Pseudonitzschia* group' within *Nitzschia*. What to do with Frenguelli's species? Well, the usual procedure in such circumstances is to transfer it to *Nitzschia*, keeping his species name, i.e. *Nitzschia australis*. However, there already was a (different) species with that name among the hundreds of named *Nitzschia* spp. In such circumstances, a new species name is created, and Hasle chose *N. pseudoseriata* as Frenguelli's species was so much like *N. seriata*.

The discovery of DA among the *Pseudonitzschia* group (three species so far but likely to be more if past history is any indication) renewed interest in these common planktonic species. This has led to the view that they really are sufficiently distinct to warrant their own genus. Consequently, Hasle revived the Peragallos' genus, and the *Nitzschia* spp. of this group were named as *Pseudonitzschia* spp., keeping the species names wherever possible. Thus, for example, *N. pungens* became *P. pungens*, this action being referred to as a new combination. Another example is *Nitzschia pseudodelicatissima*, which is now rather bizarrely referred to as *Pseudonitzschia pseudodelicatissima*, an apparent excess of pseudosity! Note that, according to the rules of nomenclature (which are published and periodically revised), the species which Hasle had renamed, *N. pseudoseriata* had to get its first name back. It is the same organism as *Pseudonitzschia australis* – they are synonyms with the latter now being the name of choice.

This may seem confusing, but it is generally understandable, I hope.

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Laboratory studies of domoic acid production by *Pseudonitzschia pungens*

To better understand the mechanisms determining bloom dynamics and speciation of *Pseudonitzschia* (previously *Nitzschia*) *pungens* in Prince Edward Island in eastern Canada (see also article by J.C. Smith, p. 1), parallel ecophysiological and cellular studies are underway in the laboratory. Field studies and monitoring programs in Atlantic Canada have been complicated by the fact that dense blooms of *P. pungens* can be present without evidence of DA production. In the Gulf of St. Lawrence, *P. pungens* appears as two morphotypes - forma *multiseries* (a DA producer), and forma *pungens*, a non-toxic organism. These forma are virtually indistinguishable by light microscopy. Scanning electron microscopy is used to resolve subtle differences in frustule morphology. Recently, an immunofluorescence assay has been developed that employs separate polyclonal antibodies that are specific to cell-surface antigens of alternative forma of *P. pungens*. No cross reactions were found between the two forma, nor with about 40 other species examined. However, very slight immunolabelling was found with *P. pseudodelicatissima* and *P. australis*, two other reputed DA producers. Work at the Institute for Marine Biosciences, NRC, Halifax, on distinguishing toxic vs non-toxic species has involved molecular biology techniques, including analysis of 18S ribosomal RNA gene sequences.

Phycotoxin research relies heavily on the availability of the appropriate cultures of toxin-producing organisms. The availability of toxigenic cultures has allowed development of chemical analytical techniques, including high performance liquid chromatography (HPLC) coupled with detection by fluorescence (FD) or UV diode-array detection (DAD), and ion-spray mass spectrometry (ISP-MS), for the analysis of DA. The manipulation of unialgal cultures in the laboratory has permitted the identification of factors influencing the growth and DA production of *P. pungens*. This work has shown that DA is not usually produced while the cells are dividing mitotically, but rather begins near the onset of the stationary phase, e.g. due to silicate (Si) limitation. Nitrogen (N) has also been found to be essential for toxin production – this is not surprising since DA is an N-containing amino acid. Other studies have shown that light is required for toxin biosynthesis and that *P. pungens* may be adapted to the low irradiance environment in which it blooms in the autumn. However, it appears that *P. pungens* has no unique adaptations for growth or toxin production at low ambient temperatures characteristic of au-

(Cont'd on p. 7)

(Cont'd from p. 6, 'Laboratory studies')

tumn bloom conditions. Recently, *P. pungens* was grown in chemostat culture at the Department of Fisheries and Oceans (Moncton, New Brunswick). In contrast to batch cultures, *P. pungens* produced DA during active cell division phases in Si- or P-limited chemostat cultures. It may be that the pathway for DA production is turned on under nutrient-stress conditions, rather than as a response to the cessation of cellular division.

The role of bacteria in DA production is under intense investigation. It has been established that cultures rendered bacteria-free using antibiotics still produce DA, but often at substantially lower levels than untreated xenic cultures. Re-addition of bacteria has been shown to enhance DA production, and this subject is an area of continuing research. Studies are also underway to determine if bacteria from *P. pungens* cultures can produce DA in isolation. Attempts to characterize bacteria associated with *P. pungens* cultures are also in progress at the Institute for Marine Biosciences, NRC, Halifax, and at the Charleston Laboratory, National Marine Fisheries Service, South Carolina. Finally, the availability of axenic clones of *P. pungens* has facilitated study of the *in vivo* DA biosynthetic pathway.

The first DA-producing diatom, *P. pungens,* was isolated into culture soon after the original ASP incident in late autumn of 1987. However, interclonal variation in morphological and physiological characteristics is also an important issue in phytoplankton research. The Southern Gulf of St. Lawrence Culture Collection of Harmful Marine Phytoplankton was established at the Department of Fisheries and Oceans, Moncton, New Brunswick, as a species bank. This collection now contains about 60 clones each of *Pseudonitzschia pungens* f. *multiseries* and f. *pungens*, as well as about 30 clones of *P. delicatissima*, and various isolates of other diatoms and dinoflagellates from various locations in eastern Canada.

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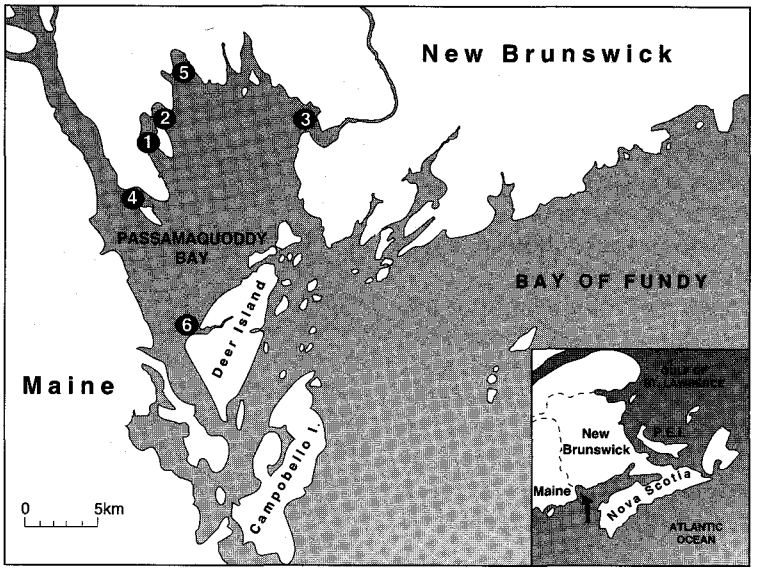
In the Bay of Fundy, eastern Canada



Bay of Fundy locations where domoic acid levels in shellfish have exceeded the acceptable regulatory limit for human consumption (20 µg/g).

In late July 1988, low levels of DA were detected in soft-shell clams (*Mya arenaria*) and blue mussels (*Mytilus edulis*) from the southwestern Bay of Fundy in eastern Canada. This early detection was a result of an extensive shellfish and phytoplankton monitoring program that was initiated in Canadian coastal regions following seafood poisonings (ASP) caused by the consumption of toxic mussels from eastern Prince Edward Island in late fall of 1987. The purpose of the study was to determine the geographic distribution of shellfish containing DA and to identify the organism(s) producing the toxin.

Closures of shellfish areas to harvesting due to high PSP toxin levels are not uncommon in the Bay of Fundy. However, prior to 1988, no shellfish toxicity had been associated with diatom blooms in this region. During 1988, after the implementation of the ASP monitoring program, DA was found only in shellfish collection areas in Passamaquoddy Bay (see figure). Although DA in shellfish was first detected in late July, shellfish harvesting areas remained open until 29 August, when levels of DA exceeded the Canadian regulatory limit (> 20 µg/g). Areas with unacceptable levels included: Chamcook Harbour (1), McCann's Cove (2), Magaguadavic Basin (3), Pottery Creek (4), Bocabec Cove (5) and Northern Harbour (6) (see figure). The highest levels of DA recorded in blue mussels were 74 µg/g measured at Chamcook Harbour (1) on 22 September, whereas maximum levels in soft-shell clams (53 µg/g) occurred on 29 September at nearby McCann's Cove (2).

Analysis of phytoplankton from the Passamaquoddy Bay region showed that the pennate diatom, *Pseudonitzschia pseudodelicatissima* (formerly *Nitzschia pseudodelicatissima*), was the dominant planktonic organism when DA was detected. Plankton were collected with a 20-µm net and analyzed for DA by HPLC. The highest cell concentrations (1.20×10^6 cells/L) were found at Chamcook Harbour (1) on 23 September 1988. Several phytoplankton species from net tows containing DA were cultured; however, only *P. pseudodelicatissima* cultures produced this toxic metabolite. The cellular concentration of DA ranged from 7.0×10^{-3} to 9.8×10^{-2} pg/cell.

Both shellfish and phytoplankton samples from the Bay of Fundy have been analyzed for DA since 1988, but this toxin has not been detected in shellfish in subsequent years. Only very low levels have been measured from plankton samples collected from 1989 to 1992. Phytoplankton monitoring since 1987 indicated that 1988 was the only year that *P. pseudodelicatissima* dominated the phytoplankton for a two-month period, reaching concentrations in excess of 1.0×10^6 cells/L. Historical phytoplankton records from the Fundy region noted *P. pseudodelicatissima* in 1931, and this species has been observed annually since 1976 – it is evidently not a recent introduction to the area.

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(Cont'd from p. 1, 'Prince Edward Isl.')

The events of 1989 were very different, in that there was no significant fall diatom bloom and *P. pungens* cell densities in the Cardigan River estuary reached only 0.6 x 10^6 cells/L. The dry summer of previous years did not occur, nor was there any runoff increase in the fall. The two *P. pungens* morphotypes followed in secession; an early non-toxic bloom was followed by a toxic event in November.

In contrast, in 1990 a bloom of the non-toxic form occurred in October, but no toxic bloom followed. After the non-toxic bloom, the meterological conditions were dominated by a series of violent, primarily northerly storms and the seaward dispersal of one incipient *P. pungens* bloom was documented at this time.

In 1991, an October bloom of the non-toxic form was observed again, and was followed by violent storms. A short period of calm weather in early November produced a diatom bloom (mainly *Chaetoceros* spp.), yet *P. pungens* f. *multiseries* was found in very small numbers in the Cardigan River system. In 1992, only a few *P. pungens* cells were found in this area and DA levels were barely detectable in phytoplankton samples.

We do not know why *P. pungens* f. *multiseries* has virtually disappeared from the Cardigan River region. One possibility is that the population was blown out to sea in 1990 before it had time to reproduce. Another possibility is that the species has been depleted by predators or parasites; we have recently observed that all bloom-forming *Pseudonitzschia* spp. in the region are subject to parasitism by a chytrid fungus, often at high rates of infection.

Large interannual variability is the most obvious characteristic of the late autumn blooms of *P. pungens* in PEI. One consistent feature, however, is that certain shallow estuaries of PEI have a propensity to produce late autumn diatom blooms, and that these estuaries are sufficiently well mixed, turbulent, and nutrient-rich at this time to sustain such blooms. If species successional processes operate such that *P. pungens* f. *multiseries* dominates the phytoplankton during the conjunction of such favourable conditions, then a toxigenic bloom can result.

Phytoplankton monitoring programs have proven effective in detecting DA events, even at unexpected locations and times. An example of this was in New London Bay (PEI) in 1991, where the pattern observed in 1988 and 1989, i.e. a large non-toxic bloom of *P. pungens* f. *pungens* in September, followed by a much smaller toxic bloom of f. *multiseries* in November, was not repeated in 1991. Instead, a large toxic bloom of f. *multiseries* occurred in early October, and was not preceded by the non-toxic form. This situation was detected by the monitoring program in place.

For monitoring, the critical variable is the concentration of particulate DA (that within cells) in the water column. This is best determined by chemical methods, which also have the advantage of detecting DA in unknown sources – *P. pungens* is not the only known producer of DA. In summary, phytoplankton monitoring has proven to be an effective and highly economical strategy in detecting DA events in eastern PEI, particularly when coupled with chemical analyses of phytoplankton and shellfish samples.

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Future events

OCTOBER 93

Sixth International Conference on Toxic Marine Phytoplankton, 18-22 October, Nantes, France. *Contact*: Cité des Congrès, 5 rue Valmy, 44041 Nantes Cedex 10, France; tel: (33) 4037 4130; fax: (33) 4037 4073.

Eleventh International Symposium of the World Association of Veterinary Food Hygienists (XI WAVFH), 24-29 October, Bangkok, Thailand. *Contact*: Dr. Vanda Sujarit, Faculty of Veterinary Medicine, Kasetsart University, Bangkhen, Bangkok 10903, Thailand; tel: (66-2) 5797539; fax: (66-2) 5798781/5790524; telex: 21957 RECOFTC TH.

The IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, 2nd session, 14-16 October, Paris, France. *Contact*: IOC Secretariat, Paris (see address below).

NOVEMBER 93

Environmental Management of Enclosed Coastal Seas (EMECS) - Change of dates from 19-21 July to **10-13 November**, Baltimore, Maryland, USA. *Contact:* Helene Tenner, EMECS Secretariat, Tawes State Office Building, Taylor Avenue, Annapolis, Maryland 21401, USA; tel: (1-410) 974 5047; or Dorothy Peterson, Sea Grant College, University of Maryland, USA; fax: (1-301) 314 9581.

12th Biennial International Estuarine Research Conference, 14-18 November, Hilton Head, SC, USA. A session will focus on all aspects of phytoplankton blooms. Individuals wishing to present papers or posters should send their abstract and fee of US $20 (made out to ERF'93 Conference) to: Kevin Sellner, Benedict Estuarine Research Laboratory, ANS, Benedict, MD 20612, USA; tel: (1-301) 274 3134; fax: (1-301) 274 3137; omnet: benedict.lab.

EDITOR'S NOTE

As indicated on p. 1, this issue of *HAN* has been dedicated to the problem of domoic acid in North America, especially in Canada. Our guest editor, Allan Cembella, is a member of the editorial team of scientists which was recently established to cover the various scientific fields and geographical areas with regards to harmful algae.

We had intended to publish in this issue the names and addresses of the members of this team. However, due to lack of space, this information will be included in the next issue (no. 7).

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 7

Highlights of Sixth International Conference on Toxic Marine Phytoplankton

Nantes, France, 18-22 October 1993

The first New Zealand outbreak of human shellfish poisoning, an 'Ambush Predator' of fish and the apparent global increase in red tides dominated the Sixth International Conference on Toxic Marine Phytoplankton, held in Nantes, France, 18-22 October 1993.

In the austral summer of 1992/93, New Zealand reported 180 cases of shellfish poisoning. This was the first outbreak of Neurotoxic Shellfish Poisoning (NSP) outside the Atlantic and it involved a complex of poisons plus a new *Gymnodinium breve*-like species. Shifting winds and altered sea surface temperatures associated with 'El Niño' were implicated.

Lurking in the murky depths of the Pamlico Estuary (USA east coast) is a strange beast indeed: amoebas, sensing warmer waters and passing fish shoals, sprout flagella, then swarm up for a gruesome kill. Aerosolization of the unidentified toxins from this mainly bottom-dwelling dinoflagellate, called *Pfiesteria piscimorte*, caused eight days of amnesia in one investigator, and researchers now wear protective shields. Large fish-kills (>1000 fish) occurred in 1991, and recently from points north (Delaware) and south (Gulf of Mexico) of Pamlico.

A total of 122 papers were reviewed describing fish-kills from harmful algal blooms (HABs). Mechanisms include anoxia, intoxication (of fish and larvae), and clogging of gills (with spiked *Chaetoceros concavicornis* and *C. convolutus* in Alaska, British Columbia and Washington State). Nations dependent on fish for food or income are most immediately threatened.

The role of ship ballast water in 'hierarchical' dispersion of alien species (see Lockwood, 16 Oct. 1993, *The Lancet*) received considerable attention. Australian authorities have begun regular monitoring for toxic algae and for

(Cont'd on p. 3)

Gymnodinium catenatum in German coastal waters

The unarmoured, chain-forming dinoflagellate *Gymnodinium catenatum* Graham is the subject of intensive research in several countries because of its obvious association with outbreaks of paralytic shellfish poisoning (PSP). As part of its sexual life cycle, *G. catenatum* produces a brown, spherical, micro-reticulate resting cyst with a prominent red accumulation body. The occurrence and distribution of dinoflagellate resting cysts is of interest because cysts represent stable populations assuring geographical maintenance in contrast to the transient bloom from which they may be derived. Because of their small size, cysts are chiefly transported with residual currents and can infect areas where they are not endemic. The fact that resting cysts can be collected and enumerated during non-bloom periods, offers a potential tool for the prediction of future toxic blooms.

As pointed out by T. Wyatt in *Harmful Algae News* no. 2, the present-day distribution of cysts and vegetative cells of *G. catenatum* in European waters extends only to the Atlantic coast of the Iberian peninsula, where it was first recorded in 1976. This species has never been recorded in any of the numerous investigations of living phytoplankton from northern European waters (Elbrächter, pers. comm.). However, large numbers of subfossil *G. catenatum* cysts where found in sediments of the Kattegat area from a period about 2000 until 300 years ago and it is suggested that in former times the species was widespread throughout

(Cont'd on p. 4)

Who produces PSP?

It is well known that paralytic shellfish poisoning (PSP) toxins are produced by *Alexandrium catenella, A. tamarense, A. cohorticula, A. fundyense, A. fraterculus, A. minutum, Gymnodinium catenatum, Pyrodinium bahamense var compressum* and *Prorocentrum minimum*, but not by other species of *Alexandrium, Gymnodinium* and *Pyrodinium.*

Silva (Proc. IV IUPAC Symp. on Mycotoxins and Phycotoxins, Lausanne. *Proc. Pathotox Publ.* 1979) suggested that dinoflagellate toxin production may be due to bacteria living in association with the dinoflagellate. Recently Kodama & Ogata (*Mar. Poll. Bull.* 19, 559-564, 1988) also pointed out the possible association of bacteria in cell interiors with toxin production by *A. tamarense*, and suggested that toxin production is not a hereditary characteristic of *A. tamarense*. We thought that this hypothesis has not as yet been supported with sufficient evidence, given that (1) the algal cultures were not axenic, and (2) there were only a few bacteria in the nuclei of 10-15% of the many algal cells present. However, we cannot deny the possible association of factors other than bacteria, such as

(Cont'd on p. 3)

Update of
International Directory of Experts in Toxic and Harmful Algal Blooms

See back page for questionnaire

Toxic shellfish event in New Zealand attributed to *Gymnodinium* cf. *breve* and *Alexandrium minutum*

By January 1993 a full-scale dinoflagellate bloom was under way in Northland, New Zealand, and reports emerged of intoxicated animals, associated with eating shellfish scraps.

At this point events escalated rapidly. There were indications of illness in human shellfish consumers. Mouse bioassays carried out by the Communicable Diseases Centre in Wellington confirmed the presence of algal toxins in aqueous extracts of shellfish gathered from right around the country. The government ordered the closure of shell fisheries around the entire New Zealand coast.

Extensive surveys of the phytoplankton were undertaken, and aerial surveys carried out to help pinpoint bloom fronts. It soon became apparent that the South Island aquaculture regions, the Malborough Sounds and Big Glory Bay, had productive waters for the time of year, but with 'harmless' species predominating. Coccolithophores and diatoms were particularly abundant. With this information, and following an adjustment to the method for preparing shellfish for the mouse bioassay (a return to the use of 0.1N HCl instead of 1.0N HCl in the aqueous extracts), false positives were minimized and South Island was reopened to shellfish harvesting.

In the north-east of North Island, several dinoflagellate species came under suspicion. *Alexandrium minutum*, a species new to New Zealand waters, was found in Tauranga Harbour, and this linked well with both the epidemiological data and the mouse bioassay results. Paralytic shellfish toxins were later confirmed in shellfish from this area by HPLC analysis. However, this species was not widespread, and did not explain the majority of the illnesses.

During the second week of February, four things occurred almost simultaneously, implicating a *Gymnodinium* species which had persisted as a sub-dominant throughout the bloom.

First an outbreak of coughing amongst the residents of Orewa Beach, on the north-east coast, followed exposure to sea-spray aerosols, generated by heavy surf at the tail of a cyclone. (This well known phenomenon in the Gulf of Mexico/Florida area in the USA is related to blooms of *Gymnodinium breve*).

Reports followed of mass mortalities of marine fauna on reefs within the more northern Bream Bay, again a classic phenomenon associated with blooms of *G. Breve*. Thirdly, a sophisticated analysis of the contaminated shellfish by Professor Yasumoto, Tohoku University, Sendai, Japan, revealed the presence of toxins closely resembling the 'brevetoxins'. Fourthly, samples taken from Orewa Beach by Dr. Hoe Chang (New Zealand Oceanographic Institute), and samples received at that time by the Cawthron Institute from Coromandel (further to the south), contained abundant *Gymnodinium* cells that on scanning electron microscope examination closely resembled *G. breve*. Although *G. breve* has tentatively been identified outside USA waters, there have never been records of an association with shellfish toxicity elsewhere, until now.

The main factor contributing to late diagnosis of the toxin, and consequently the microalgal culprit, was the failure to determine the toxicity of lipid extracts of shellfish.

Initially, because the nature of the predominant toxins involved was unknown, observation of mice following the injection of aqueous shellfish extract was continued for up to 24 hours. A positive toxicity score was regarded as causing the death of 2 out of 3 mice during that time. Subsequently it was shown that most of the toxicity was associated with a lipid soluble fraction. The delayed toxicity observed with aqueous extracts was shown to be irrelevant to human intoxications and the 1 to 24 hour observations were reduced to 1 hour, to monitor for PSP toxicity only. Once this was done the situation immediately became clearer, with lipid-soluble breve-like toxins being the predominant toxicity.

Research relating to the toxic event is still being carried out, and cyst surveys should help determine whether a seed bed exists from which further toxic blooms might arise in the future.

In retrospect, the event, involving both microalgal species and toxins new to New Zealand, was handled conservatively. This has resulted in confidence amongst consumers. New Zealand will certainly be well prepared for any future events, should they occur.

Lincoln MacKenzie and Lesley Rhodes, Cawthron Institute, Private Bag 2, Nelson, New Zealand.

IOC Manual on Harmful Marine Microplankton

A chapter on 'Algal Culture Collections' is being prepared by Robert A. Andersen, Susan Blackburn, F.J.R. Taylor and Carmelo Tomas for the forthcoming *IOC Manual on Harmful Marine Microplankton*. The goal of the chapter is to list all strains of toxic marine algae — **public and private holdings** — as well as the corresponding information: scientific name, collection site, isolator, toxins (when known), etc. If you maintain toxic strains of marine microplankton, please contact Robert Andersen as soon as possible at the following fax: (1-207) 633 9641. We are particularly interested in strains that are held privately in individual laboratories. Providing us with information does not obligate you to distribute your strains; it merely serves as a means for disseminating toxic strain information that is often difficult to locate.

(Cont'd from p. 1, 'Highlights')

Vibrio cholerae (Hallegraeff, Rigby, personal communication), and with the emergence of *V. cholerae* non-01 CT+ in Asia and new algal blooms occurring in Indonesia, Australia, New Zealand and the Philippines, the need to monitor ballast water and coastal algal blooms for vibrios is even more urgent. The International Maritime Organization is considering measures.

Marine bacterial-algal interactions were another subtheme. Included are: bacterial production of saxitoxins, bacterial enhancement of algal toxins (domoic acid), and algae as vectors of vibrios and other bacteria. In addition reports of toxic Cyanobacteria are increasing. In 1990 and 1992, in the Gulf of Finland, blue-greens produced centimetre-sized aggregates, which emphasizes the interaction of physical with biotic factors (e.g. with gels and fibres) that may enhance net growth and provide protection from grazing.

Explanations for the apparent spread of HABs could be: (1) increased awareness, (2) natural variability or (3) a real increase in the extent and the number of species involved, as a result of anthropogenic factors. The factors considered include eutrophication (nutrients), loss of wetlands, altered predation pressures (from over-fishing and fish diseases), and superimposed global change. The New Zealand outbreak and one off South Africa involving the Benguela Current involved changes in winds and sea surface temperatures associated with El Niño, whose increased frequency in the past decade (4 x) suggests increasing climate instability.

Everyone agreed that the costs of HABs to finfish harvesting, aquaculture and tourism have grown considerably. The apparent spreading of coastal algal blooms and their role as a reservoir and dispersal mechanism for cholera increases the urgency for an early warning system.

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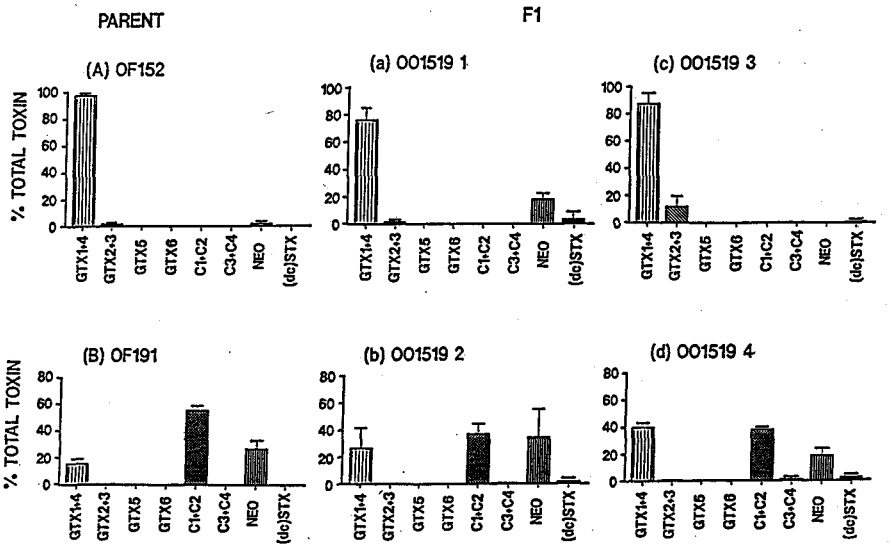
***Editors note**: If, as some forecasters feel, the El Niño system is 'locked in the on mode' (see Richerd Kerr,* Science, *262: 656-657, 29 Oct. 1993), New Zealand's troubles may be just beginning. A shortened version of this article appeared in:* The Lancet, *342, 1108, 30 Oct. 1993.*



Average toxin composition (as % total toxin, +1.0 S.D. n=8) of A. tamarense, parent strains OF152(A), OF191(B) and those 4 F1 strains (a-d).

(Cont'd from p. 1, 'PSP')

diphtheria toxin production by phage-infected cells.

We tried to determine whether the source of PSP toxins is encoded by nuclear DNA, chloroplast DNA, mitochondrial DNA, or bacterial DNA in *Alexandrium*, by studying the inheritance of toxin composition by F1 cells from parents with different toxin compositions. *A. catenella* and *A. tamarense* were examined in clonal-axenic cultures.

We noted that the mole percentage toxin composition in several strains did not change significantly until the late exponential phase. This indicates that PSP toxin composition in *A. catenella* and *A. tamarense* is a stable genetically determined trait.

Crosses of parents (mt+ and mt-) with different PSP toxin compositions were made and the toxin composition patterns of the F1 cells compared with those of the parents. Chromosome genes in crosses are inherited in a 2:2 Mendelian pattern, whereas chloroplast genes and mitochondrial genes are inherited uniparentally. Other symbiotic factors such as bacteria phages and plasmids are also inherited uniparentally or at random. Which gene codes for PSP toxin-synthesizing enzymes of *Alexandrium*?

PSP toxins composition pattern of the parents OF152(mt+) and OF191 (mt–), and a representative pair of mt+ and mt– F1 progeny (OO1519-1 and 001519-3) are shown in figure. In the parental strain OF152, the major part corresponded to gonyautoxin GTX1+4. In the other parental strain OF191(mt–), the toxin composition corresponded to C1+C2, neosaxitoxin (neoSTX) and GTX1+4. The F1 progeny OO1519-1 and OO1519-3 showed the same toxin composition as the parental OF152, and the other F1 OO1519-2 and OO1519-4 showed the same toxin composition as the parental OF191. F1 progenies from *A. catenella* parents also show either one or the other parental toxin composition (Ishida et al., *Toxic Phytopl. Bloom in the Sea*, 881-887, 1993; Sako et al., *Biosci. Biotech. Biochem.*, 56, 692-694, 1992).

Thus toxin compositions of F1 progenies in *A. tamarense* and *A. catenella* are inherited on a 1:1 Mendelian pattern, referred to as biparental inheritance, and not in a uniparental pattern nor randomly. The genes for PSP toxin-synthesizing enzymes must be coded in the chromosomal DNA of *Alexandrium*.

However, more evidence is needed before a definitive statement can be made. Experiments continue to detect and isolate PSP toxin synthesizing enzymes, especially transforming enzymes (saxitoxin → gonyautoxin → C-toxins). The isolation of these enzymes and their DNA sequences should be decisive in settling this problem in the near future.

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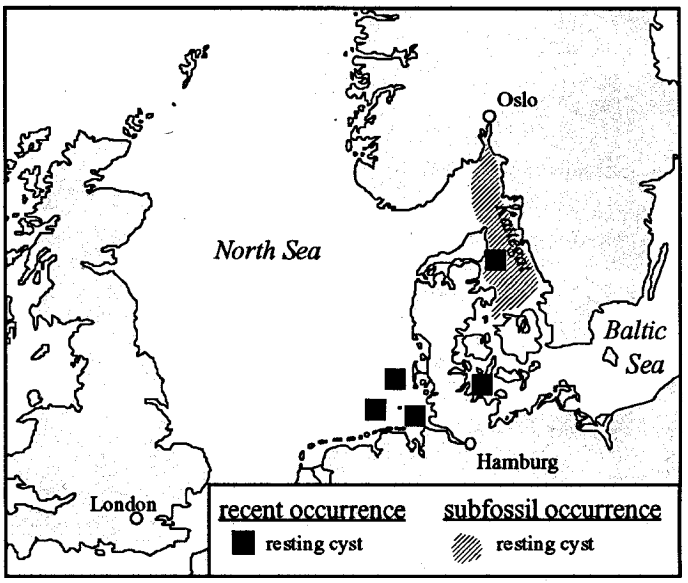
Further details appear in Sako, Y., Kim, C. H., and Ishida, Y., 1992. Mendelian inheritance of paralytic shellfish poisoning toxin in the marine dinoflagellate *Alexandrium catenella. Biosci. Biotech. Biochem.*, 56: 692-694 (eds.).

(Cont'd from p. 1, 'Gymnodinium')

the water column of the region (Dale et al., 1993. In: Smayda & Shimizu, *Toxic Phytoplankton Blooms in the Sea.* Elsevier, 47-52). Interactions between climatic change and local hydrography seem to be important factors regulating these fossil 'blooms', with the migration of relatively warmer waters contributing to the establishment of *G. catenatum.* It is suggested that the cooling of the Little Ice Age caused its disappearance in present times (Dale & Nordberg, 1993. In: Smayda & Shimizu, *Toxic Phytoplankton Blooms in the Sea*, Elsevier, 53-57).

This is in keeping with investigations from the adjacent North Sea area, especially in the German Bight in October 1991, which showed that *G. catenatum* cysts are apparently absent in recent sediments (Nehring, *Helgoländer Meeresunters.*, in press). This also holds true for other investigations of North Sea sediments (e.g. Dale, 1976, *Rev. Palaeobot. Palynol.* 22, 39-60; Reid, 1977, *Nova Hedwigia* 29, 429-463). However, in March 1992, living *G. catenatum* cysts were found at two stations in the German Bight (mentioned as 'cyst of Diplopsalid group' in Nehring, op. cit., in press) and were discovered in sediments of the Danish coast bordering the Kattegat (Ellegaard et al., *J. Phycol.*, in press). During an additional survey of the German Bight and Baltic Sea (Kiel Bight) in April 1993, living and empty cysts of *G. catenatum* were found in the topmost centimeter of sediments at numerous stations in both areas, showing maximal abundance of 17.0 living cysts/cm^3. The cysts of *G. catenatum* from Kiel Bight comply with the specimens isolated from German Bight sediments (30-35 µm in diameter) and with the description given by Ellegaard et al. (op. cit.) from the Kattegat area, but are slightly smaller than specimens from other parts of the world (for review see Ellegaard et al., op. cit.). The second important feature is that chains longer than two cells were never seen. This agrees with the comprehensive germination experiments and observations on motile cells of *G. catenatum* by Ellegaard et al. (op. cit.). Also they have found chains with only two cells under conditions where chain formation was induced in other studies (Blackburn et al., 1989, *J. Phycol.* 25, 577-590) and speculated that *G. catenatum* is in reality a species complex, with the Danish strain a different ecotype or a non-chainforming variant. The site nearest to northern Europe where motile cells of *G. catenatum* are known is the Atlantic coast of Spain (Estrada et al., 1984, *Inv. Pesq.* 48, 31-40) but Paulmier (1992, *Rapports internes de la Direction des Ressources Vivantes de l'IFREMER* – 92.007, 107pp.) recorded *G.* cf. *catenatum* from the Channel coast of France in 1983 and 1984 as single cells and chains of two cells.



Outline of Northwestern Europe showing distribution of recent and subfossil Gymnodinium catenatum *resting cysts.*

At present no motile cells or shellfish toxicity have been detected in waters of the North and Baltic Seas, but the positive germination experiments with filtered seawater from the sample locations show that the occurrence of *G. catenatum* in the water column is very likely, and has probably so far been overlooked.

These findings suggest that the toxic *G. catenatum* was introduced to German coastal waters by increased water influx through the English Channel into the North Sea and then transported with the residual currents to the Skagerrak/Kattegat area. This scenario is very likely because the current system of the North Sea should not allow infection of the German Bight with cysts from the Skagerrak/Kattegat area (see Lee, 1980, *North Sea: Physical Oceanography, II*, Elsevier Oceanogr. Ser. 24B). If so, cysts of this species must be present in recent sediments of the Atlantic and Channel coast of France as well as on the Danish North Sea coast and in the Kattegat area. In January 1993 a massive salt water influx from the Kattegat through the Great Belt into the Western Baltic took place, and may have infected this area with *G. catenatum* cysts.

It has been shown for Tasmanian waters that infection of the area with *Gymnodinium catenatum* cysts resulted in recurrent toxic blooms leading to human PSP and to temporary closure of shellfish farms (Bolch & Hallegraeff, 1990, *Bot. Mar.* 33, 173-192; Hallegraeff et al., 1988, *Aust. Fisheries* 47, 32-34). In the same manner *G. catenatum* cysts in North and Baltic Sea sediments may represent potential seedbeds for spontaneous bloom initiation. Phytoplankton monitoring programmes need to be aware of this. To get a comprehensive idea of the spreading of *G. catenatum* cysts in northern European waters, further surveys of the quantities and spatial and temporal distribution of these cysts in sediments are needed.

Cyst studies provide an important but relatively cheap additional tool for the evaluation of areas with potential toxicity problems. The potential impact of such species on commercial fisheries, aquaculture and the natural environment is of increasing concern. After three centuries of absence, a potential recolonization of *G. catenatum* in northern European waters may have taken place. With respect to global change, it is now a question of whether hydrodynamic, chemical and biological factors in the water column will favour germination of cysts and the proliferation of vegetative cells of *G. catenatum* in the future.

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HAB programme news

Advanced workshop on HAB ecophysiology

An advanced workshop on the ecophysiology of harmful algae was planned at the first meeting of the SCOR-IOC Working Group 97 on the Physiological Ecology of Harmful Algal Blooms. It was held at CREMA L'Houmeau, CNRS-IFREMER, La Rochelle, France, 23-24 October 1993. The WG is chaired by Dr. D.M. Anderson (USA).

Having outlined central questions in future research on the ecophysiology of harmful microalgae, the WG decided to organize an advanced scientific workshop to review the state of knowledge and to identify and discuss areas of future research.

The key questions identified included the roles of toxin production, bacteria, and mixo- and heterotrophy in the ecophysiology of harmful algae, as well as the importance of genetic variability in harmful algal species, and the role of UV on harmful algal succession and occurrence.

The WG decided to prepare a proposal for a NATO 'Advanced Study Institute'. A NATO ASI has a duration of 10 days and has 60 to 80 participants and includes both lectures and demonstrations. SCOR and IOC will co-sponsor the workshop, and the presentations and the results will be published.

The workshop is proposed for November 1994 or June 1996 in Bermuda. A detailed proposal was prepared and an organizing committee was identified.

A set of recommendations were adopted as follows:

(1) Convene within 3 years an advanced workshop to summarize the state of knowledge, exchange ideas, disperse new technologies, establish interaction, and identify themes of future research relating to the physiological ecology of harmful algae.

(2) Begin to contact individuals potentially interested in the workshop (at the direction of the Chairperson).

(3) Convene a second SCOR-IOC workshop in conjunction with the 7th International Conference on Toxic Marine Phytoplankton, Sendai, Japan, July 1995.

Joint ICES-IOC action planned for 1994

The joint Study Group of the International Council for the Exploration of the Sea (ICES) and IOC on the Dynamics of Algal Blooms has been re-established as an ICES-IOC Working Group on Harmful Algal Bloom Dynamics that works in close connection with the IOC Harmful Algal Bloom Programme. The Chairperson of the WG is Beatriz Reguera (Spain).

The setting up of this group was recommended at the last ICES Statutory Meeting by the Chairpersons of the Hydrography and the Biological Oceanography Committees. The terms of reference were: 'to plan and propose a programme to study the dynamics of HABs in coastal oceans'.

The activities to be carried out during 1994 are:

- ICES/IOC Workshop on 'Modelling the Population Dynamics of Harmful Algal Blooms', Vigo (Spain), 4-7 May 1994. Convenors: W. Fennel (Germany) and P. Tett (UK).
- Meeting of ICES/IOC Working Group on Harmful Algal Bloom Dynamics, Vigo (Spain), 9-12 May 1994. Chairperson: B. Reguera (Spain); including a joint session, 9-10 May, with the Working Group on Shelf Seas Oceanography, chaired by H. Dahlin.
- ICES/IOC Workshop on Intercomparison of *in situ* Growth Rate Measurements, Aveiro (Portugal), 25-29 July 1994. Chairperson: M.A. Sampayo (Portugal).

Participants from countries outside ICES, especially those interested in establishing HAB dynamic studies in other geographical regions, are invited to attend the WG meeting on Harmful Algal Bloom Dynamics and the Workshop on Modelling the Dynamics of HABs.

Intergovernmental panel sets priorities

The IOC-FAO Intergovernmental Panel on Harmful Algal Blooms held its Second Session in Paris 14-16 October 1993 to set priorities and identify resources for the Harmful Algal Bloom (HAB) Programme. Twenty-one Member States, WHO, ICES, IUPAC[1] and SCOR were represented.

The Panel decided to establish four Task Teams to address critical issues requiring specific priority and action:

- A Task Team on Algal Taxonomy will provide taxonomic recommendations, develop identification standards for preparation of manuals and training, and help coordinate activities;
- A Task Team on Aquatic Biotoxins will prepare a report on the current status of algal toxin chemistry and toxicology, and in particular facilitate the preparation and supply of algal toxins;
- A Task Team on Design and Implementation of Monitoring Programmes will assist in the preparation of a report where examples of monitoring systems on harmful algae worldwide are presented in detail, and assist in the organization of an international workshop directed toward improved design of HAB monitoring systems;
- A Task Team on HAB Project Development will work with the preparation of HAB project proposals.

The development of a comprehensive training and capacity building programme on harmful algae was agreed upon. The training programme will include courses and workshops on taxonomy, toxin chemistry, monitoring, and management of harmful algae. The first taxonomy course has already been held and both taxonomy and toxin chemistry courses are planned for 1994.

The Summary Report of the Second Session of the IOC-FAO Intergovernmental Panel on Harmful Algal blooms is available from the HAB Programme Office, IOC Secretariat.

[1] IUPAC = International Union for Pure and Applied Chemistry.

Ciguatera Management Workshop, Bribie Island, Australia

Overview

An international Workshop on Ciguatera Management was held on Bribie Island near Brisbane on 12-16 April 1993. The Workshop was sponsored by the Australian Fisheries Research and Development Corporation and the Queensland Department of Primary Industries (QDPI). Scientists, medical practitioners and fisheries managers attended and focussed on current research having implications for the management of ciguatera. *(Sources of comments are indicated in brackets.)*

A total of 56 people from Japan, mainland USA, Hawaii, France, French Polynesia, New Caledonia, Germany and Australia attended. The Workshop specifically addressed (i) the detection of ciguateric fishes and (ii) the management of ciguatera cases. P. Scheuer opened the scientific program with an historical perspective of modern ciguatera research initiated by the late A. H. (Hank) Banner and outlined some of the challenges for the future. The major themes of the Workshop were: chemical and immunological aspects of the detection of toxins involved in ciguatera; pharmacology and treatment of ciguatera; origin of the toxins involved in ciguatera; clinical aspects and epidemiology of ciguatera.

Detection of ciguateric fish

A cost-effective screen for ciguateric fish was recognized as an important management tool able to directly reduce the adverse effects of ciguatera on public health, fisheries, trade and tourism (R. Lewis, D. Park). Several different approaches to the detection of ciguateric fish were presented. Two approaches measured the interaction between ciguatoxin and the voltage-dependent sodium channel through either (i) the inhibition of brevetoxin binding to sodium channels in a rat brain synaptosome preparation (A. M. Legrand) or (ii) the cytotoxic effects of ciguatoxin on sodium channel-containing cell lines pre-exposed to ouabain and veratridine (R. Manger). Both assays were more sensitive than the mouse bioassay, and may replace *in vivo* assay in laboratories possessing the specialized equipment required. However, it is unlikely that these approaches, as they stand, can be used for the routine screening of suspect fish.

Antibody-based screens still appear to hold most promise for the cost-effective detection of ciguateric fish (R. Lewis). This approach is the basis of a potential commercial test to detect ciguateric fish being developed by Hawaii Chemtect International (Pasadena California, USA). D. Park presented a summary of the performance of this solid-phase immunobead assay (Ciguatect™) which was claimed to be able to detect most, if not all ciguateric fish. However, the test was reported to be unsuitable for detecting ciguatera toxins if the fish flesh being screened was slightly acidic (pH ≅ 6.5), a factor that may considerably limit the usefulness of the test. Y. Hokama commented that the test may not work satisfactorily because the solid-phase used in the Ciguatect™ test may not be as efficient at extracting ciguatoxins from fish as the correction fluid used for the solid-phase in the original stick test he developed (apparently the same antibody was used for both tests). When compared with the results of a well conducted mouse bioassay, predictive indices from 5% to 75% were obtained with the Ciguatect™ test in an independent study of ciguateric fish from the Caribbean (R. Dickey). These results suggest that the test may not be responding to the major toxins (as yet unidentified) present in these Caribbean fish. Lack of ready access to samples of pure ciguatoxin or its analogues, and an inability to independently validate the levels of ciguatoxins present in fish samples being screened, hamper attempts to validate (or otherwise) the Ciguatect™ test.

Pharmacology and treatment of ciguatera

Major advances are being made into how ciguatoxins cause human poisoning (J. Molgo, E. McLachlan, J. Brock, P. Hamblin, E. Beniot, K. Terao, C. Purcell, M. Capra). However, the precise mechanism by which mannitol, the present treatment for acute ciguatera, acts to relieve the symptoms of ciguatera remains unclear. A double-blind clinical study of mannitol treatment is being conducted (N. Palafox) but the results of this study were not available at the time of the meeting. Clinical experiences with mannitol therapy (D. Blythe, N. Palafox) continue to be positive, and mannitol remains the treatment of choice for acute ciguatera. Confirmation of the clinical findings would be assisted by the development of an animal model for ciguatera that responds to mannitol.

Clinical aspects and epidemiology of ciguatera

While most of the clinical features of ciguatera are well documented, the long-term effects of ciguatera and how frequently these occur are poorly understood (T. Ruff). Follow-up research on victims is required to establish the true extent of long-term effects, especially the allergy-like reactions that can persist after a single exposure to toxic fish. J. Pearn reported that ciguatera remains typically a poorly recognized and managed disease, despite the introduction of mannitol therapy. QDPI maintains a database that covers 27 years of ciguatera cases reported in Queensland. An analysis of this database using recently developed statistical modelling approaches revealed major shifts in the species and the nature of poisoning in Queensland (M. Chaloupka).

Origin and identification of toxins involved in ciguatera

Gambierdiscus toxicus is now widely accepted as the organism that produces ciguatera toxins. Indeed this organism may be the only source of these toxins. T. Yasumoto reported the structure for GTX-4A (52 epi-GTX-4B), the major gambiertoxin produced by a Rangiroa Atoll strain of *G. toxicus* grown in culture. This study confirms that *G. toxicus* is indeed the origin of the ciguatoxins isolated from fish. Long and short range inverse detected NMR of CTX-1 confirmed the structure originally proposed for ciguatoxin (R. Lewis). The structure of a maitotoxin produced by culture *G. toxicus* was also presented by T. Yasumoto. Maitotoxin is the largest non-repeating unit compound for which a structure is known and elucidation of its structure represents a significant milestone in natural product chemistry. Maitotoxin's structure is only distantly related to the ciguatoxins, thereby ending speculation that the maitotoxins may be a precursor of the ciguatoxins.

The environmental factors that cause

the upsurges of ciguatera remain poorly understood (M. Holmes, R. Lewis, Y. Hokama, D. Ichinotsubo, R. Bagnis, U. Kaly). The origin of ciguatera was questioned by J-P. Vernoux based on studies conducted in French Polynesia. A rapid extraction method for isolation of ciguatoxins from *G. toxicus* may assist studies on toxin production in culture (J. Babinchak). Studies of the vectors which transfer gambiertoxins to carnivorous fish revealed that shrimps may be involved in some communities, and herbivorous fish such as *Ctenochaetus striatus* in others (R. Lewis).

A record of the meeting will be published in a special issue of the '*Memoirs of the Queensland Museum*' by mid-1994. For further information on the published proceedings please contact: Richard J. Lewis, QDPI, Southern Fisheries Centre, PO Box 76, Deception Bay, Queensland 4508, Australia; tel.: (61-7) 203 1444; fax: (61-7) 203 3517.

Toxin action

It was shown using confocal laser microscopy that ciguatoxin causes oedema of adaxonal Schwann cells and nodes of Ranvier in myelinated nerve fibers, and that mannitol (a product successfully used in ciguatera therapy) acts via its osmotic effects (J. Molgo).

The association of ciguatoxin with specific proteins may explain its lack of toxicity to Na+ channels of fish, even though fish nerves respond to ciguatoxin in a similar manner to those of mammals (F. Capra).

Origin

For the first time ciguatera was reported (M. J. Holmes) in an area (Platypus Bay, Australia) not typically associated with ciguateric fish, without corals but with a sandy bottom covered by unattached green macroalgae (*Cladophora* sp.). Nevertheless, *G. toxicus* was attached on this algae and contained ciguatoxins, suggesting that this *G. toxicus* population is the origin of toxins found in ciguateric fishes caught in this bay. Thus the presence of corals is not essential for ciguatera development. In this area invertebrates such as shrimps were shown to contain detectable levels of ciguatoxin and to transfer these toxins in the ciguatera food chain (R. J. Lewis). It is the first time that shrimps were implicated in the ciguatera phenomenon.

Hindsight on Arctic toxic bloom

Toxic blooms of marine algae are often attributed to pollution, as in the case with *Chrysochromulina polylepis* in the North Sea in 1988. Therefore, a toxic bloom of *Chrysochromulina leadbeateri* in Norwegian coastal waters, to the north of the Arctic circle in June 1991, came as a real surprise and needed other models to explain it. The affected area was the Vestfjord, situated between the Lofoton Islands and the Norwegian mainland. The area is very sparsely populated and has little agriculture and no heavy industry. So, why here?

The case developed from late May, when farmed salmon started to die and a diver reported a layer of yellow water below the cages. Eventually, a total of nearly 550 metric tons of salmon were reported lost, from different farms isolated from each other and with unharmed farms in between. In one case, dead and dying salmon were taken out of a farm while samples were taken close to the cages, showing that cell concentrations were lower than 2 x $10^6 l^{-1}$. So, if salmon mortality was not related to the abundance of toxic algae, to what was it related?

Several research institutes in Norway became involved in the operation, mapping the distribution of the bloom and doing experiments to explain its causes. Compared to the *C. polylepis* bloom, the *C. leadbeateri* bloom had lower maximal concentrations, occurred at lower temperatures and higher salinities. Its distribution was more restricted and it did not affect the natural flora and fauna.

C. leadbeateri was present in areas to the south of the Vestfjord as well, but was then part of a flora dominated by diatoms, causing Secchi depths to be 2-7 m. Secchi depths in the Vestfjord were 8-21 m and the flora consisted almost exclusively of *C. leadbeateri*. In the Vestfjord proper, as well as in branching fjords, the mixed layer was rather deep, often causing the highest concentration of the algae to be found in depth. The mixed layer contained little nutrients and the algae seemed to optimize its growth by tactical positioning in a gradient of light and nutrients, which occurred in a deeper layer. Growth experiments with added nutri-

(Cont'd on p. 9)

Detection

Ciguatera results predominantly from the effects of the most potent ciguatoxin (CTX-1) which is present at > 0.1 ppb (10-10 M/Kg) in the flesh of carnivorous fish. Significant levels of the less potent CTX-2 and CTX-3 also accumulate in fish (R. J. Lewis).

By using the specific binding property of ciguatoxins to voltage-dependent sodium channels two detection methods were developed: the first one (A. M. Legrand) measures the ability of ciguatoxins to displace by competition ratio-labelled brevetoxins from sodium channels. The second (R. Manger) measures the enhancement of toxicity caused by ciguatoxins when added with ouabain and veratridine to neuroblastoma cells; it is simple, uses readily available reagents (MTT), is well within the scope of even modest tissue culture facilities and has the potential to serve as an alternative and complementary method to the standard mouse bioassay. A new mouse bioassay protocol adapted to 50, 100 or 200 g of fish was presented by J. P. Vernoux.

Ciguatect™, the solid-phase immunobead assay for the detection of ciguatera-related toxins is available from Hawaii Chemtect International (D. L. Park). It has high potential for screening marketplace fish for ciguatera toxicity. Nevertheless, improvement of this kit is needed since a significant number of false negative and false positive values were obtained in an FDA trial (R. W. Dickey).

Derivatization experiments with the chromophore-poor ciguatoxin-1 yielded a fluorescent coumarin-carbamic acid ester of the biotoxin. HPLC with fluorimetric detection produces a moderately intense detector response to 2 nanograms of the CTX-1 derivate (R. W. Dickey). The derivate shows potential for the detection of ciguatoxins. To separate toxic from non-toxic fish, the detection limit by HPLC should be 1 picogram, a threshold of sensitivity which has never been obtained for ciguatoxins by any described procedure. This limits the use of HPLC with fluorimetric detection of ciguatoxins to the research laboratory only.

Jean Paul Vernoux, Université de Caen, Laboratoire de Physiologie Cellulaire et Moléculaire, Esplanade de la Paix, 14032 Caen Cedex, FRANCE.

South Korean oysters gave ASP symptoms

An illness similar to Amnesic Shellfish Poisoning (ASP) beset two persons after eating smoked oysters in South Korea. The event occurred as follows: On 16 February 1992, approximately 5 hours after eating six canned oysters each from a smoked Korean oyster product, the symptoms of ASP began to show. One person, a male, suffered seizures, disorientation, confusion, vomiting, and general weakness. Tests at a hospital for stroke, brain tumour, and heart attack were negative. The other person, a female, complained of a headache, was unable to make decisions and had swollen hands and feet. Approximately 24 hours after she had ingested the oysters, she was stricken with a vomiting spell, followed by seizures, and went into a coma that lasted for 48 hours. She had extremely low blood pressure, low sodium, malfunctioning kidneys and liver, and was left with muscle damage. At present, she suffers memory loss of these events. She was tested for the same disorders as the male victim as well as for tetanus, botulism, Rocky Mountain spotted fever, Lyme's disease and bacterial infection. All tests were negative. The house where the two victims live was tested for carbon monoxide and radon gas poisoning, and the water supply was tested for numerous toxins; all tests proved negative.

On 26 March 1992, both persons ate seafood gumbo. The following day, the male had another attack with a seizure and symptoms similar to the 16 February attack. Again he was rushed to the hospital and underwent the same tests as before. All proved negative.

Both persons were in good physical condition before the illness appeared. They did not drink or take drugs, and had not shown any previous reactions when eating shellfish. Neither of the victims suffered from stomach problems, diarrhea, paralysis, or experienced tingling sensations in the mouth during the attack.

Therefore it does not seem likely that the victims suffered from Paralytic Shellfish Poisoning. The symptoms shown by the two persons, however, indicate that they suffered from ASP. Common symptoms of ASP include choking, vomiting, diarrhea, abdominal cramps, incapacitating headaches, seizures, and permanent short-term memory loss (Perl et al., 1990).

We analyzed Korean shellfish products for domoic acid (DA) contamination, the causative agent of ASP. Twenty-five cans of smoked Korean oysters and two cans of smoked Korean mussels were tested with high performance liquid chromatography methods. The cans represented different brands and lot numbers including the same brand and lot number the two persons consumed on 16 February. Domoic acid was not detected in any of the smoked Korean products.

From two of the cans, oyster gut contents were analyzed for diatom frustules from the *Pseudonitzschia* complex. To date, only phytoplankton from this group of diatoms produce DA. No forms known to be toxic were found, but the non-toxic diatom *P. pungens* f. *pungens* was present.

The HPLC analyses do not provide supporting evidence that the Korean products were contaminated with DA. However, we believe that the products we analyzed may not have been representative of what the two people ate on 16 February, despite testing cans of oysters from the same brand and lot number that were consumed. Within a can of oysters variation in oyster size was small. However, variation in the oyster size between cans was large, even when the cans were from the same brand and lot number. This implies that the oysters were harvested from different locations or times, despite identical lot numbers on the cans.

Although smoked Korean oysters tested negative, it may have been that the oysters analyzed were harvested from a non-toxic area, and that the oysters consumed by the two people were harvested from a toxic area, or even a 'pocket' of toxicity. The oyster *Crassostrea virginica* accumulates DA at low levels over short feeding periods (Roelke, 1993). It is likely that oyster species from Korea are similar to *Crassostrea virginica* in their feeding behaviour and may accumulate DA if toxic diatoms are ingested over time.

As stated earlier, a non-toxic diatom, *P. pungens* f. *pungens* was found in the gut contents of the oysters analyzed. Fryxell et al., (1992) and Dickey et al., (1992) reported that two forms of *P. pungens* are present together year-round in Galveston Bay, Texas, with the non-toxic form, *P. pungens* f. *pungens* more common in warmer months and the toxic form, *P. pungens* f. *multiseries* in colder months. *P. pungens* f. *multiseries* was found in small numbers from net hauls with many *P. pungens* f. *pungens* cells from Jinhae Vay, Korea, April 1983 (Fryxell et al., 1990). The same trend of seasonal relative dominance observed in Galveston Bay is a possibility in Korean waters as well.

During the preparation process of smoked Korean oysters, they are first shucked, then smoked, put into cans with oil, sealed in a vacuum, and finally sterilized by heating (Crown Prince, pers. comm.). Domoic acid is a heat-stable molecule. It does not appear likely that the preparation process for smoked Korean oysters would remove the toxin from the product.

The distribution of DA-producing diatoms is world-wide in temperate climates (Villac et al., in press). If appropriate environmental conditions arise that favour a bloom of these toxic species, ASP events could also occur world-wide in temperate climates. Phytoplankton monitoring for DA-producing species in Korean and Chinese waters has only just begun (Fryxell, pers. comm.). If a toxic bloom occurred in 1992 it is likely that it would have gone unnoticed.

In summary, we know that two people suffered from an illness similar to ASP after eating smoked Korean oysters. We know that a related oyster species along North America accumulates DA, and that at least one toxic diatom species occurs in Korean waters. We also know that the canning process of smoked Korean oysters is not likely to destroy DA. Finally, we know that a toxic bloom of diatoms in Eastern waters would have most likely gone unnoticed. There is, no direct evidence that an ASP event occurred due to consumption of a Korean oyster product, but these two cases serve as a warning.

As the scientific community increases its understanding of DA occurrences around North America and Europe, we also need to consider Eastern waters. It is essential that monitoring of the phytoplankton community be carried out in temperate climates world-wide where shellfisheries exist. The understanding of population dynamics and life strategy of the species we know to produce the toxin and the possible dis-

covery of other DA sources are crucial in our effort to better protect shellfisheries and consumers.

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Biology, Epidemiology and Management of* Pyrodinium *Red Tides

Limited copies of the above title, 1989 (Hallegraeff and Maclean, eds.) are available (US $25 inc. surface mail) through Dr. Rhodora A. Corrales, Marine Science Institute, University of the Philippines, Diliman, Quezon Sity 1101, The Philippines; fax: (63-2) 921 5967.

Raphidophyte algal bloom preceded New Zealand shellfish poisoning

Rhaphidophytes dominated an extensive bloom which occurred along the north-east coastline of New Zealand from August to December 1992. A dramatic increase in chlorophyll content was noted at that time in coastal waters off Leigh Marine Reserve, in Northland, New Zealand. The event was associated with the 'El Niño' phase of the Southern Oscillation Index, which resulted in unusually cold sea temperatures.

The microalgal species involved included the potentially ichthyotoxic species *Fibrocapsa japonica*, *Heterosigma akasiwo* (Raphidophytes), *Dictyocha speculum* (Silicoflagellate) and a dinoflagellate initially described as *Gyrodinium* cf. *aureolum*. On closer examination, by SEM (scanning electron microscopy), the dinoflagellate resembled the Florida red tide microalga *Gymnodinium breve*. The coccolithophore *Gephyrocapsa oceanica* was in constant and significant presence throughout the bloom. *Fibrocapsa japonica* had only been noted once before in New Zealand – it was observed in north-eastern waters the previous October. Early in the bloom, *Dictyocha speculum* occurred in its naked form, but by December, when the bloom had temporarily diminished, the unarmoured form was predominant.

Maximum cell numbers were reached in October, and although not particularly high for individual species (e.g. *Fibrocapsa japonica* reached no more than 107,000 cells per litre), the combined numbers result in a patchy discoloration of the coastal waters for over 200 km of the coastline. Bioassays for both shellfish toxins and for ichthyotoxicity were negative during this period, but there were many anecdotal accounts of unusual events. These included dieback of kelp beds, mass mortalities of penguins, skin irritations among fishermen and peppery tasting mussels in some areas.

The end of the raphidophyte-dominated bloom proved to be a brief respite. Dinoflagellates proceeded to dominate the water column along the same coastline, and in late December animal deaths and human illnesses linked to the eating of shellfish were reported. It is tempting to speculate that the conditioning of the water column by the earlier bloom, which was triggered by the unusual weather patterns, was a critical factor in the toxic event that followed.

Lesley Rhodes and Allison Haywood, Cawthron Institute, Private Bag 2, Nelson, New Zealand.

(Cont'd from p. 7, 'Arctic toxic bloom')

ents revealed that both nitrogen and phosphorus had to be added to gain growth in cultures.

A national report which sums up most of the observations, suggests that high freshwater outflow in winter led to stratification that was beneficial to *C. leadbeateri*. After the normal spring bloom in April, when diatoms and *Phaeocystis poucheti* deplete the nutrient reserves in the mixed layer, *C. leadbeateri* may have been the only species able to maintain a population under the prevailing conditions. The species seemed to become toxic in salmon farms localized in bays which exchange tidally mixed water through shallow channels. Thus, there is a possibility that admixture of new nutrients from deep water made the algae toxic. However, local eutrophication from the farms themselves should be considered, and also the saprobial faeces and feed-spill. The heterotrophic and bacteriophagous behaviour of *Chrysochromulina* genus could make fish farms feasible habitats matching its niche.

Another speculation suggests that wintering herring may have contributed to the conditions for algal growth. Most of the spring-spawning Norwegian herring spent the winter in the innermost part of the Vestfjord, leaving a water body characterized by low levels of oxygen and high levels of plant nutrients. In fact, ammonia, which was not recorded regularly during the bloom, turned up in high concentrations when measured, lending support to this view. The bloom terminated in late June, possibly due to competing algae or grazers. Thus, the research does indicate a complex matrix of causes and may have raised more questions than answers, not least because so many features deviated from previous experience.

Stig Skreslet, Kristin Heidal and Age Mohus, School of Fisheries, Nordland College, N-8002 Bodo, Norway.

Information on algal blooms and other hazardous events in Swedish Baltic

The Information Office for the Baltic Proper was established in June 1992 as a direct result of a Swedish government proposal. According to the proposal, three information offices should be established for those County Administrative Boards in whose counties Centres for Marine Research had already been established in 1989. The information offices are to report especially on the following topics:

- Algal blooms, predominantly on toxic blooms but also on the occurrence of non-toxic blooms of major extent. The reports should include information about the toxicity of the algae and how to avoid negative effects.
- Oxygen deficiency situations. It is valuable if early warnings about expected deficiencies can be given.
- Fish kills, partly in connection with oxygen deficiency and partly with other causes, like diseases. It is valuable if early warnings can be given.
- Extensive diseases and/or kills of organisms other than fish, e.g. seals and birds.
- Major spills of oil and chemicals which may cause harm to the marine environment.

The information office arranges telephone conferences with a limited number of scientists involved in, or in close contact with, field sampling programmes within the area of interest. These conferences are held at predetermined intervals (ranging from about one week up to about every second month) depending on the expected development of the situations monitored. If necessary, conferences can be held at very short notice. During the conferences the participants present available information and formulate a prognosis of the expected development. The information office staff puts the material presented, including the prognosis, into a short report or 'newsletter' (maximum about one A4 page). The report is then sent by telefax to interested receivers (all County Administrative Boards and local authorities along the coast within the area concerned, research institutes, the Coast Guard, the Navy, fishermen's organizations, mass media and others) immediately after the conference.

The Stockholm information office (Informationskontoret för egentliga Östersjön or Information Office for the Baltic Proper) is currently reporting only in Swedish. We hope to report also in English in the near future. Before that can be done we have to establish good contacts with suitable institutes/authorities in other Baltic countries in order to achieve a reliable system for the exchange of information. We are now trying to establish such contacts. Therefore, any suitable source of information in other Baltic countries is welcome to contact us. We are equally happy to be able to provide information to those interested in receiving it.

Our area of interest ranges from the northern Åland Sea to the southern part of the Sound (Øresund) between Sweden and Denmark, and includes primarily Swedish coastal waters and the open Baltic proper. The information office in Umeå (to be established) is to cover the Sea of Bothnia and Bothnia Bay. The office in Gothenburg covers the area from the southern part of the Sound up to the Norwegian border, including the Kattegat and the Skagerrak.

Contact persons are: Gunnar Anner and Kerstin Bohm, The Information Office for the Baltic Proper, Stockholm County Administrative Board, Department of Environment Protection, Box 22067, S-104 22 Stockholm, Sweden; tel: (46-8) 785 51 18 or 785 51 21; fax: (46-8) 651 57 50.

Future events

4th Canadian Workshop on Harmful Marine Algae; 3-5 May 1994; Sidney, B.C. Canada. *Contact*: Rod Forbes/Brenda Lacroix, Institute of Ocean Sciences, PO Box 6000, 9860 West Saanich Road, Sidney, B.C. V8L 4B2, Canada; tel: (1-604) 363 6443/6533; fax: (1-604) 363 6390.

5th International Phycological Conference; 26 June - 2 July 1994; Qingdao, P.R. China. *Contact*: The Secretary, 5th Int. Phyc. Conf., EMBL Institute of Oceanography, 7 Nanhai Road, Qingdao 266071, P.R. China.

Modelling & Control of Activated Sludge Processes; 21-23 August 1994; Copenhagen, Denmark. *Contact*: Mia Clausen, Conference Secretariat, c/o Dept. of Environmental Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark.

13th International Diatom Symposium; 1-7 September 1994; Acquafredda di Maratea, Italy. *Contact*: Jean Gilder Congressi snc., Via G. Quagliariello 35/E, I-80131 Napoli, Italy; tel: (39-81) 546 3779 or 545 4617; fax: (39-81) 546 3781.

Coastal Zone Canada '94; 20-23 September 1994; Halifax, Nova Scotia, Canada. *Contact*: Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N. S., B2Y 4A2, Canada; tel: (1-902) 429 9497; fax: (1-902) 429 9491.

24th International Conference on Coastal Engineering (ICCE '94); 23-28 October 1994; Kobe, Japan. *Contact*: Secretariat, c/o Inter Group, Shiroguchi Bldg., 2-15, Kakutacho, Kita-ku, Osaka 530, Japan; fax: (81-6) 372 6127.

Pollution of the Mediterranean Sea; 3-4 November 1994; Nicosia, Cyprus. *Contact*: M. Nicolaou, WTSAC, PO BOX 1735, Limassol, Cyprus.

IOC International Oceanographic Conference – 'Towards sustainable use of the oceans and coastal zones'; 7-12 November 1994; Lisbon, Portugal. *Contact:* Secretary, IOC, 1 rue Miollis, 75732 Paris cedex 15, France; fax (33-1) 4056 9316.

HARMFUL ALGAE NEWS

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International Directory of Experts in Toxic and Harmful Algal Blooms

Questionnaire

An updated and expanded edition of this international directory is being prepared as a joint effort of the Intergovernmental Oceanographic Commission (of UNESCO) and the NOAA National Marine Fisheries Service of the United States. The purpose of the directory is to assist countries facing toxic and harmful algal bloom emergencies by facilitating rapid access to scientists, fisheries managers, public health officials, and physicians who are experienced in dealing with toxic and harmful algal blooms and their consequences to fisheries, aquaculture, and public health. The directory will also serve to expedite contact among the international community of experts in harmful algal blooms and related issues.

Please complete this questionnaire if you are involved in basic scientific, fisheries, public health, or medical aspects of harmful algal blooms, and wish to be included in the directory. Please copy this form for use by other appropriate colleagues in your country. A copy of the directory will be sent to all those who reply to the questionnaire. Send the completed questionnaire to the address on the reverse side.

Surname (*last name*): .....

Given names (*first names*): .....

Title (*Prof., Dr., Mr., Ms., etc.*): .....

Academic degrees: .....

Position (*Director, Chairman, Research Scientist etc.*): .....

Institution or affiliation (*include department if applicable*): .....

.....

Postal address: .....

.....

City: .....Country: .....

State or province: .....Postal code: .....

Telephone (*include country and city codes*): .....

Telex: .....

Fax: .....

E-mail (*include system, e.g. BITNET, INTERNET, OMNET, etc.*): .....

OVER PLEASE

General expertise (*you may indicate more than one*):

Biology	_____	Taxonomy	_____	Public Health	_____
Chemistry	_____	Physiology	_____	Medicine	_____
Physics	_____	Toxicology	_____	Vet. Medicine	_____
Ecology	_____	Fisheries	_____	Management	_____
Genetics	_____	Aquaculture	_____	Other (specify)	_____

Harmful algal bloom specialty/expertise (*summarize in a few sentences your background, experience, and specific subject areas in which you feel most able to provide expert advice regarding harmful algal blooms and their effects*):

.....

.....

.....

Species of harmful algae, shellfish, fish, etc., with which you are most familiar:

.....

Algal toxins with which you have worked most:

.....

Number of years experience with harmful algae/biotoxins:

.....

Geographic areas of the world where you have worked:

.....

Regional/international programmes related to harmful algal blooms, in which you participate:

.....

.....

Languages in which you have a working knowledge:

.....

Citizenship: .....

Please send completed questionnaire by 28 February 1994 to:

Dr. Alan W. White
Northeast Fisheries Science Center
National Marine Fisheries Service
166 Water Street
Woods Hole, MA 02543 USA

The Intergovernmental Oceanographic Commission of UNESCO 1994

HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 8

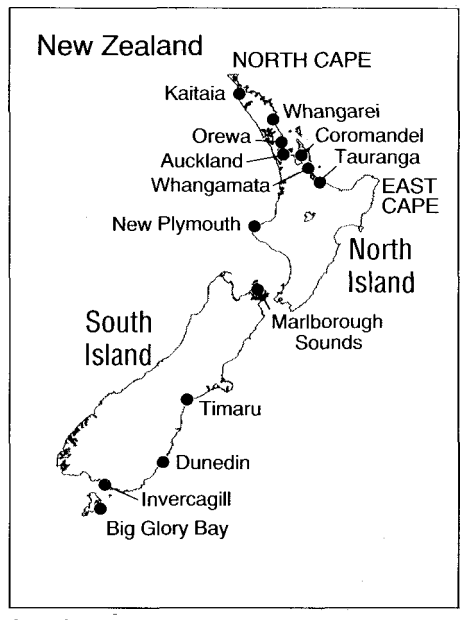
New Zealand: Major shellfish poisoning (NSP) in early '93

In early January 1993 in Whangarei, an unusual number of human illnesses was reported after consumption of shellfish. This marked the first case, ever substantiated in the region, of shellfish poisoning symptoms caused by eating contaminated shellfish. Many more cases of shellfish poisoning were subsequently reported to the Health Authorities, from areas to the north and south of Whangarei, stretching from the North Cape to the East Cape of northern North Island.

In mid-January, after the general public had been alerted to the toxic outbreaks, the first PSP-producing dinoflagellate, *Alexandrium minutum*, was identified from samples collected in Bay of Plenty. This species was immediately implicated as the culprit of the shellfish poisonings. A second species, *A. tamarense*, was also found in the Bay of Plenty area, but at very low concentrations. In late January, shellfish harvests were completely banned throughout New Zealand. The loss in export sales was estimated to be NZ $ 2 million per week.

The majority of more than 180 confirmed cases of shellfish poisoning suffered symptoms such as numbness of lips, tingling fingers, reversed sensation to hot and cold, muscular weakness, nausea, diarrhoea, and vomiting, most of which are symptoms more typically associated with

(Cont'd on p. 2)



Sampling locations around New Zealand between January and May 1993.

Harmful blooms of *Leptocylindrus minimus* in Southern Chile

In November 1989 in the centre and south of the Chiloe Archipelago, high concentrations of *Leptocylindrus minimus* were detected and unusual behavior and mortalities observed in trout and salmon (1). A literature search and enquiries amongst foreign investigators indicated that this diatom has had no harmful effects elsewhere in the world. It thus seemed to be a local problem only, that high concentrations of *L. minimus* can cause problems for cultured salmonids. We have no evidence of the mechanisms involved but suspect that the diatom causes mechanical problems in the gills.

The same species was again very abundant in October 1993 in Estero Castro and Lemuy Channel, i.e. in the same localities as in 1989, and unusual behavior and deaths of salmonids occurred again. Last summer (December 1993, January 1994), blooms were also found in Caicaen Channel and near Quellon.

Water samples are collected following the protocol of the phytoplankton monitoring programme of the Chilean Salmon and Trout Producers Association(2), and further samples are received form affected farms. Some results for *L. minimus* are given in table I.

The effects on fish are as follows. Salmon retire to the corners of their cages while trout swin right at the surface with their dorsal fins out of the water. The gills may be slightly pale in salmon, and there may be mucus secretion. There is loss of appetite, especially in trout, but also in salmon and coho. These effects are seen at cell concentrations below 10,000 cells ml^{-1}. At higher concentrations, deaths can occur.

Table I: *L. minimus* blooms in Southern Chile – counts in cells ml^{-1} for surface samples

Place	Date	L.minimus	Total phyto.	Secchi, m	Note
Castro/Lemuy/Yal	3-9 Nov. 1989	35000			(i)
Cailin	24 Nov. 1989	29770	30223	3.5	
Castro/Lemuy	7-18 Oct. 1993	17500	18945	2.5	
Caicean Channel	13-25 Oct. 1993	1961	6161	3.5	(ii)
Caicean Channel	15-30 Dec. 1993	854	1230	6.0	(iii)
Isla Coldita	4-16 Jan. 1994	33024	34195	1.5	(iv)
Cailin	9-24 Jan. 1994	4446	4514	6.0	(iv)

(i) Mortality, especially among trout; (ii) No harm to salmonids noted; (iii) Despite low concentrations, damage to salmon gills occurred; (iv) large aggregations of medusae seen.

(1) Clément, A. and G. Lembeye, 1993a. Phytoplankton Monitoring Program in the Fish farming Region of the South of Chile. In: Toxic Phytoplankton Blooms in the Sea; (Eds. Smayda, T. and Shimizu); pp. 223-228.

(2) Clément, A. and G. Lembeye, 1993b. Programa de Monitoreo y Vigilancia de Fitoplancton. Informe Técnico # 2. Asoc. de Prod. de Salmón y Trucha de Chile (A.G.); Puerto Montt, Chile.

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neurotoxic shellfish poisoning (NSP). As no NSP-producing organism had yet been identified, there was some confusion as to the cause of the January illness.

It was not until mid-February that a new *Gymnodinium breve*-like species was identified by the New Zealand Oceanographic Institute (NIWA - Marine). This breve-like *Gymnodinium* species was detected after complaints of respiratory irritation from several hundred residents and students visiting the Whangaparoa and Red Beach areas, north of Auckland. Subsequently samples received by Dr. Lincoln MacKenzie, Cawthron Institute, from Coromandel, further south of Auckland city, also revealed the presence of the same organism.

Soon after the identification of this new *Gymnodinium* species, lipid extracts from shellfish were tested positive for NSP by the New Zealand Communicable Disease Center (NZ CDC). Toxin analyses of New Zealand shellfish carried out by Prof. T. Yasumoto, Tohoku University, Japan, showed that there is a new class or classes of polyether compounds, similar but not identical to brevetoxins. *Gymnodinium breve,* found in the Gulf of Mexico and Florida, is the only species known to produce brevetoxins (potent neurotoxins). Dr. M. Poli of the Toxinology Division at the US Army, Maryland, USA, also confirmed by radio-immunoassay the presence of a brevetoxin-like chemical in New Zealand shellfish extracts.

Until now NSP outbreaks have been largely confined to the Atlantic coast of the USA. It is significant that the 1993 shellfish poisoning was the first major outbreak recorded in New Zealand, and is also the first major outbreak of NSP poisoning to be reported outside the Atlantic coast of the USA.

During the early 1993 toxic shellfish outbreaks, NSP toxins were found mainly on the northeast coast of North Island. This coincided with the distribution of the new *Gymnodinium breve*-like species (max. concentration 1.80 x 10^5 cells.l^{-1}). Shellfish extracts which have been analyzed quantitatively for NSP by the NZ CDC, showed levels ranging from 23 to 592 MU 100 g^{-1} shellfish tissue. The threshold level for NSP is set at 20 MU 100 g^{-1} in the USA, and this has been used as a guideline by the NZ CDC to close shellfish harvests ever since NSP was detected in summer 1993 in New Zealand.

Although NSP comprised the major toxic outbreak in early 1993, a moderate to low level of PSP was also detected by the NZ CDC on the northeast coast of North Island. The PSP levels ranged from 39 to 412 µg PSP equiv. 100 g^{-1} shellfish tissue. However, the majority of toxicity tests showed levels between 100 to 200 µg PSP equiv. 100 g^{-1} shellfish tissues, which is slightly over the threshold level 80 µg PSP equiv. 100 g^{-1}. In the early 1993 episode, PSP did not appear to pose much of a problem to human health. Two clonal cultures of *A. minutum* developed at the New Zealand Oceanographic Institute were subsequently tested for PSP.

Table 1
Toxic and potentially harmful phytoplankton species identified during the early 1993 toxic shellfish outbreak, and type of toxins known to be produced by these organisms

Phytoplankton taxa	Type of toxins
Gymnodinium sp. nov. (cf. *breve*)	NSP
Alexandrium minutum	PSP
A. tamarense	PSP
Dinophysis acuminata	DSP
D. acuta	DSP
D. fortii	DSP
D. tripos	DSP
Prorocentrum lima	DSP
Pseudnitzschia pungens f. *pungens*	Non-toxic
P. australis	ASP
Chrysolchromulina cf. *polylepis*	Fish-killing
Gymnodinium mikimotoi	Fish-killing
Heterosigma akashiwo	Fish-killing
Prorocentrum minutum	Hepatotoxin

Table 2
Predominant species identified in blooms between February and May, 1993

Date	Dominant species	Bloom colouration	Location
17 Feb.	*Myrionecta rubra*	Reddish-brown*	New Plymouth
18 Feb.	*Prorocentrum micans*	Brown*	Lake Ellesmere
2 March	*Prorocentrum triestinum/ Gymnodinium sanguineun*	Reddish-brown*	Timaru
10 March	*Myrionecta rubra*	Reddish-brown*	Timaru
24 March	*Myrionecta rubra*	Reddish-brown*	New Plymouth
2 April	*Asterionella glacialis*	Brown	Invercargill
18 May	*Chaetoceros armatum/ Auladiscus* sp.	Brown	Muriwai

* Sighted by Air New Zealand pilots

Like the new *Gymnodinium breve*-like species, *A. minutum* was predominantly found on the northeast coast of North Island, with a smaller incidence on the southeast coast of South Island. The bulk of this species appeared to occur in Bay of Plenty (max. concentration 1.17 x 10^5 cells.l^{-1}), coinciding with moderate levels of PSP detected in the same areas. This species is similar to the *Alexandrium* species previously isolated by Dr. Vivienne Cooper from Whangarei Harbour in 1983, but no PSP outbreak was reported then.

At the height of the toxic events, several other toxic and potentially harmful species of phytoplankton were simultaneously identified in areas where new *Gymnodinium* species and *A. minutum* were found. These are listed in Table 1. Most of these species were quite sporadic in distribution and occurred in low concentrations. Low levels of DSP (<0.5 µg OA g^{-1}) and ASP (< 19 µg DA 100 g^{-1}) were detected by the Environmental Health and Forensic Sciences in several areas around the northeast coast of North Island. These toxins did not appear to have caused any problem to human health during the toxic episode.

Between February and May 1993, several major blooms were also reported in many areas around New Zealand. Most of these blooms were harmless and were dominated mainly by a few common dinoflagellate and diatom species, and a photosynthetic ciliate, *Myrionecta rubra* (= *Mesodinium rubrum*) (Table 2). The surface discolouration caused by the build-up of these organisms ranged from brown, or reddish brown to red. Blooms of coccolithophores were also observed by Dr. MacKenzie during aerial surveys in Marlborough Sounds and Big Glory Bay in South Island in early 1993.

The early 1993 toxic algal events coincided with a prolonged period of unusual climatic conditions, an effect of El Niño. This was the second major algal event to coincide with El Niño in the last decade in New Zealand. The recent episode is distinct from the previous one in 1983, as outbreaks of shellfish poisoning were not a feature of the earlier event.

The El Niño event appeared to continue through 1993, but there are now signs of weakening of this event since the end of 1993. So far only low concentrations (< 1000 cells. l^{-1}) of *Gymnodinium breve*-like species have been found since August 1993, in Hauraki Gulf, on the northeast coast of North Island, and smaller amounts of NSP detected in shellfish in the same areas this summer compared with 1993. There are also far less areas being closed to shellfish harvesting this year. Because shellfish in New Zealand have regularly been tested for toxicity, and swift action taken by the newly established Marine Biotoxin Unit, Ministry of Agriculture and Fisheries, to close shellfish harvesting whenever toxins have been detected higher than accepted threshold levels, cases of shellfish poisoning appear to have been prevented this year.

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Sixth International Conference on Toxic Marine Phytoplankton

This event assembled 273 registered participants and some occasional visitors, in all about 300 persons from more than 40 countries, during five days of plenary sessions and round table discussions. Four scientific themes were explored: the mechanisms of harmful bloom formation, identification and detection of responsible species, cellular biochemistry and physiology, and bioaccumulation effects of phycotoxins. These themes were treated in oral communications and 160 posters, while round table discussions were devoted to subjects previously given less consideration, such as toxic Cyanobacteria blooms and the ecology of benthic dinoflagellates.

Mechanisms of harmful bloom formation

This session dealt with the theory of a global spread of blooms and outbreaks as indicated by models, trend studies and consideration of human activities and associations between microalgae and bacteria.

Some models presented can be applied on a global scale, e. g. the role of winds and weather of the southern Benguela (South Africa), the effects of advection and diffusion within and adjacent to the rías of northwest Spain and front dynamics at the entry to the Gulf of Finland. Trend studies, such as those carried out in France using data from the national monitoring network, indicate large-scale geographical specification of populations. On a regional scale, the factors involved in bloom dynamics are variable: the role of light intensity and DMS production with *Phaeocystis* blooms in the Gulf of Maine and of nutrient salts in the Gulf of Trieste. At the laboratory level, models concerning cell growth rates and the nutrient requirements of implicated species are also quite useful: the incidence of intracellular nitrogen and phosphorus quotas on the rate of cell division; adaptation to nitrogen limitation as a function of temperature; production of allelopathic substances and mucus; transition from autotrophy to mixotrophy; resistance to herbivores grazing pressure, etc. However, it should be kept in mind that these models are limited by their experimental biases. These different aspects of the bloom mechanism revealed the need for more precise definition of spatiotemporal scales and for a greater number of regional models to confirm the apparent spread of these events. In fact, two additional types of models were proposed: climatic ones (proximate diffusion) and those relating to eutrophication (hierarchical diffusion).

With respect to the influence of human activities, a considerable number of presentations and discussions were devoted to the results of introducing species from one continent into another, whether by ballast dumping from tankers or extensive aquaculture, mainly of bivalves. Effects due to eutrophication were also considered (industrial, domestic and agricultural wastes; acid rain; deforestation; soil leaching; etc.). Failure to take these factors into account when the responsible microalgae produce toxins harmful to marine fauna or the human consumer can lead to considerable economic disruption, particularly financial losses in aquaculture and the appreciable cost of maintaining monitoring networks and treating cases of human poisoning.

The round table discussion on apparent spreading of toxic blooms indicated that the situation is still not clear. Increased awareness among the public and the scientific community as well as development of extensive monitoring networks is undoubtedly providing a more complete picture than that available 20 or 30 years ago. How much of the documented increase in bloom frequency and intensity is real, and how much due to improved monitoring is still unresolved.

Confirmation of the spatiotemporal distribution of a species necessarily requires the study of cysts conserved in sediments (fossilized in the case of the oldest ones), a relatively new scientific approach. New species were reported from different parts of the world, and unknown toxins accumulated by bivalves. Moreover, the increase in the number of toxic and nontoxic blooms seems related to the practice of extensive aquaculture (fish and/or shellfish) in some countries. In this respect, the toxic outbreak in New Zealand in 1992/1993 further illustrates apparent spreading since the neurotoxic shellfish poisoning (NSP) reported there represents its first documented occurrence outside Atlantic waters.

Finally, several investigations involving the association of bacteria with microalgae emphasized the major role played by prokaryotes, in toxin production, cell growth of host algae, and the use of the latter as vectors for pathogenic microorganisms. It was thus shown that several species of marine bacteria from European coasts synthesize toxins that block sodium channels (the same mode of action as paralytic toxins or tetrodotoxins) and that the introduction of associated (extracellular) bacteria potentiates the toxin production of microalgal cultures. Moreover, even though many bacterial species are known to produce paralytic phycotoxins, few are capable of infecting marine organisms.

It was demonstrated that okadaic acid (a diarrheic toxin produced by *Dinophysis* and some *Prorocentrum*) is detectable both in the microalga, the culture medium and the associated bacteria. The biosynthesis of this toxin and its derivatives by microalgae (as indicated by labeled acetates) seems to follow metabolic pathways similar to those recently found in marine bacteria.

However, in the context of future research in this field, it will be necessary to elucidate the exact role of bacteria and viruses in the marine environment during toxic outbreaks. For example, the production of algicidal substances capable of reducing blooms would be one aspect of this approach.

It may be concluded that, in the current state of our knowledge of the mechanisms of toxic bloom formation, the conceptual tools for description and generalization of the events observed are not always available, despite spectacular advances in understanding of certain ecological interactions. Too many data are still fragmentary or related to situations concerning particular species.

Identification and detection of responsible species

One of the high points of the conference was the presentation by three participants of a new toxic species, the dinoflagellate *Pfiesteria piscimorte*, or 'ambush predator' found along the east coast of America. Depending on whether fish in an estuarine environment are alive or in a state of decomposition, this species produces no fewer than 15 different stages whose successive transformations are induced by thermal and saline shocks or the release of stimulant substances by the prey itself. Nearly all the stages are toxic and display an astonishing saprotrophic activity.

Other communications concerned the development of more efficient detection tools, especially automated ones: image analysis techniques by neuronal networks (back propagation network - BPN), immunological techniques allowing flow cytometry detection after cell labelling, or detection *in situ* of the toxin produced (e.g. brevetoxins), with subsequent determination of cell concentration by reliance on the relation between the number of cells and the quantity of toxin, or direct immunological detection of cells by spectrofluorimetry followed by separa-

tion of sample target species using magnetic processes.

Techniques based on identification of specific proteins (lectins) and fluorescence study of the pigment pattern were also considered, as well as the most suitable sampling methods. A synthesis of knowledge about the number of toxic or bloom-forming species, relative to total world-wide phytoplankton flora, provided several basic principles. In particular, the more diversified an algal class becomes, the greater its number of toxic species or blooms are. A similar result was not obtained for diversification of a genus, but it would appear that the danger of proliferation of new toxic species is far from limited.

Cellular biochemistry and physiology

The communications presented in this session were mainly concerned with biochemistry and the use of genetic engineering tools for species detection, phylogeny and the geographical dissemination of toxic or nontoxic strains of a species or genus. Studies of a physiological nature were more related to the factors involved in toxin production or the improvement of knowledge about the intoxication process. The possible role of okadaic acid on cell division was also considered. In more general terms, the allelopathic relations between toxic and nontoxic species were explored in several communications, particularly for the genera *Chrysochromulina* and *Nitzschia*. With respect to the environmental factors involved in toxin production, the critical role of amino acids (particularly arginine) in the synthesis of paralytic toxins and brevetoxins was considered, as well as the importance of the nitrogen source (NH_4, NO_3) in the toxicity of species of *Alexandrium*. For *Pyrodinium*, the toxin pattern includes STX (saxi-toxins) but not C toxins, and the total toxin content is modified by variations in temperature or salinity. Finally, toxic processes were described for the genera *Chaetoceros* (fish response to stress by mucus production), *Gymnodinium* (localization of the brevetoxin receptor on the sodium channels) and *Ostreopsis* (activation and inactivation of the sodium channels).

Detection, effects and bioaccumulation of phycotoxins

The development of detection methods is rather closely related to the elucidation of phycotoxin structures. This was clearly demonstrated in the case of maitotoxin and other ciguatera toxins as well as brevetoxins. Derivatives were tested by different types of bioassays, and their activities analyzed as a function of their affinity for binding to specific sodium channel receptors. A pharmacological test on the specific binding property (direct correlation with toxicity) is used for detection of domoic acid (the toxin of amnesic shellfish poison: ASP), whereas cytotoxicity assays recommended for quantification of maitotoxin (ciguatera) and okadaic acid (diarrheic poisoning) use hamster fibroblasts. In both cases, automation of the methods by ELISA (Enzyme-linked immunosorbent assay) provides appreciable savings in time for detection purposes.

Concerning the bioaccumulation of phycotoxins, we were reminded that bivalve mollusks are not the only vectors of intoxication. Gastropods and certain crustaceans can also accumulate toxins by feeding on contaminated shellfish. Likewise, the ecological impact of microalgal toxicity via the pelagic or benthic food chains shows evidence of contamination of herbivorous and carnivorous fishes by a variety of compounds (DSP, PSP, CFP-Ciguatera fish poisoning). In the most classic case of direct contamination of bivalves, a new okadaic acid derivative (DTX2) was recently found in considerable quantity in European mussels. The rates of accumulation and depuration in shellfish tissues were studied as well for ASP and NSP or PSP toxins. In the last case, the role played by enzymatic degradations in the formation of derivatives of varying toxicity was also demonstrated.

Conclusions

The Sixth International Conference on Toxic Phytoplankton was a valuable source of new information in different fields: the discovery of a new species of dinoflagellate (the 'ambush predator'), the occurrence of novel toxic outbreaks in New Zealand, the presence on European coasts of toxin-producing bacteria blocking sodium channels, the intracellular localization of okadaic acid, etc. Even more than at the Newport conference, we were made aware of the increasing development of detection techniques for toxic species or their metabolites. In this respect, the tools of molecular biology, detection by immunoassay, cytochemical testing and enzymatic inhibition provide investigative approaches which will very shortly improve the efficiency of monitoring networks. Concerning the global spread of blooms and outbreaks, contradictory evidence was presented during discussions. The role of environmental factors is complex and specific, but human activities, at least in introducing species, were clearly implicated.

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Phycotoxin internet

An electronic mailing list has been created on toxic phytoplankton and related subjects, at the Bedford Institute of Oceanography, Dartmouth, N.S., Canada. The list is accessible via the Internet on the address phycotoxins@biome.bio.dfo.ca.

How to subscribe to the list

Anyone interested in joining the list should proceed as follows:

- send a message via Internet to: Majordomo@biome.bio.ns.ca;
- leave the 'subject' line blank;
- in the body of the message the only statement should be: 'Subscribe Phycotoxins'
- if you require further information on the operation of the system, add the word 'help' on a new line.

The system then adds your internet address to the list. Each time a message is posted to Phycotoxins@biome.bio.ns.ca, all addresses on the list will receive a copy.

The list is potentially a very useful tool for worldwide dissemination of information on current developments related to toxic phytoplankton. Similarly, the list can be used as a 'forum' to exchange views, ask questions or advise colleagues working in the same field of interest. Of course, a reply can be sent to the 'list' or to an individual.

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The Philippines: Social aspects of red tides

Algal blooms can only be considered 'harmful' when they have direct or indirect negative impacts on man.

In 1992, a study was undertaken to (a) describe the knowledge of La Huerta (Paranaque, Manila Bay) residents about the red tide events affecting the area since 1987, (b) determine the general attitudes of the residents toward the events and (c) describe the people's coping mechanisms to the harmful effects of the events. Perceptions and reactions would, of course, be limited to the experiences of the respondents. Results might be beneficial in developing local management schemes specially pertaining to the education of the people and their social welfare/needs. The area has a population of 8,647 with 1,703 households. Ten percent of the households, which is about 170, became the basis for fielding 200 questionnaires. 164 of these were recovered but only 155 were considered, since the rest were not properly and completely answered. Field interviewers visited the respondents at random to establish a rapport with them.

General knowledge of red tide and paralytic shellfish poisoning

One group (called the Pulo residents) has been quite knowledgeable on the cause (the organism) of the discoloration and the poisoning, while the other group (the non-Pulo) attributes the phenomenon to chemical causes.

With regard to the symptoms of paralytic shellfish poisoning, the majority are well aware of the two physical effects – hallucinogenic and paralytic. Eighty nine percent of the respondents believe or know that death could follow if one eats contaminated shellfish. The majority of them, however, did not know that contaminated shellfish could still be alive and even depurate themselves of the toxin.

Concerning first-aid treatments, the majority believe in and have used coconut milk sometimes in combination with sugar. They do not really know the internal effects of these materials including the use of 'baking powder in water'. They are also aware that cooking and drying the shellfish does not lessen the toxicity.

Attitudes toward the event

More than half of the respondents believe that the 'red tides' in their area are an 'inevitable occurrence', while a minority believes that it could be a 'curse'. Reactions to the events include 'sadness' but respondents believe that there is a 'need to rise above the situation'. A few believe that 'survival' during red tide is everybody's goal because 'only when one has plenty can they help others'. This 'Kanya-kanya' or 'to each his own' system should be stopped, most respondents said, and added that cooperation is most needed during these times. The majority believe that they are not 'panicky' but 'calm' during these situations. As to the information drive regarding the occurrence of the red tides, only 25% of them believe that they have insufficient information.

Coping mechanisms

Fishermen, shellfish divers and sellers said that their incomes were reduced from half to almost nothing during red tides. Some shifted to other jobs like construction drivers, carpenters and mechanics. Others tried buying and selling items like handkerchiefs and towels. Some wives who help their husbands just 'stay at home' during red tides, while some of them sell fruits and cakes or wash clothes for other people.

With regard to the issue of why some of those in the affected areas still eat contaminated shellfish, wholesalers and middlemen take the risk since they would like to prove to buyers that their goods are 'safe'. On the other hand, the majority of the fishermen lead a 'hand to mouth existence' and have to take the chance for there is 'no other food at hand'.

Mendigo (1993) reports that in a related study, the Field Epidemiology Section of the Department of Health recounted that a number of PSP victims during the 1993 Manila Bay outbreaks had defied the ban on harvest and consumption of contaminated shellfish. One reason given was that they did not believe that the shellfish were poisonous and would like to test this on themselves. An educational campaign is now underway to correct this attitude.

These are social realities brought about by harmful algal blooms. The effects would not be the same if the events occurred in a developed place or country where there are other sources of food and income. It is evident that the people are quite well informed but the social and economic support systems are still lacking despite government financial aids given to these areas. In 1993, about 5 million pesos (approximately $196, 850 in aid was given to Manila Bay red tide affected families. At this point, there is a need for (a) more information campaigns on points not yet clear to the fishermen and other people; (b) development of permanent and feasible alternative sources of income for affected families and (c) research to determine and plan the time of closure and opening of harvest and the management of 'crops' before, during and after red tide events.

Reference

Mendigo, M. A. 1993. *Pyrodinium* Red Tides: How the Philippines Has Coped with a Recurrent Problem. Paper presented in the Pacific Science Inter-Congress held in Okinawa, Japan, June 27-July 3, 1993.

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Harmful algae blooms – Chile Working Group

Harmful algal blooms are not uncommon in Chile and PSP outbreaks in the southernmost part of the fjord ecosystem have been detected again recently. Also, we have noticed harmful effects of the diatom *Leptocylindrus minimus* to fish farms in the Xth Region Inland Sea.

Despite the long geography of our country, with a coast line larger than 4,000 km, we have made the effort to get together. On November 1993 we constituted the Chilean Working Group on Harmful Algal Blooms. The elected coordinator and secretary were Alejandro Clément and Miriam Seguel respectively.

The fundamental goals of the working group are:

- to improve communications among the expertise in the country;
- to develop a national programme taking into consideration the work done by the 'IOC-SCOR Workshop on Programme Development for Harmful Algal Blooms' (*IOC Workshop Report* N° 80); and
- to improve our national algal blooms data base.

The National Oceanographic Committee (CONA) is the representative of IOC in Chile. The Working Group is therefore under the direction of this committee whose executive secretary is Mr. Mario Cáceres.

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Pseudonitzschia Seriata – A new toxic diatom

After the serious incidents in North America with Amnesic Shellfish Poisoning (ASP), a search for potentially toxic diatoms in Danish coastal waters was initiated by the University of Copenhagen, Denmark.

Six species or forms of *Pseudonitzschia* were identified: *P. delicatissima, P. fraudulenta, P. pseudodelicatissima, P. pungens* f. *multiseries, P. pungeris* f. *pungens* and *P. seriata.* Surprisingly, *P. seriata* (clone 1877C) from Nivaa Bugt, The Sound, Denmark, was found to produce domoic acid (DA). The identification of the species was kindly confirmed by G.R. Hasle, University of Oslo, Norway. *Pseudonitzschia seriata* has previously been found to be non-toxic (Bates, 1989, *Can. J. Fish. Aqua. Sci.*, 46, 1203-1215).

The Danish material was isolated into pure culture and grown in F/2 medium at 4°C with a salinity of 20‰ and a 16:8 light:- darkness cycle at 30 µmol photons quanta $m^{-2}s^{-1}$ (Table 1). The highly sensitive FMOC-HPLC method (Pocklington et al., 1990, *Intern. J. Environ. Anal. Chem.*, 38, 351-368) was used for detection of DA.

In *P. seriata*, as in *P. pungens* f. *multiseries* (Bates, 1989), the toxin was produced only in the stationary growth phase (Lundholm et al., 1994, *Phycologia.*, 33, in press). The amount of domoic acid per cell (whole culture) was comparable to the production by *P. pungens* f. *multiseries* (Table 1). Toxicity has been confirmed in two other isolates (1877A and 1877B) from the same locality.

The following characteristics may be used to identify *P. seriata*, formerly *Nitzschia seriata* (Hasle, 1993, *Beih. N. Hedw.*, 106, 315-321): Seen in valve view, the frustules are asymmetric and 5-8 µm wide. In electron microscopy the lack of a central nodulus is characteristic and frustules possess 4 (3-5) rows of small poroids in the striae and 14-18 interstriae in 10 µm (Hasle, 1965, *Vid. Akad. Skr. I. M.-N. Kl. Ny serie*, N°18, and own unpubl. observations).

P. seriata is a cold-water species, which occurs in both neritic and oceanic areas. It has been found only in the Northern Hemisphere (Hasle, 1972, *Beih. N. Hedw.*, 39; Hasle, 1976, *Deep-Sea Res.*, 23, 319-338). Records from the Southern Hemisphere or from warm waters seem to be misidentifications (Hasle, 1972).

P. seriata is a common species in the Arctic (Smith et al., 1994, *J. Phycol.*, 30, 8-16) and the southern limit of the species in the Western Atlantic is further south (40-45°) than in the Eastern Atlantic (Kiel Bay, 54-55°N) (Hasle, 1972). Records are most numerous from winter and spring: e.g. February to May in the Skagerrak, North Atlantic (Lange et al., 1992, *Sarsia*, 77, 173-187).

Whether *P. seriata* has been involved in domoic acid poisonings is not known. In some records of domoic acid in plankton or mussel samples, the source of DA has never been identified. Thus in March 1989, domoic acid (0.3 - 1.8 ppm) was detected in mussels under the ice at Prince Edward Island, Canada, and in March 1990, domoic acid (60 ng l^{-1}) was found in plankton below the ice. *P. pungens* f. *multiseries* was not present in the samples (Pauley et al., 1993, *Toxic Phytoplankton Blooms in the Sea*, 311-316). However, one of the most prominent species in the 1990 event was *P. seriata*. Since cell concentrations were very low (max. 2.5 × 10^4 cells l^{-1}), it: 'would have to be a strong producer [of DA] (e.g. ~2 pg $cell^{-1}$, which is characteristic of *N. pungens* f. *multiseries* in culture) to account for the observed DA levels.' (Pauley et al., 1993, *op. cit.*, p. 313). These concentrations are consistent with our findings.

The finding of *P. seriata* as a producer of domoic acid has consequences for the future monitoring. Further investigations especially on the fate of toxic diatoms in the food chain are urgently needed.

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Table 1
***P. seriata* – Concentration of cells and domoic acid in stationary growth phase. The stationary phase was reached at day 20.**

Days of growth	Concentration of cells* ml^{-1}	Domoic acid $ng\ ml^{-1}$	Domoic acid $pg\ cell^{-1}$**
18	23200	4.5	0.2
25	30870	6.9	0.22
32	39728	14.3	0.36
39	48083	36.9	0.77
43	48900	51.8	1.1
48	50400	92	1.8
53	38533	126.3	3.3
62	13747	188.3	13.7

* *At least 400 cells ml^{-1} were counted.*
** *Based on whole culture (cells plus medium).*

Shellfish poisoning: Boy dies and 13 treated

In January this year, in Kota Kinabalu, Malaysia, a six year-old boy died while 13 people received emergency treatment at the Queen Elizabeth Hospital (QEH), from paralytic shellfish poisoning.

Sabah Medical and Health Services Director, Dr. M. Chan, warned the general public against collecting and consuming shellfish found at Kampung Gentisen, in Sepangar Bay.

The deceased was on a picnic with his family at the Kampung Gentisen beach where they collected some shellfish for their Sunday lunch. The father later noticed his son was sick and immediately rushed him to hospital, where he was pronounced dead on arrival.Thirteen others were admitted to the hospital for treatment.

According to Chan, one of the victims bought the shellfish from the Menggatal market, believed to have been collected from Kampung Gentisen.

The signs and symptoms of paralytic shellfish poisoning are nausea, vomitting, dizziness, weakness, numbness of the limbs, tongue and lips, and difficulty in breathing. He advised anyone suffering from such symptoms after eating shellfish to seek immediate treatment at the nearest hospital.

The department's health education unit is distributing leaflets on paralytic shellfish poisoning to residents in the affected areas. Talks on health education are also being given, with a warning against collecting and eating shellfish.

Chan said the Health Department would continue to investigate and monitor the situation, with the cooperation of the Fisheries Department.

Extract from Daily Express, 19 January 1994.

Fish kills and *Gymnodinium* cf. *nagasakiense* in Corsica (France)

A Gymnodinium cf. *nagasakiense* bloom occurred in the coastal lagoon of Diane, located on the coast of Corsica, a Mediterranean island (Figure 1). It was the cause of some fish kills, which led to the study of different aspects of the production of exotoxins by this alga.

The fish deaths occurred on 24 September 1993, preceded by strong winds on September 23rd. Histological examination of the gills of moribund fish showed a congestion, a hemorrhage and a slight subepithelial oedema.

In one day, about 500 kg each of sea bass (*Dicentrarchus labrax*) and sea bream (*Boops boops*) died. This is a low proportion of the annual production in this area (170 tons/year), but if we consider the mortalities of *Ostrea gigas* which occurred in 1987 and 1989 in the lagoon, these recent losses constitute an indicator of a persistent critical bloom situation.

Present at low levels in the summer and associated with Prorocentrales and Silicoflagellates, *G.* cf. *nagasakiense* began to dominate the phytoplankton after 14 September. The maximal density: $2.7 \cdot 10^6$ cells/litre was observed on 18 October. Then the *Gymnodinium* population decreased while *Thalassiosira* spp. prevailed at 80%. In late October the bloom declined entirely, probably because of very bad meteorological conditions. The maximum cell density followed a temperature plateau of about 21°C, and a moderate decrease in dissolved oxygen saturation (Figure 2).

During this event, seawater quality was followed in terms of its standard hydrobiological composition as well as its allelopathic and ichthyotoxic potency.

Seawater containing 4,000 *Gymnodinium* cells/litre was tested with and without filtration on GF/F filters (Whatman). The toxin affected the 9-day larvae of *Pecten maximus* inducing a 40 % death rate within 9 days of contact with the toxic seawater. The size of the surviving larvae was 30 µm, inferior to the control obtained with uncontaminated seawater.

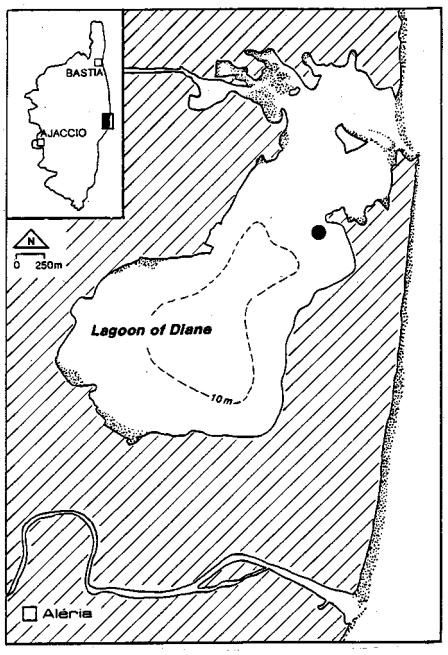

Fig. 1. ● = *Geographical site and sampling station.*

Allelopathic properties of lagoon seawater, which contained 4,000 *Gymnodinium* cells/litre, were effective towards the diatom *Chaetoceros gracile* growth.

Filtered seawater and the *G.* cf. *nagasakiense* cell were studied in terms of their lipid components. The amount of octadecapentaenoic acid, a highly toxic polyunsaturated fatty acid, was elevated in samples (filtered seawater and cellular extracts) which contained significant concentrations of *Gymnodinium* cf. *nagasakiense* cells.

This alga very closely resembles the Channel and Oceanic species but it is not definitively identified as such.

The authors also allude to the possible importance of other biological factors, including preconditioning of the water by other phytoplankton species composition, and the interaction between hydrographic features and environmental parameters (temperature and oxygen content), to explain the occurrence of high concentration of *G.* cf. *nagasakiense* cells in the surface water.

This event was studied, for water sampling and physico-chemical data, by the IFREMER Pelagic Ecology Laboratory, with the participation of the IFREMER Coastal Laboratories of Santa Maria Poggio, Concarneau and Toulon.

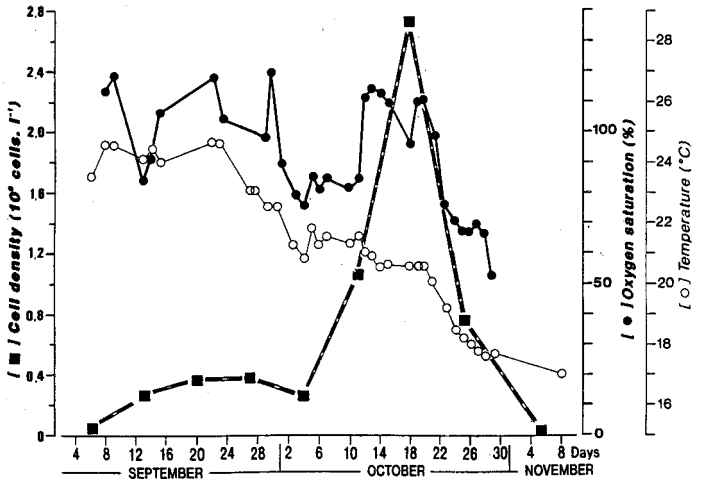

Fig. 2. Temperature, dissolved oxygen saturation and cell concentration at surface.

Geneviève Arzul, Guy Bodennec, Evelyne Erard, Patrick Gentien, IFREMER Centre de BREST-DEL, BP 70-29280 Plouzane, France.

1994 PSP Cases in the Philippines and Malaysia

Despite the imposition of a ban on shellfish gathering from affected areas, 23 people have been hospitalized for paralytic shellfish poisoning (PSP) after consuming infected 'tahong' (mussels) from Limay and Orion in Bataan, Manila Bay as of 16 June 1994.

The Governor of Bataan, Enrique Garcia, directed health officers to undertake a mass information campaign on the temporary ban on the harvest, sale and consumption of mussels from 3 towns (Orion, Limay and Mariveles). He noted that yearly, even in red tide months, many still insist on eating mussels. In the Philippines, toxicity levels for banning has been set at higher than 40 µg/100g of mussels. This increases the recorded PSP cases in the Philippines to 1,488 since 1983.

Earlier in Kota Kinabalu, Malaysia, the Daily Express reported on 19 January, 1994, that a 6 year old boy died and 13 others were treated for PSP. *Pyrodinium bahamense* var. *compressum* has been affecting the waters of Malaysia and the Philippines since the 1980's. *(See article on this case on previous page.)*

IOC Publications

Available free of charge

The publications listed below are available free of charge from the IOC. Please address requests for these publications, as well as for extra copies of issues 1-8 of Harmful Algae News to: IOC Secretariat, Harmful Algal Bloom Programme, UNESCO, 1 rue Miollis, 75732 Paris Cedex 15, France.

Summary Report of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, Second Session, Paris, 14-16 October 1993.

International Workshop on Training Requirements in the Field of Eutrophication in Semi-Enclosed Seas and Harmful Algal Blooms, Germany, September 1992. *IOC Workshop Report* No. 94.

Assessment and Monitoring of Large Marine Ecosystems. Intergovernmental Oceanographic Commission, 1993. *IOC/INF-942.*

The following copies are also available for scientists in developing countries (priority to libraries):

Proceedings of International Symposium on Marine Biotoxins, France, 1991. Ed. J. Marc Fremy. Courtesy of Centre National d'Études Vétérinaires et Alimentaires, France.

Biotoxines aquatiques (eau de mer et eau douce). Critères d'hygiène de l'environnement 37. International Programme on Chemical Safety (IPCS), World Health Organization, Geneva, 1986.

Future events

SEPTEMBER 94

13th International Diatom Symposium, 1-7 September, Acquafredda di Maratea, Italy. *Contact*: Jean Gilder Congressi snc., Via G. Quagliariello 35/E, I-80131 Napoli, Italy; tel: (39-81) 546 3779 or 545 4617; fax: (39-81) 546 3781.

4th International Symposium of the AOAC Europe Section – *'Food and Feed Analysis: A focus on methods with minimal hazards to health and environment'*, 29-30 September, Nyon, Switzerland. *Contact*: AOAC Europe Section Symposium, Dr. T. Rihs, Swiss Federal Research Station for Animal Production, CH-1725 Posieux, Switzerland; tel: (41-37) 877 111; fax: (41-37) 877 400.

OCTOBER 94

11th World Congress on Animal, Plant and Microbial Toxins, 2-7 October, Tel Aviv, Israel. *Contact*: KENES - Organizers of Congresses and Tour Operators Ltd., P.O. Box 50006, Tel Aviv 61500, Israel; tel: (972-3) 5140014; fax: (972-3) 5175674/660325; telex: 341171 kens il.

NOVEMBER 94

IOC International Oceanographic Conference – 'Towards sustainable use of the oceans and coastal zones', 7-12 November, Lisbon, Portugal. *Contact:* Secretary, IOC, 1 rue Miollis, 75732 Paris cedex 15, France; fax (33-1) 4056 9316.

JUNE 95

1995 Gordon Conference on Mycotoxins and Phycotoxins – *'Frontiers in Science'*, 25-30 June, New Plymouth, USA. *Contact*: Daniel G. Baden (Conference Chair), NIEHS MFBS Center, University of Miami RSMAS, 4600 Rickenbacker Causeway, Miami, FL 33149, USA; tel: (1-305) 361 4738; fax: (1-305) 361 4001; or Wanda M. Haschek-Hock (Vice Chair and Poster Sessions), Dept. of Veterinary Pathobiology, University of Illinois, 2001 S. Lincoln Avenue, Urbana, IL 61801, USA; tel: (1-217) 333 4755.

JULY 95

2nd International Conference on Pelagic Biogeography (ICoPB), 10-14 July, Amsterdam, The Netherlands. *Contact*: Institut voor Taxonomische Zoölogie, Zoölogish Museum, Mauritskade 57, Postbus 94766, 1090 GT Amsterdam, The Netherlands.

Symposium on Changes in the North Sea Ecosystem and their Causes: Århus 1975 Revisited, 11-14 July, Århus, Denmark. Deadline for submission of papers and posters: **1 October 1994**. *Contact*: General Secretary, ICES, Palægade 2-4, 1261 Copenhagen K, Denmark; tel: (45) 15 42 25; fax: (45) 33 93 42 15.

7th International Conference on Toxic Marine Phytoplankton, 12-16 July, Sendai, Japan. *Contact*: Dr. Takeshi Yasumoto, Conference Convener, 7th International Conference on Toxic Marine Phytoplankton, Dept. of Applied Biological Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiya, Aoba-ku, Sendai 981, Japan; tel: (81-22) 272 4321; fax: (81-22) 275 3606 or 272 1870.

Errata for HAN n°. 7

◆ The article 'Ciguatera Management Workshop, Bribie Island, Australia' was authored by Dr. Richard J. Lewis. Only that part headed 'Toxin Action' was written by Dr. J-P. Vernoux. Our apologies for the confusion.

◆ In the article 'South Korean oysters gave ASP symptoms', the oysters eaten were from South Korea. They were eaten in U.S.A.

Eds.

HARMFUL ALGAE NEWS

This issue was compiled and edited by Tim Wyatt and Yolanda Pazos, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Eduardo Cabello 6, 36208 Vigo, Spain. Tel.: (34-86) 23 19 30 / 23 19 73; fax: (34 86) 29 27 62

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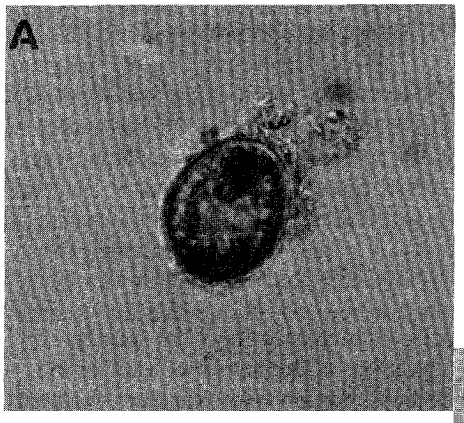
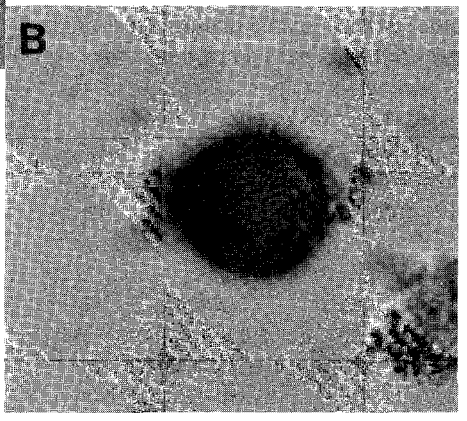
HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 9

First living *Alexandrium minutum* resting cysts in Western Baltic

On a global scale, the frequency and intensity of toxic phytoplankton blooms seem to be on the rise, and there is also some evidence of geographical spreading of nuisance species[1]. In Europe, this has been suggested for the toxic dinoflagellate *Gymnodinium catenatum (p. 1,* HAN *No. 7)*. The PSP-producing *Alexandrium minutum*, first described from Alexandria Harbour, Egypt[2], has since been reported from South Australia[3], the Atlantic coast of North America[4], Spain and Portugal[5], Italy[6,7], Turkey[8], Ireland[9], France[10], and the Netherlands[11].





Living resting cysts of (A) Alexandrium minutum and (B) Gymnodinium catenatum, both isolated from Western Baltic sediments.

In France, since 1985, toxic blooms of *A. minutum* have occurred along the Brittany coast within small embayments or shallow estuaries, especially in the northwestern area. Each time, shellfish harvesting had to be prohibited as a consequence of excess toxicity levels in bivalves[12]. At present, the region represents the main site for toxic blooms of this species in Europe.

Resting cysts of *A. minutum* were first described in 1991 from surface sediments of Port River near Adelaide, South Australia[13] and has also been recorded since then along the Brittany coast of France[12]. It was suggested that blooms of *A. minutum* are primarily initiated from their benthic seed beds[14]. During a cyst survey in the Baltic Sea (Kiel Bight, Germany) in April 1993, several living cysts of *A. minutum* were found in the topmost centimeter of sediments. The cyst is circular in apical view (21-25 mm in diameter) and reniform in lateral view. The clear cyst wall is lightly covered with mucilage and a prominent orange-red accumulation body is present. This resting cyst is similar to the descriptions and figures of specimens from Australia[13] and France[12].

As specified for *G. catenatum (p. 1,* HAN *No. 7)*, the recent occurrence of *A. minutum* in coastal waters of the Netherlands may be related to increased water influx through the English Channel, transporting *A. minutum* cells from the French coast into the North Sea. Residual currents in the region and a massive salt water influx into the Baltic Sea in 1993 may have infected the Kiel Bight with *A. minutum* cysts. However, it is not yet sure whether this scenario is true, because resting cysts of *A. minutum* were not detected in sediments of the German Bight[15] nor the Kattegat area[16]. In contrast, it is quite possible that vegetative cells of *A. minutum* have been

(Cont'd on p. 2)

Is the European *Alexandrium tamarense/ excavatum* toxic?

There have been reports of PSP in Europe for several decades, and in many of them *Alexandrium tamarense* (= *Gonyaulax tamarensis*) was considered the causative species [1,2,3,4]. Some more recent cases have been attributed to other species such as *Gymnodinium catenatum* in Spain [5] and Portugal [6], or to *Alexandrium minutum* in Spain [7] and France [8].

Since the first reports, great progress has been made, both in the taxonomy of genus *Alexandrium*, and in the improvement of culture techniques and media. The older reports were all based on bioassays of suspected shellfish, but now the development of analytical tools like HPLC and the increased number of laboratories in which this kind of analysis is routine, provide frequent reports of the toxin composition of unialgal cultures of dinoflagellates.

Reports of the toxin composition of cultures of toxic dinoflagellates isolated from European waters are for *G. catenatum* [9], *A. minutum* (= *Alexandrium lusitanicum*) [10] and *Alexandrium ostenfeldii* [11]. But HPLC analyses of strains of *A. tamarense* [11] (the Plymouth and Vigo strains) indicate that this species is not toxic [12,13].

It is surprising that the North Sea and surrounding waters are the areas where PSP reports attributable to *A. tamarense* are more frequent, yet there are no reports of HPLC analyses of cultures of *Alexandrium tamarense* isolated from those areas, in which this species appears to be toxic.

The hypothesis, that some of the PSP outbreaks reported in the North Sea area may be attributable to either *A. minutum* or *A. ostenfeldii,* should be tested. *A. minutum* on the north coast of Brittany has proved to be toxic, but the Plymouth strain of *A. tamarense* isolated not far from there is not toxic. As Brittany is the

(Cont'd on p. 3)

Early red tides

The following extract is taken from 'The Endeavour Journal of Joseph Banks, 1768-1771', Vol. I, edited by J.C. Beaglehole (1962). The entry is dated 7 November 1768, when the *Endeavour* was in latitude 21°16'S off the Brazilian Coast (spelling has been modernized):

'About noon long ranges of a yellowish colour appeared upon the sea, many of them very large, one (the largest) might be a mile in length and 300 or 400 yards wide ... we found it to be caused by innumerable small atoms, each pointed at the end and of a yellowish colour, none of them above a quarter of a line (about 50µm) in length; in the microscope they appeared to be fasciculi interwoven one within the other, not unlike *the nidi (nests) of some Phryganeus* which we call caddices' (i.e. the houses of mayfly larvae).

Banks' journal contains several other accounts of the same phenomenon. William Randolph Taylor identified these organisms as *Trichodesmium*, the genus named by C.G. Ehrenberg in 1830 to distinguish its mode of colony formation from that of *Oscillatoria* (*Trichodesmium erythraeum*: Fila septata fasciculata nec oscillantia, fasciculi discreti muco involuti sociales libere natantes). Ehrenberg himself witnessed red water caused by *Trichodesmium* in December 1823 and January 1824 near El Tor in Sinai on the Gulf of Suez. He described the sea as blood-coloured, and noted that the waves cast red slime on the beach.

It was accounts like those of Banks and many other navigators (most of which have never been published) which provoked early attempts to classify red water events. One of the most comprehensive of these was that of Camille Dareste (1855), who listed eleven different categories, the first three of which were due to different species of *Trichodesmium*.

Ehrenberg, C.G. (1830). *Annalen der Physik und Chemie*, vol. 94, part 4: 477-514.
Dareste, C. (1855). *Annales des Sciences naturelles*, Ser. 4, Zoologie, vol. 3: 179-239.

Tim Wyatt

Annual mollusc toxicity variation on Morocco Mediterranean shore

An investigation into the agent responsible for food poisoning events associated with eating seafood has led to the incrimination of molluscs collected on the Mediterranean shoreline of Morocco. Their gathering and sale are now banned.

The study was carried out on two species currently consumed in the region: the cockle, *Cardium tuberculatum* , and the hard-shell clam, *Cuterce chrome*. These species were sampled at regular intervals over a year, from May 1993 to May 1994, in the region of Tetouan at Kaa Stras and Oued Laou, in order to assess the toxicity of the bivalves there. Extracts of the molluscs were prepared according to the official method (Association of Official and Analytical Chemists, 1980).

The toxicity of the extracts was assayed on Swiss Albino mice. *Cardium tuberculatum* gave the highest toxicities at Kaa Stras, but in both cases toxicity levels remained high throughout the year and produced rapid death, preceded by PSP-type symptoms, while *Cuterce chrome* gave lower levels, whatever the period of collection.

Cardium tuberculatum:
- Kaa Stras, toxicity 1207 Mouse Units (MU)/100g of meat.
- Oued Laou, 773 MU/100g

Cuterce chrome: 190 MU/100g

Comparative investigation of the toxicity in different organs of the cockles showed that toxicity levels were clearly higher in the foot (5580 MU/100g) than in the mantle (1030 M/U/100g).

The investigation carried out has shown that biotoxin presence is permanent in cockles in the region of Tetouan, but varies over the year.

F. Tagmouti, Faculté de Médecine et de Pharmacie, Casablanca, Morocco

(Cont'd from p. 1, 'Western Baltic')

overlooked in the plankton of the region because of their scarce occurrences. This may even be the case since the genus *Alexandrium* now includes a rather long list of species, easy to confuse, which may lead to misclassification. Identification especially of the small, inconspicuous *A. minutum* requires careful study of the thecal plates. The variation of the first apical plate in *A. minutum* complicates the identification[3,6,12]. Recently, *A. ibericum* has been synonymized with *A. minutum*[4] and this may also be the case for *A. lusitanicum*[11]. Further investigations will be needed to ascertain whether its cysts can germinate and if vegetative cells can multiply under the specific conditions of this sea area, whether the Baltic *A. minutum* is toxic, and whether it is genetically distinct from other *A. minutum* populations.

In the study of harmful algae, there is an increasingly urgent need for correct identification of species. Cyst studies offer a valuable tool for an early warning on the presence and potential of toxic species in a given area and should be considered also in monitoring systems.

Stefan Nehring, Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, D-24105 Kiel, Germany.

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(15) Nehring, S. (in press). *Helgoländer Meeresunters.*
(16) Ellegaard, M. Personal communication.

(Cont'd from p. 1, 'Alexandrium tamarense')

nearest place to the east coast of England from where a toxic *Alexandrium* species is known, we think that *A. minutum* could be the causative species of PSP outbreaks in that area. The toxic *A. ostenfeldii* was observed in Norwegian waters by Braarud (named *Gonyaulax tamarensis* var. *globose*) [14], and it is probably a synonym of *Pyrodinium phoneus*, the species to which mussel toxicity was attributed in Belgium [15]. Although some cultures have been tested by mice bioassays [16], *A. ostenfeldii* is the only species that has been analyzed for toxins by HPLC in that area [11]. Thus we can also hypothesize that this species is the most probable cause of PSP in Scandinavian waters, since it is known to be toxic while *A. tamarense* from that area, as yet, is not.

The different toxin composition of the various known toxic species in Europe (see table) may help to identify the causative species if toxic shellfish samples are analyzed. For example, *G. catenatum* is rich in Cx toxins, while *A. ostenfeldii* is very rich in GTX6 (= B_2), and *A. minutum* in GTX toxins. An HPLC analysis of samples of toxic shellfish for algal toxin content could give us a first approach to these hypotheses.

More comparative studies of DNA among different isolates of *Alexandrium* are necessary in order to clarify and identify the relationships between the species of this genus, especially those considered as *A. tamarense*.

Santiago Fraga and José M. Franco, Instituto Español de Oceanografía, Apdo. 1552, 36280 Vigo, Spain.

Dinophysis bloom in West Scotland

Reports of brown-red water discoloura-tion in Loch Long, a fjord-like sea loch on the west coast of Scotland were referred to the Marine Laboratory on 31 July 1992. The discolouration extended over a 10 km strip along the eastern shore of the upper part of the loch. Water and mussel (*Mytilus edulis*) samples were collected from four sites along the shore of the loch. Water samples were examined and counts made of the dominant species and the shellfish were tested for the presence of DSP toxins.

At four sampling stations, the numbers of *Dinophysis acuminata* ranged from 8,000 cells l^{-1} (Portincaple) to 103,000 cells l^{-1} (Ardmay), 916,000 cells l^{-1} (Arrochar) and 942,000 cells l^{-1} (Finnart). *D. acuta* and *D. norvegica* were also present at all four stations sampled and at Finnart, *Scripsiella trochoidea* and *Ceratium* spp. were abundant. All shellfish sampled from the area tested positive for DSP toxins. This was the first reported incident of a *Dinophysis* bloom on the west coast of Scotland. There were no reports of any human intoxications as a result of this incident, probably as the area is not one which is regularly harvested for commercial shellfish species.

Elspeth Macdonald, Marine Laboratory, P.O. Box 101, Victoria Rd., Aberdeen AB9 8DB, Scotland.

Strain	Cx	GTX$_6$ (= B$_2$)	GTX4	GTX$_1$	GTX$_5$ (= B$_1$)	GTX$_3$	GTX$_2$	NeoSTX	dcSTX + STX
A. minutum (Brittany) (8)	++	-	+	+	-	++++	+++	-	+?
A. minutum (Vigo) (10)	-	-	++++	++	-	+	+	-	-
A. minutum (Lisbon) (10)	-	-	+++	+++	-	++	++	-	-
A. ostenfeldii (11)	+++	++++	-	-	-	++	++	-	-
G. catenatum (9)	+++	+	-	-	+	-	-	+	++

Comment

The involvement of *Alexandrium tamarense/excavatum* in the UK toxic events has been enigmatic, as was pointed out by Fraga and Franco. However, over the last few years, the Ministry of Agriculture, Fisheries and Food has funded a detailed programme of work on the causes of the regular shellfish intoxications on the British coast. As part of this work, we have isolated a number of strains of *Alexandrium* from the west coast of Scotland. One of these cultures has been the subject of detailed study. Plate dissection shows this culture to be *Alexandrium tamarense* and it has also been shown to be toxic.

The suggestion that *Alexandrium minutum* may also be involved in UK events is an intriguing one, and we have been open to this possibility. Cysts reminiscent of *Alexandrium minutum* have been found in the Firth of Forth, and hatching trials are being carried out to investigate this further.

The hypotheses of Fraga and Franco concerning the identity of organisms involved in toxic events are interesting. However, it is always risky to formulate such hypotheses in the absence of adequate information.

Jane Lewis, Applied Ecology Research Group, University of Westminster, London, UK.

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Development of a national HAB programme in the USA

As the international Harmful Algal Bloom (HAB) Programme of the Intergovernmental Oceanographic Commission (IOC) started to take shape over the last several years, it became clear that this programme should, in part, be a coalescence of national programmes established independently in different countries. The USA has never had a 'national programme', or a plan to attack these problems, despite a long history of problems associated with harmful algae and a solid history of HAB research. No single federal agency had jurisdiction over the many different types of HAB problems, and in many cases, funding fluctuated with HAB events - rising when significant impacts occurred, and decreasing during the years when problems were less obvious.

Faced with growing HAB problems in the USA and limited financial resources to deal with those problems, a series of steps was initiated to create a national programme that could help guide USA HAB activities. The first of these steps was a workshop convened in 1992 at the National Marine Fisheries Laboratory in Charleston, South Carolina. This workshop produced the report *Marine Biotoxins and Harmful Algae: A National Plan*, which identified numerous impediments to progress in the HAB field, and made specific recommendations to address those impediments. Equally important, the National Plan tried to organize the different HAB activities in a way that helped agencies identify their role in the overall programme.

This report was widely distributed within the USA scientific community as well as throughout government agencies and Congressional offices. In order to implement the recommendations of the plan, it was deemed necessary to establish a committee of programme managers from all government agencies concerned with HABs and their impacts. Accordingly, an ad hoc Task Force on Marine Biotoxins and Harmful Algae was established and now meets several times a year to discuss ways to co-ordinate agency initiatives and to more efficiently target resources on HAB issues. (At present, this Task Force includes programme managers from the National Science Foundation, the Food and Drug Administration, the Department of Defense, the National Institute of Environmental Health Sciences, the Centers for Disease Control, and several National Oceanic and Atmospheric Administration agencies (Sea Grant, the Coastal Oceans Programme, and the National Marine Fisheries Service).

During the early deliberations of this group, it became clear that certain areas (such as those associated with toxin detection or analysis) were reasonably well supported by several funding programmes, whereas others, notably those dealing with the bloom dynamics of harmful algae, were not well supported. An effort was thus initiated between two of the agencies represented on the panel (NSF and NOAA) to establish a programme on the ecology and oceanography of HABs. Although the National Plan had identified this as an important area for research, a detailed scientific agenda for this specific topic needed to be developed. A workshop was then convened in August of this year (1994) at Snow Mountain Ranch, Colorado, to develop a science plan on the ecology and oceanography of HABs. Forty participants, including academic scientists and state and federal agency officials, met to discuss those issues related to the population dynamics of HAB species and the manner in which they affect ecosystems. Oral presentations and working group discussions were organized around three topic areas: the organisms; foodweb/community interactions; and environmental regulation of blooms. Within each of these theme areas, working groups identified major hypotheses or questions, and proposed approaches that might be used to address them.

Where relevant, new technologies were identified that would greatly facilitate the attack on these questions.

The proceedings of the Workshop will be printed and distributed within the next several months. It is expected that this science plan will be used to guide agency funding initiatives, as well as to help agency officials evaluate the relative importance of proposal submissions from individual investigators. To fully achieve the goals of this Ecology and Oceanography of Harmful Algal Bloom initiative (ECOHAB), it will be necessary to form agency partnerships to share in the programme implementation costs. As with many other countries, USA HAB phenomena affect many different areas of the country's coastline, involve numerous HAB species, impact many different marine organisms and human populations, and are associated with a variety of different hydrographic systems. A programme to address all of these environmental impacts and species is clearly ambitious, and thus requires co-operation between multiple agencies for full implementation. It is expected that the Interagency Task Force will play an important role in forming these partnerships.

Another important step towards the development of a national HAB programme was the establishment of the National HAB Office at the Woods Hole Oceanographic Institution through joint funding from NOAA and NSF. This office has many a role to play in the planning and implementation of a national HAB programme. The office assists in organizing and chairing ad hoc Interagency Task Force meetings, and it serves as a direct contact point for requests for information on HABs, both technical and general. It also provides a mechanism for USA participation in numerous working groups, workshops and related activities. This office is working to co-ordinate efforts to attract funding to the HAB programme. The first activity in the latter context was to organize and convene the ECOHAB workshop. The office will also be directly involved in establishing an electronic network linking USA HAB workers. This network will also be accessible to others throughout the world via the Internet. The USA HAB office can be considered one of the 'national science and communication centres' now being established in several countries to support the IOC-FAO HAB Programme. IOC HAB science and communication centres, directed towards assisting developing countries, are planned in Denmark and in Spain.

The outlook is thus quite bright for the HAB programme in the USA. The National Plan has helped to clarify the nature of the problems we face and the steps that are needed to address them, and these issues are being further refined through supplemental workshops such as the ECOHAB initiative. High level government officials meet regularly on the Interagency Task Force to guide the activities of the programme. In the USA, at least, this type of planning has brought together the community of researchers and regulatory officials involved with marine biotoxins and harmful algal blooms, and has provided a degree of visibility at the governmental level that should lead to sustained support for this programme, and thus significant progress in understanding and managing HAB phenomena.

Those wishing to receive copies of *Marine Biotoxins and Harmful Algae: a National Plan* or the *ECOHAB Science Plan* (when published) should contact:

Don Anderson, Woods Hole Oceanographic Institution, Biology Department, Redfield 3-32, Woods Hole, MA 02543, USA (E-mail: danderson@whoi.edu).

IOC Training Course on Algal Toxins

The first IOC-WHO-FAO Training Course on Qualitative and Quantitative Determination on Algal Toxins was held from 18 to 28 October 1994, at the Friedrich-Schiller University of Jena. Twelve scientists from eleven countries participated in the course, organized by Prof. Bernd Luckas, Dr. Malte Elbächter (Biologische Anstalt Helgoland) and Dr. Helle Ravn (IOC). Invited lecturers were Prof. T. Yasumoto (Tohoku University) and Dr. A. Boenke (Commission of the European Communities). Dr. M.L. Fernandez (Vigo, Spain), a visiting researcher at the Friedrich-Schiller University of Jena, introduced the work of the European Community Reference Laboratories.



Nora Montoya (Argentina), a participant in the training course, injecting a sample in HPLC equipment for analysis of algal toxins.

The course was geared to the participants' needs, with respect to the toxins found in their countries, so as to enable them to use the knowledge gained from the course to develop methods specifically relevant to their countries. Most of the scientists had brought samples from their countries, which samples were analysed during the course.

The participants were given an overview of state-of-the-art analytical methods for determination of algal toxins. The presence of algal blooms and the most important characteristic markers for identification of algae species were discussed.

Practical experience was gained in qualitative and quantitative determination of algal toxins based on two different HPLC-methods for analysis of DSP and PSP toxins, and on one method for analysis of ASP toxins. The AOAC Mouse Bioassay for PSP toxins and the ELISA bioassay, DSP-Check Test Kit (Japan) were demonstrated respectively by Dr. Schubert (Germany) and Dr. Tubaro (Italy). The practical aspect was emphasized to enable the participants to perform analytical experiments.

All participants presented reports on toxin chemistry as well as toxicology research and monitoring in their countries. The general impression of the course was very positive, and valuable contacts for future collaboration were established amongst the participants.

Red tide flagellate rediscovered in Himalayan valley

A red tide flagellate was observed in some parts (Fig. 1) of Dal Lake, Kashmir during summer-autumn of 1991. The organism responsible turned out to be a rarely recorded flagellate, *Euglena pedunculata Gojdics* (Khan, M. A.: 1993, *Arch. Hydrobiol.* 127, 101-103). The flagellate formed a red scum layer at the surface in the proximity of the Centaur Hotel Complex, Srinagar. No such algal bloom in the lake had been documented previously.

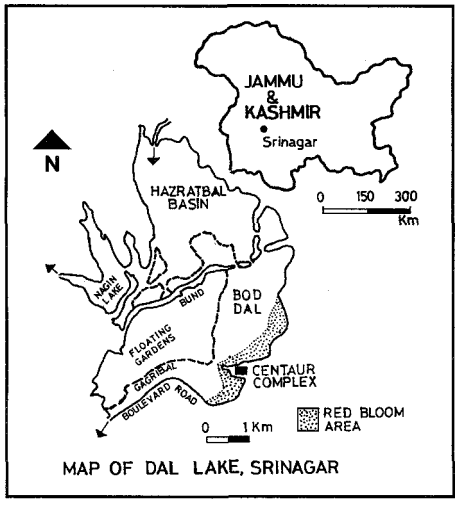

Fig. 1: Map of Dal Lake, Srinagar

The cells were mostly contracted, 20-40 mm in diameter, some slightly elongated. Few cells had flagella. Paramylon granules were round, cup-like or small and oval-shaped. Cyst formation was the most diagnostic feature; the empty cyst wall was trumpet-shaped (Fig. 2).

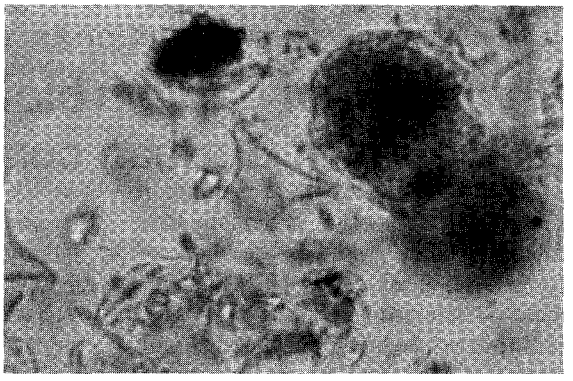

Fig. 2: Euglena pedunculata *(Microphotograph)*

The bloom was mono-specific with approximate numerical density of 15 x 10^5 individuals per litre obtained in September 1991, at the peak of the period. The bloom is attributed locally to accelerated eutrophication caused by sewage from adjacent hotels. The bloom lasted 7-8 weeks and caused no apparent fish mortality.

Further research should throw more light on the environmental implications of such algal blooms in the Kashmir Himalayan waters.

M. A. Khan, Department of Botany, University of Kashmir, Post Box 726, G.P.O. Srinagar - 190001, Kashmir, India.

Harmful algae and PSP toxicity along north Patagonia coast

There have been recurrent reports of PSP toxicity along the Atlantic coast of northern Patagonia, Chubut Province, Argentina. In 1980, values of 173,360 MU/100 g of mussel tissue were reported for sites on the Valdés Peninsula[1], in 1985 values of 173,000 MU were reported from the Chubut River estuary[2], and in 1988 values of 66,150 MU were reported for Nueva Bay[3]. These observations indicate that there has been an expansion in the distribution and increasing abundances of *Alexandrium excavatum*, a PSP-producing species in Argentine waters[4]. Since then, the Argentine Environmental Agency, in collaboration with the Department of Maritime Affairs, Chubut Province, has been monitoring shellfish toxicity by bioassay throughout these areas. High values of PSP toxins in spring and summer have resulted in closures of shellfish harvesting in these areas.

In the Nueva and San José Gulfs (Fig. 1), between November 1990 and April 1991, PSP toxicity increased, beginning in late spring, peaking in mid-summer and declining to undetectable levels in late summer (Fig 2). Maximum values were 7,900 and 22,300 MU/100 g of mussel tissue for the Nueva and San José Gulfs respectively, and the temporal pattern of toxicity in *Mytilus edulis platensis* D'Orb was similar in both locales. During this period, *A. excavatum* occurred at concentrations of 2 x 10^3 cells l^{-1} and a maximum of 16 x 10^3 cells l^{-1} of *Prorocentrum micans* were recorded during this period. Dormant cysts of *A. excavatum* were also detected in these plankton samples, but in very low

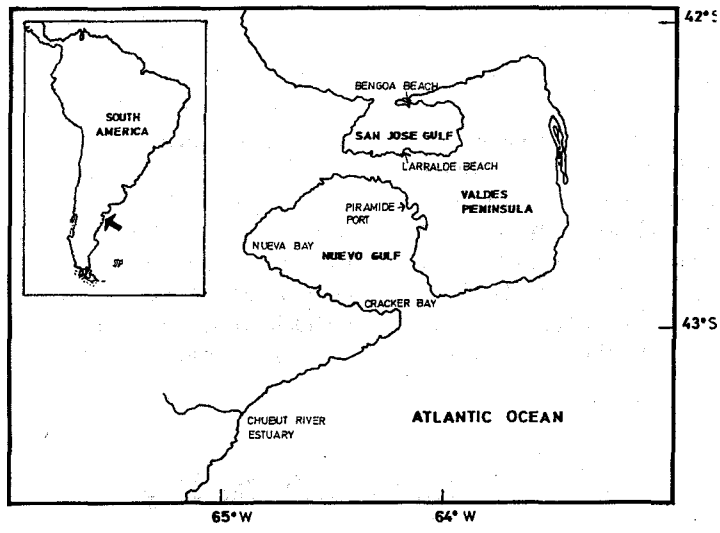

Fig. 1: Monitoring area in North Patagonian coast.

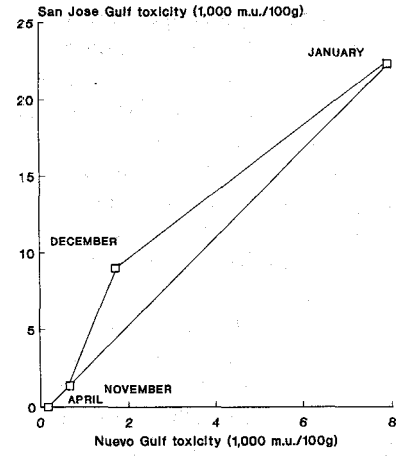

Fig. 2: Toxicity levels in mussels during spring and summer, 1990-1991.

In addition to the above-mentioned dinoflagellates, *Dinophysis acuminata* was recorded in the plankton samples, although in relatively low densities (< 200 cells l^{-1}). This dinoflagellate is of interest as it is one of the species reported to produce DSP toxins. Furthermore, *Prorocentrum lima*, another known DSP-toxin producer, was found in concentrations of 45 cells l^{-1} at Bengoa Beach, San José Gulf. More recently this species was found in relative abundance in beach sediments in Nueva Bay at Puerto Madryn (J.L. McLachlan & A. Woolaver, unpubl. results). These are the first reports of this DSP-producing species for the Argentine coast.

Our observations suggest that the northern coastal region of Patagonia is a potential danger area for shellfish toxicity, not only for PSP toxins but also for DSP toxins.

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(1) Elbusto, C. *et al.* (1981). *Acta Bioquím. Clín. Latinoam.*, 15: 447-456.
(2) Vecchio, J. *et al.* (1986). *Medicina*, 6: 705-706.
(3) Estéves *et al.* (1992). *Hidrobiol.*, 242: 115-122.
(4) Carreto, J.I. *et al.* (1985). Anderson, D.M., White, A. W. and Baden, D. G. (eds.). *Toxic Dinoflagellates.*

Prymnesium parvum blooms on the north German coast

Recurrent blooms of the potentially toxic phytoflagellate *Prymnesium parvum* Carter were observed in a shallow semi-enclosed brackish lagoon, formerly a tidal channel, located on the German North Sea coast. In 1992 maximum cell concentrations of ca. 6 x 10^6 cells per litre were reached at the end of May at a salinity around 5‰ and a water temperature of ca. 20°C. Despite persistent sunny and calm weather conditions, a decline of the bloom to ca. 2 x 10^5 cells per litre was associated with a rise in salinity and temperature at the end of July. In autumn, *P. parvum* disappeared from the plankton but in late November a locally restricted revival of the bloom, with cell numbers up to ca. 6 x 10^6 cells per litre, was observed in a small branch of the basin. Salinity was at 5‰ and water temperature about 5°C. In 1993 only a single bloom (2-3 x 10^6 cells per litre) was recorded in July at ca. 10‰ and 15°C. This is the first ascertified record (by EM) of *P. parvum* on the German West coast.

Although no toxic effects have been observed yet in this area, further attention should be paid to stagnant and eutrophic brackish water bodies in view of their potential function as seedbeds, especially for nearshore eurythermal and euryhaline coastal blooms.

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HawaiiChemtect International Aquatic-Biotoxin Research

Current aquatic biotoxin research activities at HawaiiChemtect International (HCI), focuses on the development of rapid marketplace screening methods based on immunochemical technology for toxins associated with finfish and shellfish poisoning outbreaks, i.e., ciguatoxins (CTX), okadaic acid (OA) and related marine polyethers, tetrodotoxin, and domoic acid (amnesic shellfish poisoning).

Additional areas of focus include the development of a comprehensive harvesting area and marked place monitoring programme for these toxins, evaluation of biotransfer and accumulation of ciguatera-related toxins from toxigenic dinoflagellates to fish, and the development and evaluation of clinical diagnostic methods for ciguatoxins, okadaic acid and related polyether toxins in human serum.

For an analytical method to be of value in screening marketplace seafoods, it must meet the following criteria: (a) easy to use and interpret; (b) rapid, i. e., able to test many samples quickly; (c) accurately differentiate between toxic and non-toxic products; (d) low cost; (e) available in sufficient quantity to meet private, industrial and regulatory agency testing demands; and (f) where feasible, confirm the identity of toxic products. Our research programme has been guided by these principles.

The solid-phase immunobead assay (S-PIA, Ciguatect™), soon to be available from HCI, has high potential for application to marketplace screening of fish products for CTX and DSP toxicity. Testing fish or shellfish early in a screening plan is recommended since this will minimize the cost expended for the product, and reduce potential economic losses to industry.

The kit can be used on board fishing vessels, at receiving docks, processing plants, distribution organizations, retail outlets, consumers and regulatory agencies. The self-contained assay is available as a single analysis kit designed for non-laboratory use by untrained personnel. Organizations conducting large numbers of analyses would be more inclined to use the laboratory kit, which contains sufficient material for 60 tests.

Research on ciguateric fish in the Pacific and Caribbean regions is in progress with the Food and Drug Administration (Washington, D. C. and Dauphin Island, Alabama, USA), the Canadian Health Protection Branch (Ottawa), Hancock Institute of Marine Sciences (Los Angeles, California, USA), Institut Territorial de Recherches Medicales Louis Malarde (Papeete, Tahiti), Queensland Department of Primary Industries, (Deception Bay, Queensland, Australia), ORSTOM Centre de Noumea (Noumea, New Caledonia), Ecole Nationale Vétérinaire de Nantes (France), and the research studies in Guam of the Department of Geology and Geophysics, University of Minnesota (USA). These studies are demonstrating the utility of the test kit under specific applications and that the toxins are present in a significant number of fish at levels below the sensitivity of the mouse bioassay.

Earlier studies at the Laboratoire Central d'Hygiène Alimentaire (Paris, France) focused on the extraction and purification of toxins from barracuda implicated in ciguatera poisoning. These studies confirmed the existence of three separate toxic fractions and the presence of okadaic acid in the flesh of the fish.

The Ciguatect™ test kit and the high performance liquid chromatography (HPLC) methods have also been used to evaluate DSP potential in mussels collected from Denmark and France. Such study confirmed the presence of okadaic acid and related DSP toxins in mussels implicated in a DSP poisoning outbreak (Denmark) and mussel depuration operations (France). Hence, an excellent correlation was shown between the presence of okadaic acid as determined by HPLC and the Ciguatect™ test kit for mussel samples collected before, during, and after depuration operations.

The Ciguatect™ test kit is being developed specifically as a screening tool which will allow testing of a large number of samples, thus identifying high risk products. The test kit determines the presence or absence of toxins by binding the toxins to a membrane attached to a plastic strip and exposing the toxin-laden membrane to a monoclonal antibody-colored latex bead complex which has a high specificity for the toxins of interest. The intensity potential can be determined directly on edible tissue or following specific extraction procedures.

For those products where additional testing is desired, possibly for samples testing positive, a rapid extraction method (REM™) capable of extracting and of partial purification of polyether toxins in less than 30 minutes has been developed. Toxins are extracted with a chloroform : water : methanol mixture and partitioned into select phases by varying polarity. When the REM™ test kit is used to extract and purify toxic components, the limit of detection for the Ciguatect test kit is < 0.05 ng. Also, at this point chemical methods based on thin layer (TLC) and HPLC technology can be used to confirm the presence of individual toxins.

A TLC method for okadaic acid in fish tissue and dinoflagellate cultures has been developed. Specificity of this methodology is obtained following exhaustive purification of toxins extracted from fish tissue. These methods, although not suitable for routine screening programmes, could play an important role in confirming the presence of individual toxins in fish products for laboratories not equipped with HPLC capabilities.

Seafood safety monitoring programmes: An effective food safety monitoring programme comprises primarily these components: (1) monitoring fish and shellfish harvesting areas for toxin potential, (2) establishment of regulatory limits, and (3) a screening programme for testing fish products in commercial channels designed to test a large number of samples in a short period of time so that toxic or high risk products can be identified. Unacceptable products can then, if desired, be subjected to additional testing to confirm the presence and identity of the toxin(s).

Monitoring fish harvesting areas: A key aspect of an effective seafood safety monitoring programme, where feasible, is the establishment of a programme for predicting and identifying high risk harvesting locations. This is accomplished through the testing of marine specimens endemic to fish harvesting locations. This will vary whether for CTX, DSP, or other aquatic biotoxins. Since seafoods commonly associated with ciguatera poisoning outbreaks come from highly mobile fish, collecting and testing the fish alone could provide incorrect information with both false positive and false negative predictions. This possible problem can be corrected by identifying and testing a specimen or bio-marker of limited motility, i.e. invertebrates, endemic to the fishing area.

The Ciguatect™ test kit has been

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(Cont'd from p. 7, 'HawaiiChemtect Int.')

used to screen 36 species of near-shore invertebrates off Hawaii for ciguatoxin and related polyether compounds (R. G. Kvitek, Moss Landing Marine Laboratories, HawaiiChemtect International and D. L. Park, University of Arizona). Specimens included snails, sea urchins, sea cucumbers, crabs, brittle stars, bivalves, and zoanthids. Invertebrates were collected at six 'toxic' locations having documented histories of ciguatera fish poisoning along the Kona coast and the three 'non-toxic' sites along the Hamakua coast, where there had been only one reported case of ciguatera since 1980.

A significant positive correlation between assay results and site-specific ciguatera history was found for the cowry *Cypraea maculifera*. While assay results for most other species indicated very low or no ciguatoxin present, cone snails (*Conus* spp.), ophiuroids (*Ophiocoma* spp.) and sea cucumbers (*Holothuria* spp.) tested positive frequently. However, for these genera, there was no correlation between assay results and site history. These results suggest that invertebrates – particularly grazers and deposit feeders, and especially cowries – accumulate ciguatoxins and related polyether compounds at sites known for ciguatera fish poisoning outbreaks and have the potential utility of being bio-indicators of reef toxicity. *Cypraea maculifera*, or another invertebrate native to the area under study, could be an integral part of the ciguatera monitoring programme.

The Danish and French studies mentioned earlier, demonstrate that the assay could (i) identify DSP-contaminated shellfish and (ii) serve as a useful tool to monitor shellfish on-shore or during depuration operations to determine at what point shellfish can enter the market following a *Dinophisis* sp. bloom.

Establishment of regulatory limits: An important aspect of any food safety monitoring programme is the establishment of regulatory limits designed to assure wholesomeness of the food supply. Animal toxicological and human clinical data are crucial to the determination of these values. Since multiple toxins are involved with CTX and DSP poisoning outbreaks, it is not practical to use a single compound.

Historically, the establishment of a seafood safety monitoring programme for ciguatera has been hampered by the lack of reference standards. At present, okadaic acid is the only toxin associated with both CTX and DSP poisoning outbreaks in sufficient quantities to serve as a reference standard. Therefore, the term okadaic acid equivalents (OAE) could be used to establish regulatory limits, being aware of the relative potencies of other toxins involved with the CTX or DSP phenomenon. Again, the term OAE is used because multiple toxins are involved in the poisoning outbreaks. Recent studies in collaboration with Canadian and USA governments support the hypothesis that CTX-related toxins are present in a significant number of fish, but not necessarily at levels posing a risk to humans.

Our current studies on the detection of marine polyether toxins in human serum show a significant correlation between the presence of these toxins in the serum of patients clinically diagnosed with chronic fatigue syndrome.

Screen fish in the marketplace/commercial channels: Because of the etiology of toxin accumulation in fish and shellfish, screening in the marketplace is necessary. Our efforts in this area focus on evaluating the kit for on-shore testing and commercial retail outlets for fish products originating from Hawaii, Puerto Rico, U.S. Virgin Islands and Saint Barthélemy (French Territory).

Laboratories or individuals who wish to participate in the clinical diagnosis or interlaboratory method validation studies should contact:

Dr. Douglas L. Park, Department of Nutrition and Food Science, 309B Shantz Bldg., University of Arizona, Tucson, AZ 85721, USA; tel: (1-602) 621 5107; fax: (1-602) 621 9446; or

Catherine H. Goldsmith, HawaiiChemtect International, P. O. Box 92015, Pasadena, CA 91109, USA; tel: (1-818) 568 8606; fax: (1-818) 795 6032.

Future events

MAY 95

19th International Symposium on Column Liquid Chromatography (HPLC'95), 28 May – 2 June, Innsbruck, Austria. Call for papers. Contact: Prof. Dr. W. Lindner, Institute of Pharmaceutical Chemistry, Karl-Franzens-University of Graz, A-8010 Graz, Austria; fax: (43-316) 384 6424 or 384 092?

JUNE 95

1995 Gordon Conference on Mycotoxins and Phycotoxins – *'Frontiers in Science'*, 25-30 June, New Plymouth, USA. *Contact*: Daniel G. Baden (Conference Chair), NIEHS MFBS Center, University of Miami, RSMAS, 4600 Rickenbacker Causeway, Miami, FL 33149, USA; tel: (1-305) 361 4738; fax: (1-305) 361 4001; or Wanda M. Haschek-Hock (Vice Chair and Poster Sessions), Dept. of Veterinary Pathobiology, University of Illinois, 2001 S. Lincoln Avenue, Urbana, IL 61801, USA; tel: (1-217) 333 4755.

JULY 95

7th International Conference on Toxic Marine Phytoplankton, 12-16 July, Sendai, Japan. *Contact*: Prof. T. Yasumoto, Conference Convener, 7th International Conference on Toxic Marine Phytoplankton, Dept. of Applied Biological Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiya, Aoba-ku, Sendai 981, Japan; tel: (81-22) 272 4321; fax: (81-22) 275 3606 or 272 1870.

SEPTEMBER/OCTOBER 95

Advanced Phytoplankton Course, 25 September - 14 October, Ischia (Naples), Italy. Organized by the Stazione Zoologica 'Anton Dohrn', Naples, Italy, in conjunction with faculty members of the universities of Oslo (Norway), Copenhagen (Denmark), and Banyuls-sur-mer (France), and the Florida Department of Natural Resources (USA); supported by the European Union MAST Programme and sponsored by IOC-UNESCO, ONR and ZEISS.

Participation is limited to 20 candidates with a Ph.D., M.Sc., or B.Sc. degree or equivalent, and experience in phytoplankton species identification by microscopy. The programme will consist of training in identification of marine planktonic algae, with emphasis on the use of identification literature and light microscopy.

Deadline for submission of applications: **28 February 1995**. *Contact*: Donato Marino, Marine Botany Laboratory, Stazione Zoologica 'A. Dohrn', Villa Comunale, I-80121 Naples, Italy; fax: (39-81) 764 1355.

HARMFUL ALGAE NEWS

This issue was compiled and edited by Tim Wyatt and Yolanda Pazos, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Cientificas, Eduardo Cabello 6, 36208 Vigo, Spain. Tel.: (34-86) 23 19 30 / 23 19 73; fax: (34 86) 29 27 62

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 10/11

Shellfish biotoxins and algal blooms recur in New Zealand during 1994

Since the first closures due to biotoxin contamination in the north of New Zealand in early 1993, there has never been a period when the entire coast of the country has been open for shellfish harvesting. The summer and autumn of 1994 saw a repeat of the widespread closures of 1993, however this time most of these were in the South Island and, unlike the 1993 incident, no human illness occurred.

The monitoring programme carried out by the New Zealand Marine Biotoxin Management Board has proved to be effective in detecting biotoxin contamination incidents. Currently the programme involves the analysis of shellfish for water (PSP and domoic acid) and lipid (NSP and DSP) soluble biotoxins from an average of 120 sites every week. It has prevented suspect products appearing on the market and has proved valuable in directing research efforts.

Paralytic shellfish poisoning (PSP)

1994 began with a positive mouse bioassay test for PSP being returned by a sample collected from a mussel farm in the outer Marlborough Sounds region.

This was not the first time that PSP had been detected in cultivated mussels in the Sounds (there was an earlier minor incident in November 1993), but it was the first significant incident close to the centre of the major mussel production region. For a period the mussel industry held its breath as an area of the outer Sounds was closed and it was feared a more extensive closure might follow. Fortunately this was not necessary.

The initial examination of plankton samples revealed that moderate numbers (5.5 x10^3 cells / litre) of *Alexandrium minutum* were present at the shellfish sampling site. More extensive sampling showed cell numbers up to 1.3 x10^4 cells / litre existed in nearby waters, though samples from a number of sites soon established that this organism was only present in outer Pelorus Sound. Overall the cell numbers were low and *A. minutum* was not at any time the dominant phytoplankton species.

When the first positive bioassay results became known to the industry there was a voluntary closure of the bay and surrounding areas, although the levels of toxin in the mussels were still, at that stage, below the regulatory closure level (>80 µg / 100g). As further shellfish testing was carried out in adjacent areas, it was found that there was a good correlation between the results of the plankton analyses and the toxicity bioassays.

Because of this scrutiny it was obvious at all stages how extensive the problem was and how the situation was changing over time. This allowed the industry to continue business as normal throughout most of the Marlborough Sounds.

In retrospect it was obvious that had the plankton samples from this area been examined as a matter of routine, it would have been possible to predict the appearance of the toxicity

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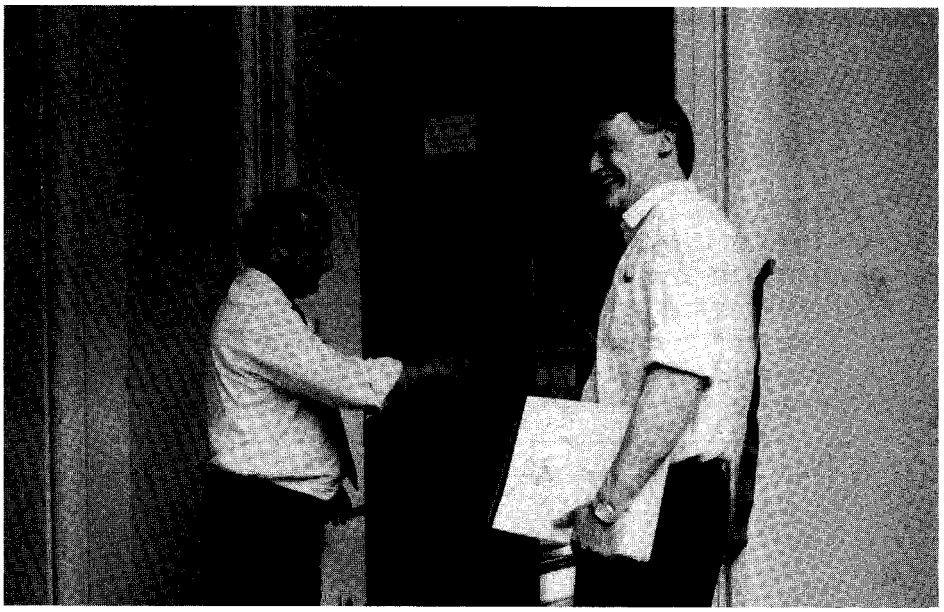
IOC opens science centres on harmful algae *(see page 14)*



The IOC Science and Communication Centre on Harmful Algae in Copenhagen is opened by the IOC Executive Secretary, Dr. G. Kullenberg, and Professor Ø. Moestrup, University of Copenhagen.

Potentially toxic algae along the Dutch coast in 1994

In 1994 high concentrations of algae were found in water samples off the Dutch Coast, on the Noordwijk transect. The seasonal sequence started with a bloom of *Phaeocystis* sp. in April. In June we observed *Pseudo-nitzchia pungens* forma *pungens* (Grunow in Cleve & Müller) Hasle with 380.10^3 cells/l. In July a *Dino-physis acuminata* bloom of long duration with maximum concentrations of 8.10^3 cell/l started. In the coloured water masses during August 1994, the Raphidophycean species *Heterosigma akashiwo* was found, a new species for Dutch coastal waters. The water column was slightly stratified (see table below).

In an extra sample, taken from Station 1 (from the pink water), large numbers of small species, like *Kato-dinium rotundatum*, solitary *Chaeto-ceros* cells and flagellates were observed. In this last group we identified the Raphidophycean *Heterosigma aka-shiwo* with the following details: two flagellae, width = 20 µm, length = 15 µm (with some size variation), numerous yellow/brown chromato-phores, more or less flat in side view. The characteristic rotating movement was recorded on video. The total numbers per litre were 2.4×10^6. This species is known to be toxic to fish[1,2], but no dead fish were observed.

The video tapes with *Heterosigma akashiwo* from the Dutch Coast were shown at a phytoplankton workshop held in Büsum, Germany, 2-3 March 1995. At that meeting, Dr. Malte Elbrächter from the List Biological Station showed cultures of *Hetero-sigma akashiwo*. Good agreement was found between the cultured material and the video taped field material. The material from Dr. Elbrächter as well as the material from the Dutch Coast could not be preserved with Lugol or Formalin: after preservation no identification could be made; the cells changed into a cluster of small unrecognizable particles.

Heterosigma akashiwo was reported by Dr. Elbrächter on 26 August and 15 December 1994, at sampling station Weststrand off List and Wadden Sea 1, respectively, in the German Waddensee.

1) Fukuyo, Y. *et al.* (eds.), 1991. *Red tide organisms in Japan. An illustrated taxonomic guide.* Uchida Rokakuho, Tokyo, 407 pp.
2) Larsen, J. and Moestrup, Ø., 1989. *Guide to toxic and potentially toxic marine algae.* Fish Inspection Service, Ministry of Fisheries, Copenhagen, 61 pp.

Marion Rademaker, Louise Janmaat, TRIPOS BV, Applied Marine Biological Research, Postbus 95125, 1090 HC Amsterdam, The Netherlands;

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Environmental data on 4 August 1994

St.	Sample	Oxygen	Salinity	Fluorimeter	Temperature
		mg/l	??? S	rel. units	°C
1	surface	10.43	26.24	3.62	21.81
1	5 m	6.83	29.92	1.64	20.53
1	10 m	6.91	31.05	1.28	19.96
2	surface	17.23	27.05	4.26	24.5
2	5 m	7.08	29.95	1.46	20.5
2	10 m	6.73	30.73	1.3	20.07
3	surface	8.27	29.35	2.93	21.75
3	5 m	7.07	29.98	1.32	20.41
3	10 m	7.24	30.37	1.34	20.21

Sampling area: Station 1 52°14'05"N, 4°17'09"E Water colour red-brown/pink
Station 2 52°13'26"N, 4°17'58"E Water colour red-brown/yellow
Station 3 52°11'22"N, 4°17'20"E Water colour red-brown

Cell numbers of some species per litre:	Station 1	Station 2
Ceratium fusus	$0.5.10^6$	13.10^6
Mesodinium rubrum	13.10^3	93.10^3
Dinophysis acuminata	26.10^3	15.10^3
Prorocentrum micans	17.10^3	84.10^3
Gonyaulax spinifera	5.10^3	not counted

Satellite imagery

When multispectral data provided by satellite sensors first became generally available in the late 1970's, various attempts were made to detect and follow algal blooms, or at least chlorophyll, in coastal waters. Not all were successful. A serious brake on some of these studies was due to the fact that the optical characteristics of coastal and especially estuarine waters are usually very distinct from those of oceanic waters, and it was for the latter that the computational algorithms with which the sensor data was interpreted had been developed.

But there have been some successes. Dundas *et al*[1] used the long-wave bands from a NOAA satellite to follow the advection of the 1988 *Chrysochromulina* bloom in Scandinavian waters, and Ligi *et al*[2] of Telespazio, Rome, are using SPOT-HRV and LANDSAT-TM (Thematic Mapper) channels to establish the distributions of gelatinous aggregations in the northern Adriatic. By combining these images with the long wavebands of the NOAA-AVHRR (Advanced Very High Resolution Radiometer), from which surface flow fields can be derived with a resolution of about 0.1°C, they have shown that the aggregations and filaments of gel are clearly related to local gyres and thermohaline fronts. Some of these features are of the order of 100 km in extent.

This group now provides periodic bulletins to authorities in the region as to where along the coast problems associated with the stranding of these gels can be anticipated. The spatial resolution of this system is about 10 km. These are just two examples which indicate how satellite data may afford short-term forecasts of use to coastal zone managers.

Trichodesmium blooms have also been monitored by means of AVHRR signals, for example by Hakansson and Moberg[3] in the Baltic. Subramanian and Carpenter[4] took advantage of the high reflectivity of *Trichodesmium* which results from its contained gas bubbles and the strong absorption at 550 nm due to its phycoerythrin, and showed that the species can be successfully detected even when mixed with other phytoplankton. They used NIMBUS-CZCS data.

(Cont'd on p. 3)

Domoic acid in Hood Canal, Washington State, USA

Domoic acid (DA) first occurred in intertidal razor clams on the Oregon/Washington coast and in the viscera of Dungeness crabs in late October 1991. Other bivalves, including commercially grown oysters and mussels were tested, and did not contain the toxin. The source of the DA in this incident in Washington was never identified, but *Pseudo-nitzschia australis*, *P. pungens* f. *multiseries*, and *P. pseudodelicatissima*, all known DA producers, have been found in local waters[1,2].

In October 1994, the Office of Shellfish Programs of the Washington Department of Health (WDH), in conjunction with the US Food and Drug Administration, started a weekly phytoplankton monitoring programme at 20 sites in coastal and inland waters of the state. Net tows are collected by WDH personnel at sites where mussel cages (used as sentinel organisms for PSP and DA determinations) are located, and by local shellfish growers, county health departments, and native tribes. The samples are analyzed for species presence and relative abundance.

The first samples collected from Hoodsport, southern Hood Canal on 8 November 1994, contained a bloom of *Pseudo-nitzschia pungens*. This species remained dominant in samples collected weekly until 15 December. On 29 November, mussels from a cage at Belfair contained about 10 µg/g DA. No phytoplankton samples were collected there before that date, but phytoplankton collected on the 29th contained about 14 µg/g wet weight toxin. Both *P. pungens* f. *pungens* and *P. pungens* f. *multiseries* were present in acid-cleaned samples examined by SEM.

Washington Department of Ecology (WDE) personnel sampled at a number of sites in Hood Canal on 1 December 1994 as part of their Puget Sound Ambient Monitoring Program. Data from some of the stations, including phytoplankton counts, are given in the adjacent table. The last few days of November were rainy with about 3.5 cm of rain falling on 30 November. Runoff was high and the water appeared brown from sediment.

Other species, including *Skeletonema costatum* and *Asterionellopsis glacialis*, increased in early December, and by early January 1995, *S. costatum* and *Thalassiosira* spp. (some forming auxospores) became dominant. The *S. costatum* bloom continued at least into mid-March (when this was written). Few *Pseudo-nitzschia* cells were present in January and February.

L. Hanson (WDH) collected weekly phytoplankton samples; L. Eisner and S. Albertson (WDE) collected the 1 December samples; C. Hatgied (National Marine Fisheries Service, Seattle) analyzed the phytoplankton samples for DA; the WDH laboratory, Seattle, analyzed the mussel samples; and R. Horner did the phytoplankton enumeration and SEM identification.

Station	Depth (m)	Secchi (m)	T (°C)	Salinity (PSU)	Chla (µg/L)	Pp* (x 10^3 cells/liter)	Sc** (x 10^3 cells/liter)	Ag*** (x 10^3 cells/liter)
10	2	2.6	10.4	28.76	0.68	32	12	6
13	1	0.9	9.2	24.38	12.14	539	363	329
14	0.5	1.0	8.7	19.56	11.48	442	520	196
	1.5		9.1	22.94	18.52	449	917	301
	2.5		9.5	26.91	16.72	573	1120	392
	25		11.1	30.73	1.38	47	16	1
16	10		10.9	30.36	5.01	394	7	54

* *Pseudo-nitzschia pungens* (incl. both f. *pungens* and f. *multiseries*)
** *Skeletonema costatum*
*** *Asterionellopsis glacialis*

(1) Horner, R. A. and Postel, J. R., 1993. Toxic diatoms in western Washington waters (U. S. west coast). *Hydrobiologia*, 269/270: 197-205.
(2) R. Horner, unpubl. obs.

Rita Horner, University of Washington, School of Oceanography, Box 359740, Seattle, WA 98195-9740, USA.

(Cont'd from p. 2, "Satellite imagery")

PC-based satellite receiving systems now provide access to AVHRR data in real time at reasonable cost. Gower[5] has now shown how the red (580-700 nm) and near infrared (720-1000 nm) wavebands of this sensor can be used to monitor at least some of the intense algal blooms of concern to the aquaculture industry in British Columbian waters. Blooms potentially detectable by this approach are those which cause increased backscatter, and the algorithm used is designed to reduce the effects of atmospheric haze and sunglint which interfere with their recognition. A good discussion of some of the technical problems associated with the interpretation of satellite images can be found in Brown and Yoder[6]. In coastal waters, increased backscatter is also due to suspended sediments delivered to near-surface waters by runoff or resuspension of bottom sediments by winds and tides. So the application of Gower's procedures to regions where these processes are very active may lead to more ambiguous results, and considerable intuition will be required to tune the algorithms for other locations. But in the relatively clear waters of British Columbia this approach is now a routine component of the red tide monitoring programme. The spatial resolution is 1.1 km at nadir and 3 to 5 km at the edges of the swath scanned by the satellite.

1) Dundas, L. *et al.*, 1989. *Oceanography*, 2: 9-14.
2) Ligi, R. *et al.*, 1992. pp. 719-722 in: "International Space Year" Conference, Munich, Germany, 30th March - 4th April, 1992.
3) Hakansson, B. G. and Moberg, M., 1994. *Int. J. Remote Sensing*, 15: 963-965.
4) Subramanian, A. and Carpenter, E. J., 1994. *Int. J. Remote Sensing*, 15: 1559-1569.
5) Gower, J. F. R., 1994. *Remote Sens. Envir.*, 48: 309-318.
6) Brown, C. W. and Yoder, J. A., 1994. *J. Geophys. Res.*, 99: 7469-7482.

Tim Wyatt

In the Philippines ...

Benthic dinoflagellates of the brown seaweed *Sargassum* spp

Sixteen dinoflagellate species were identified associated with various *Sargassum* species collected around the island of Cebu in the Central Philippines from August 1994 to January 1995. These dinoflagellates comprise nine genera in five families and three orders (all identifications are still tentative and are being sent for confirmation). The ciguatera causing *Prorocentrum* occurred most frequently, and other DSP-causing species were also found, namely: *Prorocentrum concavum*, *P. lima*, *P. minimum*, *P. rhathymum* and *Ostreopsis lenticularis*. Environmental factors (water temperature, salinity) and morphological characteristics of the *Sargassum* (age of thalli, fertility) did not seem to affect the population density of dinoflagellates.

Such baseline studies are important now to establish the occurrence of potentionally toxic species, especially since no toxin poisoning cases have so far been reported in these islands. Further health research should also be conducted to establish if these dinoflagellates are really not causing problems now. There is some anecdotal evidence of certain species (pufferfish, small tuna, sea hare, shellfish) having seasonal toxicity. Clearly, many studies still need to be done, here and in similar tropical areas. Research is slow due to lack of funds, good microscopes and literature. Collaboration is much needed in these areas.

Training on plankton and water quality methods for aquaculturists

The Marine Biology Section of the University of San Carlos held a training course on "Plankton Identification and Water Quality Methods for Aquaculture Ponds", 21-25 February 1995. The purpose was to help the aquaculture industry face up to mass mortalities of prawns, which occur in their ponds, and which are so far unexplained.

This training aims to provide them with the basic technical expertise to identify the microscopic plants and animals in their culture ponds, and to be able to assess the environmental conditions. Harmful algal species are presently the prime suspects responsible for prawn deaths, and there is thus an obvious need to obtain baseline data now before the problem affects our dollar-earning aquaculture industry. From this training, we hope to come up with appropriate management options and a comprehensive manual.

Problems expressed by the participants, who come from Cebu and Negros, are that they lack the technical and biological expertise for such work. Also, they do not have all the equipment needed. Moreover, the government agencies and consulting companies are not always up to date, and are expensive. Hence, most of the aquaculturists tend to have their own individual laboratories, and work on common problems facing the industry is poorly coordinated. This training provided a step forward towards the implementation of more standardized methods.

Perpetua Oloroso, Filipina Sotto, Jason Young, Marine Biology Section, University of San Carlos, Cebu City 6000, The Philippines.

New PSP occurrence

(Compiled from news reports from the Manila Bulletin and Daily Inquirer)

The first sign of trouble came late April 1995 when a red tide alert was raised by the Bureau of Fisheries and Aquatic Resources (BFAR), extending from the north of the archipelago (Manila, Bataan, Pangasinan, ...) to the South (Samar, Tacloban, Camiguin, Zamboanga, ...). No description of the bloom nor the causative organism, however, was included. On 18 May, the government's Red Tide Task Force ordered a ban on the harvesting, selling and eating of shellfish from the entire Manila Bay area, when a 4-year old girl died and 31 others fell ill after eating the "tahong" (mussel). In a report to the Health Secretary, the Task Force recorded patients, mostly fishermen, complaining of numbness in the mouth, lips and extremities five hours after eating mussels. The Task Force had banned earlier the harvesting and selling of shellfish. Authorities immediately released results that the "red tide poison had exceeded the allowable level of 40 micrograms per 100 gram of shellfish".

This news only merited space on page 12 of the newspaper. Radio announcers carried the warning in their daily news, and this was the best form of communication to reach the poor shellfish gatherers and consumers.

May 19 - Mussels and other shellfish were impounded by the police in markets around Manila when vendors continued to sell their harvest. Three other children, ages 2 to 12 years, are reported to die from shellfish poisoning. Ninety-three people are also admitted to hospitals due to food poisoning.

May 20 - Health authorities ask local government officials to ensure compliance of the ban. Police said those found selling shellfish will be arrested. Senator criticizes the Department of Agriculture and the Department of Health in the delay of the issuance of public warnings. No report of the causative organism and extent of the bloom is still reported.

In the Visayas region, central Philippines, the Department of Health and the Department of Agriculture quickly issued warnings to consumers to refrain from eating mussels harvested in the eastern provinces of Catbalogan and Camiguin where "red tide cells have been found in shellfish". Mussels from other areas in the region are safe.

May 21 - President Fidel Ramos declares a state of calamity in 13 coastal areas in the Manila area and neighbouring provinces. In this case, the National Disaster Coordinating Council headed by the Secretary of Defense is enabled to "implement measures to cushion the effects of the red tide phenomena on the livelihood

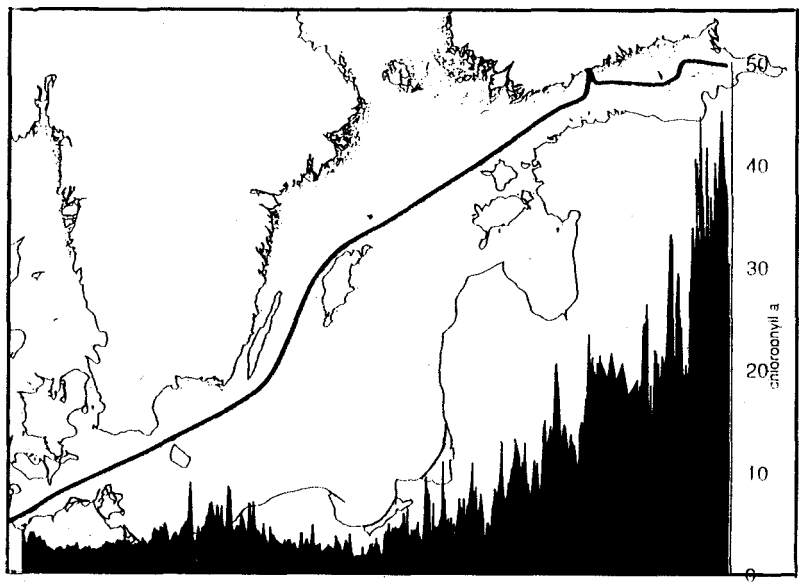
(Cont'd on p. 5)

Baltic algaline in internet

In 1992 the Finnish Institute of Marine Research started unattended measurements of phytoplankton and related parameters on merchant ships in the Baltic Sea[1,2]. The data consist of high-frequency recordings of chlorophyll *a* fluorescence, temperature, and salinity in the surface water as well as phytoplankton species composition and nutrient concentration analysis in discrete water samples. In 1993 the system was developed to serve as an early warning method for exceptional algal blooms. Based on recordings on several ferries, it has been possible to deliver almost on-line information on the development of algal blooms. In 1994 the weekly Baltic Algafax was delivered to about 40 environmental authorities around the Baltic Sea.

In 1995 the results from unattended measurements of phytoplankton on ferries are available on the Internet World Wide Web (WWW) page with URL of http://www.fimr.fi. The e-mail address of the project is <algaline@fimr.fi>. In July-September, satellite images processed by the Finnish Meteorological Institute, showing the extent of the blue-green surface accumulations, will be included too. Local information will be added on the page according to the information delivered to the project by various research institutes around the Baltic Sea. The data can be sent by e-mail or using FTP. The algaline team (Seija Hällfors, Juha-Markku Leppänen, Eija Rantajärvi) is ready to help everyone in using the Internet connection and hopes to get information in order to improve the WWW page.

In 1993, twenty-two potentially toxic phytoplankton taxa were detected in the Baltic Sea, but only the blue-green algae *Nodularia spumigena* formed extensive blooms[3]. In 1994 the situation was similar to the previous year.



Chlorophyll a concentration (mg/m³) as measured on ferry Finnjet and research vessel Aranda on 5 May in the Baltic Sea.

(1) Leppänen, J.-M. *et al.*, 1994. Unattended algal monitoring system – A high resolution method for detection of phytoplankton blooms in the Baltic Sea. In: *OCEANS-94 Proceedings*, Vol. 1, pp. 461-463. IEEE, New York.
(2) Rantajärvi, E. and Leppänen, J.-M., 1994. Unattended algal monitoring on merchant ships in the Baltic Sea. *TemaNord*, 546: 1-60.
(3) Leppänen, J.-M. *et al.*, 1995. Unattended monitoring of potentially toxic phytoplankton species in the Baltic Sea in 1993. *J. Plankton Res.*, 17(4): 891-902.

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(Cont'd from p. 4, "New PSP occurrence")

of shellfish gatherers and growers". About 20,000 fishermen are estimated to be directly affected. The NDCC Chief also added that the "spread of red tide toxins in Manila Bay was triggered by the recent rains".

May 22 - Las Pinas, Metro Manila, the Mayor "immediately responded to the urgency of the problem, most especially to the family of a red tide fatality and promised burial assistance and food aid. The Mayor also made inspection rounds in the market". In Bataan, south of the Luzon region, the chairman of the provincial Task Force points out that the "first step they will undertake is to look for alternative sources of income for the farmers dislocated by the red tide".

May 26 - The red tide continues to "wreak havoc in Metro Manila and other neighboring areas". In Bulacan, south of Manila, considered the largest producer of shellfish in Luzon, the ban is strictly observed. Health authorities order its personnel to "take shellfish samples to determine if they are contaminated with red tide toxin".

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Proceedings of the Sixth International Conference on Toxic Marine Phytoplankton

One hundred copies of the above mentioned proceedings, 1993, are available free of charge to libraries of marine science institutions in developing countries from the IOC Science and Communication Centre on Harmful Algae in Copenhagen. Please send your request to:

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Workshop on the Physiological Ecology of Harmful Algal Blooms

Plans are underway to convene an Advanced Technical Workshop on the Physiological Ecology of Harmful Algal Blooms. This is being organized by a working group co-sponsored by the Scientific Committee for Oceanic Research (SCOR) and the IOC. This working group was asked to assess the state of knowledge on the physiological ecology of harmful algae, and to identify areas for future research. At a working group meeting held in October 1993, the working group decided that the best way to accomplish these goals was to convene an advanced workshop.

A proposal is now being submitted to NATO to provide support for this meeting as a NATO Advanced Study Institute (ASI). If support is received from NATO (with supplementary funds from SCOR and the IOC), the meeting will follow the ASI format which requires a 10-day meeting with lectures, field and laboratory demonstrations, and group discussions.

The working group decided that the goal of the workshop would be to summarize major advances in two main areas: (1) The autoecology of critical groups of toxic phytoplankton, such as *Alexandrium*, *Gymnodinium*, *Heterosigma*, *Pyrodinium*, *Gambierdiscus*, and *Pseudo-nitzschia*, and (2) the ecophysiological processes and mechanisms that affect toxic bloom formation and the production of phycotoxins. The focus on "toxic" rather than "harmful" algae is deliberate. Also, given the complicated array of trophic processes involving toxic algae, it is important to narrow the focus even further by excluding such issues as toxicology, toxin biosynthesis, taxonomy, shellfish toxin uptake and depuration, management, epidemiology, ballast water transport, and physical or hydrodynamic forcings. It was agreed that a variety of techniques should be presented (e.g. measurement of *in situ* growth rates, use of molecular probes), but that physiological or ecological questions, rather than techniques, should be the driving force behind all activities. It is hoped that one outcome of this approach will be the elucidation of common factors that underly blooms of related species as well as of toxic species in general.

The purpose of this notice is to alert the HAB community that this workshop is tentatively scheduled for 27 May-6 June 1996, at the Bermuda Biological Station for Research (BBSR).

The number of participants at the workshop will be restricted as well. If NATO funding is obtained, a maximum of 80-100 participants is allowed. The organizers are targeting 80 participants, based on the housing available at the BBSR. Some travel assistance will be available, but selection of participants will in part be determined by the degree to which each individual can provide his or her own travel funding. Since it is likely that there will be more than 80 individuals interested in participating, a steering committee will select participants on the basis of the expertise that applicants can bring to the workshop, country of origin, willingness to help with demonstrations or lectures, relevance to the physiological ecology theme, professional status and funding support.

Note that this meeting is still only "tentative", as funding is not yet secured. Nevertheless, it is important to place this date on your calendars and begin thinking not only of travel support, but also about topics you might be able to offer to the workshop participants. Demonstrations of new technologies or approaches are of interest. Further details of this meeting will be announced in this newsletter at a later date, as well as through advertisements in major scientific publications, notices on e-mail bulletin boards, etc. If you have ideas, comments, or questions, please address these to the chairman of the working group: Don Anderson, Biology Department, Redfield 3-32, Woods Hole Oceanographic Institution, Woods Hole, MA 02543-1049, USA; e-mail: danderson@whoi.edu.

Ireland: Extended "toxic season" in 1994

The Fisheries Research Centre of the Department of the Marine has in place an ongoing monitoring programme for the detection of DSP toxins in shellfish in Ireland. Since monitoring began in 1984 there have been regular closures of shellfish growing areas, particularly those situated in the Southwest of the country, due to the presence of DSP toxins in both mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*). In general, the period during which closures have been in force (the "toxic season") has been the summer months of June-September, and the growers and processors have adapted their marketing strategy to take account of these events. During 1994, however, the presence of toxins was detected for a period extending well beyond the traditional "toxic season". In the neighbouring Roaring Water, Dunmanus, Bantry and Kenmare Bays, on the southwest coast of Ireland, toxins were first detected by standard rat bioassay in mid-May/early June and positive bioassay results were obtained in weekly samples from these bays until February 1995. Chemical analysis of mussel extracts by LC-MS methods confirmed the presence of DSP toxins in the samples, and showed that both okadaic acid (OA) and DTX-2 were dominant, reaching a peak of 12 µg/g of hepatopancreas in mid-August. While OA concentrations decreased to <0.1 µg/g by late September, the concentration of DTX-2 was an order of magnitude higher, and remained at concentrations > 2 µg/g throughout the winter. The high pulse of DTX-2 recorded in August was associated with a pulse of *Dinophysis acuta* of up to 20,000 cells/litre recorded in inner Bantry Bay at the same time. While both *Dinophysis acuta* and *Dinophysis acuminata* were recorded in the water column during the June-September period, no toxic species were recorded after this time, and the reason for the persistence of DTX-2 in the mussels is still unclear. It is clear, however, that a monitoring programme involving the surveillance of toxic or potentially toxic species alone is insufficient to prevent shellfish with high concentrations of toxins being placed on the market, and that bioassay and confirmation by chemical methods is essential.

Terry McMahon, Eugene Nixon and Joe Silke, Fisheries Research Centre Abbotstown, Dublin 15, Ireland.

Australia: Cyanobacterial overgrowth and toxicity in inland waters before European settlement

Cyanobacterial overgrowth in inland Australia waters has recently received attention after the development of massive blooms of toxic organisms in the Darling River basin[1,2]. These overgrowths have been attributed to increased nutrients coming from town effluents, runoff from fertilized pastures and cattle feedlot waste, together with low river flow rates and water diversion. However, this is not a recent phenomenon, for, as described by Johnstone, toxicity at the mouth of the same river system was reported as long ago as 1878[3].

There is evidence in Australia that cyanobacterial overgrowth and toxicity occurred in inland waterways well before the advent of closer settlement. Australia is dominated by old erodible soils, with prolonged dry periods broken by torrential rainfall resulting in significant transport of nutrients into aquatic systems even without the intervention of man. Where there is warmth, high evaporation with solute concentration, shelter from wind and relative stagnation, natural waters may support cyanobacterial blooms even without the further addition of waste materials.

A disease known as "Barcoo Fever" or "Barcoo Sprue" was once widespread in outback Australia and has been described by many early authors. Characteristic symptoms were nausea and vomiting, exacerbated by eating and often even by the sight or smell of food, fever, headache, initial constipation often followed by diarrhea, and variable myalgia. The disease may range in severity, all ages may be affected, and recurrent episodes were common. It does not have the features of an infectious disease and it is not due to either sun exposure or vitamin deficiency, as has been variously proposed. This disease, once very prevalent, has now disappeared from medical literature and is no longer known as such in outback Australia. However it does still exist and many habitual travellers in outback Australia, on questioning, can recall an episode of illness very similar to the old descriptions. There is good evidence that the disease is due to cyanobacterial toxicity; such an explanation would explain its original incidence and now virtual evanescence, recurrence in the one individual and marked variation in severity[4].

The early explorers found cyanobacterial and algal blooms, suffered from their toxicity, and some died from them. Capt. Charles Sturt in 1844 was the first European to discover and name Coopers's Creek, the stream in central Australia which receives the waters of the Barcoo River. He found "splendid sheets of water, some 200 yards from side to side with banks which rose up some 18 feet." The water in the stream however was vivid green. Returning to his base camp at Fort Grey, Sturt found the camp deserted, with a message form his lieutenant Harris-Browne telling him that he had been obliged to retreat to another waterhole 100 km away. The water at Fort Grey had become putrid and was causing his men dysentery. The problem was all too evident; when they examined the little pool, they found it covered with slime that was green on top and red below.

In the disastrous Burke and Wills expedition of 1960-61, death occurred not only of the leaders of the expedition but also among members of the rear party during their futile attempt to reach the base camp on Cooper Creek. Herman Beckel, the German doctor with this party described their difficulties[5]: "Mr. Wright had miscalculated this time; the waterhole which lay about a mile from here was supposed to provide us with water for four to five days, but the horses drank it all at once so that nothing remained but a layer of green slime about two inches deep. Nonetheless, I was very glad to wet my lips with it when I came to it the following day with the camels. ... The ground near this water was covered with stalactite-like stones of a strangely rounded form, coated as with varnish – possibly sintered lime or silica". (These appearances are seen when drying has occurred of water containing dense concentrations of algae or cyanobacteria.)

... "Late in the evening of 8 March, Hodgkinson returned. He had found Becker and the cook very wretched. For two days they had no drinkable water, which was not, however, our fault as the water we had taken to the camp could easily have lasted five days.

... Unfortunately Becker and the cook had become visibly more ill since I had last seen them, although the former was still in good spirits. Wright now wished to travel to Bulla with the whole party as soon as possible". It was at Bulla that the two members of the party died.

Their symptoms were those of lethargy and inanition, symptoms which may well have been due to the forced ingestion of water containing toxic microorganisms. These two were the first to become ill and it was these two who were exposed to contaminated water. Although vitamin deficiency and simple starvation may have contributed to the deaths of other members of the party, there is no reason why the members of the rear party should have been so affected as they had adequate food supplies, both for themselves and for the forward party. It would seem very likely that toxic algae caused the death of these two persons and at least contributed to the deaths of others.

Aboriginal Australians, whose forebears have lived on the Australian continent for at least 40,000 years were well aware of the dangers of contaminated water. In many areas they would dig soaks in sand beside streams and draw water from these rather than from the main stream. Experienced settlers followed their example and survived where others before them had perished.

In summary, although regarded as a recent development, blue-green algae or cyanobacterial contamination of inland Australian waters has occurred for many years if not millennia. Organisms which have evolved thousands of millions of years before man, do not require man for their propagation.

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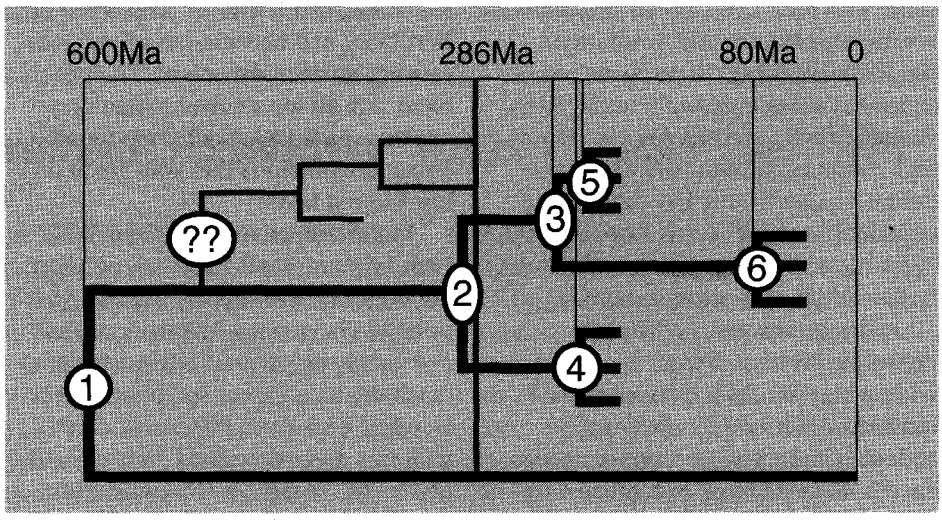
Did the majority of dinoflagellates perish in the great Permian extinction?

The dinoflagellates (*Phylum pyrrophyta*) constitute a very diverse group of protists, ranging from potent toxin producers to those responsible for the formation of red tides. The most remarkable feature of dinoflagellates is the unusual nature of their nucleus, exceptional amongst the Eukaryotes and which indicates their primitive origin (Loeblich, 1976). The dinoflagellate nucleus combines various ancestral characters representative of the Prokaryotes, and several typically eukaryotic features with other properties unique to dinoflagellate nuclei alone (reviewed by Herzog *et al.*, 1984). Consequently, dinoflagellates were widely studied at the cell biology level, and new and controversial phylogenetic interpretations were proposed, suggesting, among others, the positioning of this group among the lower Eukaryotes (Taylor, 1980), or alternatively its consideration as a sister group of the actual Eukaryotes (Herzog *et al.*, 1984).

Dinoflagellates were previously perceived to be Mesokaryotes, phylogenetic intermediaries between Prokaryotes and Eukaryotes (Dodge, 1965). However, with the onset of molecular phylogenic criteria it was demonstrated that the dinoflagellates were far from primitive, being neither eukaryotic nor afore-stated phylogenic intermediates. What was actually being considered were organisms distantly linked to ciliates and yeasts (Lenaers *et al.*, 1989). Without doubt, from a molecular point of view, the Giardias, the Microsporidios, the Kinetoplasts, the slime-moulds, the Entamoebas, the Plasmodias, and also the Euglenas had separated from the main trunk of the Eukaryotic family tree before the dinoflagellates (Knoll, 1992). The fossil record (limited to cyst-forming species) suggests an evolutionary course beginning as a primitive Gymnodinioid-like dinoflagellate. Further species evolved by plate reduction, at first originating into Peridinioid- and Gonyaulacoid-like and then later into Prorocentroid-like dinoflagellates (Eaton, 1980; Loeblich, 1984). On the other hand biological considerations support a plate increase model, where Prorocentroids represent the most primitive condition and Peridinioids, Gonyaulacoids and later Gymnodinioids evolved by a progressive increase in the number of thecal plates (Loeblich, 1976; Taylor, 1980). A further model implicates thecal plate fragmentation as the evolutive process from Prorocentroid to Gymnodinioid and plate reduction for the further development to Peridinioid-Gonyaulacoid (Bujak and Williams, 1981). Recently, the primary molecular phylogenies have shown what was suspected, that it is not possible to establish a clear phylogeny for the principle groups. In fact, Prorocentroid, Gymnodinioid and Peridinioid species all appeared intermixed (Lenaers *et al.*, 1991). A previous study indicates the Syndinophyceae (parasitic dinoflagellates) radiated much later than the free-living Dinophyceae (McNally *et al.*, 1994).

In an attempt to clarify the perplexing phylogeny of the major dinoflagellate groups, we sequenced the regions 1, 2, 9 and 10 of the LsurRNA gene, in a sense a pseudogene of the same subunit. These sequences are to be found deposited in the GenBank with the access numbers L38623 to L38640 and will soon be published (Zardoya *et al.*, in press). Using these sequences we can convert the genetic distance into a temporal distance assuming molecular change as a clock at constant velocity, which one finds to be well established for LsurRNA genes. The clock can be calibrated by means of well defined reference points in the fossil record. The figure below summarizes the results found using the average values cited by Knoll (1992) as source material to time scale the molecular change, and distance matrix methods to analyse the sequences of the LsurRNA gene regions D1 and D2. Apparently the dinoflagellates separated from the ciliates as little as 600 million years ago. In a conventional interpretation, the separation between thecal and naked species would have occurred approximately 300 million years ago, while the separation between desmokonts and dinokonts would have happened over 236 million years ago. The Gymnodinioids would have radiated some 220 million years ago, and the Prorocentroids 215 million years ago, compared to the relatively late radiation of *Alexandrium* species just 80 million years ago. However, an event of even higher importance should be mentioned, an event that occurred over 286 million years ago, culminating in the greatest mass extinction in the history of the planet. This catastrophe caused the extinction of over 90% of all marine species, being especially severe amongst planktonic organisms where diverse groups, prosperous of old, disappeared forever. Viewing the phylogenic reconstruction for the dinoflagellates, it could be considered that just two groups (thecal and naked) survived the great Permian extinction. Practically straight-



Major events in the evolutionary history of dinoflagellates: 1) separation between dinoflagellates and ciliates; 2) separation between thecal and naked dinoflagellates; 3) separation between desmokonts and dinokonts; 4) radiation of the gymnodiniales; 5) radiation of the prorocentroides; 6) radiation of the Alexandrium*; ??) possible early radiations of the extinct dinoflagellates before or during the Permian period.*

afterwards however, the dinoflagellates suffered a significant species radiation which coincides with that observed in the fossil record. Can it then be said that after separating from the ciliates, dinoflagellates existed for the greater part of their evolution without radiating, and then underwent a sudden diversification immediately after the great Permic extinction? We think not.

Thus according to our hypothesis, it seems reasonable to assume that dinoflagellates saw numerous radiations during their life history after separating from the ciliates. However, with the arrival of the Permian period they suffered an extinction of such grand proportions that it left a scarce few representatives of both naked and thecal dinoflagellates as the sole remnants of this evolutionary branch. The rest disappeared, falling foul of the Murphys law of the Permian period: extinction. In taking this secret – that many have tried to uncover – to their hidden graves, they have so deprived us of the possibility of verifying the evolution of the principle dinoflagellate groups forever.

How the radiation of the major dinoflagellate groups occurred seems ever more indecipherable with each passing moment. What is undeniable is that it is impossible to unravel the enigma of their evolution with only the dinoflagellates that have survived to this day. In this sense, it seems we are resigned to never knowing how the principle dinoflagellate groups radiated out from one another, although perhaps this polemic will survive some time yet. All is not woe however; one important fact can be indirectly ascertained from the reconstruction of the dinoflagellate phylogeny: the origin of PSP toxicity. The capacity to produce toxins appears to be unrelated to any particular phylogenic line. Toxic species can be seen for both Gymnodinioids and Gonyaulacoids, and toxic and non-toxic species can be found between species that are closely-related phylogenically. Paleontological data further complicates this problem by suggesting dinoflagellates caused high animal mortality various thousands of years ago, apparently by means of toxins similar in fashion to those responsible for PSP. Therefore it leads one to think that either the genetic base for toxicity was acquired from an external source (from bacteria?) or it is rooted in genes that manifest evolutional similarities to pseudogenes. Hence, an interesting issue can be proposed: the list of toxic species is not nor will it ever be limited. It is highly probable that we will be witness to the appearance of toxicity in species that are, for now, free from our suspicions.

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Economic consequences of toxic algae in shellfish markets

A wide variety of economic losses result form toxic algae blooms. Typically the calculated losses are a function of lost revenues by fishermen, lost revenues by other businesses, such as the retail, restaurant and hotel industries, and affected tourism revenues. There are also the medical costs of illness and consequent loss of wages, of monitoring the water and seafood for toxins, and the costs associated with public announcements and advice are not trivial either.

Another impact of harmful algae blooms arises when public announcements warn of the dangers of consuming shellfish harvested from affected areas. Such public announcements, typically reported by the news media, generally influence the demand for associated consumer products. Public announcements that shellfish from particular areas are toxic, and hence should not be consumed, generally result in consumer fear and avoidance of that product, and usually spread to related products. Often the public agency's announcements specify exactly which areas are problematic and are not meant to alarm the public. However, news media reports may not be that specific, and may even be inaccurate, creating the potential for false perceptions of elevated risk. Consumers may not be able to differentiate between safe and unsafe products, and hence assume that all supplies are unsafe. A possible result is a decrease in demand for both affected and unaffected shellfish and seafood products. This decrease in demand may continue for only a short time, a few days or weeks, or may persist for an extended period after the effects of the algal bloom are over.

In an on-going research project funded by the Rhode Island Sea Grant Program, the fraction of the economic losses attributable to the decrease in consumer demand for mussels in Montreal, following the 1987 domoic acid contamination of Prince Edward Island (Canada) mussels, was estimated. In particular, losses for one Maine firm, whose mussels were not contaminated by domoic acid, were estimated at 15 % of the firm's average annual sales to the Montreal market (actual sales numbers are confidential). These losses apply only to the one firm and only the Montreal market. Thus, these losses do not provide information on the full extent of economic losses to the Canadian and U. S. mussel industries due to a decrease in demand.

However, it does indicate that these economic losses may be comparable in magnitude to other more commonly quantified economic costs of toxic algae blooms. Quantifying these losses is necessary to compare the costs of toxic events with the benefits of reducing the frequency or severity of such blooms.

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Cont'd from p.1, "Shellfish biotoxins ...")

in the shellfish in advance of the results of the toxicity tests becoming available. In August 1994 another minor contamination incident occurred in a nearby area. In this case the plankton samples were examined immediately after collection and a warning of the presence of low numbers of *A. minutum* prevented the harvesting of mussels that, some days later, revealed traces of PSP toxicity.

The examination of a very large number of plankton samples from throughout the country over the last few years has revealed that *A. minutum* is widespread but rarely plentiful, and then only for brief periods. It seems likely that closures due to this species will be necessary, from time to time, but these will probably be rather small scale local events, representing little real hazard to human consumers and an irritation rather than a threat to the shellfish industry

The Southland "Lipo-tox" incident

The major biotoxin event of 1994 began in mid-January due to the detection (by mouse bioassay) of a lipid soluble toxin in dredge oysters (*Tiostrea lutaria*) from Fouveaux Strait. As a result, a large area of the Southland coast was closed and over the following months the closed area was extended up the east coast of the South Island, until eventually in late April a part of the northern Marlborough Sounds was affected. As well as contaminating filter feeding shellfish there was evidence of associated mortalities of other fauna such as seabirds and crayfish. There was also some evidence to suggest that it made cultivated mussels more susceptible to an infectious viral disease that also appeared at this time.

The mouse bioassay of acetone extracts of oysters first showed a toxic response of >39 MU / 100g on 14 January and these levels had risen to >850 MU / 100g by late February.

From the beginning it was apparent that the bioassay results were different from anything previously encountered. Very rapid death times of the mice (with an unusual symptomatology) resulted in high toxicity scores; however, when extracts were diluted to obtain quantitative estimates (i.e. MU values), the toxicity disappeared. Hence there appeared to be a dose-related threshold effect in the response of the bioassay mice.

The first plankton samples collected from Fouveaux Strait on 21 February revealed high numbers of a variety of *Gymnodinium* cells. The most abundant cell type observed in these samples was one resembling *Gymnodinium mikimotoi*. This cell is distinguished by a centrally located nucleus in the hypotheca, extreme dorso-ventral flattening, chloroplasts distributed around the periphery of the cell and an apical groove. Other morphotypes were represented by cells with a different chloroplast morphology and a few cells closely resembling *Gymnodinium breve* were observed. Cell numbers of *G.* cf. *mikimotoi* in excess of 100 thousand cells per litre were uniformly distributed through the water column down to the bottom at 30 metres. Recently a detailed examination of the morphology of this dinoflagellate and comparison with a Japanese strain of *G. mikimotoi* has shown that this is most probably an undescribed species.

The link between the toxicity in the shellfish and the dinoflagellate was suggested by the relationship between cell numbers and the results of the bioassays and was eventually proven by the establishment of the *Gymnodinium* species in laboratory culture and the bioassay of crude extracts of culture concentrates. These extracts gave a bioassay result identical in terms of symptoms, rapid death times and threshold effects to that observed in the contaminated shellfish.

Considerable effort has gone into investigating the nature of the toxin involved in this incident by several research groups and much progress has been made. Professor Yasumoto and coworkers at Tohoku University have succeeded in identifying a compound from the oysters that has been given the name "gymnodimine". These workers have also succeeded in isolating gymnodimine from the *Gymnodinium* sp. culture. As a result of rat feeding trials (Dr N. Towers, Ag-Research, pers. comm.), it has been established that the oral toxicity of this compound is very low if indeed it has any toxicity at all.

On the basis of these results, the Marine Biotoxin Management Board decided in September 1994 to change the bioassay procedure. This basically involves an additional confirmatory ether extraction procedure in the event of positive bioassays of acetone extracts of shellfish. The *Gymnodinium* sp. toxin is not apparent using this method and, by this criteria, gymnodimine is no longer regarded as a significant hazard to human health.

By early March, *Gymnodinium* cell numbers in Fouveaux Strait had fallen substantially and by mid March they had all but disappeared from the plankton.

One of the most unusual features of this incident has been the very long retention of acetone soluble toxicity by the Fouveaux Strait oysters. This appears to be a property of the shellfish themselves since other filter feeding molluscs have lost it rapidly in the absence of the causative dinoflagellate. One year after the initial contamination, acetone extracts of Fouveaux Strait oysters are still returning toxicity scores over 100 MU /100g. Ether extracts of the same shellfish show no toxicity.

Diarrhetic shellfish poisoning (DSP)

It has been known for many years that blooms of a variety of *Dinophysis* species are common in New Zealand waters though (with one possible exception) no actual cases of human poisoning have ever been attributed to this cause. In addition to the conventional mouse bioassay for this toxin, in 1994 the screening of shellfish using an ELISA test ("DSP-Check") for DSP toxins was begun. As a result, on several occasions indications of the trace occurrence of DSP toxins have been observed in some South Island mussels. Recently the opportunity has arisen to examine this in more detail (using bioassay, HPLC and ELISA techniques) due to blooms of *D. acuminata/sacculus* and *Dinophysis acuta*. The provisional results of these studies indicate that *D. acuminata/sacculus* appears to be a long term resident of specific locations. It does produce some DSP toxins; however, the specific toxicity per cell is extremely low. Even under worse case conditions where mussels are exposed for long periods to high *D. acuminata/sacculus* cell numbers (> 2.0 x10^4 cells / litre) the levels of toxin accumulated by the

shellfish are below the action level. A prolonged bloom of *D. acuta* and the associated contamination of mussels with okadaic acid has been followed over the last summer in some detail. There has been a good correspondence observed between the levels of toxicity in inter-tidal mussels and cell numbers in surface waters. Work is continuing on the characterisation of DSP-toxin profiles in New Zealand, *Dinophysis* species and contaminated shellfish.

Domoic acid

During 1994, the routine screening of shellfish samples for domoic acid (DA) from a large number of sites throughout the country was carried out (by Environmental Science Research Ltd.). During this period, 1,957 samples were analysed of which 7% showed traces or larger amounts of DA. DA contamination seems to be quite widespread though most results were below the action level of 20ppm.

Scallops appear to be particularly vulnerable to DA contamination. They have returned the highest results and contamination has persisted longest. Other species (except on one occasion in mussels) have only revealed very low-trace amounts of DA. DA is sequestered in greatest concentrations in the digestive glands of scallops, though the edible tissues (muscle and roe) have become contaminated to unacceptable levels at some times.

The relationship between DA acid contamination and *Pseudo-nitzschia* spp. blooms is not as clear cut as with some other biotoxins (eg. PSP). The reasons include: the lack of plankton data accompanying DA contamination incidents (and the brevity of some of these), rapid changes in specific DA production rates by the micro-algae during the growth cycle and the difficulty in the definitive identification of potentially toxic species using the light microscope.

Despite these problems there are data that indicate a relationship between *Pseudo-nitzschia* abundance and domoic acid contamination. This seems to occur during the decline phase of *Pseudo-nitzschia* blooms and these are therefore usually rather brief events.

There is recent evidence that some species or strains of *Pseudo-nitzschia* in New Zealand do produce DA. A New Zealand isolate of *P. autralis* (from Bream Bay) has been shown to produce DA in culture. This species has been found in association with DA contamination incidents in the Marlborough Sounds and the far north (Whangaroa Hbr.). Other producers of DA (eg. benthic diatoms) also remain a possibility.

Further research is required to more fully elucidate the relationship between the phytoplankton and DA contamination; however a "rule of thumb" suggests there should be extra vigilance after the peak of *Pseudo-nitzschia* blooms is reached.

"Red Herrings"

In addition to the biotoxin incidents discussed so far, there have also been several occasions where the results of bioassays for lipid soluble toxins appear to have been misleading. From an industry perspective, the most damaging incident this year was the closure of a large part of the Marlborough Sounds mussel growing region in June 1994.

This incident resulted in a large product recall, and involved considerable expense in carrying out clearance tests on detained material. The reason for the closure was a single positive bioassay on a lipid (acetone) extract of cultivated mussels. A routine plankton monitoring programme was in operation at the time which revealed no correlation between the plankton observations and the results of the bioassay. It could be said with confidence that there had been no change in the composition of the plankton for a considerable period and no potentially toxic phytoplankton were in evidence.

None of the subsequent shellfish sampling at this location, or in the general vicinity, returned positive tests and there was no means of confirming the original test. This incident had all the hall marks of a false positive. It illustrates the problem associated with reliance on mouse bioassays for lipid soluble toxins and highlights the need for some means of independently validating these results. The cause of the original positive test in this case remains unknown.

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New Zealand: Okadaic acid production in *P. lima*

Prorocentrum lima has been observed over the past three years at various sites around New Zealand, always in low numbers, but has not been linked to diarrhetic shellfish poisoning (DSP) in this country. To date, in New Zealand, all correlations of microalgal cell *in vivo* with DSP have been related to two species, *Dinophysis acuta* and *D. acuminata*. Recently, *P. lima* was isolated in moderate numbers (800 cells per Kg) from sediments collected in Rangaunu Harbour, Northland, a large tidal inlet surrounded by mangroves. Cells cultured for toxin analysis produced okadaic acid *in vitro* (6.3 ng.. 1000 cells-1) and will therefore need to be monitored in relation to oyster farming in that area.

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Bivalve mortality on southwest Atlantic shores

A mass mortality of yellow clams, *Mesodesma mactroides*, was recorded on the coast of Uruguay (Barra del Chuy) near the Brasilian frontier, beginning on 11 December 1994. It has been calculated that around 750,000 clams died, with a mean size of 44 mm and a mean weight of 12 g, equivalent to about 9 tons in total. A smaller quantity of cockles, *Donax hanleyanus*, with a mean length of 16 mm, also died. The water was clear with a temperature of 22°C, normal for this time of the year, and a salinity of 26.8 PSU, lower than that recorded 15 days earlier. Variations in salinity of this order are normal here, and do not generally affect the benthos. Mouse bioassays indicated that diarrhetic but not paralytic shellfish toxins were present.

Plankton samples contained a variety of diatoms and dinoflagellates dominated by *Asterionellopsis glacialis* and lower numbers of centric diatoms; *Dinophysis caudata* (4600 cells/l) and *D. acuminata* (3560 cells/l) and various ceratia, especially *Ceratium furca* (4000 cells/l) were the most abundant dinoflagellates. The abundance of silicoflagellates was also notable. Dense plankton blooms are common here at all times of the year, and wave energy considerable, and deoxygenation of the water can be discounted as a cause of the mortality, but it is possible that the siphons were blocked physically. The local prefecture recalls a similar event 15 years ago, but there are no records. INAPE (Instituto Nacional de Pesca) has regulated the extraction of clams from this beach since 1981 due to its commercial importance.

News of the occurrence was passed quickly to staff at FURG (Fundação Universidade do Rio Grande) in Brazil and sampling in the two countries was coordinated, following guidelines proposed during the IOC Regional Workshop on Harmful Algal Blooms, held in Montevideo, 15-17 June 1994 (IOC-UNESCO Report, N° 101). A mortality of yellow clams was noted simultaneously in Brazil, extending about 12 km to Hermenegildo. Plankton samples taken there yielded similar results to those from Barra del Chuy. A massive mortality of yellow clams in southern Brazil took place in 1993, and extended for approximately 350 km. The present event was smaller since the population has not yet recovered.

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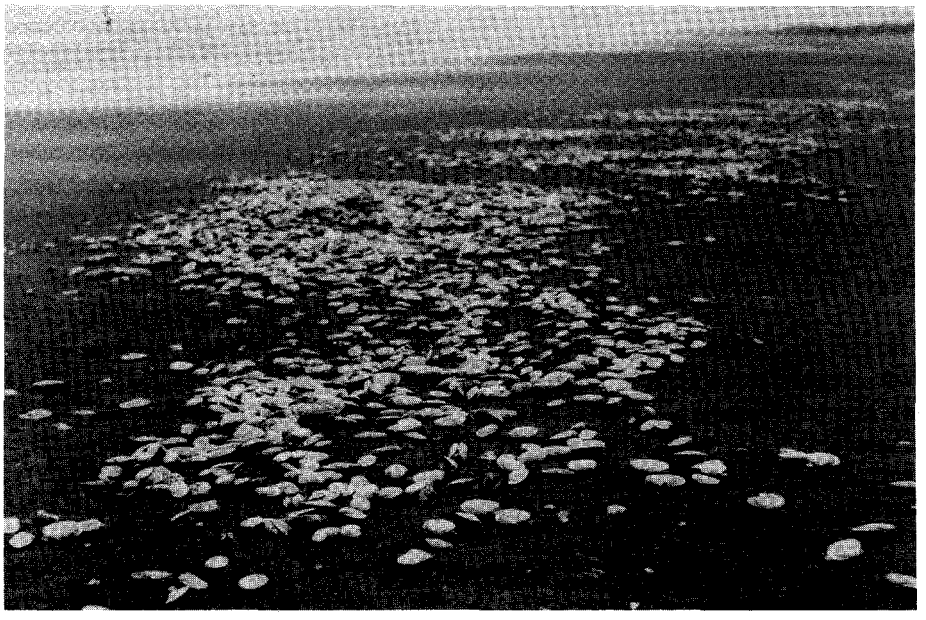

Dead Mesoderma mactroides *on Barra del Chuy beach, 13 December 1994. Photo: Silvia Méndez.*

Bibliographic notes

Dr. Felipina Sotto and her colleagues in the central Philippines recently suggested that their nationally planned red tide management programme is not flexible enough to respond rapidly to regional events, due to "the lack of coordination among agencies and the inadequate response of local government units", and also the fact that "active involvement of both regional and municipal governments ... was not implemented ..."[1]. This newsletter has already drawn attention to the difficult political and social challenges faced there[2]. The Philippines archipelago has a total area of around 300,000 km^2, roughly the same as Italy or the Ivory Coast. But its coastline is amongst the longest in the world – only high latitude regions with fjords like in Chile or Norway are in the same league. Coasts like these are obviously nightmares for the agencies responsible for comprehensive nationwide monitoring programmes, given the spatial and temporal scales of toxic phytoplankton events.

A red tide in November 1992 which affected green mussel farms in Samar provoked the establishment of a regional task force in Cebu, headed by the Department of Health there, and a rapid response to the threat of contaminated imports from the neighbouring region. According to this account, a local credibility problem between exporters and health authorities was quickly resolved, and some technical constraints on more effective regional monitoring were highlighted.

The court life of the Heian period of Japanese history is familiar to many through the widely translated 11th century novel, *The Tale of Gengi*. This period saw the establishment of Kyoto as the capital and the rise to power of the Fujiwara clan. Less familiar will be the red tides recorded then (8th and 9th centuries of the western calendar) and summarized by Akashio Takano[3]. A thorough search of ancient Japanese sources would surely reward us with much more of interest in this context, as has been done for the Pacific sardine fishery, documented in a semi-quantitative way as far back as the 14th century.

Modern Japan, as is well known, sometimes suffers severe economic losses due to red tides. The main species responsible are *Gymnodinium*

nagasakiense, *Chattonella antiqua*, *C. marina*, and *Heterosigma akashiwo*. Tsuneo Honjo of the Nansei Fisheries Institute has recently provided a concise precis of the biology of these four organisms[4], together with an assessment of some empirical methods for anticipating when they might bloom. These methods are site-specific. In another paper[5], Honjo runs briefly through the kinds of evasive actions which are used to protect cultured stocks when algal threats are imminent.

It is a long way from the 9th century Kyoto court to 17th century England, but the longest available instrumental temperature record, the Central England time series or CET, began there in 1659 A.D.[6,7] A recent analysis of this time series by David Thomson of the AT&T Bell Labs in New Jersey[8] suggests that the dominant seasonal cycle is timed in accordance with the anomalistic year which runs from perihelion to perihelion (when the sun is closest to the earth), rather than with the tropical year (which runs from equinox to equinox and measures the normal calendar year). Since the difference between these two ways of measuring the year is only one part in 26,000, the distinction may at first sight appear rather esoteric. Not so. It is important to recall that the position of perihelion "precesses", that a complete circuit of the earth from one perihelion to the next is not exactly 360°. There is thus a small phase change, called the precession constant, which amounts to about 50 arc seconds per year. The startling feature of Thomson's analysis of the CET is that the phase of the temperature data follows that of precession for 300 years, and then alters dramatically, in about 1940 A.D. Some other records show similar features, while others do not, and there is some evidence of phase capture triggered by solar forcing. Interested readers should consult Thomson's paper for details. What has all this to do with harmful algal blooms, or mediaeval Kyoto for that matter?

For more than a decade, there has been a fascinating debate, initiated by Ted Smayda, which is based on his contention that there has been a worldwide increase in the frequency and intensity of algal blooms. The debate is about whether it is true, and if so, what is causing it. Changes induced by climate change or greenhouse forcing provide an attractive alternative to competing hypotheses. Thomson concludes his analysis with the comment that "the effects of increasing greenhouse gases may be worse than previously thought", since removal of the precessional signal from temperature time series increases the importance of the increase observed in recent decades. What we need then from our special perspective is reliable time series of HAB events covering at least the last 100 years, to compare with the trends being uncovered by meteorology and other disciplines. Archival studies like that of Takano[3] are one route to the compilation of such time series, and if they become available, will help us to understand better what is going on. But for the time being political and technical innovations such as those being dealt with by Sotto and her colleagues in the Philippines and by Honjo and others in Japan are of more immediate practical concern.

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**We are grateful to Dr. Honjo for supplying us with this paper.*

Tim Wyatt

English usage

Professor Enrique Balech wrote to us on 27 March as follows:

"I see... that PSP toxins are still often named paralytic. However, the correct adjective is paralysing. English is the only language which introduces some doubts about the correctness of using 'paralytic' instead of 'paralysing', though it is clear that paralysing is quite correct, and paralytic is, at best, dubiously adequate ... Anyway it is beyond doubt that in other languages the equivalent of paralysing is the only correct adjective. Thus the Spanish Academy of the language says that 'paralitico' is the organism suffering paralysis (not the producer of paralysis). The same is true for French, Italian, German, etc. Therefore on behalf of international understanding and linguistic logic the name of the toxin should be paralysing."

This is a perennial complaint, but I am not aware that it has ever been addressed in print. In the phrase 'paralytic shellfish poisoning' (PSP), the adjective 'paralytic' refers to poisoning caused by shellfish, not to the shellfish per se, and is used correctly. 'Neurotropic' and 'encephalitic' are two more general adjectives used in the same way. The present participle 'paralysing' is an adequate alternative for 'paralytic' but is no more correct. Some would argue that it is more mannerist, and others that it should be spelt 'paralyzing'. The precise Spanish medical equivalent is in fact 'paralizante', not 'paralitico'. In English, the word 'paralytic' is used colloquially to describe the effects of alcoholic polyneuritis (dead drunk), with which PSP is sometimes confused diagnostically. It is of course not uncommon to suffer from both simultaneously, and it is a comforting thought that the polyuria induced by the former reduces the severity of the latter.

When the third edition of the famous American dictionary, *Webster's*, appeared, a leading article in the equally famous *New York Times* deplored its attitudes to American English, and maintained that it would continue to follow the recommendations of the second edition. In the course of saying so, the critic employed around 200 usages listed in the third but not the second edition! William Paley (1785), in *The Principles of Moral and Political Philosophy*, wrote that "All senses of all words are founded upon usage, and nothing else." I do not think the academies will ever be able to alter that. Many native speakers of English, when writing, employ the word 'utilize' rather than 'use'. As far as I know, they are exactly equivalent in meaning. But I do not feel that it is my mission to insist on the more economic of the two. In Spanish, in contrast, 'usar' is more colloquial, so that 'utilizar' is perhaps better for formal communications. Usage will tell.

Tim Wyatt

IOC opens science centres on harmful algae

To provide a better service to Member States facing serious problems caused by harmful algae, the IOC at its Seventeenth Assembly in 1993, decided to establish Science and Communication Centres on Harmful Algae. The Centres will in particular facilitate the implementation of the HAB Training and Capacity Building Programme. In 1993, Denmark and Spain offered the IOC to establish Centres at the University of Copenhagen and at the Instituto Español de Oceanografía, Centro Vigo, respectively. The Centre in Copenhagen was opened by the IOC Executive Secretary, Dr. G. Kullenberg, and Professor Ø. Moestrup, on 5 May 1995. The Centre in Spain is expected to be operational early 1996. The two Centres are established for a five year period, are planned to be complementary and will be coordinated by the IOC Secretariat.

The Centre in Copenhagen is jointly sponsored by IOC, the Danish International Development Assistance (DANIDA), the University of Copenhagen, the Danish Institute for Fisheries Research, and the National Environmental Research Institute, and the activities of the Centre will be directed specifically towards developing countries. The Centre will offer practical and direct assistance with identification of problematic species of harmful algae, establish a data base on harmful algae literature, and build up a reprint collection which will be available free of charge to research institutions in developing countries. A major activity will be recurrent training courses on the taxonomy and biology of harmful marine phytoplankton in particular to train trainers. A first course was held already in 1993 at the University of Copenhagen. The second course is scheduled for 31 July - 11 August this year. Up to 15 phytoplankton scientists will attend each course. Courses will also be given regionally. In 1995, a course for the Northern and Western Indian Ocean is planned for 4-14 September 1995 at the University of Mauritius.

The Centre will also offer supervision of PhD students from developing countries. PhD projects will be offered in collaboration with the sponsoring Danish institutions.

The research activities of the Centre will concentrate primarily on applied aspects of the biology and toxicology of harmful algae. Research institutions in developing countries will be invited to take part in both the planning and implementation of the projects. Activities are expected to include: (i) publication of material for identification of toxic algae; (ii) establishment of cultures and reference collections of harmful algae to be used for teaching purposes and for species intercalibration in difficult taxonomic fields; (iii) investigations on the effects of algal toxins on caged fish.

The Centre will initially have three staff members: PhD Jacob Larsen, Associate Professor, MSc Henrik Enevoldsen, IOC Project Coordinator, and a third post which has not yet been filled. The focal point at the Botanical Institute is Prof. Ø. Moestrup.

For more information, please contact: IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Botanical Institute, Dept. of Mycology and Phycology, Øster Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; tel.: (45-33) 13 44 46; fax: (45-33) 13 44 47; e-mail: hab@bot.ku.dk.

In the next issue of *HAN*, we will bring more news on the Centre in Vigo, Spain.

HAB Panel meets in Paris

During the first week of June (6-9 June 1995), the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms had its third meeting at UNESCO/IOC Headquarters to evaluate the results of the IOC-FAO HAB Programme, to decide on new activities, and to work on the possible funding of those activities. Some highlights from the Session: (i) The Harmful Algal Bloom Programme Office at the IOC Secretariat presently has one staff member, seconded by Denmark. The Panel strongly encouraged Member States to second staff members to the HAB Programme Office. (ii) The Panel welcomed the establishment of IOC Science and Communication Centres on Harmful Algae in Copenhagen and Vigo. The Panel also welcomed the proposal to investigate the possibility of establishing centres in the USA and Japan. (iii) The Panel recommended that a working group on harmful algal blooms in South America (COI-FANSA) be established. (iv) The Panel recognized the need for a WESTPAC Project Coordinator for developing regional projects and recommended IOC support for the coordinator. (v) Sweden and Chile offered to assist in the production of an information brochure on the IOC HAB Programme. (vi) The Panel recommended that the IOC Task Team on Aquatic Biotoxins should continue until its tasks can be charged to a permanent working group. (vii) The Panel considered it to be of the highest priority to facilitate the provision of toxin standards and reference material to developing countries. (viii) The Panel decided that the IOC Task Team on Design and Implementation of HAB Monitoring Programmes should continue, finalize a report on monitoring systems on harmful algae and assist in organizing an international conference/training workshop on this subject. The Panel stressed that monitoring of the occurrence of harmful algae should be seen as an integral element of the Global Ocean Observing System (GOOS). (ix) The Panel elected Dr. A. Zingone, Italy, as the new Chair, and Dr. R. Corrales, the Philippines, Vice-Chair. The Panel expressed its gratitude to the outgoing Chair, Dr. Bernt I. Dybern, Sweden, for his considerable efforts in promoting the Panel's work, as well as their thanks and appreciation for the hard work and dedication displayed by the staff of the IOC Secretariat. (x) In March 1995, FAO announced that it would cease to co-sponsor the Panel because of financial constraints and re-prioritization of activities, but confirmed its general interest in the activities of the Programme.

Major HAB Programme activities 1995

- IOC-DANIDA Training Course on the Taxonomy and Biology of Harmful Marine Phytoplankton, University of Copenhagen, Denmark, 31 July - 11 August.
- IOC/WESTPAC-Japan Training Course on Field Sampling Methods, Kitasato University, Japan, 17-21 July.
- IOC/WESTPAC-ASEAN-Canada Training Course on Harmful Algae, Ambon, Indonesia, October.
- IOC-SAREC-DANIDA Training Course on the Biology and Taxonomy of Harmful Marine Microplankton, University of Mauritius, Mauritius (postponed).
- IOC-UNEP-WHO-FAO-Italy Training Course on Toxin Chemistry and Toxicology related to Harmful Algal Blooms, University of Trieste, Italy, 3-12 September.
- MAST-ONR-IOC 6th Advanced Phytoplankton Course, Taxonomy and Systematics, Zoological Station Anton Dorhn, Napoli, Italy, 24 September - 14 October.
- ICES-IOC Working Group on the Dynamics of Harmful Algal Blooms, Helsinki, Finland, 17-19 May.
- Theme Session on "Ballast Water Ecological and Fisheries Implications", cosponsored by the IOC and IMO, 1995 ICES Annual Science Conference, September, Aalborg, Denmark. Chair: Prof. J. T. Carlton, Williams College, USA. IOC has invited Dr. G. Hallegraeff, Australia, to give a lecture on transfer of harmful algal species via ballast water.

Analytical procedure to avoid interference of PSP in mouse bioassay for DSP

The Galician rias in northwest Spain support the largest raft cultivation of blue mussels in the world. It is quite common towards the end of summer, that *Gymnodinium catenatum* and species of *Dinophysis* are present in the same plankton samples, and the mussels may therefore contain both paralytic and diarrheic toxins. The same problem has been raised by scientists in Chile and Uruguay, and was discussed at the IOC-UNESCO Regional Workshop on Harmful Algal Blooms held in Montevideo, 15-17 June 1994 (Report N° 101).

The mouse bioassay is the method usually used to detect marine biotoxins in shellfish for public health purposes, and most countries follow the official method (AOAC, 1990) for the paralytic shellfish toxins (PSP). For the diarrhetic shellfish toxins (DSP), biological methods are also used, but there are wide differences between countries in the type of bioassay employed and in the toxicity criterion used. The commonest test for DSP is the mouse bioassay described by Yasumoto (1978). In this method, the digestive glands of the shellfish are extracted with acetone. After evaporation of the acetone, the residue is resuspended in Tween 60, and 1 ml samples injected into each of three mice. Different times of death are used as the toxicity criterion. The method is not very selective, and for this reason is very suitable for public health purposes. Its main disadvantage is the possibility of analytical interference which can confuse the interpretation of the results and lead to false positives.

PSP toxins can alter the results of this method even when they are present in concentrations too low to be detected by the standard mouse bioassay. These toxins are extracted together with the DSP toxins during the acetone treatment, and the final extract is highly toxic to mice. They can therefore die with neurotoxic symptoms, and it is not then possible to observe the possible presence of DSP toxins in the extracts. This problem can be avoided by using a modification of the method (Yasumoto, 1984) in which the aqueous residue obtained following acetone evaporation is made up to a fixed volume with water and extracted three times with diethyl ether. The PSP toxins are removed on the water phase. The combined ether solutions are backwashed with water and then evaporated. The residue from this procedure is then resuspended in Tween 60 and 1 ml samples injected into mice as before. With this procedure, the possible PSP interferences are removed in the water layer. This assay is also very suitable in the case of commercial presentation of shellfish without glands. Shellfish must be tested anyway, and a higher amount of shellfish meat is required. The high amount of salts present in the muscle might produce mice deaths, unless water washing to remove them are carried out during the procedure. A disadvantage of this assay is the poor solubility of Yessotoxins in the diethyl ether, that could lead to false DSP negative when they are present in shellfish samples. By using dicloromethane instead of diethylether, all the DSP toxins, even the most polar as YTXs, are detected (Yasumoto, personal communication). Another quite different debate is whether Yessotoxins as well as Pectenotoxins, that do not produce diarrhetic symptoms, must be excluded from the DSP group.

AOAC (1990). *Official Methods of Analysis*, 15th ed. K. Helrich. pp. 881-882. Association of Official Analytical Chemists, Arlington, USA.
Yasumoto, T., Oshima, Y., Yamaguchi, M. (1978). *Bull. Jap. Soc. Scient. Fish.*, 44, 1249-1255.
Yasumoto *et al*, 1984. In: *Seafood toxins*. Ragelis, E.P. (ed.) ACS: Symp. Sr. 262, ACS, Washington DC, 207-214.

Ana Martinez y Aurea Miguez, Laboratório de Sanidad Exterior de Vigo, Community Reference Laboratory on Marine Biotoxins, Estación Maritíma sn. 36271, Spain.

Just published:

UNESCO secondary school field guide curriculum on tropical marine environments

Study No. 7: The Tropical Marine Environment: Field Exercises for Teacher Training and Class Work, in the Series *Contending with Global Change,* was just published by and can be obtained from: UNESCO Office, Jakarta, Jln. M.H. Thamrin No. 14, Jakarta 10002, Indonesia (send orders to the attention of: Ms Nuning Wirjoatmodjo). *Cost*: US$ 25 per copy. A limited number of complimentary copies are available to institutions in developing countries.

Included is a section on practical work in the study of phytoplankton, which at the same time produces results usable in the study of phytoplankton in the target location, e.g. on phytoplankton responses to nutrient loads in the region of Jakarta Bay (*Noctiluca scintillans*) into the Pulau Seribu chain since 1990 and also involving *Oscillotoria* sp.

Framework: The objective of this issue within the series of publications, produced by the UNESCO Jakarta office, is to present an inter-disciplinary field studies curriculum that can prepare today's and future generations by giving them an understanding of marine, terrestrial and associated systems as they relate to ecologically sound and sustainable development in a continually changing world. The programme also encourages the inclusion of training in navigation, basic and advanced outboard boating skills, snorkeling, basic Scuba practices, as well as direct environmental monitoring using techniques such as shoreline debris calculations, resource assessment strategies, plankton sampling, secci-disk and water quality readings and so forth.

Case study: The publication includes an exemplary or model field trip structure involving a launch trip from Jakarta to a coral atoll in the Java Sea, about 85 km northwest off the north coast of Java. Here, over a period of three days the group conducts land-based forays into the island and related reef system in order to: (i) collect physical information and measure associated biological responses over distinctive environmental trends and (ii) carry out observations of in situ ecosystem elements. In cruising from the mainland city of Jakarta to the island, an activity schedule is defined for the sampling and counting of phytoplankton and zooplankton populations growing on the sewage and nutrient discharges from the city. Class data obtained annually over a seven-year interval (during the period 1986-1992) showed an apparent significant increase in population densities and the extent of the standing algal bloom out to about 60 km from Jakarta.



Shown above are participants and lecturers at the Second IOC-DANIDA Training Course on the Taxonomy and Biology of Harmful Marine Microalgae, held at the IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Denmark, 31 July - 11 August 1995. Trainees came from twelve countries, and lecturers were: Prof. K. Matsuoka, University of Nagasaki; Prof. Ø. Moestrup, University of Copenhagen; Dr. J. Larsen, IOC Centre; Dr. R. Corrales, University of the Philippines; and Dr. M.L. Fernandez, EU Community Reference Laboratory for Marine Biotoxins. Also, an IOC/WESTPAC-Japan Training Workshop on Monitoring PSP Plankton and Shellfish Toxicity, was held at Kitasato University, Japan, 17-21 July 1995. Source of photo: Henrik Enevoldsen.

Future events

Second IOC Regional Science Planning Workshop on Harmful Algal Blooms, 30 October - 1 November 1995, Mar del Plata, Argentina. Contact: Dr. J.I. Carreto, INIDEP, Casilla de Correo 175, 7600 Mar del Plata, Argentina; tel.: (54-23) 517818; fax: (54-23) 517442.

IUPAC-UNESCO/IOC-FAO International Workshop on Ciguatera, 8-10 November 1995, Santo Domingo, Dominican Republic. Contact: Prof. D. Park, Food Science Department, Louisiana State University, Baton Rouge, LA 70893-4200, USA; fax: (1-504) 388 5300.

IXth International IUPAC Symposium on Mycotoxins and Phycotoxins, 27-31 May 1996, Rome Italy. Contact: Dr. Marina Miraqlia, Instituto Superiore di Sanita, Viale Regina Elena 299, I-00161 Roma, Italy.

Fifth Canadian Workshop on Harmful Marine Algae, September 1996. Contact: M. A. Paranjape, NAFC, Science Branch, Division of Ocean Ecology, PO Box 5667, St. John's, Nf. Canada A1C5X1; fax: (1-709) 772 3207; e-mail: mparanjape@nflorc.nwafc.nf.ca.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 12/13

Florida red tides

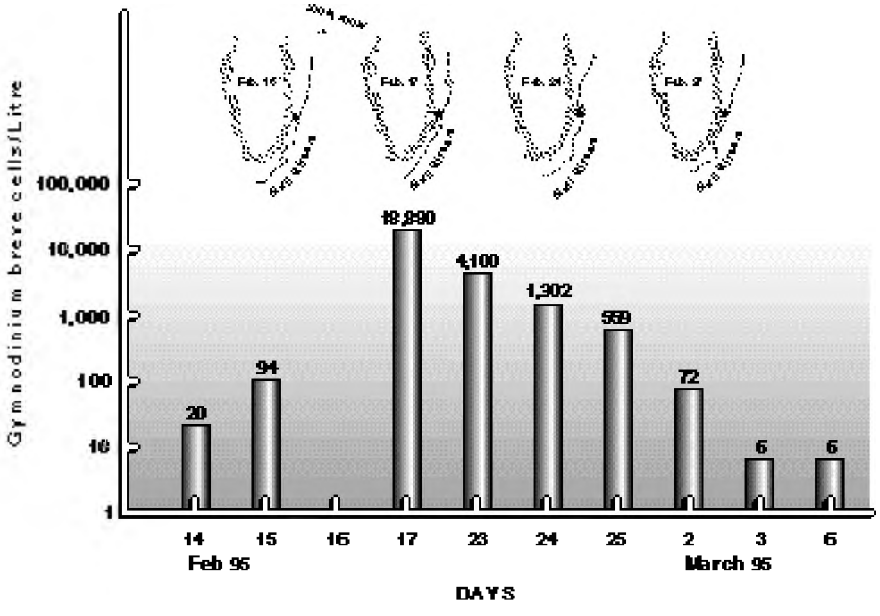
Red tides in Florida caused by *Gymnodinium breve* start 18 to 74 km offshore and take several weeks to develop in coastal waters. Typically *G. breve* is present all year in Gulf of Mexico[1] and Gulf Stream waters[2] 10-100 cells L^{-1}. When counts are above 5,000 cells L^{-1}, a bloom is either developing or one is in progress. At a count of 5,000 cells L^{-1} in inlets, bays, or lagoons, open shellfish-growing waters are closed to harvest by the Florida Department of Environmental Protection.

Gymnodinium breve blooms can become entrained in the Florida Current–Gulf Stream system and transported from Gulf of Mexico to Atlantic waters. Although most *G. breve* blooms remain in the Gulf, four occurrences of this species have been documented along the Atlantic coast of Florida[3,4,5]. In each of these instances the blooms were short-lived (± 1 month). In fall and winter 1987-88, a large *G. breve* red tide bloom persisted along the coast of North Carolina for 4-5 months[6]. This occurrence was the first record of *G. breve* north of Florida, a range extension of more than 800 km. The Florida Current–Gulf Stream system was the suggested transport mechanism for *G. breve* cells from a late summer/fall bloom off the southwest coast of Florida. The estimated transit time for cells around the peninsula northward to the North Carolina coast was 22-59 days.

1994-1995 outbreak

Although the 1994-95 Florida *Gymnodinium breve* red tide was reported in the national press as the most virulent red tide ever, this outbreak started off as a typical red tide on the west coast of Florida in September 1994. In the initial stages of a red tide, *Gymnodinium breve* concentration of 250,000 cells L^{-1} can kill fish; cell counts in waters where fish were dying 26 km off Cape Sable were 280,000 cells L^{-1}. Cell counts made by the Florida Department of Environmental Protection personnel along the west coast of Florida, from Tampa Bay to Sanibel Island, ranged from normal to 4,700,000 cells L^{-1}. The red tide was atypical, however, in three aspects: 1) extensive fish kills occurred in open waters off the Florida Keys, 2) two unconfirmed cases of neurotoxic shellfish poisoning were reported from consumption of surf clams in closed waters, and 3) the red tide was transported from Gulf to Atlantic waters in February 1995. The extensive fish kills, which were reported in January and February, covered over 5,000 km^2 of open water westward from the Florida mainland to the Dry Tortugas. Dead and dying fish included grunts, snappers, groupers, mullet, tarpon, cobia, sharks, and other fishes. Octopi, stone crabs,

(Cont'd. on p. 2)



Gymnodinium breve *cell counts near West Palm Beach, Florida (26.55 N, 80.00 W) and the position of the western edge of the Gulf Stream.*

Note on an unknown poison

HAN issue N° 5 (page 1) carried an account of a toxic event of unknown aetiology in French coastal waters, which lasted from November 1992 until May 1993. No toxic organism had been identified at the time, although the mouse test suggested the presence of PSP. But the toxin was unstable, and chemical analysis failed to detect it. Further details have been made available by Françoise Marco[1] of the Laboratoire côtier in Toulon. Mussels from Toulon screened by J. Minet of the Faculté de Pharmacie, Rennes, revealed a flora dominated by *Bacillus cereus*. Other common pathogens (*Salmonella*, *Staphylococcus*, *Clostridium*) were absent. Acetone extracts of a pure strain of *B. cereus* injected into mice provoked the same symptoms as mussel extracts.

The Editors

(1) Marco, F. *La Gazette des microbes*, October 1994, 63.

Trichodesmium bloom in Gulf of Mexico, summer 1995

During a recent cruise in the Gulf of Mexico on board the University of Texas' R/V *Longhorn*(1), blooms of *Trichodesmium* were observed like never before (c.f. Sargasso Sea, Arabian Sea). What follows is a preliminary and purely observational report on this major but episodic event. The study of the abundance and composition of dissolved organic matter in the Gulf of Mexico was the primary goal of this cruise, and unfortunately we were not set up to do primary production measurements or even chlorophyll estimates.

Colonies of the cyanobacterium (2-3 mm size bundles of trichomes) could be seen with the naked eye in the surface water of every station that we occupied. It would not be wrong to surmise that the entire Gulf of Mexico surface water was blooming with this cyanobacteria, possibly the dominant primary producer during this period. The abundance of *Trichodesmium* increased as the cruise progressed, from < 10 colonies per bucket of surface water during the Texas-Florida leg, to > 100 colonies per bucket of surface water during the Florida-Texas leg of the cruise. It appeared from swimmer observations that *Trichodesmium* was concentrated in the upper 3 meters of the water column, and underwater aggregates became abundant towards the latter part of the bloom.

Towards the latter part of the return leg (Florida to Texas), the water became visibly thick with the bloom. In the late evenings, we could practically smell the emissions (Dimethylsulphide) from decaying *Trichodesmium* in the air. The surface water attained a brownish color, and sea conditions remained flat calm for several days. Several plankton tows were made using a small hand net and several litres of this exclusively *Trichodesmium* sample were concentrated for biochemical analysis. From observing how viscous these samples were, it is suggested that the exudates/lysis products of the *Trichodesmium* were responsible for prolonging the calm weather we had by enhancing surface wave dampening. It is noteworthy that *Trichodesmium* blooms occur in the warm (summer) nutrient poor tropical/subtropical ocean only under very calm conditions.

On the morning of 20 August, in 27° 14.441 N, 94° 14.032 W, we observed a gigantic accumulation of the bloom along a windrow ≈ 100 m wide and extending from horizon to horizon. Occasionally there were streaks of pink-red colour on the brown bloom where the phycoerythrin pigment from dying cells must have leached out. Satellites over this region must have picked up such mesoscale features as well as general and substantial increase in chlorophyll fluorescence over the entire Gulf of Mexico during the month of August, accompanying an increasing abundance of *Trichodesmium* in surface waters. Discussions with the ship's crew who have a decade of experience sailing these waters indicated that *Trichodesmium* bloom sightings in this region are not an uncommon phenomena during the summer.

It is suggested that such episodic blooms of this nitrogen fixer go largely unnoticed because of their brevity, and not only bring in large pulses of organic matter into the system, but may also shift the system temporarily from one that is N-limited to one that is P-limited. The role of *Trichodesmium* blooms, that are episodic but large scale events in the world ocean, must somehow be incorporated into the study of oceanic C and N cycles. Otherwise we will continue to miss a key element in the global elemental cycles puzzle.

Acknowledgments: The captain and crew of R/V *Longhorn* of the University of Texas helped enthusiastically with both observation and sampling. Ship-time and research support was provided by a National Science Foundation grant to Ronald Benner and John Hedges.

(1) Cruise# 648; 2-22 August 1995; Port Aransas, Texas to Key West, Florida and return, following an almost straight line transect across the entire Gulf of Mexico.

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(Cont'd from p. 1, "Florida red tides")

and top snails also suffered mortalities. Coincident with these mortalities were several stranded dead dolphins and sea turtles. At times, floating dead fish were estimated to be one every 1 to 3 m of water and they varied in size from 15 cm to over a meter. Fish kills off the Florida Keys have been reported in 1878, 1880, 1916?, 1946/47, 1953/54, 1972 and 1976.

The two cases of probable neurotoxic shellfish poisoning occurred 2 January when two 39-year-old men, visiting Florida, consumed surf clams collected at Sanibel Island; dead fish were beached near the collection site. Within an hour, both men felt tingling in their tongues and finger extremities. Two hours later, one of them was nauseated, weak, and dizzy. During the following few days, they continued experiencing nausea, muscular pains, anxiety, and diarrhea. They were treated in the Lee County Health Park Medical Center, but NSP was not then and has not since been confirmed by medical personnel as the cause of their illness.

During the 1994-95 Florida west coast red tide, concern over the possibility of *G. breve* cells being transported to the Atlantic prompted sampling off West Palm Beach, Florida from September 1994 through early March 1995 (Fig. 1). In February, coincident with fish kills in southwest Florida waters, a water sample about 12 km off the Florida Keys on the Atlantic side had almost 10 million cells L^{-1}. This high count prompted further concern about transport of a red tide bloom in the Gulf Stream. Samples from September-December 1994 ranged from 0-6 cells L^{-1}, but by mid-February cell counts rose to nearly 20,000 cells L^{-1} at the West Palm Beach sample sites where the continental shelf is very narrow and the Gulf Stream can move close to shore. By early March the cell counts were once again below 10 cells L^{-1}. The cell counts were proportional to the degree of Gulf Stream influence in the West Palm Beach area (Fig. 1). As the western edge of the Gulf Stream impinged on the sampling area and formed a meander (17-20 Feb.), *G. breve* cell counts increased. As the Gulf Stream moved offshore, away from the sampling site, the cell numbers decreased (27 Feb.). Fourteen days later, *G. breve* cells were noted in outer

shelf samples from Onslow Bay, North Carolina.

The red tide along the west coast of Florida reappeared in April with observations of human respiratory irritation and *G. breve* concentrations above background. Shellfish harvesting areas were closed again from Pinellas to Lee counties. Respiratory irritation and fish kills affected communities as far north as Tarpon Springs. Venice to Tarpon Springs were still affected in early June. A patch of red tide (10^6 *G. breve* cells L^{-1}) about 18 km west of Anclote Key near Tarpon Springs was detected in early May and this offshore bloom no doubt inoculated Tarpon Springs and Clearwater inshore waters. If a red tide remains offshore when inshore concentrations of *G. breve* decrease to below background levels, the offshore population can reinoculate inshore areas and red tide conditions may be reestablished.

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IOC Training course on detection methods for algal toxins



Participants in the training course held at University of Trieste, 3-12 September 1995.

A training course on chemistry and toxicology related to harmful algal blooms was held at the Department of Biomedical Sciences of the University of Trieste (Italy), from 3-12 September 1995. It was sponsored by IOC, UNEP, WHO, FAO and Italy.

The purpose of the course was to impart to the participants theoretical and practical knowledge about the methods of detecting the toxins that are produced by microalgae and that are responsible for the most widespread intoxications (DSP, PSP and ASP).

During the course, basic information was also provided on the taxonomy and distribution of these microalgae. Seven different methods were demonstrated for use by the participants. In particular, DSP-contaminated samples were analyzed by the participants, using three different methods: the mouse bioassay, an ELISA assay and the protein phosphatase inhibition assay.

The HPLC method for DSP toxin was demonstrated by Dr. C. Casadei (Centro di Ricerche Marine di Cesenatico, Italy). PSP-contaminated mussels were analyzed using the AOAC mouse bioassay. The same samples, as well as other contaminated specimens (i. e. urine of PSP patients) were analyzed also by a receptor assay. An ELISA assay for PSP was demonstrated. The results obtained by each participant were compared and a satisfactory correlation was found. The practical aspects of all the applied methods were emphasized to enable the participants to perform the analyses in their laboratories. Furthermore, all the participants presented a status report on harmful algal blooms in their countries. The participants' general impression of the course was very positive.

Thirteen scientists from 12 countries participated in the course, organized by Dr. Aurelia Tubaro and Prof. Roberto Della Loggia (University of Trieste, Italy), Prof. Giorgio Honsell (University of Udine, Italy) and Dr. Helle Ravn (Intergovernmental Oceanographic Commission of UNESCO, Paris, France). Invited lecturers were Prof. T. Yasumoto (Tohoku University, Japan), Dr. A. D. Cembella (National Research Council of Halifax, Canada), Dr. M. L. Fernandez (European Community Reference Laboratory of Vigo, Spain), Dr. J. S. Ramsdell (Marine Biomedical and Environmental Science, South Carolina, USA).

A visit to the mussel farms of the Gulf of Trieste concluded the activities of the training course.

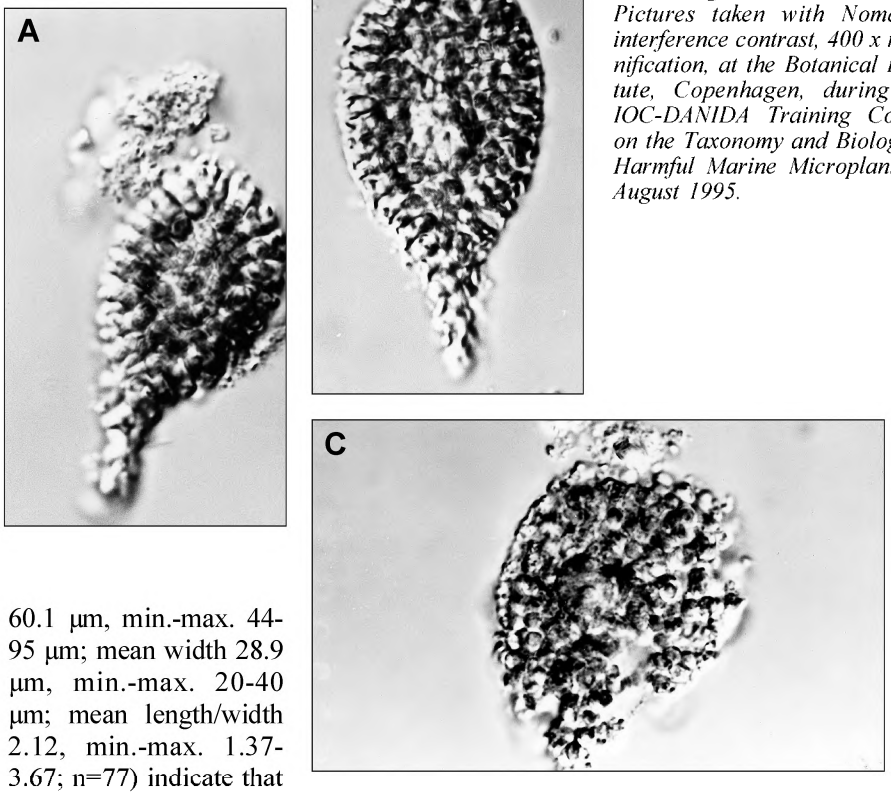
Raphidophycean in southern Brazil

During a routine sampling program in the estuarine region of the Patos Lagoon (32° 10' S; 52° 06' W), conducted on 4 May 1995, cells of *Chattonella* sp. were observed for the first time in southern Brazil. Also present was the raphidophycean *Fibrocapsa japonica*, though this species was previously observed in live samples from coastal shelf waters off southern Brazil (Odebrecht, unpubl.). The phytoplankton community was dominated by the diatoms *Leptocylindrus minimus*, *Pseudo-nitzchia pseudodelicatissima* (confirmed by Ø. Moestrup, using electron microscopy), cf. *Cylindrotheca closterium* and nanoflagellates (> 10^5 cells l^{-1} and 10^6 cells l^{-1} respectively). The presence of several dinoflagellates (*Ceratium furca*, *C. tripos*, *Dinophysis caudata*, *D. acuminata*, *Scripsiella* sp., *Gymnodinium splendens*, *Gyrodinium spirale*, *Polykrikos*, *Noctiluca scintillans*, *Protoperidinium* spp.), the ciliate *Mesodinium rubrum* and the silicoflagellate *Dictyocha fibula* indicate that low turbulence conditions prevailed in the water column.

Water temperature and salinity during the study were 19°C and 19.5 respectively, typical of autumn conditions when the estuary presents euhaline conditions. Chlorophyll *a* concentration was 15,2 mg m^{-3}, and concentrations of nitrate + nitrite (0.9 µM), ammonia (2.4 µM), phosphate (1.25 µM) and silicate (35.2 µM) resulted in Si:N and N:P ratios of 10 and 2.8, respectively, which indicate that nitrogen could be the main nutrient limiting phytoplankton growth.

Counts of cells in Lugol-fixed surface samples, using an inverted microscope, indicated *Chattonella* abundance of 9,000 cells l^{-1} (lanceolate organisms, Figure A, B). However many cells (34,000 cells l^{-1}) were rounded, contracted and/or disrupted (Figure C), so the actual concentration was probably higher. The abundance of the species *Fibrocapsa japonica* was 48,000 cells l^{-1}, thus together the raphidophycean species summed approximately 100,000 cells l^{-1}.

Although live material is considered necessary for reliable identification of raphidophycean species (Throndsen, 1993), the shape and large size of the more intact organisms (mean length 60.1 µm, min.-max. 44-95 µm; mean width 28.9 µm, min.-max. 20-40 µm; mean length/width 2.12, min.-max. 1.37-3.67; n=77) indicate that these probably were *C. antiqua*, the largest known species of *Chattonella*, recorded from Japanese waters (Fukuyo *et al.*, 1990).



Lanceolate form of Chatonella: *(A) 56 µm x 27 µm, (B) 72 µm x 34 µm, and (C) disrupted organism from the estuarine region of Patos Lagoon, southern Brazil. Pictures taken with Nomarski interference contrast, 400 x magnification, at the Botanical Institute, Copenhagen, during the IOC-DANIDA Training Course on the Taxonomy and Biology of Harmful Marine Microplankton, August 1995.*

There are no indications so far of a harmful effect of the raphidophycean species in southern Brazil, although shellfish mortality has been associated with harmful algae in this area on several occasions (Garcia *et al.*, 1994; Odebrecht *et al.*, 1995). In Guanabara Bay, Rio de Janeiro (22° 50' S; 43° 10' W), raphidophyceans (probably *Chattonella*) were observed in June/July 1978 forming a typical red tide (17,000 cells ml^{-1}), though no fish mortality was observed (Sevrin-Reyssac *et al.*, 1979).

In the Patos Lagoon, problems related to harmful algal blooms might be expected in the future, since anthropogenic eutrophication is increasing, and efforts to establish shrimp and fish culture are underway. Thus, phytoplankton monitoring programmes and detailed studies must be enhanced in this area.

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Mussel production and toxic algal blooms in Santa Catarina State, southern Brazil

Various possible causes have been suggested for the global increase in recent years of harmful algal blooms (HABs), ranging from atmospheric ozone layer depletion to increase in scientific awareness concerning HAB events and their effects (Carreto, 1994; Hallegraeff 1993). Not only increase in the occurrence area, but also in frequency, toxicity and quality, have been observed. In a global distribution map, the Brazilian coast is regarded as a non-occurrence area for all of the known phycotoxins. This does not mean that the Brazilian coast is free from toxic algal bloom events and related human poisoning problems (Odebrecht *et al.*, 1995; García *et al.*, 1994; Yunes *et al.* 1994). The lack of historical data on toxic algal blooms in Brazil arises from the fact that most of the observed cases were not reported, or when published, they did not lead to scientific concern.

An important factor of concern is the wide programme to stimulate mussel production in Santa Catarina State (south Brazil) as an economic alternative for artisanal fishing communities, faced with problems of a fall in captures and competition with the industrial fleet. The programme, which began in 1989, consists of the spread of mariculture benefits, technology transfer, stimulation of cooperative formation, and selection of areas suitable for the activity (Agropecuaria Catarinense, 1993). As a result, cultivated mussel production, mainly *Perna perna*, increased from 190 to approximately 5000 ton.year^{-1}, in 6 years, turning Santa Catarina into the largest producer of cultivated mussels in Brazil. Although obvious benefits have been generated, possible HAB events and their impact on community health were not considered in the initial programme despite at least one official case of shellfish poisoning registered in Santa Catarina (Zenebon and Pregnolatto, 1992). The event occurred in 1990 when various cases of diarrhea in the population were registered in Florianópolis (Santa Catarina State's capital) as a result of DSP poisoning.

The mouse bioassays (Zenebon and Pregnolatto, 1992) and the presence of *Dinophysis acuminata* (unpublished data) confirmed the presence of DSP in the water. Other unofficial evidence is found among several regular mussel consumers who suffered poisoning symptoms (DSP), suggesting that DSP is widespread. In some littoral areas, people do not consume mussels during August and September (austral winter), either because it can be dangerous or, as it is said, they do not taste as well as in other seasons. In an attempt to minimize possible public health and economic losses, a HAB monitoring programme in Santa Catarina was developed at the Universidade do Vale do Itajaí (UNIVALI). Apart from natural and methodological difficulties, a crucial element has been for authorities to acknowledge that the problem exists. So far, all actions directed to face the issue have come from universities rather than from public health or environmental offices. It seems that it will take a while until the situation is properly assessed and the population fully benefits from the programme to stimulate mussel production in Santa Catarina.

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Harmful algal blooms in South-East Asia: *Common problems and networking*

Red tides can extend over wide areas, often involving the waters of adjacent countries. South-East Asia, which is currently undergoing rapid economic growth, is one of the regions negatively impacted by toxic red tides / harmful algal blooms. Brunei, Indonesia, Malaysia, the Philippines, Singapore, Thailand and Vietnam compose the Association of South-East Asian Nations (ASEAN). Geographically, however, the region includes Laos and Cambodia, which have not yet applied for membership of the association.

Since 1993, ASEAN has implemented a marine science programme in collaboration with Canada. A red tide component which partly aims to train personnel in undertaking monitoring and research programmes and educating the public on the phenomenon, includes an "ASEAN–Canada Red Tide Network". A fax-based "Awareness Network" serves to inform member nations of the occurrences of harmful algal blooms / red tides through the regional coordinator*. Authorized country anchors and alternate anchors feed and receive data and information to and from the network. An "Information Network" provides materials useful for the understanding and the management of HABs in the region.

Pyrodinium bahamense var. *compressum* remains the major Paralytic Shellfish Poisoning (PSP) causative organism in the region. To date, in the Philippines alone, it has caused about

(Cont'd on p. 6)

a hundred deaths and 1,618 illness cases, and has affected nine bays all over the country based on accounts from 1983 (Corrales and McLean, 1995). The organism has also been reported in Sabah (Malaysia), and in Brunei Bay and several bays in Indonesia, causing negative impacts on many occasions in most of these areas. Occurrences of the harmful species of *Chattonella* sp., *Alexandrium* sp., *Dinophysis* sp. and *Gymnodinium* sp. have also been noted in many countries in the region. Blooms of *Noctiluca scintillans* and *Trichodesmium erythraeum* have been observed in several areas, with Thailand reporting the most frequent occurrence. Other red tide organisms found in the Gulf of Thailand are *Ceratium* spp., *Cochlodinium* spp., *Mesodinium rubrum* and diatoms, *Coscinodiscus* spp., *Rhizosolenia* spp., *Chaetoceros* spp., *Bacteriastrum* spp., *Skeletonema costatum* and *Nitzchia* spp. (Piumsomboon *et al.*, 1995).

ASEAN members have expressed their desire to continue the HAB networking beyond the ASEAN–Canada programme implementation period (1993-1996) and be further linked to the rest of the world. The need to undertake regional collaborative research on a major HAB problem has also been recognized. Foremost and the ultimate goal of these efforts should be the development and implementation of the seafood safety programme in all the countries of the region in order to meet the requirements of seafood importing countries. These efforts and cooperation will be significant to the regional maintenance of food security and economic growth; and fundamental to the sustainable utilization of the region's marine resources.

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Phycotoxins on the internet: an update

Harmful Algae News No. 8 (1994) indicated how to subscribe to an electronic mailing list called "Phycotoxins" which deals with local, national and international issues concerning all aspects of toxic phytoplankton. There are currently about 250 subscribers and the topics are wide-ranging, from reports of toxic blooms to discussions of laboratory techniques.

Here is updated information on how to join the list:

- send an e-mail message to: Lists@scotia.dfo.ca
- the 'subject' line can be left blank
- in the body of the message, write: "subscribe phycotoxins" on a line by itself (leave out the quotation marks, of course).

This mailing list is managed by Bill Silvert (Silvert@scotia.dfo.ca) and is located at the Habitat Ecology server of the Bedford Institute of Oceanography in Dartmouth, Nova Scotia, Canada. The server also has a World Wide Web page which provides access to the archives of postings to the mailing list as well as links to other sites relevant to phycotoxins research. Following is a sample of the type of information available from the BIO server on phycotoxin issues (the URL is: http://biome.bio.dfo.ca/science/toxins.html):

- Phycotoxins mailing list archive
- IOC Science and Communication Centre on Harmful Algae
- *Harmful Algae News* ON-LINE
- Domoic Acid and *Pseudo-nitzschia* Bibliography
- Canadian Federal Food Inspection Paper (July 1995)
- Phycotoxin Research in the Habitat Ecology Section at BIO
- PSP Toxins in Whelks

There are two other mailing lists that may also be of interest. ALGAE-L is maintained by Mike Guiry (mike.guiry@seaweed.ucg.ie) at the National University of Ireland, Galway, Ireland. It has discussions about any and all aspects of algae, but seems to be of interest especially to cyanobacterial fans. DIATOM-L is maintained by Roger Sweets (sweets@ucs.indiana.edu) at Indiana University, Bloomington, Indiana, USA. This list has had recent queries on domoic acid and *Pseudo-nitzschia*. Be prepared to receive cross-listings if you subscribe to all of these lists.

To subscribe to ALGAE-L:

- send an e-mail message to: Listserv@irlearn.ucd.ie
- leave the 'subject' line blank
- in the body of the message, write: "subscribe algae-l yourfirstname yourlastname" (without the quotation marks)

To subscribe to DIATOM-L:

- send an e-mail message to: Listserv@iubvm.ucs.indiana.edu
- leave the 'subject' line blank
- in the body of the message, write: "subscribe diatom-l yourfirstname yourlastname" (without the quotation marks)

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Seventh International Conference on Toxic Phytoplankton

The Seventh International Conference on Toxic Phytoplankton was held 12-16 July, 1995, in Sendai, Japan. More than 270 participants from 36 countries attended. Sixty talks and 151 posters were scheduled along with 5 evening round-table discussions/demonstrations, an afternoon excursion to the Matsushima Islands and the conference banquet.

The presentations were divided into a number of topics, the largest of which dealt with toxin chemistry, toxin production, and new methods for identifying toxins or toxic species. A variety of methods, including chemical and molecular techniques, are being used and new methods, or applications of old ones, are being devised and tested. Perhaps the most exciting new method for a taxonomist is the use of oligonucleotide probes to identify species of *Pseudo-nitzschia* in field samples and in near real-time. The method could be used for other toxic taxa, as well.

Another large topic was that of regional blooms and toxicity events. These are being reported from many places now, sometimes based on observations over several years. One new area where PSP toxins were found in shellfish was King George Island, Antarctica. Mortalities of fish and other marine organisms are also being reported from more areas. These are caused by a variety of organisms, principally dinoflagellates, and with kills often due to hypoxia as blooms decay.

Bloom dynamics provided another theme for lively discussion. A number of factors – including initiation of blooms by cyst germination; effects of temperature, salinity, and nutrient levels; local hydrographic conditions; and cell cycles – are all known contributors to bloom formation and maintenance.

The diatom genus *Pseudo-nitzschia* was poorly known until 1987 when *P. multiseries* was shown to produce the powerful neurotoxin, domoic acid. It was strictly an eastern Canadian phenomenon until 1991, when domoic acid appeared on the US West Coast. The Lund and Newport meetings in 1989 and 1991 featured papers that discussed domoic acid and detection methods, but they were few and primarily by Canadian authors. In Nantes (1993), there were three presentations from the US West Coast and mention of a *Pseudo-nitzschia* sp. from New Zealand. In Sendai, 16 papers/posters from 11 geographic regions treated *Pseudo-nitzschia* spp., primarily distributions, but also domoic acid production and food web effects. Domoic acid is no longer a strictly North American problem, although no human illnesses have been reported outside of eastern Canada and Washington State.

Other topics discussed included the effects of toxic species on aquaculture (*Dinophysis*, *Alexandrium*, and *Heterocapsa* spp.); monitoring/management programmes (with the exception of the Bay of Fundy, the other programmes discussed are relatively short-lived); bacteria/symbiosis (stationary phase dynamics and bacterial occurrences, co-occurrence of bacteria and toxin producers); heterotrophic and mixotrophic dinoflagellates (*Gymnodinium breve*, *Dinophysis* spp., *Chrysochromulina polylepis*, *Alexandrium* spp.); cyanobacteria (microcystins); and interactions between toxin producers and other organisms (domoic acid, bacteria, *Heterosigma*, *Gymnodinium sanguineum*, *G. catenatum*, and *Alexandrium* spp.).

At one workshop, the taxonomy of unarmoured dinoflagellates provided interesting discussion since the distinction between species is not as clear as originally thought. Although proposals were made for a more accurate classification, it was decided to continue as is until taxonomists have more time to study the species through live mounts and SEM (scanning electron microscopy). There were a number of beautifully hand-carved dinoflagellates to complement the session.

At the business meeting, it was decided to hold the conference after Vigo (June 1997) in Hobart, Tasmania, probably in January 2000. It was also decided to start the organization process for a formal society for the study of harmful algae, something that was suggested in Lund in 1989 and again in *HAN* No. 2 (1992). Max Taylor volunteered to do the initial organization (*see pages 12-13 of this issue*).

The conference was held in a small part of the large Sendai Civic Auditorium. It was well-organized and our Japanese hosts did a splendid job of making the conference a pleasant and successful one.

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First report of *Gymnodinium catenatum* from Atlantic Morocco

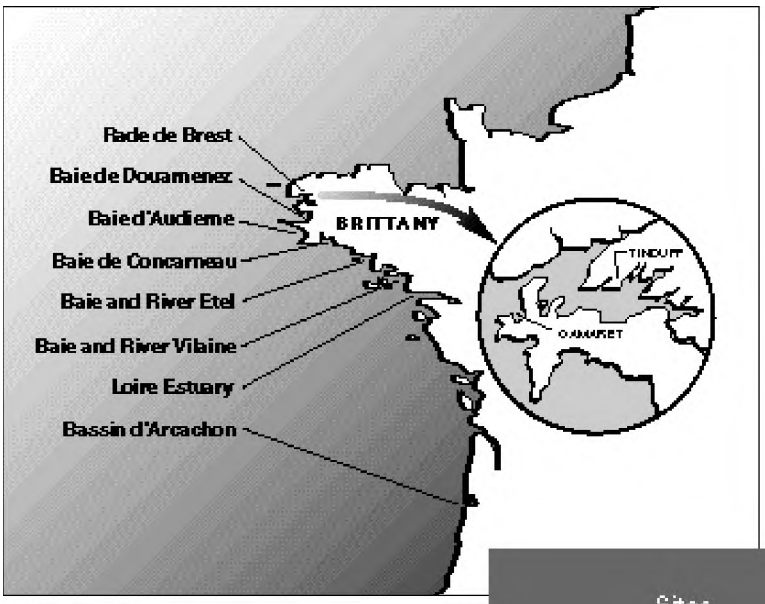
The first red tide records on the Atlantic coast of Morocco date from 1966[1]. Since then, they have appeared several times in late summer and early autumn. The years 1971, 1975 and 1982 were marked by shellfish contamination in mussels and clams, with PSP levels exceeding the international norm of 400 M.U. Consumption of shellfish was banned on those occasions. The toxic species responsible for the episodes were not identified.

In October-November 1994, a bloom appeared on the coast between Larache (35° 12' N) and Essaouira (31° 30' N) and led to human poisoning in the Casablanca area, where 74 people were hospitalized and 4 died. PSP levels in some shellfish samples reached 28,000 M.U.[2]

The species identified as responsible for this outbreak was the dinoflagellate *Gymnodinium catenatum*, which was observed in chains 6 to 32 cells long during the first two weeks of November. Maximal concentrations of about 30,000 cells/l were found near Casablanca (33° 39' N). The bloom occurred after some days of heavy rainfall and decreased surface salinities following the dry months of May to September. Surface temperature in October was about 17°C, the optimal temperature for growth of *G. catenatum*[3].

Ichthyotoxic events associated with *Gymnodinium* cf. *nagasakiense* on the Atlantic coast of France

A series of important blooms of the dinoflagellate *Gymnodinium* cf. *nagasakiense* occurred along the Atlantic coast of France, between May and August 1995, with sporadic developments remaining until September. Blooms were associated with spectacular kills of worms, urchins, gastropods, shellfish and later, fish. The blooms affected a long stretch of the French coast from the north coast of Brittany to the Arcachon Lagoon (Bassin d'Arcachon), primarily southern Brittany and around the Loire estuary (see map above). Important blooms of this dinoflagellate were already observed in this area in 1987, but cell densities (maximum 3 x 10^6 cells/L) and damages reported were lower.



The first dense population of *G.* cf. *nagasakiense* (3 x 10^4 cell/L) associated with common phytoplanktonic species was observed in the Concarneau Bay on 9 May. Subsequently, the cells spread rapidly along the northwest coast of Brittany, reaching Audierne Bay (22 May), Camaret (30 May), Douarnenez Bay (6 June) and the Brest Roads (Rade de Brest) (12 June); and extending southward to the Loire estuary (19 June). Populations tended to monospecificity, and densities reached 9 x 10^5 cells/L in coastal zones, and 6 x 10^6 cells/L in shallow areas. The highest concentration (48 x 10^6 cells/L) was observed in dense patches of a seawater tank at a scallop hatchery in the Brest Roads, on 27 June.

At the same time, massive mortalities of urchins, *Echinocardium cordatum*, worms *Arenicola* and later the bivalve *Donax vittatus*, were observed on beaches of Douarnenez Bay (densities of *G.* cf. *nagasakiense* were higher than 10^6 cells/L), and the whole trout production of a fishfarm in this bay died. In Concarneau Bay, wild and reared bivalves and gastropods died: *Cardium*, *Venerupis*, *Tellina*, *Glycimeris*, *Ostrea*, *Crassostrea*, *Solen*, *Mytilus*, *Patella*, *Haliotis*, *Littorina* etc. Mortalities of fish (congers and finfish) were reported. Shellfish kills accompanying *G.* cf. *nagasakiense* blooms extended to the Loire estuary, including the Etel and Vilaine rivers and bays. In the south of the Loire estuary, mortalities occurred in reared and wild mussel (100% in some places) and oyster beds, and provoked great quantities of stranded fish (congers) and finfish (*Pleuronectes*, *Solea*, eels). Surviving shellfish remained weaker.

Losses were important for shellfish producers, in scallop hatcheries, shellfish and fish nurseries. As a consequence severe reduction in future production is likely to occur. Anoxia was probably the first cause of mortalities in shallow areas and in the deeper layers during the blooms, but aeration in farm basins did not prevent damage. During this event, a second cause of mortality was also suspected: the toxicity of *G.* cf. *nagasakiense* cells. Consequently, the haemolytic property of seawater contaminated by *G.* cf. *nagasakiense* development was studied. The results of the haemolytic test conducted on horse red blood cells suggest between 800 and 1600 equivalent Haemolytic Units of Saponin/L, to be the toxic limit inducing mortalities of shellfish (see table below). These observations concerned scallop larvae in the metamorphosis stage, with a low metabolism excluding feeding and filtration. The highest values of haemolytic capacity measured in this study was 8000 eq. HU/L, in a seawater sample containing 4.4 x10^6 cells/L, sampled in Douarnenez Bay (6 June). The haemolytic property of seawater was not always correlated to the density of *G.* cf. *nagasakiense* cells in the samples.

Sites	Cell densities/L	Haemolytic Units/L	Losses
Baie de Douarnenez, 6 June	4,400,000	8,000	worms, urchins
Camaret, near fish farm, 13 June	311,900	1,600	no
Tinduff hatchery basin, 27 June	4,000,000	800	no*
Concarneau shellfish farm, 20 June	1,000,000	1,600	shellfish

* *in this case, metamorphosing scallop larvae*

Even though blooms of *G.* cf. *nagasakiense* are now a common occurrence along the coast of France during

(Cont'd on p. 9)

A safe and clean coast for Europe

All too often, local governments are taken by surprise when decisions are taken which have a major impact on their regions. This is true with regard to planned development as well as unforeseen disasters such as oil spills. For this reason, a series of workshops was held over the last few months in several European coastal cities. The results of these workshops will culminate in an action plan to be agreed upon during the ECOTOP Conference in Lille, 3-4 April 1996.

Europe's coasts are under siege. We're all familiar with the sight of burning or wrecked oil tankers and oiled seabirds; and news reports about near or actual marine disasters are increasingly common – ferry accidents, poison bags washing ashore, nuclear waste transport and disposal, to name a few. It has been reported that since 1981, the Belgian coast (only 65 kilometers long) narrowly avoided major environmental disasters on at least 43 occasions (*see insert*). However, there is a more systematic destruction of the coastal and estuarine environment which rarely gains press attention. With 50% of Europe's population living in coastal areas, and an increasing number of tourists flocking to European beaches, the competition for space and natural resources is intensifying. Every day, approximately 30 hectares of sand dunes and beaches disappear as a result of human activities. At the same time, the coastal environment is endangered by activities originating far away from their sites of impact. Agricultural runoff, litter deposition, oil spills and intentional discharges, discarded fishing gear, and the transport and disposal of hazardous materials at sea have long-range, transboundary impacts.

A wide variety of public and economic interests are directly affected by coastal disasters. Coastal towns and provinces, governmental coastal authorities, environmental and conservation organizations, tourist interests, and the fishing and insurance industries all benefit from preventing coastal disasters. However, there is little cooperation amongst these diverse groups to do so.

The prevention of coastal and estuarine disasters is commonly undertaken through two influence lines: political and diplomatic consultations and environmental protest actions. But so far, not enough has been achieved through these channels. Even when potentially strong and far-reaching resolutions are adopted, there is often a lack of consistent action in implementing them. This may be explained in part by the insufficient participation of a broad base of local and regional coastal authorities in developing conservation initiatives. Yet it is inevitably local governments which shoulder the cost of cleaning up messes after the fact.

For this reason, the European Union for Coastal Conservation (EUCC) has launched a project called European Regions for a Safe and Clean Coast (ERSCC), sponsored by the EC and several partner organizations. Its primary purpose is to enhance cooperation and facilitate the exchange of information between local and regional governments on the one hand, and scientific and ecological experts, non-governmental organizations, industry representatives, and intergovernmental organizations on the other.

Between August and November 1995, a series of workshops were held under the auspices of ERSCC. These workshops took place in Norway, Italy, Spain, the UK and The Netherlands, with different coastal problems emphasized in each workshop.

Vital information was gained concerning the role that local governments can (and in many cases, do) play, and the tools they need to do so, in addressing the problems described above. Most of the workshops proposed a series of recommendations aimed at solving these problems at the source. These will be presented at a pan-European conference to be held 3-4 April during ECOTOP '96 in Lille.

The Lille conference will try to bring together all existing coastal networks and platforms of local and regional authorities, individual coastal municipalities, provinces and regions, national authorities and services, and international non-governmental networks. In short, it is expected that the key players in coastal management will be present to lend their experience, and benefit from the experiences of others, in ensuring a sustainable future for European coasts.

Kelly Rigg, European Union for Coastal Conservation (EUCC), PO Box 11059, 2301 EB Leiden, The Netherlands; fax: (31-71) 512 4069.

Environmental disasters narrowly avoided

The following information is from an article in the Belgian newspaper Belgisch Dagblad *from 20 October 1994, written by Eddy Surmont.*

Between 1981 and 1994, at least 43 environmental disasters along the Belgian coast were narrowly avoided, according to oceanographer Thierry Jacques. During a presentation at a symposium in Oostende in October 1994, Dr Jacques reported that a total of 135 "incidents" occurred, 43 of which were considered to have presented a real environmental danger. Of these, 14 were ships that had run aground, 9 were collisions involving petroleum tankers, and at least 20 were accidents involving chemical tankers or ships with chemical cargoes. Each of these could have resulted in enormous environmental and economic consequences.

(Cont'd from p. 8, "Ichthyotoxic events")

the summer, a development of such magnitude this year and the resulting damage were unexpected.

This event was monitored following the phytoplankton monitoring network REPHY of IFREMER coastal laboratories: Concarneau, La Trinité, Nantes, La Rochelle, La Tremblade, Arcachon. The studies on toxicity were performed in the Laboratoire d'écologie pélagique of Brest, and information on local events provided by colleagues from the Comité local des pêches of Tinduff hatchery and SEMMI fishfarm.

Geneviève Arzul, Evelyne Erard-Le Denn, IFREMER, BP 70, 29280 Plouzané, France.

Catherine Belin, IFREMER, BP 1105, 44311 Nantes Cedex 03, France.

Elizabeth Nézan, IFREMER, 13 rue de Keroze, 29900 Concarneau, France.

Role of vascular land plants in late-Devonian oceanic anoxic events and biotic crises

During the mid- to late Devonian period, vascular land plants began to have an increasing impact on weathering processes and global geochemical cycles. This was a consequence of: 1) the advent of arborescence (tree-sized stature) which increased the depth of soil formation and surface area of chemical weathering and 2) evolution of the seed habit, which freed vascular land plants from reproductive dependence on moist habitats, and allowed colonization of drier upland areas. There was thus a major increase in the areal extent of deeply-weathered soils. These developments led to profound changes in fluvial particulate and nutrient fluxes, and to eutrophic conditions and anoxic events in the coastal ocean. These late Devonian events are recorded in black shales, many of which contain large amounts of algal remains.

Among marine organisms, about 21% of families and 50% of genera were eliminated during this interval. This was one of the "Big Five" mass extinctions of the Phanerozoic. This biotic crisis was unusual in two respects. It spanned an interval of about 30 million years, and extinctions were largely restricted to tropical marine invertebrates. High latitude and cold water species survived disproportionately.

The origin of the Late Devonian biotic crisis is unknown. Much recent research has been devoted to the possibility of a bolide impact, because evidence for such an event at the Cretaceous/Tertiary boundary has spurred considerable interest in bolides as a general mechanism for mass extinctions. Minor iridium anomalies and small concentrations of microspherules have been identified at or near the Frasnian/Framennian (F/F) boundary at several locales. However, siderophile-element ratios are incompatible with those of meteorites, and such anomalies are more likely the product of changes in redox conditions or concentration of metals by cyanobacteria. Other mechanisms have been proposed, e.g. climate change associated with global tectonics oceanic overturn, and sea-level elevation changes, although none of these theories have successfully accounted for the unique aspects of the Late Devonian biotic crisis: duration and selectivity.

The Late Devonian is also characterized by an unusual combination of major excursions or permanent shifts in a variety of sedimentologic and geochemical systems. Laminated organic-rich black shales record episodic development of widespread oceanic anoxia in many cratonic sequences during this interval. Marine evaporites of this age exhibit a +8 to +10 ‰ δ^{34} S excursion as a consequence of large-scale bacterial reduction of dissolved sulfate in association with burial of large quantities of organic carbon. Deposition of organic-rich black shales sequestered large quantities of carbon in the sedimentary reservoir, drawing down atmospheric CO_2 levels from *ca.* 12-16 PAL in the Early and Middle Paleozoic to *ca.* 1 PAL in the Permo-Carboniferous. Changes in atmospheric chemistry are reflected in +3 to +4 ‰ shifts in marine carbonate δ^{13} C and δ^{18} O values during the Late Devonian. The δ^{13} C shift resulted from increased burial of isotopically light organic carbon, causing carbon remaining in the dissolved inorganic reservoir to become isotopically heavier whereas the δ^{18} O shift was probably the result of cooling of tropical seawaters by *ca.* 10-12°C. Global cooling is recorded in continental glacial deposits of Late Devonian age.

Vascular land plant evolution

Although land plants appeared in the Late Ordovician or Early Silurian and vascular plants diversified in the Late Silurian and Early Devonian, full colonization of land surfaces is likely to have been a protracted process that continued through the Devono-Carboniferous. Initially, the impact of land plants on their physical environment was negligible owing to small size, limited biomass, and shallow depth of rooting. Land plants exerted a progressively stronger influence as they increased in size and became abundant and geographically widespread. In this regard, two evolutionary developments are of major significance: 1) the advent of arborescence and 2) the appearance of seeds.

Close temporal relationships exist between the advent of arborescence and the seed habit on the one hand, and Late Devonian anoxic and extinction events on the other. The correspondence is especially good between the appearance of seeds and black shales and extinctions of the Hangenberg Event, a temporal lag of less than 1 m. y. within the Late Famennian. The relationship between arborescence and earlier anoxic events is not as sharply defined because the development of tree-sized stature was a protracted event. It may be significant, however, that the onset of persistent oceanic anoxia in the Late Givetian coincided with the advent of large progymnosperms, and that the Kellwasser Event at the F/F boundary coincided with the peak abundance of archaeopterids. With regard to both trees and seeds, it is important to note that first appearances are less important than increases in abundance and biomass, which are harder to quantify but far more important in terms of geochemical consequences.

Development of modern weathering patterns

The appearance of arborescence and the seed habit resulted in large increases in the depth and geographic extent of "modern" soils (as opposed to pre-Devonian microbial protosoils; and precipitated a series of secondary effects during the Late Devonian. Transient effects are related to increased particulate and nutrient fluxes resulting from short-term environmental disturbances. Although dense vegetative cover generally exerts a stabilizing influence on landscapes, sparse vegetation is likely to be destabilizing owing to a low resistance to wildfires, drought, and landslides, all of which would lead to mechanical disruption of thin juvenile soils. Episodic disturbances of this type during the progressive development of mature soil profiles would be likely to cause regionally or globally elevated fluxes of particulates and nutrients.

Indirect evidence of elevated Late Devonian nutrient fluxes is provided by

high concentrations of marine algal matter in coeval black shales, implying high rates of primary productivity, and by enigmatic fossils of wide geographic but restricted stratigraphic occurrence, e.g. *Protosalvinia*, which may be the product of algal blooms. Widespread anoxia and eutrophication commonly result from pulses of nutrients to shallow epicontinental seas, as in modern semi-restricted marine basins such as the Black and Baltic Seas.

Long-term effects of the development of "modern" soils derive from the increased intensity of chemical weathering due to vascular land plants, approximately 7 times greater than that due to pre-vascular land plants. Changes in weathering processes are reflected in coeval shifts in the composition of clay mineral assemblages and in carbonate mineralogy. The dominant components of clay mineral assemblages shift from illite-chlorite in the pre-Late Devonian to smectite-kaolinite in the post-Devonian, reflecting an overall increase in the intensity of pedogenic chemical weathering. Carbonate sediments exhibit an abrupt step-wise decrease in dolomite abundance during the Late Devonian, from 40-80% in the Early-Middle Paleozoic to 30% in the Permo-Carboniferous, probably as a consequence of sequestering of Mg in smectites and reduced riverine Mg/Ca ratios. Thus, land plant evolutionary events have probably played a key role in determining the rate and direction of changes in terrestrial weathering processes, fluxes of particulates and nutrients to marine areas, and global atmospheric and oceanic chemistry.

T. G. Algeo, Department of Geology, University of Cincinnati, OH 45221, USA.

ERRATA FOR *HAN* NO. 10/11

In the last issue of HAN, under the title "Satellite imagery", it was suggested that Trichodesmium blooms occur in the Baltic. This is not so, and the article should have said *Nodularia spumigena*, which is a frequent bloom species there. We thank Susanna Hadju of Stockholm University for drawing attention to this error.

The Editors

VIII International Conference on Harmful Algae

Vigo, Spain, 25-29 June, 1997

First Announcement

The above mentioned conference will be hosted by the Instituto Español de Oceanografía (Spanish Oceanographic Institute), the Ministerio de Sanidad y Consumo, Laboratorio Comunitario de Referencia (Ministry of Health, European Union Reference Laboratory), and the Conselleria de Pesca, Marisqueo y Acuicultura de la Xunta de Galicia (Ministry of Fisheries, Shellfisheries and Aquaculture of the Galician Government).

Contributions are invited on all aspects of toxic and harmful algae (including brackish and freshwater cyanobacteria, and ciguatera agents). Papers which address the following problems will be especially welcome:

- Empirical and model studies of the population dynamics of harmful algae blooms, including the coupling of physical and biological processes, harmful algae-zooplankton interactions, growth patterns etc.;
- Ecophysiological, biochemical and toxicological aspects of harmful and toxic algae species;
- Problems related to monitoring, aquaculture, public health and the management of harmful algae events and their economic impacts.

Travel information
Vigo is the most populous city of Galicia (NW Spain), and lies about 1 hour by road from the province's capital, Santiago de Compostela, and 2 hours from Oporto in Portugal. Santiago and Oporto both have international airports, and there is a daily (except Sundays) air link between Vigo and Paris, as well as several daily flights between Vigo, Madrid and Barcelona.

Please provide the following information ***before 15 March 1996***:

Name: .....

Affiliation: .....

Full mailing address: .....

.....

Tel.: ..... Fax: .....
(country code) (city code) (number) *(country code) (city code) (number)*

E-mail: .....

I intend to present: (i) a paper ☐ (ii) a poster ☐

on the following topic: .....

Tentative title(s): .....

For further information, contact:

Beatriz Reguera,
Conference Coordinator,
VIII International Conference on Harmful Algae,
Instituto Español de Oceanografía,
Aptdo 1552, 36280 Vigo, Spain.

The Second Announcement will be sent in May 1996.

Unfinished business from Nantes and Sendai:

An international society for the study of harmful algal blooms

by F.J.R. "Max" Taylor

One of the primary concerns of those of us who look to the future of the study of HABs is continuity and a non-governmental umbrella to bring together scientists and others who work in this highly multidisciplinary area. So far, this initiative has been kept alive remarkably well by the series of seven or eight conferences that have focused on this area of research. They have been supported by a relatively stable, but arbitrarily appointed, International Advisory Committee. The volumes that have been published as proceedings of these and related meetings serve as a central archive of the scope and progress of this field and provide an invaluable unifying resource and key to the articles in the primary literature. Whenever I have a student who wants to begin a study in any aspect, I use the proceedings as a first reference. Unfortunately, these volumes have become very expensive, especially for libraries and those that don't attend the conferences, and so it is very welcome news to learn of IOC-UNESCO's plans to publish the Sendai proceedings.

The conferences have happened largely due to willingness to take on this onerous task by a succession of sacrificing organizers, most recently Dr Patrick Lassus in Nantes and Prof. Takeshi Yasumoto in Sendai, together with their faithful local helpers. The next one will be in Vigo, Spain, in 1997, followed by Hobart, Tasmania, in the auspicious year 2000. However, can we count on such generosity forever? Some offers in the past were made only after a blank silence when requests were made.

There is little doubt that the field will remain a dynamic, highly multidisciplinary and exciting area of scientific endeavor and it needs an organization that reflects the direct desires of those active in the area of study. It would also be beneficial if high priority research areas could be identified (for funding purposes) and scholarships and prizes could be awarded. An important feature to remember is that this society can do whatever the membership collectively wants it to do: endorse new approaches, create a journal (unlikely at present and we are very fortunate to have the *Harmful Algae News* to communicate with), offer travel assistance, give awards, etc. Such an initiative has been supported by the IOC-HAB Programme as an essential step towards preserving this as a multidisciplinary research area.

At the conference on red tides and other harmful algal blooms in Nantes, the statutes associated with the formation of an international society was presented in writing by Dr Ted Smayda, ably assisted by six other colleagues. There was a near-unanimous endorsement that such a society should be formed, but there were three problems that prevented a resolution of the proposal at the conference: participants were caught unawares and hadn't thought the implications through, there was not enough time to consider such an important proposal, and the scope and officers of such a society were not defined. A call for officers was made, but who was to vote on such persons?

In my mind there is no question that this is an initiative that should not die. In my scientific lifetime I have watched this area grow from simple phenomenological investigations (by those faced with such an outbreak) to a multidisciplinary effort involving time series field studies aimed at anticipating such events (whose scope expands annually), and laboratory experiments aimed at elucidating the autocology of harmful species, and the chemistry and toxicology of natural products. In addition, public health authorities and epidemiologists have become involved. Aquaculture has added a significant dimension, both in terms of avoidance of impact and increasing the risk of human exposure. From the observed pattern so far, it is my opinion that there is no coastline in the world that does not experience one or more of the phenomena that we term HABs, absence of reports being a reflection of absence of awareness. This does not mean that some areas are not worse than others, not that eutrophication, climate change or introductions might not aggravate such problems. These are important questions that such a society should focus upon.

So, to summarize, the Nantes conference voted for the formation of a society and started to vote for officers, but the issue was not resolved, largely due to a lack of clarity as to who should vote. What has become clear is that the officers of a society can only be chosen, as usual, by the membership of such a society and so the first step is to establish such an entity.

The scope of the society has not been formally defined, although at Sendai there was a vote by the International Organizing Committee for Conferences to include both benthic phenomena, leading to ciguatera and related problems, and freshwater and marine cyanobacterial blooms. My own view is that this decision should be driven by the scientific benefits: if inclusion leads to better science, so be it, even if the community gets less cosy. In reality, while country representation is obviously influenced by where the conferences are held, the degree of participation by subdisciplines, such as pharmacology or epidemiology, fluctuates dramatically. The society can appoint section leaders whose responsibility would be to report on advances in all areas.

Various suggestions for the name of the organization were made, including the Society for Harmful Algal Blooms (which sounds like we support and/or promote such events), the International Harmful Algal Bloom Society (IHABS, The Smayda committee choice) or my own suggestion, the International Society for the Study of Harmful Algal Blooms (ISSHAB), a search for acronyms involving HABs having failed to produce anything catchy. Toxic is nice but too exclusive. Members will be asked to suggest alternatives (it would be helpful if they had acronyms like DOOM; DEATH; APOCALYPSE; SAVIOURS OF SOCIETY, etc.).

In order to progress further I have been asked to informally act as the *animateur*, i.e. the individual charged with bringing life (not caricature) to this con-

(Cont'd on p. 14)

Society use Only
Membership No
Section(s)
Dues received, current?

Application for founding membership in International Society for the Study of Harmful Algal Blooms (ISSHAB)

Name: .....

Affiliation: .....

Address: .....

Fax: ..... *E-mail*: .....

Are you on the mailing list for *Harmful Algae News*?	Yes	No
If not, do you wish to be added?	Yes	No
Are you in the IOC Directory of HAB Experts?	Yes	No
If not, do you wish to be in the next edition?	Yes	No

Please indicate, to a maximum of four, which of the following potential sections (to be confirmed, with chosen leaders, at future meetings), you are most interested in:

- ☐ I. Taxonomy/ genetics-cycles,
- ☐ II. Ecology (marine planktonic),
- ☐ III. Ecology (marine benthic, ciguatera),
- ☐ IV. Ecology (freshwater, including cyanobacteria),
- ☐ V. Biogeography/Introductions,
- ☐ VI. Toxicology,
- ☐ VII. Pharmacology,
- ☐ VIII. Epidemiology/Public Health etc.

(Regional sections will be added later)

Information on your background experience will be requested. Meanwhile, would you rate yourself as:

- ☐ very experienced
- ☐ moderately experienced
- ☐ beginning professional research in the field
- ☐ a graduate student in the field
- ☐ an enthusiastic amateur
- ☐ other

Please enclose a cheque or money order for US$ 20.00 to:

Henrik Enevoldsen
IOC Science and Communication Centre on Harmful Algae,
University of Copenhagen, Botanical Institute,
Dept. of Mycology and Phycology,
Øster Farimagsgade 2D, DK-1353 Copenhagen K., DENMARK.
Tel: (45) 3313 4446; fax: (45) 3313 4447
E-mail: henrike@bot.ku.dk

(Cont'd from p. 12, "ISSHAB")

cept. I do so happily – aided considerably by the *Harmful Algae News* to communicate with you (thanks to IOC-UNESCO and Tim Wyatt, the Editor) and by Henrik Enevoldsen, IOC, who will provide initial help with informal Membership registration and fee accountancy – as I firmly believe in this as a project with lasting value. The process I suggested, and which was endorsed by a planning session of the Sendai conference committee, is an initial membership registration in this and the next issue, to produce a core of serious foundation members, followed by a call for nominations of officers (President, Secretary, Treasurer, with written agreement of their willingness to serve) and a vote (by members only), so that at the next conference my role will have been completed successfully and we will have three key officers and a membership identified. Objectives and procedures will be the focus of the plenary meeting in Vigo.

Let's go!

F. J. R. "Max" Taylor, Department of Oceanography, University of British Columbia, Vancouver, B.C., Canada.

(Cont'd from p. 7, "Gymnodinium catenatum")

This species may have been transported across the Gulf of Cadiz from the Iberian Peninsula, where blooms occurred in Galicia in September and Portugal in October the same year. Alternatively, it may be autochthonous to Moroccan coastal waters[4], with resting cysts germinating under favourable climatic conditions, and may have been responsible for the PSP events of 1971, 1975 and 1982. Cyst distribution studies could provide support for this hypothesis.

References

(1) Essaid el Feydi, A. 1977. L'intoxication paralytique par les fruits de mer. Thèse no. 44, Fac. med. phar., Rabat, 106 pp.
(2) Inst. Scient. Pêches Marit., Casablanca, 1994, Internal Reports.
(3) Blackburn, S.I. *et al.*, 1987. *J. Phycol.*, 25:577.

Tahri Joutei Laila, Institut scientifique des pêches maritimes, 2 rue Tiznit, Casablanca 01, Morocco.

ANNOUNCEMENT

NATO Advanced Study Institute

Jointly with SCOR and IOC

"The Physiological Ecology of Harmful Algal Blooms"

Bermuda Biological Station
27 May to 6 June 1996

A NATO Advanced Study Institute (ASI) on the Physiological Ecology of Harmful Algal Blooms will be convened at the Bermuda Biological Station for Research (BBSR), 27 May to 6 June 1996. This activity is organized by a working group co-sponsored by SCOR (Scientific Committee for Oceanic Research) and IOC (Intergovernmental Oceanographic Commission) of UNESCO. Funding for the meeting is provided by NATO, SCOR and IOC.

The objectives of the ASI will be to assess our understanding of the fundamental physiological and ecological issues underlying harmful algal blooms (HABs), to identify inadequacies, impediments, and promising areas for future research, and to advance and disseminate new approaches and technologies. The ASI will follow the format specified by NATO, which requires a 10-day meeting involving many different types of activities (e.g. lectures, field and laboratory demonstrations, group discussions). Efforts will be made to summarize major advances in two main areas: (1) The ecology of critical groups of toxic phytoplankton (= "Autoecology"); and (2) the ecophysiological processes and mechanisms that affect toxic bloom formation and the production of phycotoxins (= "Ecophysiological Processes and Mechanisms"). It is hoped that one outcome of this approach will be the elucidation of critical factors that regulate blooms of related species as well as of toxic species in general.

If you feel you can contribute to this ASI and wish to participate, please return the completed "Application" (on facing page) to: Don Anderson at the address given below. Funds for travel will be provided to some participants, but selection will in part be determined by the extent to which each individual can provide his or her own travel funding. Other criteria that will be considered in participant selection include restrictions on the number of non-NATO participants, the need to balance attendance among NATO countries as much as possible, and the need to provide opportunities for students as well as advanced workers. The number of participants at the workshop will be restricted as well, as organizers are targeting 80 to 85 participants given the facilities at the BBSR.

Since it is likely that there will be more than this number interested in participating, a steering committee will select participants on the basis of the above constraints, as well as the expertise that they will bring to the workshop, their willingness to help with demonstrations or lectures, their relevance to the physiological ecology theme, and funding support. The ecophysiology theme is rather narrowly defined and thus not all HAB disciplines will be appropriate for the meeting.

For further information, please contact:

Donald M. Anderson, ASI Director,
Biology Department, MS #32,
Woods Hole Oceanographic Institution,
Woods Hole,
MA 02543-1049, USA.
Fax: (1-508) 457 2134
E-mail: danderson@whoi.edu

Deadline for submission of applications is **1 February 1996**. Participants will be notified soon thereafter.

APPLICATION

NATO Advanced Study Institute

Jointly with SCOR and IOC

"The Physiological Ecology of Harmful Algal Blooms"

Bermuda Biological Station

27 May to 6 June 1996

Name: ..... **Nationality:** .....

Institutional affiliation:.....

Address: .....

.....

Tel: ..... **Fax:** ..... **E-mail:** .....

Educational status: .....
(student, professor, postdoc, industry, researcher, other)

Gender: (for accommodation purposes) .....

Scientific interests: (brief sentence) .....

Your proposed contribution to this ASI:
(Examples include demonstrations of techniques, posters, group discussions, videos, models, etc. Oral presentations will be by invitation only. If you have no specific contribution, indicate *"plan to participate as student".*)

.....

.....

.....

Funding: On-site costs for the ASI will be US$ 900 for housing and meals. Indicate below the amount of financial support you are requesting to travel to and attend the ASI. This information will be considered in selecting participants, as space and budget are both limited.

I am prepared to contribute $ _____ from my own sources, and request $ _____ for travel, plus $ _____ for housing and meals.

If you have proposals pending that might provide financial assistance, please provide details, including the date when you will know of final decisions on funding:

.....

To assist the committee that will be selecting participants, please submit a short curriculum vitae. If you are a student, please substitute a statement of interest or qualifications and furnish the name of your professor.

All students and lecturers must attend the entire Advanced Study Institute. A non-refundable deposit will be required upon acceptance to the ASI. Notification of your participation will be in early February 1996.

Please return this form no later than 1 February 1996 to Donald M. Anderson, MS #32, Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA. Fax: (1-508) 457 2134.

New HAB Publication Series

"Amnesic Shellfish Poisoning (ASP)" has just been published as Volume 1 of the new HAB Publication Series in *IOC Manuals and Guides* No. 31.

During the Second Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, a Task Team on Aquatic Biotoxins was established. The Task Team on Aquatic Biotoxins was formed in order to initiate, catalyze and activate interaction between relevant organizations and Member States with interest in the chemistry and toxicology of algal toxins. This document is a product of the collaboration between organizations and IOC Member States participating in the Task Team on Aquatic Biotoxins.

In this manual, a review is provided of the chemical and toxicological aspects of Amnesic Shellfish Poisoning (ASP). The document contains information on chemical structure, chemical data, on where to obtain standards and reference materials, origin and occurrence, chemical analysis, mouse bioassay, epidemiology, mechanisms of action, symptoms and therapeutics. The practical use of the document has been highlighted in agreement with the Members of the Task Team on Aquatic Biotoxins.

Volume 1 in this series is a supplement to the IOC Manual on Harmful Marine Microalgae (in preparation).

Other volumes on Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP) and other important toxins are planned to be printed in the future.

This document is free of charge and can be obtained by contacting: Dr Helle Ravn, The Harmful Algal Bloom Programme Office, Intergovernmental Oceanographic Commission (IOC) of UNESCO, 1, rue Miollis, 75732 Paris Cedex 15, France; fax: (33-1) 40 56 93 16; e-mail: hab.ioc@unesco.org

Future events

IOC/SAREC-DANIDA Training Course on the Taxonomy and Biology of Harmful Marine Phytoplankton, University of Mauritius, Mauritius, 5-14 February 1996. Lecturers include Dr F.J.R. Taylor (Univ. of British Columbia) and Dr Jacob Larsen (Univ. of Copenhagen/IOC Centre). Organized by the IOC Science and Communication Centre on Harmful Algae, Copenhagen, and co-sponsored by SAREC (Swedish International Development Agency) and DANIDA (Danish International Development Agency).

IX International IUPAC Symposium on Mycotoxins and Phycotoxins, Rome, 27-31 May 1996. Contact: Istituto Superiore di Sanità, Segreteria per le Attività Culturali, Viale Regina Elena 299, 00161 Rome, Italy.

Third IOC-DANIDA Training Course on the Taxonomy and Biology of Harmful Marine Microalgae, University of Copenhagen, Denmark, 15-26 July 1996. This advanced course will focus on identification and preparation techniques supplemented by lectures on different aspects of the biology of harmful algae. Teaching staff will include: Dr Yasuwo Fukuyo (Univ. of Tokyo), Prof. Øjvind Moestrup (Univ. of Copenhagen), Dr Rhodora Corrales (Univ. of the Philippines), Dr Jacob Larsen (Univ. of Copenhagen/IOC Centre), Dr Enda Graneli (Univ. of Lund). Organized by the IOC Science and Communication Centre on Harmful Algae and the Botanical Institute, Dept of Phycology, and co-sponsored by DANIDA (Danish International Development Agency). Priority will be given to applicants from developing countries.

For application forms, contact: the IOC Secretariat, or the IOC Science and Communication Centre on Harmful Algae, Botanical Institute, Ø. Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; fax: (45-33) 13 44 47.

1st European Phycological Congress, Cologne, 11-18 August 1996. There will be a session on "Red tides and algal-bacterial interactions" convened by Drs Malte Elbrächter and Karen Steidinger. Congress Secretariat: Prof. Dr Michael Melkonian, Botanisches Institut, Universität zu Köln, Albertus-Magnus-Platz, D-50923 Köln (Germany); tel.: (49-0) 221 470 2475; fax: (49-0) 221 470 5181; e-mail (internet): mmelkon@biolan.uni-koeln.de

14th International Diatom Symposium, 2-8 September 1996, Tokyo, Japan. Convener: Dr Hiromu Kobayasi, Tokyo Diatom Institute, Honcho 3-8-9-813, Koganei-shi, Tokyo 184 Japan. Contact: Shigeki Mayama, Department of Biology, Tokyo Gakugei University, Konagei-shi, Tokyo 184, Japan; e-mail: mayama@u-gakegei.ac.jp; tel.: (81-0) 423 25 211; fax. (81-0) 423 24 9832.

Fifth Canadian Workshop on Harmful Marine Algae, St. John's, Newfoundland, Canada, September 1996. (*More information will be given in next issue of HAN.*)

Literature for developing country libraries

Limited copies of the following titles are available from IOC's HAB Centre in Denmark, to libraries of marine science institutions *in developing countries only*:

- ***Proceedings of the Sixth International Conference on Toxic Marine Phytoplankton***. P. Lassus *et al.* (eds.), 1995.
- ***The Genus Alexandrium Halim (Dinoflagellata)***. E. Balech, 1994.
- ***Marine Phytoplankton. A Guide to Naked Flagellates***. C. Thomas *et al.* (eds.), 1994.
- ***Marine Phytoplankton. Identifying Marine Diatoms and Dinoflagellates***. C. Thomas *et al.* (eds.), 1996.

Applications must be submitted and signed by the responsible librarian, and should indicate why the requested title is of specific interest to your institution and its ongoing research or teaching. *No requests from individuals will be honoured.* Address applications to:

IOC Science and Communication Centre on Harmful Algae,
University of Copenhagen,
Botanical Institute,
Dept. of Mycology and Phycology,
Øster Farimagsgade 2D,
DK-1353 Copenhagen K, Denmark;
tel.: (45-33) 13 44 46;
fax: (45-33) 13 44 47;
e-mail: hab@bot.ku.dk

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

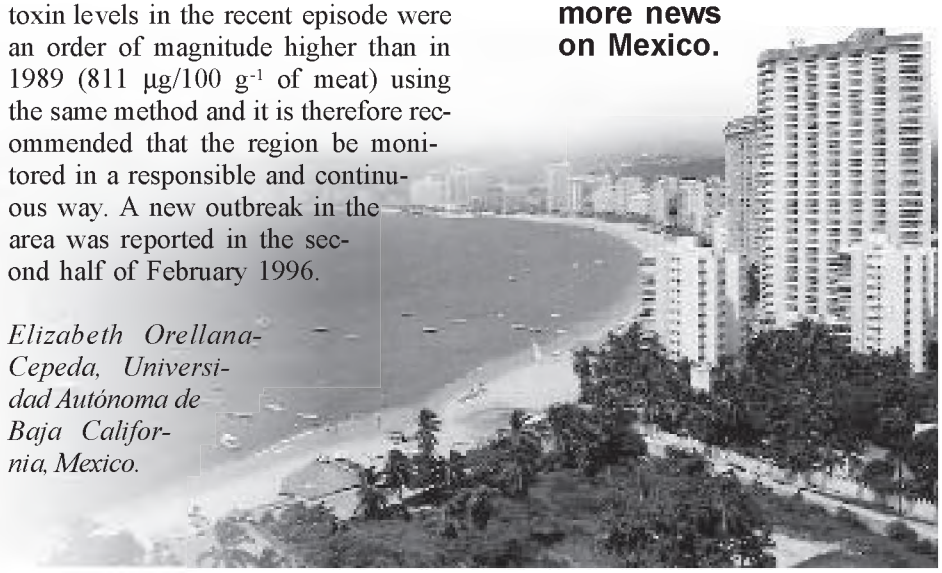
An IOC Newsletter on toxic algae and algal blooms No. 14

Pyrodinium toxicity reappears near Acapulco

In November 1995, a bloom of *Pyrodinium bahamense* var. *compressum* was recorded off Acapulco in the state of Guerrero, Mexico. It subsequently moved southeast along the Mexican and Guatemalan coasts in the early days of December. Toxin levels estimated by bioassay reached levels of 8,549 µg/100 g^{-1} in oyster (ostión) meat, and measures to protect public health were taken. The saxitoxin was identified by HPLC and compared with the results of the bioassay using the same extracts.

This bloom was not completely unexpected, since high concentrations of the same species (up to 1.7 x 10^6 cells l^{-1}) occurred in November 1989 in the same region,(1) and off the coast of Guatemala in summer 1987.(2) The toxin levels in the recent episode were an order of magnitude higher than in 1989 (811 µg/100 g^{-1} of meat) using the same method and it is therefore recommended that the region be monitored in a responsible and continuous way. A new outbreak in the area was reported in the second half of February 1996.

See also article on page 4 for more news on Mexico.



Elizabeth Orellana-Cepeda, Universidad Autónoma de Baja California, Mexico.

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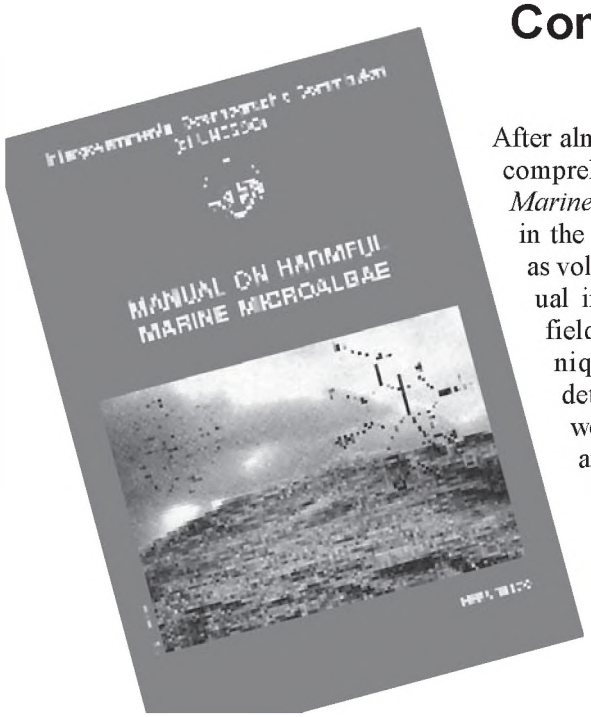
The Intergovernmental Oceanographic Commission announces HAB manual



After almost four years of preparation, a comprehensive *Manual on Harmful Marine Microalgae* has been published in the IOC Manual and Guides series as volume No. 33. The 500-page manual includes chapters on taxonomy, field techniques, culturing techniques, algal toxin chemistry, detection and quantification, as well as description of monitoring and management practices.

The manual is edited by Drs. G. Hallegaeff, University of Tasmania (Australia), D. Anderson, Woods Hole Oceanographic Institution (USA), and A. Cembella, Institute for Marine Biosciences (Canada). Forty-two leading scientists in the field of harmful algae have contributed to the various chapters.

The manual is available from the IOC Science and Communication Centre on Harmful Algae, University of Copenhagen (see address on back page). The manual is free of charge but a fee of US$30 will be charged to cover postage and handling. A check issued in US$ for Mr. H. Enevoldsen, IOC, should accompany all orders.

Excepted are marine science libraries and scientists in developing countries which can apply for delivery free of charge.

◆ WEST COAST OF IRELAND

Winter toxicity of unknown aetiology in mussels

In November 1995, a shellfish poisoning event in The Netherlands, in which at least eight people became ill after eating mussels, was traced to a consignment of mussels from a site on the west coast of Ireland. The consignment of mussels was dispatched from Ireland on 10 November, 1995. The symptoms displayed by those who became ill included nausea, vomiting, severe diarrhoea and stomach cramps. DSP rat bioassays carried out in The Netherlands by Dr P. Hagel (RIVO-DLO) on the leftovers of one of the meals gave a strongly positive (+++) response indicating high toxicity.

The illness of the shellfish consumers and the positive rat bioassay results were surprising as mussel samples from the same area which were tested by rat bioassay at the Fisheries Centre, Dublin, on 12 October 1995 and 31 October 1995, had given negative results, and the DSP toxins commonly found in shellfish in Ireland, okadaic acid (OA) and DTX-2, were present at low levels (4.9 and 2.0 µg/100 g of tissue respectively on 12 October and 3.7 and 2.6 µg/100 g of tissue respectively on 31 October). Furthermore, water samples from the area taken at the same time did not contain any toxic phytoplankton species and the phytoplankton associated with DSP toxicity in Ireland, *Dinophysis acuta* and *D. acuminata*, had not been observed in water samples from the area since mid-September.

When advised of the illness in consumers, the harvesting of shellfish from the affected area was prohibited and a further sample analysis was undertaken. From 27 November 1995 to 13 February 1996, rat bioassays on samples taken weekly from several locations in the area gave positive results (+++) even though no known toxic phytoplankton species were detected in water samples. OA and DTX-2 were, however, detected at low levels, e.g. 1.4 and 2.3 µg/100 g of tissue of OA and DTX-2 respectively in samples taken on 27 November 1995. DSP mouse bioassays on samples tested by Dr Margaret McDonald at the Central Science Laboratory in Aberdeen, Scotland, have also given positive results with mice survival times of approximately 90 minutes. The mouse bioassays suggested a "neurotoxin-like" response but samples tested by Dr Marisa Fernández at the European Union (EU) Reference Laboratory on Marine Biotoxins (EU-RLMB) in Vigo, Spain, did not reveal any PSP toxins, and PSP mouse bioassays carried out at the Fisheries Research Centre, Dublin were negative. HPLC analysis of samples at the EU-RLMB also confirmed the presence of OA and DTX-2 at only trace concentrations. Domoic acid was undetectable.

Bacteriological examination of samples by Dr Mary Seavers at the BIM (Irish Sea Fisheries Board) laboratory in Dublin showed that the common pathogens, such as faecal *Streptococci*, *Staphilococcus aureus*, *Salmonella* spp., and *Vibrio* spp., were not detectable. Rat bioassays on samples which had been boiled for 30 minutes and samples which had been processed in a certified shellfish depuration facility also gave positive results.

At present neither the toxin involved nor the organism responsible for production of the toxin have been identified, and harvesting of shellfish from the affected area is still prohibited. While both the rat and mouse bioassays have been criticized for their lack of specificity, in the present case their usefulness is clear in detecting the presence of an unknown toxin which can produce DSP symptoms in shellfish consumers.

Terry McMahon and Joe Silke, Marine Institute, Fisheries Research Centre, Abbotstown, Dublin 15, Ireland.

◆ ALEXANDRIA WATERS

First recorded toxic *Alexandrium minutum* Halim bloom

Almost 40 years ago in the summer of 1957, marked discolouration of the neritic waters of Alexandria Harbour (Egypt) was observed, and the causative organism, a small dinoflagellate, proved upon examination to be a new genus and new species, *Alexandrium minutum* Halim[1]. Since then, progressive eutrophication has encouraged the development of algal blooms in this area, and they are now a recurrent phenomenon in the warm season. Other common species, in addition to *A. minutum,* are *Prorocentrum triestinum*, *P. minimum* and *Skeletonema costatum*[2,3]. So far however, no cases of toxicity have accompanied these blooms, although *A. minutum* is known to produce PSP elsewhere[4]. Fish kills have occurred occasionally, for example in the Eastern Harbour in May 1987, when they were attributed to localized oxygen stress and/or gill clogging.

During the first week of October 1994, a bloom of *A. minutum* extended along 20 km of coast with a peak concentration of 24 x 10^6 cells/L. The surface cell density showed a diurnal periodicity, decreasing from sunset to dawn due to nocturnal downward migration, and the population density tended to become quasi-homogeneous down to the bottom at 4.5 m. The bloom appears to have been triggered by the input of domestic sewage to a stratified water column. It dissipated after a few days under the mixing effect of NW winds. Unlike earlier records, the bloom was accompanied by widespread toxic events: (a) massive fish mortality occurred along the area affected by the bloom. The dead fish included pelagic (*Epinephelus* spp) and benthic species (*Murena* sp); (b) all fish and invertebrates inside the public aquaria of the National Institute of Oceanography and Fisheries were killed in spite of continuous aeration. The aquaria are supplied with seawater from the Eastern Harbour where the bloom started;

◆ GREEK SEAS

Algal bloom monitoring is needed

The red tide phenomenon has been known since antiquity and was recorded by Roman authors like Strabo and Pliny as early as 208 BC in fresh waters. It was also recorded in the Bible (Exodus, Chapter 7, lines 17-21) but it is impossible to find out the causes of red tides in those remote times.

In modern times, red water has appeared periodically in Greek coastal areas since 1956 and perhaps a little earlier (see map). The first reliable list of micro-organisms found in red blooms was recorded by Anagnostidis[1] in the Gulf of Thessaloniki (N. Greece). In this extensive list, there are species of dinoflagellates (*Protoperidinium* spp., *Gonyaulax* spp., *Heterodinium* spp., *Ceratium* spp.), diatoms (*Rhizosolenia* spp., *Chaetoceros* spp., *Licmophora* spp.), and sulphur bacteria (*Chromatium vinosum*, *Macromonas* spp., *Thiospira* spp., *Beggiatoa alba*, *Spirillum* spp.). Among the algae there were no toxic species. The harm observed in these cases was probably due to deoxygenation of the water, which led to fish deaths. Sulphur bacteria might contribute to creating a toxic environment, particularly when they appear in massive concentrations as Anagnostidis[1] observed. This, however, has not been proved.

In the Gulf of Saronikos (Southern Greece), a red tide was recorded in 1983[2] and attributed to the massive growth of the dinoflagellate *Gymnodinium breve* (10^7 cells/L). Eutrophication, calm waters and high temperatures were thought to be responsible at the time.

Red blooms also appeared in 1978 in the Gulf of Kavala (Northern Greece), associated with a shipwreck which was full of phosphate fertilisers. There are no records of the micro-organisms found in that bloom. In August 1986, following an effort to haul up this ship, the bloom phenomenon reappeared and lasted 20 days causing fish deaths. The dominant species in the bloom was *Alexandrium tamarense* (10^7 cells/L) observed at depths of 0-7 m.[3] There were no records of human poisoning. Associated species were the diatoms *Skeletonema costatum*, *Rhizosolenia fragilissima* and *Chaetoceros curvisetum* and the dinoflagellate *Protoperidinium stenii*.

An extensive algal bloom associated with fish deaths was again observed in the Gulf of Kavala in May 1994. Again there were no records of human poisoning. The only available information concerning the algal composition is that the dominant species was *Noctiluca scintillans*.

Nobody has examined red tides in detail in Greek waters, although they are not rare, particularly during recent years. There is certainly progressive eutrophication, but there are no monitoring programmes.

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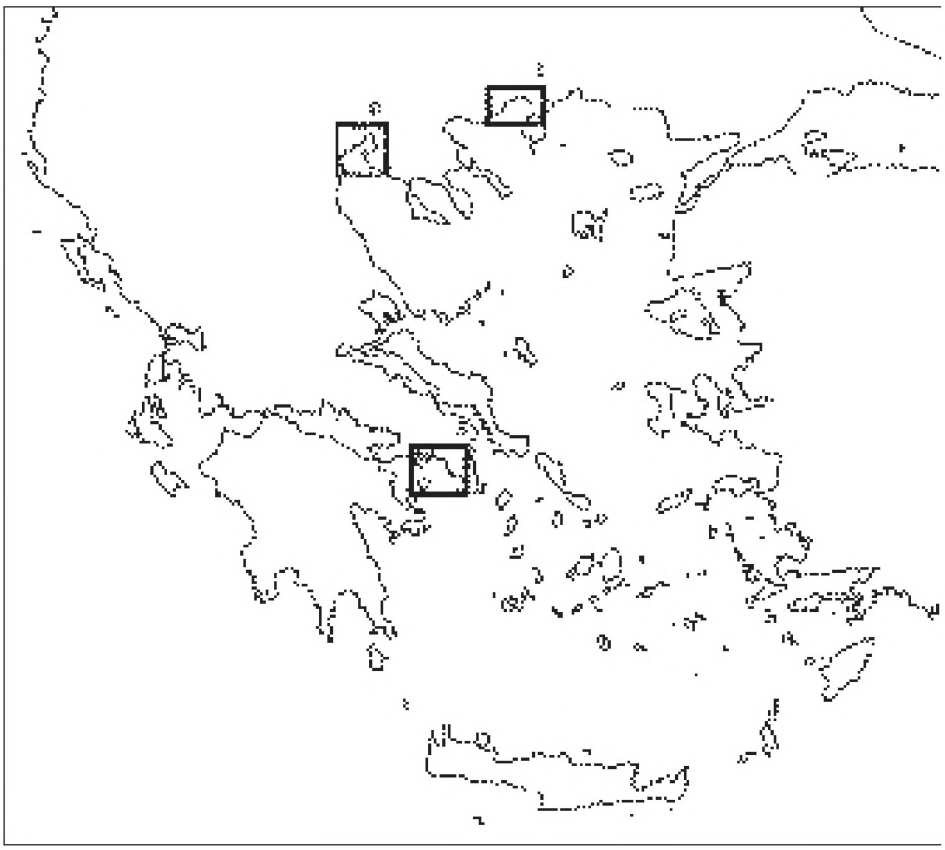
Dr. Alexis Tsangridis, National Agricultural Research Foundation, Institute of Fisheries Research, N. Peramos, 64 007 Kavala, Greece.



Map of Greek localities where red tides occurred: (1) Kavala Gulf; (2) Thessaloniki Gulf; (3) Saronikos Gulf.

(Cont'd from p. 2, "Alexandria waters")

(c) dead fish exhibited symptoms of toxicity with yellowish colouration of the body and gills; others appeared to have lost their equilibrium, swimming on their side or upside down; (d) hundreds of rock crabs migrated out of the water onto the beach (*Eriphia* sp., *Pachygrapsus* sp., etc.).

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◆ MEXICO

Recent observations of HABs and toxic episodes

The Pacific coast of Mexico has experienced a number of harmful algal blooms in the past. The year 1939 witnessed the first record of what was identified as *Gonyaulax catenella* (later identified as *Gymnodinium catenatum*) in the Gulf of California, Mexico (Graham, 1943). Unfortunately, the lack of a suitable system to monitor and follow up events in the area has always precluded the consistency and accuracy of all previous observations and studies. In 1991, the Center for Biological Research (La Paz) was invited to participate in a task group dedicated to the study of marine biotoxins in Baja California waters. It is no surprise that first reports were incomplete and even wrongly addressed. For example, we were looking for tetrodotoxin (TTX) in shellfish meat as a cause of a massive death of clams at Concepción Bay, Baja California, Mexico, in summer 1993, but of course our assays turned out to be negative. A year later, we used the mouse bioassay for PSP detection in clams and obtained the first positive results with values as high as 23,000 mouse units per 100 g of clam tissue. Suspected dinoflagellates involved were *Prorocentrum* sp. and *Alexandrium* sp., together or in succession (Sierra-Beltran *et al.*, 1995). Yet we could not relate the presence of such toxins to clam mortality.

Similar findings were reported in a twelve-year study carried out at Mazatlán Bay by Cortés-Altamirano and Núñez-Pasten (1992), in which the appearance of PSP-like toxins was confirmed. The phenomenon took place preferentially during winter, and was persistent throughout that season. Therefore, it was assumed that toxic outbreaks of PSP in the area are periodic and frequent phenomena on the Pacific coast of Mexico. In this study, the most common toxic species, although neither the most abundant nor the most frequent, was *Gymnodinium catenatum*, to which two cases of intoxication with human casualties have been linked, one in April 1979 with two dead, and another in 1988 with 10 dead.

During autumn 1995, the famous port of Acapulco and neighbouring southern states (Guerrero, Oaxaca and Chiapas) on the Pacific coast of Mexico were affected by a "red tide" event that covered several miles of coastline. The PSP toxin concentration was officially reported to be between 120-2000 µg per 100 g of meat, and the organism responsible presumably belonged to *Alexandrium* spp. Six people died and many more were sick (136 in November) as a result of the event. In addition, fish and turtles in great numbers were found dead on the beaches. Other microalgae previously involved in severe intoxication events in the area are *Pyrodinium bahamense* v. *compressum* in two separate events in 1989, as well as *Gymnodinium catenatum* with as many as 99 poisoning cases and 3 deaths in the region the same year. In 1995, and farther north on the California Peninsula, two persons died after eating puffer fish fillet presumably contaminated with TTX. Although this event is not related to a HAB, it should be considered within the context of human exposure to marine toxins. Unfortunately, when human casualties occur, it turns out to be difficult to obtain samples from the Health Authorities in Mexico (as indeed in other countries) that may help to corroborate the hypothesis. It seems that, more than neglecting the event, authorities are afraid of recognizing that we live in areas which are susceptible to toxic episodes, and therefore fail to provide education and orientation. A recent outbreak of domoic acid in the southern tip of Baja California in early 1996 was the culprit of more than one hundred dead brown pelicans (*Pelecanus occidentalis*) in the area. No dramatic change in weather conditions, nor in the aquatic environment were associated with this mortality. Sardines and mackerel, both fresh and canned, were analyzed for the presence of diatoms (*Pseudo-nitzschia* sp.) and domoic acid. Only the fresh specimens showed positive results to both factors. The hypothesis thus is that the pelicans ate the fish and, feeling sick, they returned to their shelter where they finally died. On their way, they regurgitated the food, so when captured, both dead and sick animals had in their stomachs only small amounts of toxin, as determined by the mouse assay and chromatographic techniques. Again in January 1996, a quite unrelated toxic event was observed in a reservoir located in Sonora, Mexico. In this case, thousands of freshwater fish appeared dead on the water surface, with their gills and eyes invaded by mucilaginous cyanobacteria including *Micrococcus* spp. This is a common phenomenon in many water reservoirs around the world and requires the application of prevention measures to avoid economical losses and, more important, human fatalities.

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J.L. Ochoa, A. Sierra-Beltrán , A. Cruz-Villacorta, A. Sánchez-Paz, E. Núñez-Vázquez, Centro de Investigaciones Biológicas del Noroeste, Box 128, La Paz 23000, BCS, Mexico.

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***Harmful Algae News* is now available on Internet at http://www.unesco.org/ioc/news/newslet/han.htm**

Second IOC regional scientific planning workshop on harmful algae blooms in South America

This workshop was held in Mar del Plata (Argentina) from 30 October to 1 November 1995, at the National Institute for Fisheries Research and Development (INIDEP). The workshop was organized by IOC, with the logistic support of UNESCO's Regional Office for Science and Technology in Latin America and the Caribbean (ROSTLAC) in Montevideo (Uruguay).

The meeting was convened to: (a) present and discuss national reports on Harmful Algal Blooms in South America (FANSA, Florecimientos de Algas Nocivas en Sudamérica); (b) update progress in understanding the dynamics of FANSA, including thematic and methodological aspects; (c) evaluate the most promising strategies to study FANSA and reduce their effects on marine ecosystems; (d) develop proposals for a regional training programme, and regional research objectives, integrated within the existing IOC international programme on HAB; and (e) establish collaborative mechanisms between the operational programmes (monitoring, resource protection, public health) being developed in each country.

There were 34 participants, from the countries which comprise the southern cone of South America (Argentina, Brazil, Chile, Peru, and Uruguay) together with observers from Colombia and IOC. The meeting was chaired by José I. Carreto (Argentina). Beatriz Reguera (Spain) acted as rapporteur and representative of the IOC Intergovernmental Panel on Harmful Algal Blooms. As in the previous meeting, this workshop was an excellent forum for the interchange of information on the region and showed that significant progress had been made in the intersessional period. Amongst the more important items, we emphasized the establishment of a monitoring programme in the state of Santa Catarina (southern Brazil), an area of increasing mussel cultivation, the functioning of a modern laboratory for toxin analysis in the University of Chile, the submission of a scientific project by Argentina and Chile (in cooperation with France and Germany) to the European Union programme for cooperation with third countries (INCO), and the beginning of cooperation between Uruguay and Brazil regarding the mass mortality of benthic organisms.



Participants in front of the National Institute for Fisheries Research, Mar del Plata, Argentina.

The most important results of this workshop were:

(1) *educational elements*: A recommendation that IOC should support two regional courses, one on the taxonomy and culture of harmful algae (in Brazil), and another on biochemical techniques for the study of HAB (in Chile). There was general agreement on the need to establish long-term educational programmes which would allow countries in the region to develop the appropriate expertise to deal with all aspects of coastal zone management, including studies related to HABs.

(2) *scientific elements*: Plans were made for two regional projects, one on the ecology of *Alexandrium tamarense* and its associated toxicity in the southwest Atlantic (Argentina, Brazil and Uruguay), and a second on the ecology of *Alexandrium catanella* in the Austral region with the aim of identifying the possible causes of its recent geographic expansion (Argentina and Chile). The importance of international cooperation for the development and financing of these projects was stressed, and the international programme between France, Germany, Chile, and Argentina on "Phytoplankton responses to environmental disturbance caused by salmon cultivation in Latin America" was cited as an example.

(3) *operational elements*: several needs were identified: (a) consolidate nascent monitoring programmes in southern Brazil and Peru, and assist expert training at a regional level; (b) organize regional intercalibration of toxin determination by bioassay, with the help of the European Union Reference Laboratory for Marine Biotoxins.

Finally, it was proposed that the next meeting of the IOC-FANSA Working Group should be held in Chile in 1996 following the annual marine science meeting there. The meeting concluded with a Creole barbecue, and camaraderie in which participants enjoyed the delights of Argentinian meats and wines.

The full report of the meeting is available in Spanish and English from the IOC Secretariat (*IOC Workshop Report* No. 123).

José Ignacio Carreto, INIDEP, C.C. 175, 7600 Mar del Plata, Argentina.

Advanced phytoplankton course



Mast-IOC-ONR Training Course, Naples, Italy, 25 September to 14 October 1995.

An advanced phytoplankton course was organized by the Marine Botany Laboratory (Naples) in co-operation with the Marine Botany Section of the Department of Biology of the University of Oslo and held in Casamicciola (Island of Ischia, Naples) from 24 September to 14 October 1995. This was the sixth in a series of courses which started in 1976 following a recommendation of the SCOR Working Group on Phytoplankton Methods (SCOR WG33). UNESCO supported the recommendation and made it possible to organize the first three courses (1976, 1980 and 1983) in Norway at the Marine Botany Section of the University of Oslo. The fourth, fifth and the present course were organized by the staff of the Marine Botany Laboratory of the Zoological Station in Naples. The main goal of the course was to train and upgrade qualified students on the identification of phytoplankton species by light microscopy. Financial support for the course was provided mainly by the European Community-MAST programme. Other funds were provided by the Naples Zoological Station and by the Intergovernmental Oceanographic Commission of UNESCO. Carl Zeiss S.P.A. kindly lent 22 microscopes for the practical sessions.

Amongst the faculty were some of the former teachers of the course in 1976 (G.R. Hasle and J. Throndsen) and 1983 (K.A. Steidinger), plus the staff of the Marine Botany Laboratory (D. Marino, M. Montresor and A. Zingone). Other teachers and lecturers included M.J. Chretiennot-Dinet, J.D. Dodge, C.B. Lange, J. Larsen and C.R. Tomas, almost all of whom had participated as students in one of the previous courses.

The course consisted of a theoretical part, including lectures on the general morphology and taxonomy of the different marine phytoplankton groups, followed by practical sessions, with examination of fixed, cultured and slide material in the light microscope. The relevant techniques for collecting and culturing phytoplankton specimens, as well as displaying the essential taxonomic characters, were presented, and everybody was given the possibility to practise. The course also included a series of seminars covering several aspects of phytoplankton taxonomy, phylogeny, biogeography and ecology. The identification of harmful algae received particular attention during the practical sessions, and three workshops dedicated to harmful and nuisance diatoms, dinoflagellates and other phytoflagellates, respectively, were also held to further assist in species recognition and cover other aspects relevant to the topic.

All the participants took the opportunity to improve their skills in species identification and the application of different techniques, as well as to go through the most up-to-date taxonomic literature. A pleasant collaborative atmosphere was established during the sessions, which promoted a full exchange of knowledge and experience. A complete report of the course is available as *IOC Training Course Report* No. 36, from the IOC secretariat.

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Harmful Algae News – use it!

As many of you already know, this newsletter is published up to three or four times a year by UNESCO's Intergovernmental Oceanographic Commission in Paris. The present circulation is around 3,000. The initial idea for this newsletter emerged during discussions at a meeting of the IOC-FAO/OSLR *Ad hoc* Group of Experts on Harmful Algal Blooms, held in Paris, 31 January to 2 February 1990. The first issue was circulated in early 1992, and since then about 130 articles from 31 different countries have appeared.

The 8th International Symposium on Harmful Algae will take place in Vigo, Spain, 25-29 June, 1997. So far, more than 500 people from 64 countries have registered their interest in attending.

The recently published *IOC International Directory of Experts in Toxic and Harmful Algae and their Effects on Fisheries and Public Health* (UNESCO, 1995) lists 578 people from 59 countries.

These numbers show that we belong to a large and active community, and that we like to talk to each other.

But very few are using the opportunity the newsletter provides to let us know their news and views!

This note is to encourage you to do so. We welcome articles on all aspects of harmful algae from descriptions of events to public health and aquaculture issues, including taxonomy, ecology, monitoring, epidemiology, management strategies, new techniques, models, ... the list is almost endless in such a multidisciplinary field as ours.

Articles can be of any length from 25 to 1500 words, and can include black and white photographs or illustrations. The newsletter appears in English only at present, but if you do not feel sufficiently confident to write in English, we are prepared to translate short articles from the following languages: Chinese, Danish, French, German, Italian, Norwegian, Philippino, Portuguese, Spanish and Swedish.

Articles should be sent to the *HAN* Editor: Tim Wyatt, Instituto de Investigaciones Marinas, Eduardo Cabello 6, 36208 Vigo, Spain; fax: (34-86) 292762; e-mail: twyatt@nautilus.iim.csic.es

ICI/IEO practical course on toxic phytoplankton

A common situation in developing countries is that a few experts, sometimes in quite remote places, have to deal with varying aspects related to the occurrence of harmful algae events. Bearing this in mind, an ICI (Instituto de Cooperación Iberoamericana) practical course on toxic phytoplankton was held in the Instituto Español de Oceanografía (IEO) in Vigo, Spain, from 13 to 29 February 1996.

The course was addressed to Iberoamerican phytoplanktologists from governmental institutions who are in charge of establishing or improving regional programmes for the investigation and control of harmful algae. The objective of the course was to give the participants a broad perspective of the different aspects of harmful algae that need to be dealt with in these programmes, and to train them and provide practice in the basic techniques which are applied in contingency plans for the occurrence of toxic/noxious algal events. Course topics included the multipurpose sampling of "red patches" for taxonomic identification of the causative organisms, the isolation and culturing of the suspect microalgae, and the preparation and preservation of extracts of field plankton populations, bivalve meat, and dead fish viscera for mouse bioassays and HPLC analyses.

The course included theoretical classes, but special emphasis was given to practical workshops in which participants had the opportunity to collaborate in various activities. These included: (i) the identification of noxious or toxic algae from their own samples or from cultures maintained in the IEO culture collection in Vigo; (ii) monitoring the development of freshly isolated single cells and the subsequent establishment of monoalgal cultures from them; (iii) joining in routine monitoring cruises in Ría de Vigo on board the R/V *J.M. Navaz*, and further storage of CTDs and plankton data; (iv) preparing extracts of concentrated algae cultures and bivalve meat for PSP, DSP and ASP toxin detection by mouse bioassay at the European Union (EU) Reference Laboratory for Marine Biotoxins; and (v) demonstrations of toxin analysis of algal and bivalve meat extracts by high performance liquid chromatography (HPLC).

The instructors in the course were from the Spanish Oceanographic Institute (B. Reguera, S. Fraga, I. Bravo and J.M. Franco), the EU Reference Laboratory (A. Martínez, M.L. Fernández, A. Míguez, E. Cacho) and the Galician Department of Fisheries, Mariculture and Aquaculture (J. Mariño, J. Blanco), together with an invited speaker from the Marine Sciences Institute in Barcelona (M. Delgado). The participants visited the Galician Monitoring Center and the Laboratory of the Association of Canning Producers (ANFACO, Asociación Nacional de Fabricantes de Conservas). They were also taken in a traditional mussel raft boat for a demonstration of the seeding and thinning of mussel ropes in Ría de Arosa, which is the "pearl" of the Rías Bajas of Galicia and produces about three quarters of the total of Spanish mussel production.



The ICI/IEO Course participants: (from the left) J. Franco (IEO), M. Frangopulos (Chile), L. Rorig (Basil), G. Ferrari (Uruguay), B. Reguera (IEO); (front row, from left) L. Morquecho (Mexico), N. Santinelli (Argentina), E. Delgado (Peru).

Beatriz Reguera, Instituto Español de Oceanografía, Aptdo. 1552, 36280 Vigo, Spain.

Spanish commitment to the IOC HAB Programme

On 28 March 1996, an agreement was signed between Spain and the IOC to establish a science and communication centre on harmful algae at the Instituto Español de Oceanografía in Vigo, Spain. The centre is expected to open in January 1997, and will help implement the IOC HAB Programme with respect to research and capacity-building activities. The Vigo centre will be complementary to, and work closely with, the IOC Science and Communication Centre on Harmful Algae at the University of Copenhagen, Denmark, as well as with the HAB Programme Office in Paris. More news on the activities of the centres will be published in coming issues of *HAN*.

Editor's bibliographical notes

■ Some years ago I received an over-exposed nth-generation photocopy of an editorial in the *Philippines Journal of Science*, from 1908.(1) During the copying and recopying, it has been marked by at least five different hands, and rubber stamped twice. The names of Jay (or Fay? probably John!) MacLean and Rudolf Hermes appear amongst these "graffiti". It has been "indexed" three times. The text has been marked at some time with one of those blunt felt pens with which some readers persuade themselves they can extract the essentials from a document (and mutilate textbooks). The title, "PERIDINIUM", was type-set in capitals; nevertheless, someone found it necessary to mark the initial "P" with "c/", meaning – capitalize it! On the copy before me is written in my own hand "From Rhodora, 23 July 1990", the same Rhodora Corrales who contributes regularly to this newsletter. And it bears the stamp of the ICLARM library. All this in only one and a half pages – these are the hallmarks of a classic!

Authorship is commonly attributed to H.M. or A.M. Smith, both of which are technically incorrect but reasonable, since six of its seven short paragraphs seem to be taken from a report he wrote. The first paragraph, by the anonymous editor, informs us that Smith was Deputy Commissioner of the United States Fish Commission steamer *Albatross*, so that H (for Hugh) is meant. The putative quotation from Smith's report begins: "There have been at least three visitations of Peridinium in Manila Bay during the current year..." A few details follow. The blooms were monospecific, due to luminescent chlorophyll-bearing organisms, and formed red or rust-coloured "wavy bands several yards wide and hundreds of feet long" at the surface. They were associated with the disappearance of fish and birds. Fish and molluscs in an aquarium on board the *Albatross* were mostly killed when the ship entered Manila Bay, but some were saved by filtering the water supply through bolting cloth.

The last paragraph of this editorial begins: "During the prevalence of this pest, the Manila markets contain much less fish than normally, and many stalls are entirely vacant. Inquiries among the fishermen show that there is a decided falling off in the catch and that some dead fish are reported in the baclods", which are cages built of canes with funnel-shaped entrances designed to allow fish to enter but not escape again.

Does the wording suggest that the pest was then a recurrent problem? Be that as it may, the introductory paragraph mentions "a great mortality among fish" attributed by the residents to "dumpings from the sanitary barge Pluto...". Brongersma-Sanders(2) wrote: "SMITH (sic) demonstrated that this mortality is caused by red water of dinoflagellates". He did no such thing, of course, but merely raised the possibility. The editorial (last sentence) in fact says: "..the known mortality among aquatic creatures being so small as to afford a noteworthy contrast to the ravages of Peridinium in America and Japan".

So far as I know, the possibility that the editorialized events in Manila Bay in 1908 may have been due to *Pyrodinium* has not been specifically raised. One apparently vital piece of information is lacking – no paralytic shellfish poisoning (PSP) was recorded. Is this detail so vital? Several points suggest not entirely: (i) there are surprisingly few planktonic species in the West-Pacific region which are both fish killers and "Peridinium", and records from recent decades indicate that *Pyrodinium* is the only commonly occurring species associated with such events; (ii) the main vector of PSP in the Philippines is the green mussel, *Perna viridis*, which in 1908 was consumed in trivial quantities. Most green mussels then were in fact taken from the canes with which the baclods were constructed. It was not until 1962 that this mussel was cultivated, and then only experimentally. Substantial production did not begin until some years later. The first major PSP episode was in 1983;(3) (iii) in Papua New Guinea "There are some villages where the link between red tides and food poisoning is well recognized and tradition dictates that shellfish are not consumed for several months after the occurrence of red tides".(4) Perhaps equivalent traditions formerly existed in the Philippines, even if the danger were infrequent. It could be argued that the many cases of PSP which have been reported in the Philippines in recent decades have been caused at least in part by the adoption of new dietary habits and the loss of traditional taboos – a consequence of urbanization.

■ This year's meeting of the ICES-IOC HAB Working Group was held in Brest, France, from 17 to 20 April. An issue of a regional newspaper for 19 April 1996 informed us (amongst other items) that "Au Danemark, 1er exportateur européen de moules, une toxine diarrhéique fait des ravages". There are only two errors in these thirteen words, "1er"– third or fourth would be more accurate, at least according to FAO statistics; and "ravages" (again!)? At least nobody told me that Denmark has been laid waste recently. The report continues "En Alaska, l'*Alexandrium* a provoqué trois décès, à la fin des années 80". Not very startling, but not very accurate either. The problem in Alaska is paralytic rather than diarrhetic, and *Alexandrium* is in the paralytic class. "Trois décès"? Possibly true. My records say five. Further on: "On a découvert des kystes d'algues fossiles datant de 350,000 millions d'années" which is getting on for about 100 times some estimates of the age of life on earth. The estimates may not be reliable.

The last sentence of this report maintains that a toxin from USA introduced by a ship into Australian waters caused damage to the Black Sea anchovy! ("...une toxine, venue des USA par bateau, a commis des dégâts énormes, sur les anchois de la mer Noire"). C'est magnifique la confusion! Two stories have probably been conflated here, first, the invasion of the Black Sea by the comb jelly *Mneniopsis leydii*, a native of the western Atlantic, and the associated "eco-catastrophe",(5) and, second, the introduction of the toxic dinoflagellate *Gymnodinium catenatum* to Tasmania.(6) "La France n'est pas épargnée" the writer said, meaning from toxic algae, and, we must add, "du problème de communication entre les scientifiques et les journalistes."

References:

1) Anon. 1908. *Philippines J. Sci.*, Ser. A, 3: 187-188.

2) Brongersma-Sanders, M. 1948. Verhand. d. Koninkl. Nederland. Akad. v. Wetensch. afd. Natuurk., Sect. 2, 45(4): 112 pp.

3) Estudillo, R.A. 1984. Bureau of Fisheries and Aquatic Resources, Tech. Pap. Ser., 7: 8 pp.

4) Rironiro, K., Sims, L.D. 1989. pp.149-151. In: G.M. Hallegraeff, J.M. Maclean (eds.), *Biology, Epidemiology and Management of Pyrodinium Red Tides* (ICLARM).

5) Sorokin, Yu. 1993. *FAO Fisheries Rep.*, 495, Annex II, 45-59.

6) Hallegraeff, G.M. and Bolch, C.J. 1992. *J. Plankton Res.*, 14: 1067-1084.

Tim Wyatt

VIII International Conference on Harmful Algae

Second announcement and call for papers

The first circular announcing the VIII International Conference on Harmful Algae (Vigo, Spain, 25-29 June 1997) has met with an enthusiastic response: more than 500 experts from 67 countries have pre-registered. The present announcement requests participants to register formally, and to submit abstracts of communications to be presented orally or in poster sessions. Once more, the title of the conference has been changed to adapt itself to the increasing interest of the international community to marine and freshwater microalgae, planktonic or benthic, and their toxicity and other harmful effects. The topics of the conference will be:

I. Ecology and oceanography of harmful algae events

- Models
- Population dynamics
  - Physical-biological interactions
  - Harmful algae-zooplankton interactions
  - Interactions with viruses, bacteria or other algae
  - Growth
- Physiology (with special emphasis on toxin production)
- Biogeography

II. Taxonomy

- Species description by conventional optical methods
- Alternative methods: molecular probes, DNA sequencing, image analysis, neural networks and others

III. Toxins

- New toxins and advances in analytical methods
- Alternative methods: molecular probes, cytotoxic assays
- Transmission of toxins through the food web – Uptake and elimination by bivalves and other organisms

IV. Management of toxic and harmful algae events

- Descriptions of events – Impact on public health and on resources
- Monitoring of harmful algae and phycotoxins
- Mitigation of the harmful effects – Prediction

V. Pharmacological and epidemiological studies

The conference will comprise: keynote lectures; oral and poster presentations; and round-table discussions. Professor Ramon Margalef will give the opening address. Professor F.J.R. "Max" Taylor will organize the inaugural meeting of the International Society for the Study of Harmful Algae. At this meeting, the officers of the Society (President, Secretary and Treasurer) will be elected, and the goals and statutes of the Society decided. A call for nominations will be included in a forthcoming issue of *Harmful Algae News*. Persons nominated must indicate their willingness to stand. Only paid-up members of the Society will be eligible to vote, although everybody will be welcome to attend the session. For membership (fee of US$20), see p. 11 in this issue of *HAN*.

Registration

Up to 1 March 1997, the registration fee is 48000 ptas (accompanying person: 18000 ptas). After 1 March 1997, the fee rises to 60000 ptas (20000 ptas). The fee for the accompanying person includes the banquet and all social activities of the participants, plus two other excursions of cultural interest in the Galician region. Payment should be made in Spanish pesetas. Please make payment by wire transfer, or by credit card, carefully following the instructions in the form. The registration form can be requested by e-mail: insovigo@cesga.es; or by fax: (34-86) 492351.

Submission of abstracts

Papers submitted for consideration as oral or poster contributions within the topics outlined in this announcement are welcome. Authors should submit a one-page abstract of 200-300 words in one original and two copies to the Conference Secretariat for review and selection by the organizing committee. Abstracts accepted will be accessible to the harmful algae community via the web page of the IOC HAB Programme (http://www.unesco.org:80/ioc).

Abstracts will be photocopied for distribution in an abstract volume. Please confine the text to a width of 170 mm and a height of 115 mm. The title should be given in capital letters followed by full names and addresses of authors on the 2nd line below. The text should follow on the 2nd line below the address and be single spaced. If using a typewriter, 10 pitch/inch font is preferred. If you are using scaleable fonts, do not use anything smaller than 10 points. To facilitate correspondence, please ensure that your full name(s) and address(es) appear on the abstract. If you are not sure that the mailed abstracts will reach us on time, please send a copy by fax or e-mail.

The organizers reserve the right to decide whether a contribution will be presented orally or as a poster, and may suggest that a proposed poster contribution be presented orally if helpful in organizing thematic sessions.

Submission of manuscripts

The Conference Proceedings containing accepted papers (oral contributions and posters) will be published and made available to participants as part of the registration fee. One of the main objectives of the organizers is to have the proceedings published before the end of 1997. To facilitate this, three double-spaced typed copies of the manuscript, based on talks or posters, must be submitted to the conference organizer not later than 20 April 1997. Each manuscript should be in English. Make sure your manuscript is linguistically acceptable when sending it for preview and within the limited length when retyped in camera-ready copy. A selection of the papers will be made on the basis of criteria related to their relevance to the themes of the conference and their originality. Manuscripts, as corrected or amended by two referees, will be returned to their authors before, during or after the Conference. The final version of the typed (single-space) manuscript should be prepared according to instructions which will be circulated at a later date. The editorial committee reserves the right to refuse any manuscript not meeting their requirements.

Posters

Posters should be prepared to occupy a space measuring 80cm in width and 120cm in height. Posters must be legible from a distance of 2 metres, which means your font should have a minimum height of 5mm. All contributions, including those presented orally, must be displayed as posters.

Deadlines

15 December 1996	Submission of abstracts
1 March 1997	Registration
20 April 1997	Submission of manuscripts for review
31 July 1997	Submission of manuscripts in publication format

IOC/SAREC-DANIDA Training course on taxonomy and biology of harmful marine microalgae

University of Mauritius, 5-14 February 1996

A regional course specifically on the identification and biology of harmful marine microalgae was requested by the IOC Sub-commission for the Western and Northern Indian Ocean (IOCINCWIO). Occurrences of harmful algae and harmful incidents have been known in the region for centuries, but so far harm has been relatively restricted. With the growing interest in shellfish and fish farming, monitoring for harmful species becomes essential in relation to export of seafood products as well as in relation to protection of local consumers.

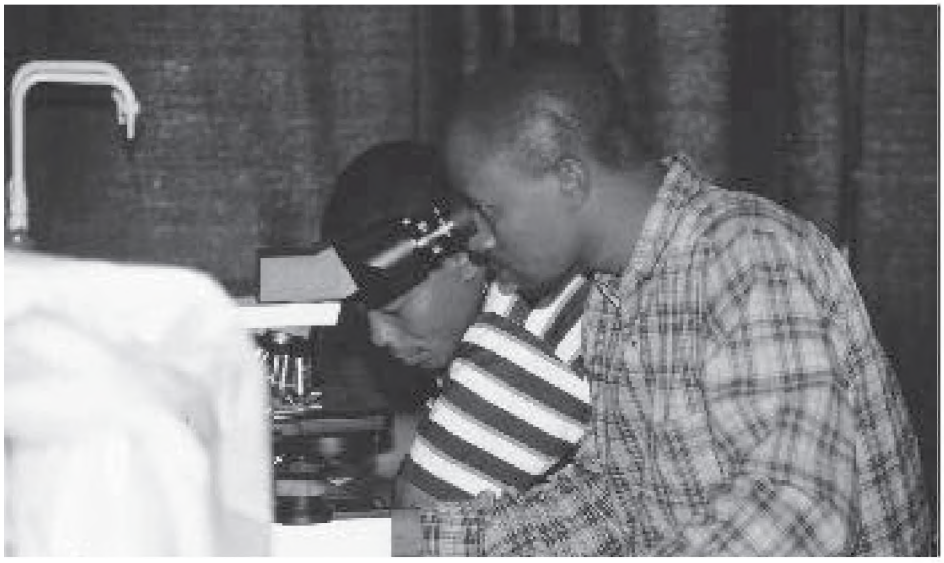
The local organizer was Professor I. Fagoonee, Dean, Faculty of Science, University of Mauritius, in collaboration with the IOC Science and Communication Centre on Harmful Algae (Denmark). The Course was funded jointly by SAREC (Sweden) and DANIDA (Denmark).

Eleven participants from Kenya, Mauritius, South Africa, and Tanzania were selected from 25 applicants.

The objective of the course was to improve the participants' taxonomic skills in order to enable them to make reliable identification of phytoplankton species causative of harmful algal events. The course was aimed at participants who had a basic knowledge of phytoplankton taxonomy (main taxonomical groups and their characteristics) and were familiar with the basic use of microscopes. Applicants who were themselves trainers were given priority.

The course was opened by the Hon. S. Obeegadoo, Minister of Fisheries and Marine Resources, by Prof. I. Fagoonee, Dean, Faculty of Science, University of Mauritius, and by H. Enevoldsen, IOC.

The course was a combination of practical work in the laboratory, microscopy, lectures, and demonstration of methodology in the field. Light microscopes were used for demonstration of specimen features and preparation techniques. A selection of modern and classical taxonomic literature was available during the course. Lectures were given by Dr Max Taylor, University of British Columbia, Canada, and by Dr Jacob Larsen and Henrik Enevoldsen, IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Denmark. They focused on taxonomy, but also included ecology, sampling techniques, and toxicity tests. Dr. J.P. Quod, ARVAM, La Réunion, was a guest lecturer on ciguatera problems in the region.



Mr. Wycliffe Shimbira (KMFRI, Kenya) and Charles Lugomela (IMS, Tanzania) working on the identification of dinoflagellates.

Each participant was provided with a 400-page "take-home" training course compendium, summarizing the state of the art with respect to harmful microalgae taxonomy and identification, as well as a summary of the lectures given during the course. After the course, the participants were given a copy of the *IOC Manual on Harmful Marine Microalgae*, which partly replaces the compendium. Each trainee was provided with a 20 µm mesh plankton net, a Sedgewick Rafter Counting Slide for quantitative work, and basic equipment for simple Artemia toxicity tests.

Most participants had brought their own samples, and time was reserved for them at the end of the course to undertake work according to their individual wishes. This provided an opportunity to address specific questions, and many trainees had an opportunity to prepare and bring home quality scanning electron or light microscopy photos of specimens from their own samples.

Preliminary discussions were held on regional follow-up action with the Chair of IOCINCWIO, Prof. Ragoonaden and the Dean of the Faculty of Science, University of Mauritius. Such action included possible research projects, as well as Ph.D. and master's degree projects in the framework of cooperation between the IOC Centres and research institutions in the region.

At the end of the course, a questionnaire for assessment by trainees was distributed. In general, the participants were very satisfied, and felt that the objectives of the course had been met. Many participants noted that they would feel more confident in their future work in identifying toxic algal species. Several indicated that, upon returning home to continue their monitoring work, they would reconsider their previous species identifications. Some participants expressed the need for better library facilities. The University of Mauritius provided excellent accommodations for the course, and the modern and well-equipped laboratories helped to ensure the success of the training course.

The report is available as *IOC Training Course Report* No. 40.

Society use Only
Membership No
Section(s)
Dues received, current?

Application for founding membership in the
International Society for the Study of Harmful Algal Blooms (ISSHAB)

Name: .....

Affiliation: .....

Address: .....

Fax: ..... *E-mail*: .....

Are you on the mailing list for *Harmful Algae News*?
If not, do you wish to be added?
Are you in the *IOC Directory of HAB Experts*?
If not, do you wish to be in the next edition?

Please indicate, up to a maximum of four, which of the following potential sections (to be confirmed, with chosen leaders, at future meetings) you are most interested in:

- ☐ Taxonomy/genetics-cycles
- ☐ Ecology (marine planktonic)
- ☐ Ecology (marine benthic, ciguatera)
- ☐ Ecology (freshwater, including cyanobacteria)
- ☐ Biogeography/introductions
- ☐ Toxicology
- ☐ Pharmacology
- ☐ Epidemiology/public health etc.

(Regional sections will be added later)

Information on your background and experience will be requested. Meanwhile, please rate yourself as:

- ☐ very experienced
- ☐ moderately experienced
- ☐ beginning professional research in the field
- ☐ a graduate student in the field
- ☐ an enthusiastic amateur
- ☐ other

Please enclose a cheque or money order for US$ 20.00, issued in the name of:
(Credit cards not accepted)

Henrik Enevoldsen
IOC Science and Communication Centre on Harmful Algae,
University of Copenhagen, Botanical Institute,
Dept. of Mycology and Phycology,
Øster Farimagsgade 2D, DK-1353 Copenhagen K, DENMARK.
Tel: (45-33) 134446; fax: (45-33) 134447
E-mail: henrike@bot.ku.dk

Future events

1st European Phycological Congress, Cologne, 11-18 August 1996. There will be a session on "Red tides and algal-bacterial interactions" convened by Drs Malte Elbrächter and Karen Steidinger. Congress Secretariat: Prof. Dr Michael Melkonian, Botanisches Institut, Universität zu Köln, Albertus-Magnus-Platz, D-50923 Köln (Germany); tel.: (49-0) 221 470 2475; fax: (49-0) 221 470 5181; e-mail (internet): mmelkon@biolan.uni-koeln.de

14th International Diatom Symposium, Tokyo, Japan, 2-8 September 1996. Convener: Dr Hiromu Kobayasi, Tokyo Diatom Institute, Honcho 3-8-9-813, Koganei-shi, Tokyo 184, Japan. Contact: Shigeki Mayama, Department of Biology, Tokyo Gakugei University, Konagei-shi, Tokyo 184, Japan; e-mail: mayama@u-gakegei.ac.jp; tel.: (81-0) 423 25 211; fax. (81-0) 423 24 9832.

Fifth Canadian Workshop on Harmful Marine Algae, St. John's, Newfoundland, Canada, 11-13 September 1996. The workshop will promote exchange of new and unreported information and plan for future research on aspects related to harmful marine algae. The proposed programme will include contributed oral presentations, poster papers, a review of relevant work done by different agencies, working group sessions, including a session on harmful marine algae and aquaculture site management, and a plenary session. Contact: M.A. Paranjape, Ocean Ecology Division – Science, Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, NF A1C 5X1, Canada; tel: (1-709) 772 6184; fax: (1-709) 772 3207; e-mail: mparanjape@nflorc.nwafc.nf.ca

IOC Regional Training Course on the Taxonomy and Biology of Harmful Marine Microalgae, Rio Grande, Brazil, March 1997. This course will focus on identification and preparation techniques supplemented by lectures on different aspects of the biology of harmful algae. Teaching staff will include: Dr Yasuwo Fukuyo (Univ. of Tokyo), Prof. Øjvind Moestrup (Univ. of Copenhagen), Dr Jacob Larsen (Univ. of Copenhagen/IOC Centre), Dr. Clarisse Odebrecht (Univ. of Rio Grande). Organized by the University of Rio Grande and the IOC Science and Communication Centre on Harmful Algae, Copenhagen.

For application forms, contact: the IOC Secretariat (Paris), or the IOC Science and Communication Centre on Harmful Algae, Botanical Institute, Ø. Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; fax: (45-33) 13 44 47.

7th East Coast Protistology Conference, University of Rhode Island, 21-23 May 1997. Open to all who are interested in eukaryotic unicellular organisms. The programme will include contributed oral and poster presentations, a keynote speaker and a special workshop on Ecological Methods for Protistology. Conference coordinator: Dr. Linda Hufnagel, Department of Biochemistry, Microbiology and Molecular Genetics, University of Rhode Island, Kingston, RI 02881, USA; e-mail: aun103@uriacc.uri.edu; or Lucie Maranda, Department of Pharmacognosy and Environmental Health Sciences (same address); tel: (1-401) 874 5048; fax: (1-401) 874 2181.

Literature for developing country libraries

Limited copies of the following titles are available from IOC's HAB Centre in Denmark, to libraries of marine science institutions *in developing countries only*:

- ***Proceedings of the Sixth International Conference on Toxic Marine Phytoplankton***. P. Lassus *et al*. (eds.), 1995.
- ***The Genus Alexandrium Halim (Dinoflagellata)***. E. Balech, 1994.
- ***Marine Phytoplankton. A Guide to Naked Flagellates***. C. Thomas *et al*. (eds.), 1994.
- ***Marine Phytoplankton. Identifying Marine Diatoms and Dinoflagellates***. C. Thomas *et al*. (eds.), 1996.
- ***Algae. An Introduction to Phycology.*** C. van den Hoek *et al.*, 1995.
- ***Guide to Toxic and Potentially Toxic Marine Algae.*** J. Larsen & Ø. Moestrup, 1989.
- ***The Biology of Dinoflagellates***. F.J.R. Taylor (ed.), 1987.

Applications must be submitted and signed by the responsible librarian, and should indicate why the requested title is of specific interest to your institution and its ongoing research or teaching. *No requests from individuals will be honoured.* Address applications to:

IOC Science and Communication Centre on Harmful Algae,
University of Copenhagen,
Botanical Institute,
Øster Farimagsgade 2D,
DK-1353 Copenhagen K, Denmark;
tel.: (45-33) 134446;
fax: (45-33) 134447;
e-mail: hab@bot.ku.dk

New IOC publications

- International Directory of Experts in Toxic and Harmful Algae and their Effects on Fisheries and Public Health. Published jointly with the US NOAA. *IOC INF Document* 1015.
- Design and Implementation of Some Harmful Algae Monitoring Systems. Co-published with ICES. *IOC Technical Series* No. 44.
- Summary Report of the Third Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, Paris, 6-9 June 1995. Available in English, French, Spanish.
- Amnesic Shellfish Poisoning (ASP), Volume 1, *IOC Manuals and Guides*, No. 31.

These publications are free of charge and available from the IOC Secretariat (Paris) and the IOC Science and Communication Centre, Copenhagen (see addresses elsewhere on this page).

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 15

Noxious *Gymnodinium* species in South African waters

At the height of the 1995-96 summer holiday season, beachgoers and seaside residents in South Africa's largest bay, False Bay (Fig. 1), were overcome by the discomfort of coughing, burning of the nasal passages, difficulty in breathing, stinging eyes and irritation to the skin. Microscopic examination of water samples from the bay, which had turned a dirty olive-green colour, revealed the presence of a species of *Gymnodinium* first recorded in False Bay in 1988. The noxious gases associated with this plankton bloom were initially reported in the Fish Hoek, St. James and Muizenberg areas, but later the effects were also felt at Monwabesi and Koeelbaai on the eastern side of False Bay before spreading to the coastal resort of Hermanus in Walker Bay (Fig. 1). These conditions persisted for several weeks. Although the discomforts experienced were considerable, symptoms were usually relieved on leaving the area, and no long-term effects were noted.

Rapid growth of the human population on and near the margin of False Bay has placed increasing demands on it, both as a prime recreational area, and as a site of disposal of solid and liquid waste. Media interest in this noxious bloom was therefore intense and the event received considerable exposure. Many unsubstantiated reports attributed the bloom either to the increased discharge of sewage into the bay, or to the introduction of the species through ballast water discharge by damaged oil tankers seeking shelter and undergoing repair in the bay.

(Cont'd on p. 2)

Gymnodinium catenatum and autumnal toxicity in Mar del Plata

Outbreaks of paralytic shellfish poisoning (PSP), some of high intensity, are well known in Argentina. They are normally due to blooms of *Alexandrium catenella* in the fjords of Tierra del Fuego and to *Alexandrium tamarense* on the Atlantic coast. In recent years, both species seem to have expanded their range. The first report of a toxicity outbreak in southern South America was that of Segers (3) who described a mass intoxification with deaths of aborigines following mussel consumption in the Ushuaia in 1886. Curiously, no further outbreak was recorded in this region until 1972, and others have occurred in 1981, 1989, and 1991. The last was unusually intense and persistent, and extended the range of *A.catenella* northwards to 45°15'S in the Pacific and to the Beagle Channel (1, 4).

In the South Atlantic, the first toxic outbreak of *A.tamarense* was detected in 1980 in an area near Peninsula Valdez, and subsequently expanded to almost the entire coastal ecosystem at least be-

(Cont'd on p. 3)

This issue includes a special section on the NATO-SCOR-IOC Advanced Study Institute on the Physiological Ecology of Harmful Algal Blooms, Bermuda Biological Station, 27 May-6 June 1996

See p. 13-23

Gymnodinium catenatum on west coast of India

Gymnodinium catenatum is a toxic dinoflagellate involved in paralytic shellfih poisoning (PSP). It has been reported from western North America, Spain, Australia and Japan. During a study of dinoflagellates and their cysts on the coast of Karnataka, India, we detected both the planktonic (Fig. 1) and cyst forms of *G.catenatum*. This is highly significant in view of earlier reports of PSP in this region. At the time planktonic cells and cysts were located, there was no toxicity in shellfish in the region tested by mouse bioassay. The planktonic cells were present in very low numbers. Whether this accounts for the lack of toxicity is not known. However this finding highlights the need for close monitoring of the coastal waters, sediments and shellfish of Karnataka.

Anna Godhe, Indrani and Iddya Karunasagar
College of Fisheries, P.B.527, Mangalore-575 002, Karnataka, India



Fig. 1. Chain of Gymnodinium catenatum from coastal waters of Karnataka, India.

This issue of Harmful Algae News is sponsored by the Danish Institute for Fisheries Research

(Cont'd from p. 1.
"Noxious Gymnodinium species in South African waters"

In 1988, when the *Gymnodinium* species responsible for this event was first recorded in local waters, surfers, swimmers and fisherman suffered eye, nose and throats irritations. The following year a bloom of the same species was responsible for extensive mortalities of mostly sub- and intertidal fauna, including an estimated 40 tons of abalone in the H.F. Verwoerd marine nature reserve (Fig. 1, Horstman et al., 1991).

Despite this species having bloomed on several occasions since it was first observed, never have the noxious effects to humans been so evident as during the 1995-96 event. However, faunal mortalities were small, with the exception of the larval mortalities experienced by several land-based abalone farmers in the Walker Bay area. Furthermore, mussels collected from Muizenberg and tested by mouse bioassay (Delaney, 1985) were found to be toxic, and a warning was issued against the collection of shellfish.

Attempts to identify the *Gymnodinium* species present in False Bay were interesting in that alhough it possesses some characteristics of several other toxic marine dinoflagellates, it also has features which distinguish it from these species. For example, the presence of aerosol toxins which result in respiratory distress in humans has been associated with *Gymnodinium breve* Davis blooms off the west coast of Florida for many years. The False Bay species is, however, morphologically dissimilar in that it does not possess the apical crest so distimetive of *G.breve*. Instead it bears a greater resemblance to *Gymnodinium mikimotoi* Miyaki *et* Kominami *ex*

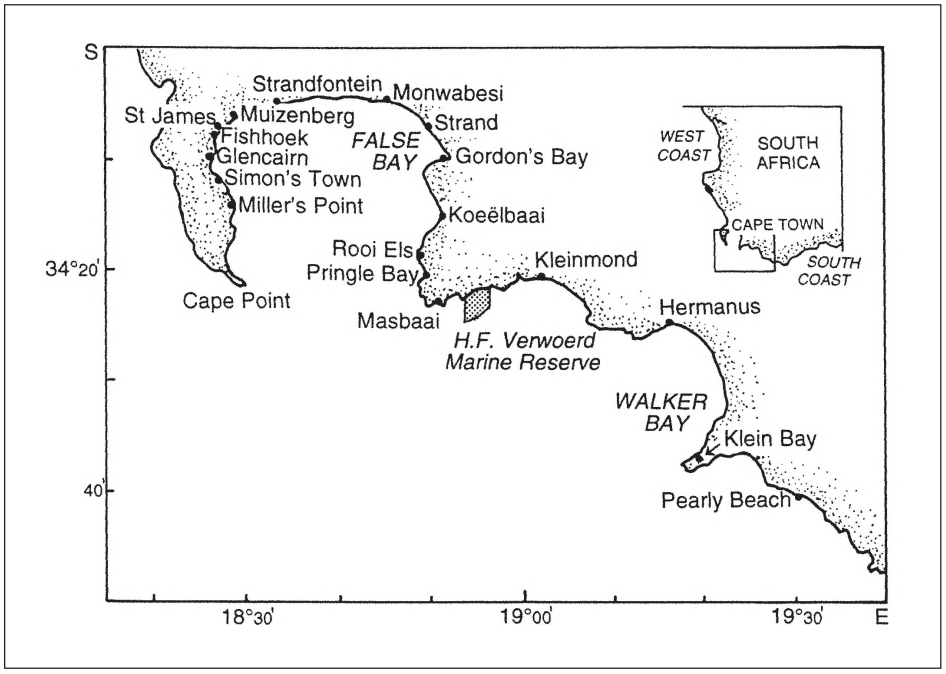

Fig. 1. False Bay and Walker Bay.

Oda (*G.mikimotoi* is conspecific with *G.nagasakiense* Takayama and Adachi and is a senior synonym for the latter, Takayama and Matsuoka, 1991) common in Japanese waters, and to the north European species *Gyrodinium aureolum* Hulbert. Morphologically the resemblance to *G.mikimotoi* is particularly strong in that the cells are similar in size, are dorso-ventrally flattened, an apical groove is sculpted on the epicone, and girdle displacement and the number and shape of the chloroplasts are essentially identical. The local species, however, differs from *G.mikimotoi* in the shape and position of the nucleus. In *G.mikimotoi* an ellipsoidal and reniform nucleus is longitudinally orientated in the left hand side of the cell, whereas the nucleus of the local species lies horizontally in the hypocone (Fig. 2). Furthermore, although *G.mikimotoi* and *G.aureolum* have been associated with mortalities of marine fauna, the presence of aerosol toxins as encountered in False Bay has apparently not been associated with these species.

The *Gymnodinium* species described here therefore appeared unique until 1993, when New Zealand experienced its first harmful algae bloom problems. Initial indications of toxic alga in New Zealand waters were the mortality of marine organisms on reefs, an outbreak of coughing due to exposure to sea spray aerosols, and the identification of *breve*-like lipid-soluble toxins in affected shellfish (MacKenzie, 1995). The *Gymnodinium* species responsible was also found to bear a strong resembance to *G.mikimotoi* but, as with the False Bay species, the nucleus was horizontally

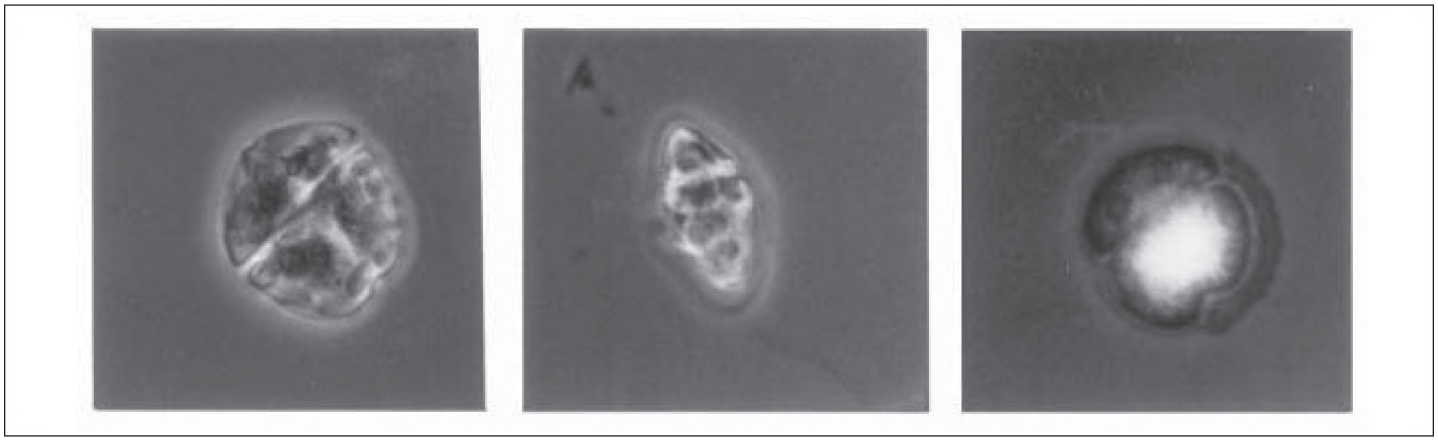

Fig. 2. Light micrographs of the Gymnodinium *species from False Bay: (a) dorsal view; (b) lateral view; (c) visualization of the nucleus under UV fluorescence after staining with acridine orange, to show its shape and location.*

orientated in the hypocone, and not in the left lobe of the cell as in *G.mikimotoi*. This feature according to MacKenzie et al. (in press) is a distinctive and invariable characteristic of the species.

Together these observations indicate the presence of a southern hemisphere *Gymnodinium* species which shares certain features with toxic northern hemisphere *Gymnodinium* species, but has other characteristics which may be sufficient to discriminate it from those species.

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(Cont'd from p. 1.)
"Gymnodinium catenatum and autumnal toxicity in Mar del Plata"

tween San Julian in 49°15'S in Argentina and the coast of Uruguay (2, 5). Since then blooms of *A.tamarense* have recurred in the Buenos Aires area during spring (6). In this region too in some years, as well as the spring toxicity maximum, there has been a secondary peak in autumn (6), the origin of which has not been established. In autumn 1962, Balech (7) reported the presence in this area, sometimes in abundance, of *Gymnodinium catenatum*, a well known PSP producer, but it was not until 1992 that a toxic outbreak in this region was associated with this species, on the Uruguayan coast of the Plate estuary (5). The repetition of these events in the following years suggests that there may have been earlier outbreaks which were not detected. It is also probable that this species was responsible for PSP at the end of summer 1980 on the Uruguayan coast (8), since it has not yet been demonstrated that the present species, *Alexandrium fraterculus,* is toxic (9). Our study of the annual phytoplankton cycle (1994-95) in the coastal Mar del Plata is in agreement with that of Balech (7), and indicates that *G.catenatum* appears in autumn, which would explain the secondary peak in mussel toxicity in the area. It must be pointed out that the maximum development of *A.tamarense* is in spring, and it is present in the plankton from late winter to late spring. It is interesting to note that Balech (7) did not observe this species in his study of the annual cycle of this region, which lends support to the idea that it has only recently colonized or been introduced to the area (9).

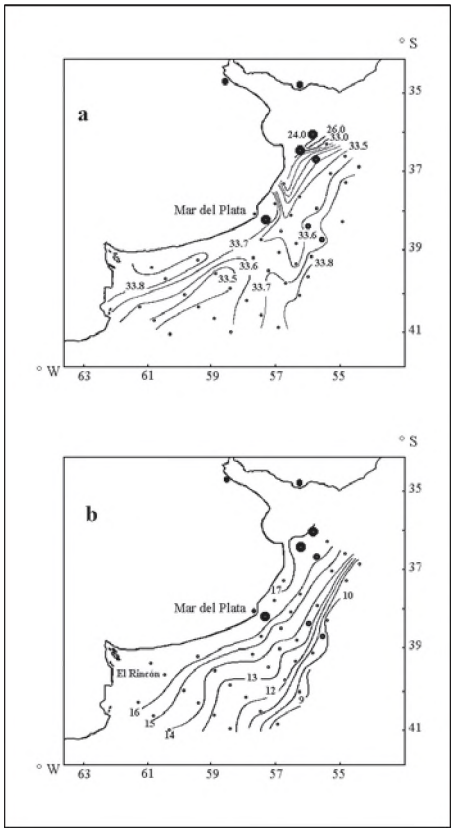

Fig. 1. Distribution and abundance (•: <100 cells l⁻¹; •: 100-300 cells. l⁻¹; •: 1500-2400 cells l⁻¹) of Gymnodinium catenatum *(May 1981) in relation to distribution of salinity (ppt, Fig. 2a) and temperature (°C, Fig. 2b).*

In the sediments of the Mar del Plata area, resting cysts of *A.tamarense* are very abundant, and are present throughout the year. The cysts of *G.catenatum* have only been identified once. Thus while blooms of *A.tamarense* could be initiated *in situ*, the presence of *G.catenatum* seems to be linked to advection of populations from nearby areas, probably from the Plate Estuary. Retrospective analysis of the distribution of *G.catenatum* in a broad area of the shelf in autumn 1981 indicates that although the main concentrations are associated with the estuarine front of the Plate River (Fig. 1), the distribution is discontinuous and is associated with different water masses. In the coastal region of the Mar del Plata, which seems to be the limit of its distribution, the waters of El Rincon (33.7 ppt, 16.5°C) predominate, while further offshore near the continental slope, *G.catenatum* is associated with subantarctic water (33.7-33.8 ppt, 10-14°C). This discontinuity seems to indicate the existence of more complex phenomena than the simple transport of *G.catenatum* from the Plate Estuary by a stable flow. The plume of the Plate River flows preponderantly to the NNE, but persistent winds from the northerly sector give rise to a countercurrent along the coast (10).This alternation of transport directions could provide a mechanism for inoculating *G.catenatum* into a broad area, where it might subsequently persist and grow only under favourable conditions.

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◆ Scotland

Awareness of Cyanobacterial or Algal Blooms at the Premonstratensian Monastery of the Green Loch, Soulseat Scotland, from the Twelfth Century, and Cattle Poisonings Attributed to Cyanobacterial Hepatotoxins at this location Eight Hundred Years Later

Are toxic blooms of cyanobacteria and algae becoming more common? This question is one of the most often-received by those who carry out research on toxic blooms and associated poisoning incidents in fresh-, brackish- and marine waters. The question arises from water-users at freshwaters, where drinking supplies and recreational activities are adversely affected by toxic cyanobacterial blooms, and at estuarine and coastal waters where, for example, shellfish harvesting and sales are proscribed if levels of marine algal toxins exceed statutory limits. It has been argued that blooms appear to be more common because researchers are looking more widely and intensively at the problems, that consumers are eating more seafood so cases of illness are more evident, and that statistical data on bloom occurrences are improving (1). In freshwaters, state or national monitoring programmes have shown high occurrences of cyanobacterial blooms with toxicity being detected in at least 50% of the blooms analysed (e.g., 2, 3).

Evidence appears to indicate that freshwater and marine blooms are indeed increasing in distribution, frequency and duration (1, 4, 5). Palaeolimnological analyses of 210Pb-dated sediment cores are making major contributions to the understanding of the history of increasing eutrophication (4, 6). Perhaps of secondary value, but of human interest, are the glimpses of past human awareness of cyanobacterial and algal blooms and scums, and in some cases, of associated poisoning hazards.

A tribal custom among western Queensland Aborigines of Australia of digging soaks for drinking water, rather than taking the water directly from sources thought to contain cyanobacterial scum, indicates a traditional awareness of health hazards presented by such water (7). The written record is perforce fragmentary. Algal and cyanobacterial blooms are not the stuff of great literature, although there are some exceptions. As suggested by Fogg et al. (8), the comment by Shakespeare's Gratiano in *The Merchant of Venice*, that "There are a sort of men whose visages do cream and mantle like a standing pool" indicates an assumption by the playwright that his audiences would be familiar with blooms and scums. Other indications of the early awareness of these manifestations in Europe are discussed elsewhere (9, 10).

In ongoing research into the toxicity assessment and toxin content of cyanobacterial and algal blooms in UK and other waters (2, 11), this laboratory annually encounters a small number of animal poisonings and human health problems associated with blooms and scums, e.g. (12-14). In late November and early December 1994, at Soulseat Loch, a eutrophic freshwater lake near Stranraer, SW Scotland, six young cattle died, with liver abnormalities, after drinking from the lake which contained a scum-forming bloom of *Oscillatoria*. Two further cattle died in May 1995 at the same waterbody. On the latter occasion it was possible to carry out an integrated investigation of the event with veterinary colleagues (in prep.). In agreement with the signs of hepatotoxicosis in the cattle was the presence of a hepatotoxic bloom and scum of *Oscillatoria agardhii* in the lake and of partially broken down filaments of *O.agardhii* in the animals' rumen contents. Two microcystin hepatotoxins have so far been identified and further work is in progress. The 1994 and 1995 Soulseat Loch cattle deaths are, in sum, attributable to cyanobacterial hepatotoxicosis.

An interview with the farmer who owned the poisoned cattle indicated that Soulseat Loch has a history of annual blooms and scums. Whilst the farmer can attest to this personally for the past 50 years (Mr. D. Little, pers. comm.), evidence suggests that human awareness of blooms and scums at Soulseat Loch also existed in the twelfth century. The lake is partly bisected by a peninsula, upon which a monastery was founded by Fergus, Lord of Galloway, for monks of the Premonstratensian* order in 1175 A.D.or earlier (15). The monastery became known as the *Monasterium Viridis Stagni* and as the Monastery of the Green Loch, or Stank. Dick (15) suggests that the Latin name is "traceable to the green appearance of the water from time to time by the presence of spore-like vegetable growths". It can be assumed that the monks of the monastery at Soulseat were familiar with cyanobacterial or algal blooms which developed sufficiently to discolour the water and cause offensive odours.

Although cattle deaths at Soulseat eight centuries later are attributed to the ingestion of microcystins in *O.agardhii*, the sceptical may wonder what has been happening in the intervening eight hundred years at this eutrophic site. Surely a few cattle deaths in the 1990's would not have been the only outward signs of toxic blooms over the years? In today's era of "data-sets", we have to acknowledge that those for Soulseat over this period are incomplete.This would have been partly assured by the decline of the *Monasterium Viridis Stagni* as a seat of literacy after the Scottish Reformation of the 1560's, into a ruinous state by 1684.

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* this unnecessarily lengthy name is derived from that of the mother house of this monastic order, Premontre near Laon NE of Paris, founded by St. Norbert at the desire of Calixtus II in 1120. (ed.).

Society News

The members of the international society for the study of harmful algae (provisionally ISSHA), have been sent a call for nominations for officers for the society. The deadline is 1 April 1997 and the vote will be conducted at the 8th International Conference on Harmful Marine Algae, Vigo, Spain, June 1997. Members not attending will be invited to write-in voting on beforehand.

We have also asked for logo design suggestions. Other items to be discussed at Vigo will include the statues of the Society, its registration, goals, and general structure.

If you are interested in joining the Society and take part in the decisions concerning statues, goals and structure please use the application form included in Harmful Algae News No. 14 and send it to:
Fax.: (43) 33 13 44 46,
E-mail: hab@bot.ku.dk

Can we predict harmful algae blooms

Studies carried out at the Institute of Marine Sciences in Erdemli, Turkey, since 1994 suggest that *Emiliania huxleyi* blooms are predictable. A long term atmospheric sampling programme of mineral dust on the harbour jetty of the institute has indicated sporadic dust intrusions into the eastern Mediterranean originating from deserts (1). Each pulse of dust is associated with an order of magnitude increase in the atmospheric concentrations of elements like aluminium and iron. These observations are supported by satellite data (NOAA-AVHRR). Corresponding 3-D air-mass back trajectory analyses allow us to model dust uplift and transport with a regional scale atmospheric model which includes dynamical and transport components.

An extraordinary dust storm which originated in the Sahara was observed on 6th April, 1994, and yielded a maximum dust load of 1600 mg m^{-3}, the largest ever recorded since the sampling programme started. The Saharan origin of the dust pulse can be seen clearly in AVHRR data for 6 and 7 April 1994. Satellite data from the eastern Mediterranean obtained a week after the event (14-15 April) indicated high reflectance of visible light from the sea surface in regions coinciding with the dust intrusions in the Black Sea, Sea of Marmora, and Mediterranean, suggesting blooms of coccolithophorids. The post-bloom phases of these, when the coccoliths become detached, can increase the reflectance by 25% or more, easily detected by contemporary AVHRR sensors in the visible band (2, 3). This high reflectance can only be observed as the coccoliths are released 7-8 days after a bloom (4) by the present broad spectrum visible channel of AVHRR.

By coincidence, the research vessel of the institute, *R.V. Bilim,* was in the Sea of Marmora at the time the high reflectance patch was seen on the satellite images of 14-15 April 1994, and reported an unusual and very dense red tide. It was so thick that it was almost impossible to observe the wake of the ship. *Noctiluca scintellans* appeared to be the dominant organism, but the presence of *Emiliania huxleyi* could not be established since the samples, which could not be analysed on board, were preserved in formaldehyde which dissolves carbonate shells. During 1995 it was possible to demonstrate by SEM that the samples collected from the high reflectance region in the Mediterranean contained *E.huxleyi.*

These observations from such diverse environments, from oligotrophic to brackish and from almost pristine to highly polluted, suggest that a common triggering mechanism is involved, and that it was the week old atmospheric dust intrusion. J.H. Martin's hypothesis on iron limitation of phytoplankton productivity has been tested in the equatorial Pacific by fertilizing an area with iron.The result was a doubling of the plant biomass, chlorophyll and production (5). It is important to note that the biologically effective species of iron is Fe II (6). Faust (7) reported that rapid photo-oxidation/reduction reactions of iron species in cloud droplets, with reaction times of the order of minutes, determine the balance of Fe II and Fe III. A balance in favour of Fe (II) is maintained during the daytime, especially when humidity is present together with sunlight. These facts suggest that the formation of photoreduced iron within cloud droplets during daytime and wet dust intrusions lead to the blooms, and that the diel differences in the balance between different forms of iron, combined with the dust transport and humidity patterns of moving storms, lead to the patchy distribution of the blooms.

Having observed the rather unexpected features of dust, Saharan dust transport and its ocean effects in 1994, we planned to detect similar events in 1995. With experimental dust forecasts kindly supplied by the Land-3 project, we had a better chance to detect them. A synoptic scale meteorological event carrying dust was predicted to effect the region on 27-29 March, 1995. Satellite data indicated an area of high reflectance of visible light on 6 April, 1995, near Cyprus. A ferry sailing from Cyprus to Turkey at our request gathered samples from the high reflectance patch, and SEM analyses of the samples confirmed the presence of detached coccoliths. The high reflectance areas appear about a week after the dust events because the formation of detached coccoliths typically requires 6-8 days to develop after the initiation of a bloom.

Thus the photo-reduced iron in the cloud droplets of humid dust intrusions may result in phytoplankton blooms at the sea surface. It is probable that diel variations in the balance between different forms of iron combined with the dust transport and humidity patterns lead to the patchy distribution of blooms, i.e.the patchiness depends on synoptic scale meteorological events. The 1996 dust transport campaign was planned to demonstrate the diurnal characteristics of the iron, and we designed a sampling system which allows us to fix the delicate bioavailable Fe II that may be present in rain samples. In 1996, a dust pulse event was recorded on 8th February by AVHRR in the eastern Mediterranean, and a diurnal cycle of Fe II was demonstrated in rain samples on 8-9 February as predicted. The rapid reoxidation of Fe (II) makes it very reactive, and it should be taken up by the biota immediately. Although it has not been demonstrated at this stage, this nutritional influx is believed to be responsible for the formation of coccolithophores only, or of both these and red tides. This view is based on the simultaneous observation of both *E.huxleyi* and red tide blooms in the Mediterranean and Sea of Marmora during 1994.

It is expected that dust transport and its interactions with water vapour can lead to global effects. The increased cloud albedo due to enhanced production of cloud condensation nuclei (CCN) during coccolithophore blooms could potentially reduce radiation in higher latitudes where clouds are more abundant. On the other hand the suspension of dust in the atmosphere by erosion of desert regions is a function of aridity in these regions, and would decrease with southerly shifts of rain associated with the polar front. Furthermore, coccolithophore blooms release CO_2 and release its absorbtion by the ocean, so that atmospheric CO_2 would tend to increase with dust induced blooms. As a result, negative feedback is suggested between atmospheric dust fluxes and global temperatures, leading to cyclic regulation of climate such as occurred during the interglacial periods. It might be argued that iron limitation is not to be expected in land-locked seas like the Mediterranean, and that insoluble complexing of iron with the scarce phosphates is the reason for the oligotrophic nature of these waters. As mentioned, we think that desert dust is a necessary but not sufficient condition, and that the daytime deposition of the dust under cloudy conditions and above some threshold light intensity is required for the onset of phytoplankton blooms. In fact cyclone tracks over the Mediterranean show that Saharan dust spreads over the eastern Mediterranean during April and May, and the main track then becomes northerly to reach Norway and the North Sea from June to August. This may be the reason for the sporadic increases in DMS and MSA in the North Atlantic from June to August. The growth of *E.huxelyi* through such processes would throw light on climate variability via the formation of DMS, SO_4, and other chemical species and rates of formation of cloud condensation nuclei (9).

Finally, it is suggested that the next iron experiment should not seed the sea water with iron, but inject it directly over clouds with tracers during daytime when solar radiation is above the threshold intensity.

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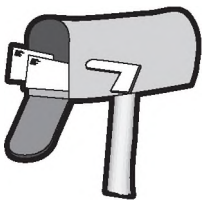
Identification Service



The IOC Science and Communication Centre on Harmful Algae, Copenhagen, and the Asian Natural Environmental Science Center offers an identification service. If you have problems with identification of harmful algal species, the Centres may assist you. You should send samples to one of the two addresses given below. It should be noted that this assistance is not given for routine identification/monitoring of samples, but applies to particulary difficult species, or to species that require special techniques e.g. electron microscopy, for identification.

IOC Science and Communication Centre on Harmful Algae,
Univeristy of Copenhagen,
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Asian Environmental Science Center,
The University of Tokyo,
Yayoi 1-1-1,
Bunkyo-ku, Tokyo 113,
Japan.

10 Young Scientist Grants to Attend the VIII International Conference on Harmful Algae (Vigo, Spain, 25-29 June 1997)

The "Federation of European Microbiological Societies" (FEMS) has accepted co-spnsor this conference by supporting (partially) the participation of 10 young scientists.

To qualify for the award of a Young Scientist Grant the applicant must:

i) be 35 years old or under.
ii) belong to microbiological society affiliated to FEMS

In the event that the applicant does not belog to a FEMS affiliated society (e.g. a research student), a grant may be awarded on the recommendation of a Sponsor who must be a member of a FEMS society.

Young scientists interested to apply will be sent an application form. Please contact:

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◆ France

Filamentous and mucilaginous algal blooms in a Corsican Bay (Calvi-France)

In recent decades there have been profound changes in the benthic algal vegetation off the coast near Calvi (Corsica, France). These are probably linked to the disturbing effects of domestic sewage discharge which continually increase along with urbanisation and tourism.

The Oceanographic Research Centre "STARESO" is located in the bay of Calvi and provides an excellent base for carrying out long term studies of algal vegetation. The first part of this study involved mapping the most important seaweeds of the bay (1, 2). The phenology, ecology and epiphytism of *Cystoseira balearica*, the most common fucoid in this part of the Mediterranean have been studied (3, 4). Other studies have dealt with microscopic algae (5; epiphytic Cyanophyceae and Bangiophyceae), the influence of pollution on the distribution and ecology of some epiphytic Cyanophyceae (6), and the distribution of the Bangiophyceae in the bay of Revellata (near Calvi Bay). (7, 8, 9).

Other *Cystoseira* species were present in the mediolittoral (*C. compressa* and *C. stricta)* and medium infralittoral zones (*C. spinosa*). Since 1984, the various species of *Cystoseira* and some other algae have shown a clear regression, while certain nitrophilous species (*Cladophora prolifera, Halopteris scoparia, Colpomenia sinuosa* and *Enteromorpha* sp.) tend to thrive. Fucoid algae and especially *Cystoseira* are known to exhibit a marked sensibility to organic wastes and gradually disappear from polluted sites (10). Now other algal associations, including *Halopteris scoparia*, *Dictyota* sp. and *Padina pavonica*, have replaced or interrupted the *Cystoseira* stands in Calvi Bay. In the northern Adriatic, these nitrophilous species are associated with moderately polluted sites (11). In addition, since 1992, we have noted the extensive development of mucilaginous and filamentous aggregates in some parts of the bay. In early spring, large amounts of filaments grow on rocks, macroalgae, gorgonians, and seagrass (*Posidonia oceanica)*. These new associations are found in all photophilic zones from 5 to 30 m with some preference for substrates with a southern exposure. The predominant species in these filamentous aggregates is *Nematochrysopsis roscoffensis* var. *giganteus* (Chrysophyceae). This species was first described in the bay as *Tribonema marinum* (Xanthophyceae). *Chrysonephos lewisii* is also found in these associations. It is a tropical genus first described in the Mediterranean by Sartoni (12).

In 1995, the first of these filamentous patches appeared near STARESO between 15 and 28 March, 1996. By mid-May, they could be found almost everywhere in Revellata Bay. In some places, their development is very important and affects the growth of the rest of the vegetation by shading the water below and preventing photosynthesis. We have also observed growth of this filamentous association on artificial structures (i.e. metallic structures and ropes). At the "crique de l'égout", some filaments more than 2 m long grow on submerged ropes. On uncovered substrates, a lack of competition with other species and fast growth in these filamentous forms seems to favour the development of new associations. The filamentous species grow preferably as epiphytes on *Dictyota dichotoma* or *Cystoseira balearica* when present, rather than on other macroalgae. *D. dichotoma* appears in various associations in early March. At the "La Bibliothèque", the *Cystoseira balearica* association still exists but is less healthy. During May 1996, the filamentous associations was so prevelant that filaments could be found floating in the water column, free from any substrate. As a result, most of the macroalgae, and some *Posidonia oceanica* leaves are totally shaded and exhibit a markedly reduced growth.

At the end of spring, mucilaginous aggregates also begin to grow in high densities. The first globules were observed on May 29th 1996 at Punta Revellata and in the STARESO area. They mainly contain Chrysophyceae, *Pulvinaria giraudii*. This species can be found on macroalgae and on seagrasses, but is most commonly observed on uncovered rocks in small streams. Concurrently we noticed patches of blue-green algae and diatoms developing on the macroalgae. The blue-green algae are mostly *Hydrocoleum* sp. and *Pseudanabaena* sp. Due to the lack of competition with other macrophytes, these blue-green algae can develop in the upper infralittoral zone during the summer. Often the layer of blue-green algal filaments trap bubbles of oxygen from photosynthesis and are lifted to the sea surface.

The development of these aggregates is a peculiar phenomenon that changes benthic associations that are of potentially great importance when considering the general ecology of the bay. These changes often involve the occurence of new and rare algal species, indicative of the need for further taxonomic and ecological studies.

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◆ New Jersey

The first *Aureococcus anophagefferens* brown tide in New Jersey

We report the first known bloom of the "brown tide" picoplankter *Aureococcus anophagefferens*, in New Jersey. In early May, 1995, personnel at Biosphere, Inc., a commercial aquaculture facility in Tuckerton Bay, New Jersey, noted reduced growth of juvenile hard clam, *Mercenaria mercenaria*, in the nursery system. Feeding and development of hard clam larvae were interferred with at all stages; growth cessation was complete by mid-May. Growth recovery of survivors was partial in early July and complete in mid-July.

The observed clam growth reduction coincided with widespread presence of a picoplankton bloom in the general area. Tuckerton Bay comprises the southwestern-most portion of Little Egg Harbor, New Jersey central coast (Fig. 1). Water pumped into hatchery aquaria typically was so turbid that visibility was lowered dramatically. The bloom was prolonged, being most evident from about the first week in May through mid-July. Based on light microscopy, the same phytoplankter apparently persisted, essentially as a monoculture, during this time. Cell counts on June 5, 10 and 15, respectively, were 1.8, 1.5, and 1.1 × 10^6 ml^{-1}. Effects of the bloom on flora and fauna in the affected bay areas were not determined.

The problem prompted Biosphere, Inc. Biologist G. Zodl to send water samples to the Suffolk County, New York, (Long Island), Department of Health Services (SCDHS). SCDHS has surveyed occurrence of *A. anophagefferenns* brown tide since it first appeared in Suffolk County bays in 1985. Brown tide has had severe detrimental effects on flora and fauna in affected Long Island areas, especially the bay scallop, *Argopecten irradians*. Catastrophic brown tide also occurred in Narragansett Bay, Rhode Island in 1985, but not since.

Analysis by SCDHS of June 1995 New Jersey samples by immunofluorescent microscopy (1) revealed cell numbers approaching 10^6 ml^{-1}, well above the minimal levels (2-2.5 × 10^5) found detrimental to shellfish in laboratory experiments (2) and suggested by field observations (3). Light microscopy picoplankter counts for the same time by G. Zodl approximated the *A. anophagefferens* numbers confirmed by SCDHS. Subsequently, samples were collected in early July from lower Barnegat Bay to Great Bay (just south of Tuckerton Bay), a distance of approximately 20 miles, by New Jersey Department of Environmental Protection (NJDEP) and National Marine Fisheries Service (NMFS) personnel. Analysis of these by SCDHS, detected *A. anophagefferens* in levels of 2.7 to 4.5 × 10^5 cells ml^{-1}. Additional interagency cooperative surveys in mid-August, with samples collected by helicopter by the U.S. Environmental Protection Agency (USEPA), Region II, spanned the entire New Jersey intercoastal system from the Hudson-Raritan Estuary to Delaware Bay. Counts, again by SCDHS, indicated that the bloom was in collapse at this time. The organism was found at various sites in Barnegat Bay and to the north, but cell numbers ranged only to a maximum of 2 × 10^3. A 1990 survey had found similar *A. anophagefferens* distribution in New Jersey, and had not detected it south of Barnegat Bay, most notably in Delaware or Chesapeake bays (1).

Subsequent to intense (>10^6 cells ml^{-1}) and widespread catastrophic brown tides in 1985 and 1986 in eastern Long Island, the blooms have recurred annually, with varied level and distribution, e.g. only in Great South Bay in 1994. Incidence in Long Island was more widespread in 1995, when brown tide occurred in south shore and Peconic Bay systems. Although moderate incidence of *A. anophagefferens* in

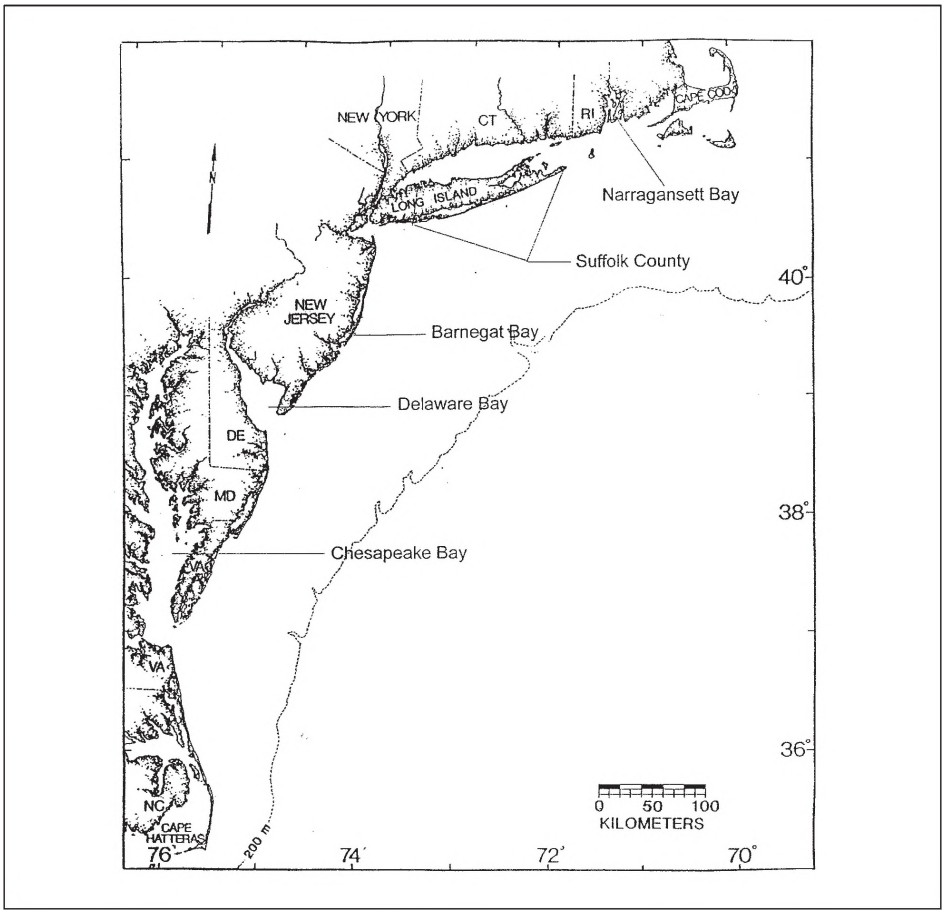

Fig. 1. Locations in the northeastern United States where Aureococcus anophagefferens brown tides have occurred, and of Delaware and Chesapeake Bays where the species has not been detected.

New Jersey waters has been known since 1987 (4), the major 1995 lower Barnegat Bay *A. anophagefferens* bloom is the only one outside of eastern Long Island since 1985. Chronic summer-long blooms of another picoplankter, *Nannochloris atomus*, responsible for murky, yellowish-brown water in the Barnegat Bay system since at least 1985, may have masked rising abundance of *A. anophagefferens*. The major brown tide in New Jersey in 1995 reaffirms the regional nature of the phenomenon. NJDEP and NMFS, with the assistance of USEPA and SCDHS plan to monitor for brown tide in New Jersey on a regular basis in the future.

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New www site on the microplankton species of the Black Sea, Sea of Marmara Aegean Sea, and Eastern Mediterranean. The web site also includes HAB species and events from the Turkish Seas. I hope you will find ny www site interesting. Please visit the www on

http://bornova.ege.edu.tr:80/~korayt/plankweb/index.html

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◆ Argentina

Massive mortality of yellow clams, *Mesoderma mactroides*, in Monte Hermosa Beach, Argentina

The yellow clam *Mesoderma mactroides,* an inhabitant of sandy beaches in Buenos Aires province, was an important economic resource for Argentina between 1940 and 1957. Arguing an apparent overexploitation, commercial harvesting was prohibited in 1958, and this legislation has remained in force until the present. In 1995, a study of the population dynamics was begun at the 32 km long Monte Hermosa beach in southern Buenos Aires province, to establish the state of the resource after 37 years of prohibition. During this work, a massive mortality of the species took place between 11 and 17 November. This event resulted in the almost total disappearance of yellow clams from the area. The number of dead animals was estimated in the order of 45 millions, with a mean size of 50 mm, equivalent to about 35 tons total dry weight. Similar mortalities were recorded for this species in Uruguay and southern Brazil in 1994 and 1993-4 respectively, and attributed to phytoplankton blooms (1, 2).

Analyses of the Monte Hermosa samples showed that water parameters were within the normal ranges (3). No traces of heavy metals were found in the clam tissues, nor were diarrhetic, paralytic or haemolytic toxins detected, despite a coincident red tide on the Buenos Aires coast that month (3). Water samples of November 19 contained mainly centric diatoms, and the dominant species in clam stomachs was *Paralia sulcata*. Strong S-SE winds blew on 10-11 November, with gusts of 40-50 km per hour. Water temperatures varied between 18-19 C. (4).

This is the first record of this phenomenon in Argentina, and although it is possible to discard contamination as the cause, virological and bacteriological studies still need to be made before we can postulate a plausible hypothesis to account for this catastrophe.

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◆ New Zealand

New Zealand update on HABs, 1992-1996

Shellfish biotoxin events in New Zealand have continued to be managed through 1995 and into 1996 by the NZ Marine Biotoxin Management Board. As of July 1st, responsibility for closures passed from the centralized Marine Biotoxin Surveillance Unit to local Health Protection Officers, though central government (MAF Regulatory Authority) will remain responsible for quality assured export products. The flesh testing will continue, and phytoplankton monitoring is included in the management plan. Phytoplankton monitoring continues for shellfish and caged fish, mainly salmon, and mortalities have been avoided. However, wild fish and shellfish were not so lucky during blooms of *Gymnodinium mikimotoi* during 1995 and 1996.

Pseudonitzschia blooms and DA

Blooms of *Pseudo-nitzschia australis* were responsible for domoic acid (DA) contamination of scallops in Northland in 1993, and DA was again detected in shellfish in the far north in summer (November to January) 1994-95 and 1995-96. A bloom of predominantly *P.australis* was also detected in March this year in the far south of NZ, at Big Glory Bay, Stewart Island, using whole gene probes provided by Chris Scholin, Monterey Bay Aquarium Research Institute, USA. *P.multiseries* was also detected but in negligible numbers. The use of tese probes will assist in building up a flora of the genus *Pseudonitzschia* in NZ waters, and has already proved to be a useful monitoring tool.

Pseudonitzschia pungens commonly blooms in the Coromandel and throughout the Marlborough Sounds from spring to autumn, and was responsible for DA in greenshell mussels in Pelorus Sound in December 1994. *P.turgidula* blooms, reaching 6.0×10^6 cells l^{-1} were recorded early to mid- summer 1994, in the Bay of Plenty, causing DA contamination of scallops in that area, and again reached 1.7×10^6 cells l^{-1} in November 1995. Such blooms are not unusual; *Pseudonitzschia* has been recorded in NZ waters for the past 40 years.

Dinophysis blooms and *Prorocentrum* mats

Dinophysis is common throughout NZ waters, and blooms of *D.acuta* (max. $>4.0 \times 10^4$ cells l^{-1} in integrated samples) occurred in Queen Charlotte Sound from late December 1994 to May 1995, and from October 1995 to April 1996. The blooms caused closures to recreational harvesting of blue mussels at West Point in the sound, with okadaic acid (OA) peaking at levels of >95mg/100g. The closure level is 20mg/100g. A non-toxic strain of *D.acuminata* which commonly occurs with *D.acuta* bloomed in Port Underwood in summer 1994.

Prorocentrum lima has been responsible for OA in oysters at Rangaunu Harbour in the far north. A mat of this alga occurs in channels of the muddy mangrove estuary and when disturbed, e.g., during storms, cells are suspended in the shallow water. This was reflected by the presence of OA in farmed oysters there in May this year.

Raphidophytes in Hauraki Gulf

1992 was a key year for blooms in NZ. At that time raphidophyte dominated blooms spread through Hauraki Gulf, to be followed by a neurotoxic shellfish (NSP) event in January 1993. Raphidophyte blooms have recurred in the gulf every year since, from early spring (August) to summer. Both *Heterosigma akashiwo* and *Fibrocapsa japonica have* caused extensive yellow-brown blooms, accompanied at times by a peppery taste in mussels which temporarily stalled shellfish harvests in the Coromandel. Further south in Timaru Harbour, in January this year, *Heterosigma* reached 66×10^6 cells l^{-1} and divers reported that oily tea-coloured water left a peppery taste in their mouths. That bloom occurred in calm waters with surface temperatures of 18 C, following heavy rainfall.

The 1992-94 raphidophyte blooms occurred during an unusually long-lived El Niño climate pattern. Germination of *Fibrocapsa japonica* cysts in the cool temperatures (>1.0 C lower than the average in 1992) was postulated as a factor in its dominance. However in 1995 the climate pattern changed, and a weak La Niña pattern produced warmer weather over NZ. The continuing dominance of raphidophytes during the past year could be due to the build up of major cyst beds, particularly in northeastern waters.

... and *Myrionectra*, *Noctiluca* et al

Deep purple blooms of *Myrionectra rubrum* are reported around New Zealand coastline each year. August 1994 saw massive blooms extending >200 km from Northland to the Coromandel, and over large areas in the Marlborough Sounds between October and December, and these were followed in the Gulf by dense brick-red blooms of *Noctiluca scintillans. Noctiluca* again bloom in the Coromandel in mid-winter 1995. None of these extensive and long lasting blooms were linked to fish or shellfish mortalities. Pilchards died in Wellington Harbour in December 1993, due to suffocation caused by clogging of the gills with *Tetraselmis* (1.9×10^6 cells l^{-1}). These deaths were not related to the pilchard deaths that occurred around Australia and NZ in 1995, the cause of which is thought to have been a virus infection.

Prorocentrum micans is another common bloom species in NZ, both on the southwest and northeast coasts. January 1994 saw counts of 4.5×10^8 cells l^{-1} in Hawke Bay. The unialgal bloom caused a slimy coating on fish and looked like an oil slick at the surface, with layers seen on echo sounders well below 50 metres.

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◆ Brazil

Liver failure and human deaths at a haemodialysis centre in Brazil: microcystins as a major contributing factor

At a haemodialysis centre in Caruaru, Brazil, 110 (84%) of 131 patients experienced visual disturbances, nausea and vomiting following routine treatment in February 17-20, 1996. On February 20, one patient died with seizure following dialysis. By March 6, twelve patients had died from seizure and/or acute haemorrhage. Subsequently 83 patients developed cholestatic liver disease and of these 32 died from compliations of liver failure by mid-April. By August 4, fifty-five patients died, with 44 of these having a common syndrome associated with liver haemorrhage or liver failure.

At the beginning of March, initial reports from medical doctors in the State of Pernambuco implicated hyperchloremia from the chlorinated water supply of the city's Tabocas Reservoir. On reading newspaper articles and watching television reports of the outbreak during late March, Dr. Sandra Azevedo of the University of Rio de Janeiro recognized that the symptons were not those of chlorine poisoning and suspected that cyanobacterial toxins might be involved. She contacted the State Health authorities with her suspicions and visited Caruaru the same week to collect phytoplankton samples from the reservoir. Phytoplankton counts were not made by the water company at the time of the outbreak but examination of previous year's counts showed that cyanobacteria had dominated the reservoir since 1990, with the most common genera being *Microcystis, Anabaena* and *Anabaenopsis*. Dr. Azavedo's samples showed that the most common cyanobacteria present at the end of March were *Aphanizomenon* and two species of *Oscillatoria*. She analyzed the dialysis centre's in-house water treatment system for microcystins by high performance liquid chromatography (HPLC). Chromatograms of methanol extracts from the activated carbon filter demonstrated peaks with the characteristics of microcystins. The largest peak had a retention time corresponding to microcystin-LR, the leucine/arginine variant of these potent cyclic peptide liver toxins, produced by several genera and species of cyanobacteria. Further examination with sand and anion/cation exchange filters showed microcystin residues in the ppt to ppm range. Samples sent by Dr. Azavedo to Wayne Carmichael's (Ohio) laboratory confirmed these findings using HPLC, enzyme-linked immunosorbant assay (ELISA) and a protein phosphatase inhibition assay (PPIA). Carmichael's laboratory also examined blood sera provided by Brazilian health authorities and the Center for Disease Control in Atlanta, Georgia, from affected patients who were still living and from the liver tissues of deceased patients. The sera and tissues of all patients who had been exposed were positive for microcystins. The ongoing analyses of sera and liver tissue from deceased patients, many of whom were recently exhumed by Brazilian authorities, is providing valuable on dose/effect data that will help establish risk assessment criteria for microcystins in humans. A clinic for survivors set up in Brazil will help monitor their progress and serve as a data base to assess long term health effects including cancer rates, since microcystins are potent liver tumour promoters.

In Carmichael's laboratory, ELISA guided HPLC seperation followed by amino acid analysis of microcystins from liver tissues is providing a profile of the microcystins present. In addition, K.L. Reinhart's laboratory (Illinois) is performing mass spectrometric analysis of liver tissues and of isolated microcystins from liver and sera, to provide structural confirmation of the microcystins present. To date, the most common found are microcystins-CR, LR, and possibly WR. Pathological examinations of affected liver tissues by Dr. Victorino Barreto at Pernambuco University showed a uniform pathological picture for the 16 initial victims. The pattern of liver plate disruption was identical with that found in experimental animals exposed to microcystins.

All biological and chemical evidence to date supports the view that microcystins were the major cause of death for at least 44 of these dialysis patients. One important question is how could lethal levels of microcystins occur in waters receiving standard treatment procedures such as sedimentation, coagulation, filtration, and chlorination. The answer to this question is that the haemodialysis centre did not have water piped directly to it, but had relied on water trucked to the centre and treated in its own sand-filtration carbon-filtration anion/cation exchanger followed by a microfilter. Due to poor removal of chlorine residues, later recognized as due to inadequate maintainance of the in-house water treatment system, it was decided to use untreated reservoir water to avoid chlorine residues in the water used for dialysis. This water contained high levels of microcystins which were not removed by the small inadequately maintained in-house treatment facility of the haemodialysis centre.

There are many important public health lessons and much scientific information to be learned from this first confirmed outbreak of human poisoning involving cyanotoxins. Since most of the world's resevoir and lake based water supplies are subject to increasing nutrient levels, it is highly probable that repeated episodes of cyanotoxin poisoning will occur unless measures are taken to improve our understanding of the role of cyanotoxins in water-borne diseases. These measures should include watershed management to reduce nutrient inputs, cyanotoxin monitoring to alert authorities to the presence of cyanotoxins, and improvements in water treatment techniques to reduce or remove cyanotoxins from water supplies.

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◆ Oman

Early Red Tides

The Sultanate of Oman has not previously been mentioned in this newsletter, and to many readers may appear remote both geographically and from our professional interests. Michael Gallagher of the Natural History Museum in Muscat has prompted me to correct these views. He recently sent me a short note by Thabit Zahran (1), now director of the Marine Science and Fisheries in Muscat, about fish mortalities in Omani waters.

Western knowledge of Oman perhaps begins with the 5th century Greek historian Herodotus who called the coastal inhabitants of the NW Arabian Sea, from Makran to Somalia, *Ichthyophagoi* or fish eaters, an indication of fertile coastal waters. The Arab geographer El Idrisi in a desciption of the world prepared for the King of Sicily in 1040 A.D. wrote of the coastal tribes there in much the same terms, and so did Lorimer in his *Gazeteer of the Persian Gulf, Oman, and Central Arabia* published in1915 in Calcutta. These people lived on fish, fed them to their camels, and roofed their dwellings with the skins of whales.

Portuguese commercial motives in the 16th and 17th centuries kindled interest in Oman and two fortresses in Muscat record their presence. They commissioned the Danish botanist Carsten Niebuhr to write a description of the country. The red hair and some oddities of dialect of the Shihuh who inhabit parts of the southern coast are sometimes attributed to these incursions. Dutch, French, and British imperialists coveted control of Oman, and the last persuaded Omani rulers to cede the Kuria Muria Islands srategically placed between Suez and Bombay, in return for support against their nominal Ottoman overlords. Fortune seekers have been tempted to Omani waters by rumours of guano and ambergris, further indications of high productivity. Famous research ships have explored these waters, including the *Challenger* in 1839, the *John Murray* in 1933-34, and the *Discovery* in 1963.

Thabit Zahran (1) reported three fish mortalities which he attributed to red tides, one on the Salalah coast in August 1976 in which an estimated 7 to 10 thousand tons of *Sphyraena, Siganus, Lethriaus,* and *Epinephelus* were killed, and two smaller events near Ras al Madraka in October 1976, and north of Muscat in February 1978. Red tides and fish mortalities are probably very common in this region. Brongersma-Sanders (2) recorded red tides here in September 1936 and October 1950, and linked them to the fish cemeteries discovered during the *John Murray Expedition.*



Fig. 1. Red water near Qalhat, Oman (22°49'N, 59°16'E) in March, 1993.

Accounts of "discoloured water" in this region, always of concern to navigators, are recorded in early editions of the *Gulf of Aden Pilot* (1st edition 1863). Such events are undoubtedly more common than these few episodes indicate. Mr.S.H.G.Bennett (pers.comm.), on behalf of Commander Mugaddam Bahry of the Oman Royal Navy, writes that their Hydrographic Department has been keeping records of red tides since 1991. He says "Red tides seem to occur from February to March, principally from Ra's al Hadd (22°30'N, 59°48'E) to Ra's Abu Daud (23 19 N, 58 56 E) decreasing in intensity towards the NW." Colour photographs supplied to me by the Navy show stunning patches of discoloured water (Fig. 1). In Mr. Bennett's words, an event in March 1993 "was thick and orange ... and had been forced towards the coast by diurnal onshore winds", and another in March 1994 "was more jelly-like and a reddish brown colour". These were in 22°45'N, 59°18'E, and 22°45'N, 59°21'E respectively.

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Ed.



Check out the HAN on WWW!

Harmful Algae News is now available on Internet at http://www.unesco.org/ioc/news/netslet/han.htm

NATO ADVANCED STUDY INSTITUTE
Held jointly with SCOR and IOC
"The Physiological Ecology of Harmful Algal Blooms"
Bermuda Biological Station
27 May-6 June, 1996



Bermuda discussion groups:

Zooplanktion grazing measurements

Measurements of particle ingestion are frequently reported in dinoflagellate blooms, with clearance rates often highly variable. Discussions focused on outlining routine procedures that should be employed in grazing experiments. Manipulation and handling of the grazer populations and prey were intensely discussed, from collection, concentration, acclimation, enclosure, duration and sampling procedures. Similar discussion was initiated for prey. Short discussions of several routinely used techniques were also made, including experiments using changes in prey concentration, labelled fluorescent prey, gut filling and the dilution technique. The group concluded that meticulous and careful handling of grazer and prey would eliminate many of the artifacts likely to lead to the high variability noted in the literature, and that some techniques must be cautiously employed in blooms where initial prey densities might be below the detection limits of the procedure. To further lower variability in grazing measurements, future studies should use HAB species from the same geographic region as the grazers and, if possible, use natural communities at ambient concentrations in one of the treatments. Further, zooplankton abundance needs to be assessed in the field so that estimates of grazing pressure on blooms can be determined.

Kevin Sellner, Jeff Turner

Bulk-phase rheology and small-scale processes

Rheology is study of the deformation of materials, particularly those materials showing both viscosity, the mechanical property characteristic of fluids, and elasticity that is characteristic of solids. Such materials include colloids, particularly polymers. In seawater, they increase the viscosity and provide elasticity. Measurements, recently made of this thickening, indicate that at certain scales, particularly in dense phytoplankton blooms and under conditions of little turbulence, such polymeric thickening dominates the viscosity contributed by the small molecules of water and salt. Then the polymeric component largely determines how whole seawater deforms and flows.

An apparatus for measuring water flow rate through fish gills as a function of hydrostatic pressure difference, from 5 cm to 0.1 cm water (approx. 500 to 10 Pa) was demonstrated. A similar apparatus had previously been used to show reduction in flow (increase in viscosity) produced by HAB species such as *Gymnodinium* cf *nagasakiense*, and thereby

confirm a theory that even though flow is rapid between fish-gill lamellae, it can be significantly reduced by the increased viscosity produced by algal mucus. This reduction of gill ventilation may contribute to the death of fish and other organisms in blooms of some species of ichthyotoxic algae. The advantage of using a freshly killed fish as a rheometer is that it exactly duplicates the scales, geometry and perhaps gill-surface effects occurring in living fish during a HAB attack.

After the demonstration a discussion followed on rheology and small scale processes in relation to HAB ecophysiology, during which the following points were made.



Tery Cuci (left) conducts flow cytometry demonstration.

State of the art

i) Exceptionally, particularly during dense blooms, exopolymeric material produces observable mechanical changes to the water, such as apparently increased viscosity, oiliness, etc. Normally, however, any such changes are not obvious to the casual observer, except in surface slicks.

ii) At relevant length-scales and at the mechanical deformation forces *in situ*, recent evidence suggests that the water that bathes particles, especially phytoplankton and bacterial cells, is frequently not an ideal (i.e., Newtonian) liquid. Colloidal, organic material imparts "excess" viscosity as well as elasticity to the intercellular continuum.

iii) This exopolymeric matter comprises glycocalyxes, looser slime surrounding cells, organic aggregates, and more diffuse material. It acts as an adsorption medium for some substances, thereby reducing their molecular diffusion. By viscous and perhaps also elastic effects, it can reduces or even stops flow and thus also turbulent diffusion.

iv) Over the last decade, classical rheological measurements (i.e., using mechanical spectra) on phytoplankton cultures and seawater have shown: a) a polymeric "excess" viscosity negatively related to shear rate, generally by a power law exponent; b) a polymeric elasticity. Under the conditions of measurement used for the most part, the elastic modulus is typically <20% of the "excess" viscous modulus, but the time scale considered and the amounts of shear might change this; c) both "excess" viscosity and elasticity are positively related to chlorophyll content by a power-law exponent between 1.0 and 1.5; d) both "excess" viscosity and elasticity are approximately log-normally distributed.

v) Somewhat analogous measurements of the rheological properties of material at the air/sea surface show that the material responsible for these properties (polymers consisting of polysaccharides with associated peptides/proteins and lipids) is largely similar to those responsible for the bulk-phase rheological properties. This material is often in exchange between the air/sea surface, no doubt other surfaces, and the bulk phase.

vi) Some organisms are likely to have evolved to use exopolymeric secretions, sometimes along with differential solar heating in layers, to modify: a) dispersion dynamics for nutrients, toxins and allopathic substances; b) inter-organism recognition and control of adhesion. Such recognition and adhesion control is used in predation, and both parasitic and pathogenic infection. It is also used to avoid such predation and infection, and to manage both sexual and trophic encounters. The potential for such employment of polymers increases with population density.

vii) When seawater passes through fish gills, plankton nets and filters, clogging is frequently correlated with phytoplankton abundance. Filter-clogging, which can be produced either by hard particles (e.g., some cells) or by soft ones (e.g., organic aggregates), represents a potential low-tech method to measure polymeric viscosity at different shear stresses (hydrostatic pressure difference across a filter) and length scales (both filter pore size and flux per unit area of the filter). However it is still not known how to relate such clogging to the distributions of mechanical and adhesive properties in the seawater continuum.

Areas requiring more attention

i) Three-dimensional flow trajectories of particles relative to each other in the sea, particularly at length scales from mm to dm, are largely unknown. These should be studied as a function of stratification and rheological properties.

ii) Secretion of polymers by HAB phytoplankton and other species, should be investigated as a function of the abundance and the physiological state of the organisms concerned.

iii) Future work should target the overall structure of dissolved/colloidal organic matter (including recently produced exopolymers) and of aggregates (including transparent exopolymeric particles). This requires characterisation in much more detail than hitherto.

iv) Structure at the primary (make up

of oligomers), secondary (oligomer arrangement in polymers), tertiary (polymer geometry, including folding and cross-links) and quaternary (aggregate structure and inter-aggregate bridging) levels should be determined at the same time as the foregoing points i) and ii).

v) The mechanical constraints exerted by colloidal organic matter on ensembles of both sinking and swimming cells, and their effects on swimming, sinking and fluxes, should be determined at scales from mm to 1000s of km.

vi) There is a need to determine the relationships between contents of phytoplankton and dissolved/colloidal organic matter and clogging of filters, nets and gills by seawater over different length-scales (both mesh size and flow/unit area) and deformation pressures (cross-filter hydrostatic pressure differences).

vii) Physical oceanographers should be urged always to bear in mind that their studies concern a mixed polymeric/aquatic, inter-cellular continuum.

Different approaches

i) Classical rheometry: determination of mechanical spectra using rotating (essentially viscosity only) or oscillating (viscosity and elasticity) Couette-system rheometry.

ii) Fish-gill, net and filter clogging.

iii) Molecular techniques, including fluorescence depolarisation.

iv) Characterisation of primary to quaternary structure of dissolved/colloidal organic matter.

Proposals for future research

i) Investigation of rheology (viscoelasticity) over different deformation regimes and length-scales (measuring gaps), both in HABs and in non-HAB water, as far as possible combined with other investigations (toxicity, nutrient and other physiological states of the algae, C:N ratios of the dissolved/colloidal organic matter and the algae, abundances of phytoplankton, bacteria and other organisms, flocculation and stickiness of phytoplankton and exopolymeric matter, characterisation of primary to quaternary structure in dissolved/colloidal material).

ii) Development of low-tech methods to measure polymeric viscosity at different deformation stresses, suited for ship-board use. Such methods must be calibrated against classical (i.e., hi-tech) rheometric methods.

iii) Characterisation of turbulence in vitro and in situ as modified by various concentrations of HAB and non-HAB phytoplankton species and their mucus.

iv) Investigation of the roles of viscoelasticity, flocculation, adhesion and surface forces, as functions of species make-up and density, on:

v) Vertical and lateral fluxes of: a) chemical elements; b) viable genetic materials.

vi) Toxic and allopathic action, by HAB and non-HAB species.

vii) Organism behavior.

viii) Alteration of predator-prey interactions in the plankton food web.

Although this section addresses marine environments specifically, it is likely to be broadly applicable also to bodies of fresh water.

Ian Jenkinson, Patrick Gentien

Advanced Imaging and Flow Cytometry

A brief review of advanced fluorescent cytometric techniques was presented with special reference to methods most applicable to eco-physiological studies of phytoplankton. All these approaches are amenable to rapid, automated, single-cell analysis by either flow or imaging cytometry.

Phylogenetic stains, including both antigen-antibody and oligonucleotide approaches, were discussed. These techniques allow the identification of species or specific groups of cells in mixed natural samples. Significant work is required to make these methods practical and easy.

Constitutive fluorescent probes were reviewed for algae. These are fluorochromes with affinities to specific cell constituents including the nucleus (nucleic acids), cell wall, membranes, mitochondria, dinoflagellate thecal plates, storage products, and vacuoles.

A third type of fluorescent technique that is potentially useful is the intracellular functional probe. These are fluorochromes that indicate processes or biochemical states within cells such as mitosis, pH, enzyme acitivity, free glutathione or protein -SH groups, transmembrane potential, hydrogen peroxide, oxygen radical formation, or general oxidation state.

Discussion following this brief review focused on ideas of cellular constituents and processes that might be important to measure for understanding phytoplankton eco-physiology. Ideas proposed included total protein, membrane lipids, food vacuole presence or activity for mixotrophy, redox state of the quinone pool, toxin effects on membranes of potential target cells, and other life cycle indicators. It was not known by this group whether fluorescent indicators of many of these processes yet exist. Several members of the group were interested in attending an advanced workshop such as the one that has been held at Bigelow Laboratory on functional fluorescent probes for phytoplankton.

The next major topic of discussion was the use of imaging and flow cytometry for measurements of mixotrophy using fluorescently-labelled prey. Discrepancies between flow cytometric methods and microscope methods were reported. Possible explanations for this were discussed including detection by flow cytometry, overlapping of fluorescently-labelled prey with mixotroph cells in microscope preparations, and fixation artifacts. An experiment to test some of these hypotheses was done using the instruments at the workshop. Results (obtained after the Discussion group meeting) suggested that autofluorescence by *Chrysochromulina* in the green light emission band interfered with detection of ingested fluorescently-labelled prey using flow cytometry. This led to an underestimation of ingestion using flow cytometry relative to microscope methods.

The last topic discussed was the detection of rare events by flow and imaging cytometry. This issue is important in detecting harmful algal species in natural samples before they achieve bloom concentrations. Topics discussed included the number of cells statistically necessary to detect, the effect of flow rate on sample analysis time, and various methods of concentrating samples.

Mike Sieracki

Dinoflagellate cell cycle and cell regulatory mechanisms

During the NATO-ASI on HABs, a number of issues emerged that were addressed in part at the ecological level, but which would benefit from a better understanding of the molecular mechanisms regulating phytoplankton growth and behaviour. A fundamental question which remains unresolved for most HAB species is whether blooms result from events which "trigger" explosive growth rates of HAB species, or whether blooms result from the advection of cells and maintenance of dense populations by either physical or biological mechanisms. A group of approximately 20 ASI participants met to assess the current status of our knowledge of cellular mechanismswhich regulate growth and cell division in dinoflagellates and to discuss how insight into cellular regulations can contribute to the understanding of bloom dynamics.

Two presentations provided focus for the discussion. The first, presented by Fran Van Dolah, provided recent evidence identifying the presence of a typical eukaryotic cell cycle regulatory machinery in dinoflagellates. The cell cycle in eukaryotes is regulated by two classes of proteins, the cyclins and cyclin dependent kinases, which together control progression of a cell into both DNA synthesis and cell division. The presence and mitotic activation of a cyclin dependent kinase in *Gambierdiscus toxicus*, as well as specific inhibition of cell cycle progression by inhibitors of cyclin dependent kinases, indicates that this dinoflagellate posseses at least some of the normal eukaryotic cell cycle machinery, and supports the notion that the eukaryotic cell cycle regulatory machinery was acquired quite early in evolution of eukaryotes.



Fran Van Dolah demonstrates neuroreceptor assay metods. Neuroreceptor Binding Assays, *(See p. 18).*

Gaspar Taroncher-Oldenburg next presented his recent findings on cell cycle dependent toxin biosynthesis. Working with synchronized cell populations, he found that production of saxitoxins by *Alexandrium fundyense* is restricted to the G1 phase of the cell cycle. Using the technique of differential display of mRNA, he has found a number of genes under transcriptional regulation, which are differentially expressed during different cell cycle stages. This is a significant finding in that many dinoflagellate proteins studied to date appear to be regulated at the translational level. It is anticipated that probing the differentially expressed genes may yield components of the saxitoxin biosynthetic pathways and/or cell cycle regulatory proteins.

The general conclusion of this discussion group was that understanding dinoflagellate growth regulation at the molecular level will help to elucidate the mechanisms involved in bloom initiation and progression. Furthermore, it will also allow the development of probes for determination of *in situ* growth rates or other cell cycle events which could prove useful in monitoring and predicting blooms.

Fran van Dolah,
Gaspar Taroncher-Oldenburg

NATO-ASI demonstration:

Demonstration on dinoflagellate mixotrophy and heterotraphy

A two-hour laboratory demonstration on methods for detecting and studying mixotrophy, phagotrophy, and parasitism in toxic and non-toxic dinoflagellates was attended by approximately 70 NATO-ASI students. The demonstration was chaired by Dr. Edna Graneli (Lund Universitet, Sweden), with presentations by Drs. Per Juel Hansen (University of Copenhagen, Denmark), Catherine Legrand and Per Carlsson (Lund Universitet) and Wayne Coats (Smithsonian Institution). Dr. Hansen discussed feeding mechanisms and preferences of heterotrophic and mixotrophic species, and described methods for direct observation of phagocytosis. He also presented a 25-min video showing ingestion of prey by selected dinoflagellate taxa and documenting lethal and sublethal effects

of a toxic strain of *Alexandrium tamarense*on the algivorous ciliate *Favella ehrenbergii*. Drs. Legrand and Carlsson reported methods for determining ingestion of prey and dissolved/colloidal substrates using a variety of fluorescent tracers. Their demonstration included a 30-min video and a 20-min slide presentation that reviewed procedures for labeling potential prey (FLA and FLB), documented ingestion of phytoplankton by *Heterocapsa triquetra* and *Chrysochromulina polylepis*, showed uptake of fluorescently labeled dextran (200K m.w.) by *Alexandrium catenella*, and provided evidence of food vacuoles in *Dinophysis acuta* and *D. acuminata* using DAPI staining. Dr. Coats described cytological staining procedures and fluorescent techniques to reveal the presence of parasites and ingested prey inside photosynthetic and heterotrophic dinoflagellates. His 25-min slide/video presentation considered methods for estimating in situ feeding rates of mixotrophic dinoflagellates and described the development and impact of parasitic dinoflagellates (*Amoebophrya ceratii*) on several host species.

Optical Oceanography & Remote Sensing Demonstration

John Cullen reviewed the principles of optical oceanography, detailed the value of optical measurements in long term monitoring studies, research and aquaculture. He also demonstrated the a spectral radiometer buoy in the field and discussed it applications and versatility (Cullen et al. in press Limnol. Oceanogr.). Pat Tester reviewed the application and availability of sea surface temperature (AVHRR data from the NOAA COASTWATCH Program) and ocean color data sets (CZCS data files on line at http://daac.gsfc.nasa.gov/). CCOAST software designed for viewing NOAA SST files was distributed to workshop participants.

Molecular Probes

This demonstration had three elements. Chris Scholin demonstrated DNA probes for identifying Pseudo-nitzschia species, Jane Gallagher discussed the use of RAPD to differentiate closely related organisms, and Jose Cordova discussed antibody detection of algal species.

DNA Probes

Nucleic acid probes are cited as tools with great potential to speed the detection and quantification of HAB species. Realization of this goal, however, has been difficult for many reasons. Chief among these are a paucity of sequence data and corresponding lack of species-specific probes. Another significant and often overlooked challenge is to develop a probe application strategy that is simple, rapid, and does not demand specialized laboratory facilities and highly trained personnel to execute. In a step towards meeting these demands, whole cell and sandwich hybridization assays were devised for detecting a variety of toxic and nontoxic Pseudo-nitzschia species. In all cases the probes are targeted towards species-specific sequences of large subunit ribosomal RNA (rRNA).

Whole cell (in situ) hybridization begins with a chemical fixation to preserve macromolecular structure followed by an alcohol rinse to dehydrate the specimen and reduce autofluorescence. Fixed cells are subsequently exposed to a solution containing a fluorescently labeled oligonucleotide, rinsed to remove unbound probe, and finally viewed by epifluorescence microscopy. End products of this procedure are intact cells that contain fluorescent probes bound to complementary sequences of rRNA in the cytoplasm. Filter-based sample processing is used to collect cells from a known volume of water and to apply a series of labeling reagents sequentially. After requisite reactions are completed, filters are mounted on standard microscope slides to view and enumerate the labeled cells. This method requires about three hours to execute.

In contrast, sandwich hybridization begins by homogenizing a concentrated sample of living cells in a chaotropic solution that both liberates nucleic acids and protects them from degradation. Two separate hybridization reactions then ensue: capture of target rRNA sequences from the crude lysate using an oligonucleotide tethered to a solid support, and binding of an enzyme-tagged signal probe to a sequence near that of the capture site. Visualization of the captured rRNA/signal probe complex is accomplished enzymatically yielding a macroscopic, colorimetric product on a plastic "dipstick". The extent of color development can provide a measure of the abundance of target species in the original sample. By coupling this technique with existing instrumentation one can detect a variety of Pseudo- nitzschia species in less than 1 hr; approximately 15 min are required of the person performing the assay. Simultaneous detection of multiple species within the same sample is possible.

RAPD Analysis

RAPD's stand for random amplified polymorphic DNA. The technique is a variant of the polymerase chain reaction (PCR) using single primers of 10 base pairs with random sequences. Wherever complimentary sites are located on opposite strands, amplification will occur. The amplification products are then visualized on gels.

Problems with replication of RAPD bands can be caused by impure DNA, small differences in concentrations of reactants in the amplification mixture, and variation among batches of chemicals provided by manufacturers. It was recommended that reactions be repeated more than once.

Empirical data show that the method is only effective as a phylogenetic tool for resolving relationships among very closely related groups, such as populations. It's main use for HAB organisms is for tracking individual genotypes and may be of utility for tracking introductions of organisms. RAPD bands specific to individual genotypes could be cloned and made into probes for very closely related organisms in natural populations.

Antibody Detection of HAB Species

Marine biologists and taxonomists responsible for monitoring programs around the world face every day with the challenge to maintain an accurate record of the organisms that cause algal blooms (red tide). This information is becoming a crucial factor to take the appropriate measures to mitigate the impact of red tide in our society. However, this objective is hampered and sometimes difficult to fulfill due to laborious analysis of field samples under the microscope, by the increasing number of organisms that cause HABs and by the limitation of the morphotaxonomy to distinguish them. A method that could efficiently respond to these problems is the generation of monoclonal antibodies (MAb).

The MAb application in research as well as in biomedicine is notorious due

to their accessibility and accuracy. Perhaps one of the most important properties of MAbs, is their ability to identify a specific antigen regardless of its distribution, localization and number within a complex mixture. An example of this unique feature is the distinction of lymphocytes subsets by specific identification of a membrane antigen.

In this laboratory demonstration the first MAbs generated to the Chilean strain of *Alexandrium catenella*, organism that produces PSP were presented. These MAbs primarily recognize antigens that are theca specific with a specific discrete distribution, suggesting a polarization of the theca. At present, evaluation of their species-specificity is underway. Also, they were coupled to different sized beads that are able to "precipitate" fixed *Alexandrium* cells, indicating their potential use to isolate them from a phytoplanktonic cells mixture. Preliminary attempts to use them in monitoring samples soon will be performed by employing magnetic beads.

MAbs for other *Alexandrium* species are reported. However, limited information is reported to overcome the problems encountered during the process that could help other researchers to generate MAbs for a different toxic species. Therefore, during this laboratory demonstration, special emphasis was made to discuss these problems and some alternatives were provided.

Jane Gallager

Summary of Flow and Imaging Cytometry Demonstrations

Three sessions for approximately 15-20 people each introduced the use of flow and imaging cytometry methods for plankton analysis. Imaging cytometry was demonstrated using a system with two different imagers: a cooled CCD, slow- scan camera used for picoplankton analysis, and a 3-chip color CCD with frame integration used for nanoplankton analysis. Principles of image analysis were introduced with special reference to analysis of fluorescing cells. Methods for automated image analysis of pico- and nano-plankton samples were demonstrated and methods for microplankton analysis were discussed. Examples of natural plankton samples from local Bermuda waters were used to demonstrate the methods.

The basic principles of flow cytometry were reviewed using a FACScan flow cytometer. This included sample flow rate, excitation light sources and beam size, hydrodynamic focusing of the sample stream, data display, and measured parameters. Demonstrated techniques included the analysis of groups of phytoplankton such as cyanobacteria, cryptophytes and *Prochlorococcus* from local Bermuda waters; analysis of mixotrophy in *Prymnesium* ingesting fluorescently labelled algae; and DNA analysis of live and dead cells of both cultures and natural assemblages using new fluorochromes.

One advanced session was held for more detailed discussions of particular problems and analysis of samples brought by participants. Samples included 1) *Alexandrium* stained with both an oligonucleotide, large subunit rRNA probe, and a cell surface immunological probe; 2) *Pseudonitzchia* stained with dual oligonucleotide probes; 3) comparison of *Gambierdiscus* and *Prorocentrum* cells stained with propidium iodide and a new DNA fluorochrome for cell cycle work.

Mike Sieracki and Terry Cucci

Neuroreceptor Binding Assays

The toxins of interest to researchers and regulatory agencies concerned with harmful algal bloom (HAB) events encompass a diverse array of structures, activities, and relative potencies. Attempts to develop reliable as well as efficient means of detecting and quantifying these compounds and their toxicity represent an ongoing effort in many laboratories throughout the world. The neuroreceptor binding assay approach to the selective detection and quantitative estimation of marine biotoxins, including the saxitoxins, brevetoxins, domoic acid, and ciguatoxin, is a promising technique anticipated to find application in both research and regulatory settings, and was among the Demonstration Sessions conducted at the NATO-ASI on the Physiological Ecology of Harmful Algal Blooms held at the Bermuda Biological Station for Research (27 May-6 June, 1996).

A total of about 50 ASI participants attended the receptor assay demonstrations, which were held over a period of four evenings. The primary aims of the demonstration were to introduce participants to the basic principles and procedures of neuroreceptor binding assays, and to encourage members of the HAB scientific community to incorporate this method into research/regulatory activities requiring determination of sample toxicity. Participants were provided with detailed write-ups on individual assays, including background information, protocols, and technical/purchasing information for the necessary supplies and equipment. Each demonstration session consisted of an introductory lecture followed by the performance of assays in a laboratory setting. The lecture was used to outline the rationale for employing the receptor binding approach, discuss the theoretical basis of the assays, provide an overview of the protocols, and summarize the sample types with which the receptor assays are compatible. Participants were encouraged by the robust/rapid nature of these assays, especially their utility in diverse sample matrices (e.g., shellfish, algae, cyanobacteria, bacteria, and mammalian serum and urine) and the prospect of obtaining near-real time toxicity data associated with HABs.

In the laboratory, receptor assays for several toxins, including domoic acid, saxitoxin, and brevetoxin, were either demonstrated or discussed. Participants were shown the steps required to perform these straightforward, radioisotopic-based assays, all of which are conducted in a 96-well microplate format. It was pointed out that the versatility of these assays permits results to be quantified on either a microplate scintillation counter or a conventional rack-type liquid scintillation counter, depending on the type of 96-well format used. Those assays demonstrated involved filtration of samples onto a 96-place filter mat into which a solid wax scintillant was melted prior to counting on a model 1450 Microbeta Trilux scintillation counter, kindly provided by Wallac, Inc. (Gaithersburg, MD) for the demonstration. Many participants commented on the obvious advantages of using the solid scintillant rather than conventional liquid scintillants. A highlight of the demonstration was the performance of the Microbeta scintillation counter which is capable of analyzing 96 samples in about 15 minutes, while the Windows-based controlling software shows a real-time display of the activity for individual samples. The laboratory part of the demonstration concluded with a discussion of what is required for data

processing and interpretation in order to obtain results of the highest possible quality.

By the end of the demonstrations, considerable interest in the neuroreceptor binding assays among the participants was apparent. Based on the numerous requests for additional information and discussions of collaborative research projects involving these assays, this approach to assessing toxicity has the potential to become an integral tool in the study of HAB toxins and their effects.

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Conclusions and recommendations:

1. Mixotrophy by hab species

Justification:
It is now clear that many HAB species have the potential for mixotrophic nutrition. The ability to utilize both photosynthetic and heterotrophic pathways may give species of mixotrophic HAB a competitive advantage over strictly autotrophic members of the phytoplankton. Thus, we cannot understand bloom dynamics, the effects of eutrophication on plankton communities, or life cycle dynamics of key HAB taxa without thoroughly understanding the forces driving mixotrophy and the extent to which autotrophic processes are supplemented by heterotrophy.

Research Priorities:
- Develop reliable methods to detect and quantify mixotrophic nutrition
- Determine the mixotrophic potential of key HAB species
- Identify factors that induce or enhance phagotrophy in photosynthetic organisms
- Investigate the effects of natural and anthropogenic eutrophication on HAB mixotrophy
- Evaluate the effects of mixotrophic nutrition on toxicity of HAB species
- Assess the significance of mixotrophic HAB on the structure and function of marine food webs.

2. Thin layers and microphysics of the pycnocline

Justification:
Different strategies lead some species, amongst which a large proportion of the harmful species, to accumulate into thin layers (some tens of centimeters) associated with the pycnocline for long periods of time. Spatial scales in these features have not yet been investigated but there is evidence that they can extend, at least, to the 10 km scale.

For different species, greater knowledge of behavioral responses responsible for such accumulations or confinement would allow better understanding of HAB dynamics. Detailed studies of a few target species might lay the basis for typifying functional groups of species. Such detailed studies would thus also provide insights into biomodification of the physico-chemical environment.

Although all the detailed processes leading to and maintaining these thin layers are not yet understood or even described, the displacement and maintenance of these layers are of paramount importance to the prediction of harmful events. The behavior of these boundary layers has not yet been sufficiently investigated by physicists (for example in cross-shelf and along-shore transport).

Recommendations:
Studies of the microphysics at the appropriate scales, involving physicists, should be encouraged in order to:

- Characterize the microphysics and chemistry at the viscous and intermediate sub-ranges in order to quantify the various processes influencing the development of HABs.
- Characterize the processes leading to the formation and the erosion of thin-layers.
- Develop an understanding and modeling of mesoscale advection in thin layers in relation to cyclic forcing, weather events and biologically mediated reduction of turbulent viscosity (through turbulence suppression, e.g. by differential solar heating of phytoplankton layers, and/or by viscoelastic polymer secretion).
- In order to understand 3D-movements of these features, development of appropriate instruments is necessary (density-adjusting floats, fine-scale profilers, particle analyzers, video systems, in situ rheometers).

3. Macronutrients

Justification:
There has been an increase in anthropogenic nutrient inputs to many coastal areas. Correlative evidence indicates that HABs have increased in response to elevated nutrient loading in localized regions (e.g., North Sea, Baltic Sea, Seto Inland Sea, Tolo Harbor). Some HABs have also been related to natural nutrient dynamics (e.g., upwellings) in coastal areas. The relationship between macronutrients and mechanisms of HAB formation/maintenance remain to be determined. In particular, it is unknown whether HAB species have unique nutrient utilization capabilities and strategies which allow them to out compete other species, or whether, in the presence of high nutrient availability, the occurrence of HABs is controlled by grazing pressure. Other unresolved issues include the role of nutrient supply ratios in selecting for HAB species, and the importance of DOM either directly, as a nutrient source, or as an indirect promoter of HABs.

Future research priorities:
- Determine the nutrient utilization capabilities and strategies of selected HAB species in comparison with co-occurring phytoplankton. Interactive field and laboratory studies are essential. Field studies may require the development of new technologies for focusing on individual species in conjunction with more traditional approaches.
- Determine how nutrient concentration, composition, ratios, and cycling influence the occurrence of HABs. Retrospective analysis of long-term data available in some regions or evidence preserved in cores can provide

information about the past occurrence of HAB in areas where nutrient inputs have increased. Process-oriented field studies of current HABs, especially recurrent, predictable HABs where all phases of the bloom dynamics can be studied, are essential.
- Evaluate the role of DOM as either a direct source of nutrients which HAB species can utilize or as an indirect stimulant of HABs through interaction with bacteria and the microbial loop.
- Investigate the relative importance of top-down control in regulating species composition of HABs.

4. Populatin genetics/ biogeograhpy

Justification:
Morphological features and life histories are the primary criteria used to distinguish phytoplankton species. However, a single group of organisms defined in this way a species may include multiple genetic variants or strains. Definition of species and their associated infraspecific variants is critical for understanding the basis of biodiversity, toxin production, physiological optima and tolerances and origins of HABs on local and global scales. The presence of genetic variation even within populations of apparently unspecific blooms creates serious problems for studies based on clonal cultures because it is unclear how representative these cultures are of natural populations. There is an urgent need to expand the study of genetic variation in HAB species with particular reference to the roles of biogeography, genetic isolation and long-term changes in genetic diversity.

Research Priorities:
- Molecular tools are particularly useful for these studies. Currently available techniques are limited and progress is slow because of the small number of investigators in this area. More technology needs to be developed, particularly those techniques that bridge the critical gap between lab investigations and natural populations. More training opportunities need to be provided for phytoplankton ecologists to learn these tools.
- The requirement of morphotaxonomy to provide a platform for species identification and genetic comparisons necessitates a renewed effort in this area. The impending loss of expertise in morphotaxonomy creates a need for training new people in traditional as well as advanced methods of recognition of morphospecies.
- Support for culture collections is declining. However, a primary requirement for the study of genetic variation among HAB organisms is the availability of multiple isolates of the same species from different geographic areas. Recognition of the importance of culture collections is required and more financial support is needed to maintain and expand them.
- Physiological studies should recognize the potential for variability for such parameters as environmental optima and tolerance ranges, quantity of toxins and toxin profiles because the patterns of variation may yield useful insights into the relative importance of adaptive factors controlling the distribution of organisms. More technology, such as automated counting methods, need to be developed that facilitates the examination of larger sample sizes.
- Multidisciplinary approaches to the study of genetic variation can yield critical insights. Support for multidisciplinary investigations that examine HAB species from both morphological and subcellular perspectives should be encouraged.
- There is provocative evidence that anthropogenic dispersal of HAB species has occurred. The introduction of harmful organisms may be accompanied by the introduction of harmful genes that may be incorporated into native populations. Further assessment of the extent of role of human-assisted dispersals and their occurrence in the past needs to be conducted.

5. Freshwater/ stratification

Justification:
Some degree of vertical stability is essential for the development of phytoplankton blooms. Such stability can be provided by density gradients caused by heat or by low salinity inputs. Examples of the latter include estuaries and buoyant coastal currents. However, freshwater input also brings with it a supply of poorly characterized, land-derived nutrients: macronutrients, such as nitrogen and phosphorous from agricultural and industrial/domestic sources; micronutrients including metals and vitamins; and, dissolved and particulate organic materials. The latter may serve as growth promoters (e.g. chelators) or directly as nutrients.

The linkage between HABs and freshwater input has been established in many areas (e.g. *Phaeocystis* blooms associated with Rhine River discharge, *Heterosigma* blooms in the Strait of Georgia, *Alexandrium* blooms in the southwestern Gulf of Maine coastal current, and *Pyrodinium* blooms along river plume fronts). Research is needed to understand the mechanisms underlying this linkage and to elucidate the relative importance of the many factors which may be operating.

Research priorities:
- determination of in situ algal species growth rates and physiological status within and outside areas of freshwater influence as they relate to the chemical constituents of the water;
- determination of the relative importance of physical effects and algal behavior vs. direct growth stimulation;
- elucidation of the mechanisms underlying blooms occurring within low salinity water masses vs. those occurring at the boundaries of water masses;
- develop an understanding of the influence of the timing of freshwater inputs and stratification to bloom dynamics

6. Small scale physics, behavior, and photosynthesis

Justification:
We have a conceptual model, often termed the Margalef mandala, that provides a framework for describing how physiology, behavior and hydrography interact to promote or maintain algal blooms. We also have good descriptions of algal blooms in several well-studied regions. However, we are not yet able to couple sufficiently detailed information on algal physiology and behavior into realistic hydrodynamic models to describe single species bloom events in natural water columns. Consequently, our present numerical models are not robust in the real world.

Research Priorities:
- Incorporate physics into all HAB initiatives to provide information on the environmental conditions that support bloom development, maintenance and decline.
- Develop effective methods to characterize how organisms behave under

the influence of variable environmental factors.
- Develop appropriate methods to characterize photosynthesis and nutrition under relevant, natural hydrodynamic variability. Conventional culture techniques are not sufficient.
- Improve coordination among physiological/behavioral characterizations, biological-physical modeling, and quantitative descriptions of hydrography and natural communities.

7. Ecophysiology of toxin productin in hab species

Justification:
Toxic secondary metabolites are produced by several photosynthetic eukaryotic and photosynthetic/nonphotosynthetic prokaryotic groups of microorganisms. To date, research on these compounds has focused on structural elucidation, detection methods and mechanisms of action. The next major areas for understanding the physiological function of toxin production should be a rigorous investigation of their biosynthetic pathways and genetic regulation. Some data is available on these topics but we have only begun to really understand these processes.

The effect of environmental factors on production of phycotoxins has not been investigated in detail for any of the toxin groups but some information is available for a limited number of strains and key species, e.g. PSP, NSP, and DSP toxins in *Alexandrium* spp., *Pyrodinium bahamense, Gymnodinium catenatum, G. breve Prorocentrum spp.,* anatoxin production in *Anabaena* spp., and domoic acid production in *Pseudonitzschia* spp. We know some information on saxitoxin and neosaxitoxin biosynthesis yet the biosynthetic pathway and its genetic regulation among the different toxin-producing organisms is unknown. In contrast, the structure and biosynthesis of domoic acid is better understood and we can now ask questions concerning its regulation and physiological importance. A total understanding of the function of these toxins will only be achieved when we understand their structure, biosynthesis, genetic regulation and ecophysiology. Emphasis should be on the functional roles of toxins in the primary source organisms e.g. do these toxins serve an intrinsic or an extrinsic function?

Research Priorities:
- Determine the kinetics of growth, toxin biosynthesis, and interconversions.
- Identify the cellular location of the toxins and the timing of their production in the cell cycle.
- Characterize the components of the toxin biosynthetic pathways.
- Determine if toxins are directly involved in predator-prey interactions.
- Determine if toxins serve an allelopathic function against other algae, bacteria or fungi, including the role of other compounds in stimulating or inhibiting the effect of the toxins.
- Determine the critical interactions between extrinsic environmental factors and the genome in regulating toxin biosynthesis, catabolism and sequestration (phenotypic vs. genotypic variation in HAB species).
- Model the production of toxins for extrapolation to natural populations.
- Determine the evolutionary origin of the toxin biosynthetic genes.
- Develop an international source for toxin standards and reference materials.

Understanding the genetic basis for toxin production will provide valuable insights into their ecophysiological role in bloom dynamics.

8. Bacterial-algal interactionsin hab population dynamics, cellular growth, and toxicity

Justification:
Co-existing with algal communities containing HAB species are microbial assemblages which, like the algae, undergo changes in species composition, exhibit nutrient competition, and produce bio-active compounds, including toxins. These and other microbial processes are influenced by the algal community and, in turn, can potentially influence the population dynamics, cellular growth, and toxin characteristics of the algae. It is now clear that bacteria can produce both intracellular and extracellular compounds with algicidal as well as growth inhibiting or stimulating effects. Moreover, available evidence demonstrates that bacteria not only can modify the toxicity of algal cells, but can themselves synthesize certain 'algal' toxins.

Research Priorities:
We must now characterize natural bacterial assemblages associated with HABs, and establish whether production of growth/toxicity modifying substances and/or toxins occurs on temporal and spatial scales permitting them to influence algal bloom dynamics, cellular growth, and toxicity. It is also essential to determine whether bacteria constitute an important source of 'algal' toxins in the context of trophic transfer and seafood safety issues. Suggested approaches to achieve these objectives include:

1. Apply classical and molecular approaches to characterize the temporal/spatial association of bacterial assemblages with HABs, and define the systematic as well as functional relationships between bacteria and these algae.
2. Isolate and characterize bacterial and algal bio-active metabolites, and determine their respective effects on bacterial and algal growth and/or toxicity, including elucidation of the mechanisms involved and the patterns of synthesis in natural communities.
3. Evaluate the role of toxigenic bacteria as a point of entry for the transfer of 'algal' toxins between trophic compartments in aquatic food webs, and assess the contribution of these microbes to the toxification of fisheries resources.
4. Determine the distribution of toxin genes among bacteria and extrachromosomal elements (e.g., plasmids, viruses) as a means of explaining the phylogenetic diversity of organisms capable of synthesizing certain 'algal' toxins, and identifying potential mechanisms for the lateral transfer of these genes.

9. Trace Metals and Chelator Interactions

Trace metals including iron, manganese, cobalt and selenium have been hypothesized to limit the production of harmful algal blooms. Examples include limitation of *Alexandrium tamarense* (Gulf of Maine), *Aureococcus anophagefferens* (Peconic Bay), *Heterosigma sp.* (Osaka Bay) *and Chattonella antiqua* (Seto Inland Sea) by iron, the limitation of *Chrysochromulina sp.* (Kattegat) by cobalt and the inhibition of *A. tamarense,* and *C. antiqua* by copper. In most cases, the importance of a single trace met-

al was determined when the addition of that particular metal stimulated the growth of a HAB organism in culture.

In recent years, the importance of chemical speciation and organic complexation in the open ocean has revolutionized our view of how trace metal chemistry affects phytoplankton growth dynamics. Current data on trace metal concentrations in coastal waters, along with information on chemical speciation and biological interactions indicate that it is plausible that trace metals influence the growth and species composition of coastal phytoplankton communities. This new information on trace metal availability now needs to be applied to the development, density and frequency of harmful algal blooms.

Areas for future research include:
lObtain more detailed information on trace metal concentrations and speciation in coastal waters.Determine techniques to estimate their bioavailability to harmful algal bloom species.

- Examine the mechanisms of uptake.
- Examine the effects of multiple nutrient limitation.
- Determine the minimum cell quotas for trace metals.
- Examine the effects of trace metal limitation on cellular biochemistry including toxin biosynthesis.
- Examine the importance of bacteria trace metal interactions.

10. Life Cycles

Knowledge of life cycles and cell cycles is fundamental to the understanding of formation, maintenance, and decline of harmful algal blooms. The water column or benthic origins of bloom inocula, cell growth, bloom termination and long-term species are mediated by poorly understood or largely unknown life cycle event. Most HAB species have benthic phases, which are believed to be important in species survival and persistence, but control on interactions between planktonic and benthic phases remain to be elucidated. The following information on the basic biology of these organisms will be vital to furthering our understanding of bloom dynamics:

- full elucidation of life histories for all HAB species (including asexual and sexual reproduction);
- the development of rapid and reliable molecular/immunological probes for detection of life cycle stages, leading to improved and/or automated recognition;
- description of cell cycle morphologies (for example, division stages) facilitating calculation of in situ growth rates;
- determination of endogenous cell cycles, for example, the gating of cell division on a diurnal basis and annual cycle of excystment;
- identification of the specific nutritional and environmental triggers as well as genetic controls involved in stage transformation, and the conditions required for successful transformation;
- determination of the survival and metabolic activity of benthic stages;
- understanding the physics of warm column – sediment transport and subsurface sediment mixing and burial in deep sediment and their influence on life history.

11. Emerging techniques and technology

Justification:
An understanding of HAB phenomena requires the detection of causative species and the toxins they may produce. Analogous problems exist in biomedical diagnostics, and many techniques and technologies used for those purposes are applicable to HAB research. For example flow cytometry, antibody and DNA probes, phycotoxin-specific receptor binding assays have been transferred and used successfully in the laboratory and, to varying degrees, in field studies. The interfaces between traditional disciplines have often yielded scientific breakthroughs, thus interdisciplinary collaborations should be fostered to solve the difficult issues relevant to understanding HAB phenomena. Programs should be developed that foster cross-training and collaborations between biomedical and HAB researchers.

Research priorities:
Inexpensive, simple, efficient, and accessible methods for identification and detection of all HAB species and toxins must be developed. Methodologies and tools should be made widely available to the field as a whole as they are developed. Confirmation of the efficacy and utility of new techniques in the field requires multi-disciplinary investigations that could include: taxonomy, physical oceanography, ecology, and biochemistry.

- *Probes for species identification.* DNA and antibody probes have been developed and tested in the laboratory for a limited number of HAB species. These probes must now been tested in the field.
- *Toxin detection.* Receptor assays for toxins in HAB species have been demonstrated in the laboratory. These assays now need to be tested for utility in natural populations.
- *Measurement of in-situ growth rates.* Methods for determining in situ growth rates are needed in order to understand the mechanisms driving HABs. Flow and imaging cytometric



PHOTO: DR. SERGE MAESTRINI (CREMA, L'HOUMEAU, FRANCE).

"East meets West" at the Bermuda NATO ASI, Dr. Maija Balode (University of Latvia), and ASI Director Dr. D.M. Anderson (WHOI)

methods have been demonstrated to be useful in the field and show promise for HAB species.

- *Probes for physiological status*. Many fluorescent probes for measuring intracellular conditions have been developed for mammalian systems. Application of these tools to algal physiology may yield new understanding of the regulation of phytoplankton population dynamics.

Since the choice of reference taxa is critical due to potential genetic variability, culture repositories should be supported and expanded internationally.

Reccomendations from Graduate Student Participants at the ASI

Reflecting upon the scientific interactions which brought about the formation of this NATO-ASI and the fruitful exchange of ideas which occurred throughout it, we would like to see the continuation of such cooperation on the following levels:

i) Integrated Monitoring of HABs: monitoring of harmful algal system dynamics to include aspects such as the microbial loop and grazing measurements.
ii) Collaboration, to promote the integration of laboratory and field studies, to facilitate the cross-fertilization of scientific fields to further our understanding of HABs on a multidisciplinary level (i.e. physicists, chemists, limnologists), to develop an "international laboratory" to encourage the exchange of knowledge and technology, and to disseminate information regarding HABs to all levels of society
iii) Establishment of Graduate/Post-Doctoral fellowship programmes for the study of HABs

Now on WWW!

The IOC Directory of Scientists and Managers in Toxic and Harmful Algae and Their Effects on Fisheries and Public Health, is now available as a searchable data-base at

http://www.unesco.org/ioc/isisdb/html/habd.htm

HARMFUL AND TOXIC ALGAL BLOOMS

The IOC and the Laboratory of Bioorganic Chemistry, Tohoku University is happy to announce that the Proceedings of the Seventh International Conference on Toxic Phytoplankton, Sendai, Japan, 12-16 July 1995 are now available.

Through joint effort of the Organizing Committee, University of Tohoku and the IOC it has been made possible to publish the Proceedings at low cost. With the cooperation of Instituto Espanol de Oceanografia and Woods Hole Oceanographic Institution is has been made possible to ensure distribution also at a very low cost.

We can therefore offer you the Proceedings for 30 US$ per copy. It can be ordered from the addresses below according to where in the world you live.

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Future events

IOC-ICI-IEO Training Course on Toxic Phytoplankton, Analytical Techniques for Marine Phycotoxins, Vigo, Spain, 29 June-16 July 1997. The IOC, the Institute for Iberoamerican Cooperation (ICI) and the Spanish Institute of Oceanography (IEO) are organizing a course on toxic phytoplanton with special emphasis on the detection and quantifucation of marine phycotoxins (bioassays, in vivo and in vitro, chemical, immunoenzymatic and biochemical techniques). Lecturers will include staff from IEO, Laboratorio de Sanidad Exterior (European Community Reference Laboratory on Marine Biotoxins), and Conselleria de Pesca, Marisqueo e Acuicultura (Xunta de Galicia), plus other guest lecturers from Spanish and foreign institutions. The Course will be given in Spanish and priority will be given to applicants from Latin America. Deadline for applications is 30 March 1997. Contact: IOC-IEO Science and Communication Centre on Harmful Algae, Instituto Espanol De Oceanografia, Cabo Estay, Candio, Apartado 152, 36280 Vigo, Pontevedra, Spain, Fax.: +34 86 49 23 51, E-mail: insovigo@cesga.es

IOC Danida Training Course on the Taxonomy and Biology of Harmful Marine Microalgae, August 1997. This course will focus on identification and preparation techniques supplemented by lectures on different aspects of the biology of harmful algae. Teaching staff will include: Dr Yasuwo Fukuyo (Univ. of Tokyo), Prof. Øjvind Moestrup (Univ. of Copenhagen), Dr Jacob Larsen (Univ. of Copenhagen/IOC Centre), Organized by the University of Copenhagen, and IOC Science and Communication Centre on Harmful Algae, Copenhagen. For application forms, contact: the IOC Science and Communication Centre on Harmful Algae, Botanical Institute, Ø. Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; fax: +45 33 13 44 47. Deadline 7. April 1997.

IOC-NorFA Nordic-Baltic Course on the Taxonomy and Biology of Harmful Microalgae, Tvärminne Zoological Station, Finland. 17-24 August 1997. The objective of the course is to improve the taxonomic skills of the participants in order to enable them to make reliable identification of phytoplankton species causative of harmful algal events in the Baltic region. The Course will cover also ecology and toxin chemistry particularly of blue-green algae. A number of seats are available for participants from the Baltic countries and from Northwest Russia. Course lecturers will include: Dr. G. Cronberg, University of Lund, Dr. K. Kononen, Finnish Institute of Marine Research, Dr. J. Larsen, IOC Science and Communication Centre on Harmful Algae, Professor Ø. Moestrup, University of Copenhagen, Denmark; Dr. K. Sivonen, Finnish Institute of Marine Research. Deadline for applications: 1 April 1997. Application form can be obtained from the address below and from the WWW at http://www.unesco.org:80/ioc/oslr/oslr.htm. Contact: IOC Science and Communication Centre on Harmful Algae, Copenhagen. (See below).

International Symposium on Marine Cyanobacteria and related Organisms. Instiute de Océanopgraphique, Paris, 24-28 November 1997. Organizers are ORSTOM, University of Sidney, CNRS, French Ministry of Foreign Affairs. The symposium will focus on new techniques such as molecular phylogeny and cell sorting. Contact: Dr. Loïc Charpy, ORSTOM, Centre d'Océanologie de Marseille, Rue de la Batterie des Lions, F-13007, Fax. (33) 491 01 16 35, E-mail: charpy@orstom.rio.net

Second International Conference on Molluscan Shellfish Safety (ICMSS II), Iloilo City, Panay Island, the Philippines, 13-17 November 1997. A forum for scientists from various disciplines; and shellfish growers, processors and regulators. Discussions will focus on the latest research, commercial practices and standards concerning the contamination, purification and overall safety of molluscan shellfish with respect to chemical, biological and microbiological contaminants. Contact: Dr. Rhodora A. Corrales, Chairman, Organizing Committee, Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, the Philippines; fax: (63-2) 9215967; E-mail: hod@msi.upd.edu.ph

IOC publications

- International Directory of Experts in Toxic and Harmful Algae and their Effects on Fisheries and Public Health. Published jointly with the US NOAA. *IOC INF Document* 1015.
- Design and Implementation of Some Harmful Algae Monitoring Systems. Co-published with ICES. *IOC Technical Series* No. 44.
- Summary Report of the Third Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, Paris, 6-9 June 1995. Available in English, French, Spanish.
- Amnesic Shellfish Poisoning (ASP), Volume 1, IOC *Manuals and Guides*, No. 31.

These publications are free of charge and available from the IOC Secretariat (Paris) and the IOC Science and Communication Centre, Copenhagen (see addresses elsewhere on this page).

- Manual on Harmful Marine Microalgae, IOC Manual and Guides No. 33. The Manual is free of charge, but a fee of US$ 30 will be charged to cover postage and handling. A cheque issued to H. Enevoldsen, IOC should accompany all orders. Excepted are marine science libraries and scientists in developing countries which can apply for delivery free of charge.

Literature for developing country libraries

Limited copies of the following titles are available from IOC's HAB Centre in Denmark, to libraries of marine science institutions *in developing countries only*:

- The Genus Alexandrium Halim (Dinoflagellata). **E. Balech, 1994.**
- Algae. An Introduction to Phycology. **C. van den Hoek et al., 1995.**
- Guide to Toxic and Potentially Toxic Marine Algae. **J. Larsen & Ø. Moestrup, 1989.**
- Proceedings of the Sixth International Conference on Toxic Marine Phytoplankton. **P. Lassus et al. (eds.), 1995.**
- The Biology of Dinoflagellates. **F.J.R. Taylor (ed.), 1987.**
- Marine Phytoplankton. Identifying Marine Diatoms and Dinoflagellates. **C. Thomas et al. (eds.), 1996.**

Applications must be submitted and signed by the responsible librarian, and should indicate why the requested title is of specific interest to your institution and its ongoing research or teaching. No requests from individuals will be honoured. Address applications to:

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Harmful Algae News

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 16

◆ USA, Washington

Heterosigma bloom and associated fish kill

A bloom of the harmful marine phytoplankton, *Heterosigma carterae* occurred in upper Case Inlet, south Puget Sound, Washington in late September, 1994, correlating with the presence of at least 35 dead salmon. This marks the first time that this alga has been closely correlated with a wild fish kill; in the past it was thought to be associated with kills of penned fish at fish farms only. We were informed of the presence of a possible harmful algal bloom and dead salmon near the town of Allyn on 27 September and a team was formed to investigate. We arrived at the Allyn waterfront at 17:30 hours the same day. Prior to our arrival, state agency personnel walked approximately two miles of shoreline from the powerlines north of the dock, to the mouth of Sherwood Creek and conducted the only official count of dead fish present along the shore consisting of 12 coho salmon *(Oncorhynchus kisutch),* 11 chum salmon *(Oncorhynchus keta),* 12 chinook salmon *(Oncorhynchus tschawytscha),* one flat fish, and one sculpin on the morning of 9/27. Since previous harmful blooms of *Heterosigma* have resulted in the majority of net penreared salmon sinking to the bottom of pens, and only approximately two miles of shoreline were sampled, it is suspected that many more exposed fish may have succumbed than were counted. Witnesses who explored the east side of the bay reported seeing many dead salmon there as well, but no counts were made. State agency personnel who observed the fish kill reported seeing "dying fish coming to the beach, gulping at the surface, trying to get out of the water." Scavengers were seen consuming the salmon carcasses; these included two harbor seals, a house cat, and Hymenopteran insects. None suffered any noticeable acute ill effects. Although precise cause of death has not been ascertained, visual inspection of the reproductive organs from a deceased male chum salmon found on the shore at Allyn confirmed that the fish was not yet reproductively mature and therefore did not die from completion of the spawning process.

(Cont'd on p. 4)

◆ Brazil

PSP Outbreak in Brazil

This communication reports the first appearance of PSP toxins in cultured mussels from Santa Catarina Coast, in southern Brazil, observed during a pilot HAB monitoring program. The program was set first in a culturing area in order to start HAB studies to support mussel culturing, which has been growing fast along Santa Catarina's coast in the last decade (HAN No. 12/13, 1995). Due to algal distribution and former cases of massive intoxication characterized by gastrointestinal disorders, our first attempt was to verify the occurrence of DSP toxins. In august 1995 we first detected mussel toxicity by the mice bioassay in acetone extract. The extract was analyzed by HPLC and a small amount of okadaic acid was detected in the sample (1) and later analysis showed the presence of traces of PSP in the same sample. After this finding, we started to carry out regular analysis of mussel toxicity by the mice bioassay in a pilot HAB monitoring program at Armação do Itapocoroy (27° S). On the afternoon of Friday 26 of

(Cont'd on p. 3)

◆ USA

ECOHAB Program underway

The U.S. has just initiated its first national research program on the ecology and oceanography of harmful algal blooms (ECOHAB). ECOHAB, a partnership of four Federal agencies, the National Oceanic and Atmospheric Administration, National Science Foundation, Environmental Protection Agency, and Office of Naval Research, is coordinated by Dr. Kevin Sellner in NOAA's Coastal Ocean Program and is designed to support long-term research on the physiology, behavior, ecology and toxicity of HAB species and the environmental factors responsible for bloom expression. There are two types of research considered in ECOHAB, large, multidisciplinary regional studies that provide a regional understanding of physics, basin characteristics and water quality that support the physiology and ecology of the HAB species, in the end generating a predictive model for forecasting regional blooms. Targeted studies comprise the second research element. These projects include one to several researchers and focus on identifying specific characteristics of a bloom species that will assist in the general understanding of the organism's cellular characteristics, ecology or behavior.

Following competitive peer review, two regional and seven targeted projects were selected for support. The regional studies focused on *Alexandrium tamarense* in the Gulf of Maine (D. Anderson

(Cont'd on p. 2)

Funding for the Harmful Algae News is provided by the Coastal Ocean Program of the U.S. National Oceanic and Atmospheric Administration.

(Cont'd from p. 1. "ECOHAB Program underway"

et al., WHOI) and on *Gymnodinium breve* in the Gulf of Mexico (K. Steidinger et al., Florida Department of Environmental Protection). Targeted studies included projects on brown tide population genetics (J. Stabile and I. Wirgin, New York University), trophic effects of *Prorocentrum minimum* and *Gyrodinium aureolum* (G. Wikfors et al., National Marine Fisheries Service) and *A. tamarense* (E. Durbin et al., U. Rhode Island), including sea otters and shorebirds (R. Kvitek, California State University Monterey Bay), ecophysiology of *Pseudo-nitzschia* (D. Garrison et al., U. of California, Santa Cruz), algicidal bacteria and *G. breve* (G. Doucette, U. of South Carolina) and macroalgal overgrowth in tropical reefs (V. Paul, U. Guam).

A second ECOHAB announcement has just been published providing support for regional and targeted studies as described above and a new area addressing *Pfiesteria* and related species. These taxa have been identified in the Mid-Atlantic and South Atlantic states of the eastern U.S., associated with fish lesions, fish kills and in some cases, human health problems. In 1997, *Pfiesteria* and related species severely impacted fish in several small tributaries on the eastern shore of Chesapeake Bay and more dramatically, caused acute short term memory losses and skin rashes in people exposed to *Pfiesteria*-containing water or aerosols. This threat to human safety resulted in a Federal response, providing immediate funding for state monitoring and assessment teams as well as public health studies. Using the U.S. national HAB plan as a model (*"Marine Biotoxin and Harmful Algae: A National Plan"*), a comprehensive strategy was developed for *Pfiesteria* and related taxa and HABs in general providing priorities for immediate, short term and long range efforts in monitoring, assessment, research and public health and education (*"National Harmful Algal Bloom Research and Monitoring Strategy: An Initial Focus on Pfiesteria, Fish Lesions, Fish Kills and Public Health"*). Proposals are due in late February, 1998 and following peer review, the second round of ECOHAB awards will be issued in mid- to late summer.

The U.S. ECOHAB Program provides a foundation for a long-term national program to support research on the increasingly frequent HABs in U.S. coastal waters. Through ECOHAB, a national predictive capability for HABs along the U.S. coast is anticipated, providing coastal communities with forecasting abilities to mitigate bloom impacts on coastal resources and local economies and ensuring public safety. ECOHAB research should also enhance development of cell and toxin assays, extremely important in pro-active monitoring programs in coastal waters. Finally, ECOHAB might be applicable for designing investigations of HABs common in other parts of the world, as exemplified by recent IOC support of ECOHAB as a model for its participating members (Paris, 1997) and IOC-SCOR discussions of an international workshop for development of an international HAB program along the lines of the ECOHAB Program. With increasingly frequent HABs all over the world, international multi-disciplinary investigations of blooms and their causes would greatly enhance our global predictive capabilities, potentially providing bases for predicting benefits from bloom control strategies throughout our coastal ecosystem once effective control strategies have been identified.

Additional information on the U.S. ECOHAB Program, including Announcements of Opportunity, as well as other relevant information on U.S. HAB activities can be obtained from our web site (under "Projects" on the HAB page at http://hpcc.noaa.gov/cop/projects/HAB.htm), the National Office on Marine Biotoxins and Harmful Algal Blooms at http://www.redtide.whoi.edu/hab/ or Kevin Sellner, ECOHAB Coordinator, at ksellner@cop.noaa.gov.

◆ Global

27 countries meet to discuss HAB priorities

On 30 June - 2 July 1997 the IOC Intergovernmental Panel on Harmful Algal Blooms held its fourth session (IPHAB-IV) in Vigo, Spain. The Panel was hosted by Instituto Español de Oceanografía, and more than 40 participants from 27 countries met to review activities completed since the Third Session of the Panel in 1995, discuss priorities, and to decide upon new initiatives to be taken.

The Panel noted that the awareness of problems caused by harmful algae is constantly increasing on a global basis. It was therefore proposed to develop an international science agenda on the ecology and oceanography of harmful algae to help IOC Member States in setting national priorities and in particular to promote the establishment of national and international research programmes. The Panel made a number of recommendations to promote science and capacity building in relation to harmful algae; (i) the Panel encouraged a stronger participation of scientists from IOC Member States in the ICES-IOC Working Group on the Dynamics of Harmful Algal Blooms; (ii) it was recommended to support on-going long-term studies and inclusion of phytoplankton species composition analysis as a routine part of monitoring programmes (including GOOS and LOICZ); (iii) the regional aspect was emphasised, and a strengthened coordination between harmful algae activities of organizations in the Pacific (IOC/WESTPAC, ASEAN, APEC, PICES) was stressed; (iv) The formation of an IOCARIBE working group on harmful algae in the Caribbean and adjacent regions was recommended; (v) The necessity of enhancing an integrated and coordinated research effort in Europe was stressed; (vi) Improved management and mitigation of the effects of harmful algae was referred to as a high priority; (vii). It was recommended that the IOC, in cooperation with APEC, organizes an international workshop, directed towards dialogue and improved design of management and monitoring of harmful algae and algal toxins; (viii) To ensure continuity in the implementation of the HAB programme, and to follow up on the recommendations of the IPHAB, it was reiterated that the HAB Programme Office requires one additional staff member to be seconded by Member States. Dr. Adriana Zingone, Italy, was re-elected Chair and Dr. Rhodora Azanza, the Philippines, was re-elected Vice-Chair of the Panel.

A full report of the IPHAB-IV is being published and can be obtained from the IOC Secretariat and from the IOC WWW-site.

◆ Argentina

Ceratium hirundinella blooms in Argentine reservoirs

Ceratium hirundinella (O.F. Müller) Schrank is a widespread freshwater dinoflagellate ocurring in temperate and subtropical lakes and reservoirs of the Northern Hemisphere. Records for Southern Hemisphere are, in contrast, relatively scarce, with a few mentions for Africa, southeastern Australia and Patagonia (1). In fact, this species has previously been recorded in South America as extremely rare in several Andean lakes of Southwestern Argentina (2, 3) and Chile (A. Boltovskoy, pers. comm.).

In recent years, however, dense blooms of *C. hirundinella* were detected in western Patagonia, mainly in reservoirs located along the rivers Limay and Neuquen. The highest population densities (275 cells.ml^{-1}) were recorded in E. R. Mexia and Arroyito Reservoirs (November 1996 and December 1996 respectively). Unfortunately, there are no quantitative data for Neuquen River reservoirs. All these man-made lakes are used for hydroelectric power generation; nevertheless, no harmful effects have been so far reported.

During early summer 1996-1997 *C. hirundinella* was first recorded in Paso de las Piedras Reservoir (located in southeastern Pampean Plains), the drinking water supply for the towns of Bahia Blanca (pop. 270,000) and Punta Alta (pop. 56,000). *Ceratium* population increased very rapidly and attained a maximum density of 2,000 cells.ml^{-1} by mid-summer (February 1997), raising chlorophyll a concentration from 38 to 130 mg.m^{-3}, the highest since the reservoir was built. The bloom of this large-sized plankter resulted in the clogging of the sand filters in the treatment plant and the consequent shortening of filter runs. This event was accompanied by a quite noticeable fishy to septic odour.

Massive developments of *C. hirundinella* and its rapid expansion in Argentine water-bodies are recent phenomena whose determining causes are still not clear. During their life cycle these dinoflagellates usually form resting cysts under unfavorable conditions and then sink down to the sediments. When favorable conditions return, the nutrients stored in the cysts allow the germinating vegetative cells to iniciate a bloom, independently of the nutrient concentration in the water (4). This competitive strategy suggests that *C. hirundinella* will persist as a troublesome algae for reservoir management and water quality in an early future.

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(Cont'd from p. 1. "PSP Outbreak in Brazil")

July of 1997 (austral winter), we detected by mice bioassay the first PSP positive in cultured mussels (*Perna perna*). Although positive, sample toxicity was under 400 MU.100g^{-1}. The presence of PSP was latter confirmed by post column oxidation FLD-HPLC analysis and toxicity was attributed to GTX_1, GTX_2, GTX_3, GTX_4 and STX. Concentrated net samples showed a similar toxin profile. Several dinoflagelates were present in the water, including species of *Alexandrium*, *Gonyaulax*, and *Gymnodinium*. Action was taken in order to notify local public health authorities which stayed on alert, and to sample and analyze samples from other culturing areas. All samples produced visual symptoms in the mice but toxins were only detected in samples from Armação do Itapocoroy. The toxicity increased a little and after two weeks it decreased to well under 400 MU.100g^{-1} following a the passage of a cold front system. These results have confirmed the necessity of a more comprehensive monitoring program in the region. From an ecological point of view, we still have to find the "guilty" alga or algae and attribute its toxicity. One possibility is that the causative organism was *Alexandrium tamarensis* which causes PSP in Argentina and Uruguay, and was observed on the high abundance in 1996 at Rio Grande Coast (2), about 700 km south of Armação do Itapocoroy. Other PSP producers in the southern Atlantic include *Gymnodinium catenatum* and *A. catenella*. These findings brings new elements to the discussion: is PSP spreading from the southern cast Atlantic to northern waters or was it always there but not detected?

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(Cont'd from p. 1.
"Heterosigma bloom and associated fish kill"

Noticeable red algal streaks were present in the water during the sampling period on 9/27, which coincided with vertical salinity and temperature stratification of the water column and Secchi disk depths of 0.5 m. The water appeared clear the following morning, 9/28 with Secchi disk depths of 3 m until the tide began to flood, at which time vertical temperature and salinity stratification became established, characteristic red algal streaks reappeared, and Secchi disk depths decreased to 1 m. Unusually low nitrate concentrations of 0.22 µM were recorded throughout the water column during low tide on 9/29, however ammonium and orthophosphate concentrations were relatively normal. Numerous dead salmon carcasses, presumably not included in the fish count, were noted floating past the dock during the sampling period from 9/27-9/28.

Investigation of the area encompassed by the bloom on 9/28 suggested that it extended north to the end of the bay and south as far as Sherwood Creek and Gills Cove. Red discoloration in the water column and dead chum salmon, presumably not included in the fish count were seen on the bottom of Gills Cove and around the mouth of Sherwood Creek. Reports from residents around Allyn suggest that the bloom began on 9/24, and 9/28 was the last day we saw evidence of the bloom.

A review of climatological data provided by the National Oceanic and Atmospheric Administration for Seattle Tacoma Airport provides insight regarding conditions that may have promoted bloom development and cessation. In general, September, 1994 was 1.5 to 2.3°C warmer than normal at westem Washington recording stations. The warmest day of the month was September 22, just two days prior to the bloom's first appearance near Allyn. September 23 was the second warmest day of the month and the entire period of the bloom was marked by relatively warm air temperatures. Cessation of the bloom coincided with daily high temperatures dropping to below 29°C. The air temperature regimes correlated directly with the percent of possible sunshine index from the Weather Bureau. This index indicates that sunshine percentage was at least 88% of the maximum from 9/21-9/28 and dropped to 25% on 9/29, the day after the bloom. Rainfall was 1.69 inches in September at the nearby airport reporting station. This was only 0.19 inches less than normal, but most of the rainfall occurred in the first half of the month. Wind velocity did not change markedly in the period before to after the bloom, but wind direction changed from northerly to southerly on September 29. This is significant because the inlet is oriented almost north and south and hence is more susceptible to wind-driven mixing when winds are from the south. Additionally, a construction site was located approximately one mile north of the town of Allyn which was scarred with deep runoff trenches that had occurred as a result of a heavy thunderstorm approximately 2 weeks prior to the appearance of the bloom. Unconfirmed reports of grass seed being planted and fertilized the afternoon prior to the storm were stated by a local resident. If this were the case, such a storm may have introduced a large pulse of nutrients into nearby Case Inlet and provided the appropriate nutrient seed for an algal bloom. It should be noted that the unusually low nitrate levels reported here were sampled the day after the bloom and algal cells may have assimilated the available nutrients prior to and during the bloom.

In the past we have observed two general time periods for the occurrence of noticeable *Heterosigma* blooms: late spring/early summer and the fall. Rensel (in press, Proceedings of Research '95, Puget Sound Water Quality Authority) discusses past blooms in Puget Sound pointing out that these blooms require quiescent weather conditions, often coupled with vertical stratification of the upper column. A major fall bloom in northern Puget Sound during 1989 occurred during a calm, warm period, but freshwater runoff at that time of the year from the nearby Fraser River was well past the annual maximum. Similarly in Case Inlet, riverine discharge during the late summer is typically very low, although a minor reduction of salinity was noted at the surface when the bloom was present. The very warm air temperatures and influence on surface water temperatures, high percentage of sunlight occurrence, lack of south winds, and potentially elevated nutrients from the construction site possibly combined to encourage formation of this bloom. The cessation of these conditions coincided with the termination of the bloom.

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◆ Spain

The unique microreticulate cyst?

Since 1976, *Gymnodinium catenatum* blooms have been frequent in the Rias Baixas of northwest Spain, and the principal cause of PSP toxicity in this region. The appearance of this species in Iberian waters is not confined to the Rias Baixas, and it also occurs along the Portuguese coast and the south coast of the Iberian Peninsula. With the aim of studying the cyst distribution of *G. catenatum*, sediment samples were collected from 27 stations on the Galician-Portuguese continental shelf between 40° 30' N and 42° 20' N in 1993.

The cyst of *G. catenatum* was first described by Anderson et al (1), and at that time (1988) was the only cyst of a naked dinoflagellate known to have a microreticulate external surface reflecting features of the vegetative cell. Since then, examples of this cyst have been found in sediments from various parts of the world, and in most cases have been identified as *G. catenatum* cysts due to the characteristic wall reticulation.

Two types of cyst can be differentiated in sediments from the Galician-Portuguese shelf, both similar to the cyst of *G. catenatum* (brown wall with microreticulation), but of different sizes. Fig. 1a shows the frequency distribution of cyst diameters, and the two clear modes correspond to the two types mentioned. *G. catenatum* cysts produced in laboratory cultures have a size range (fig. 1b) which corresponds with that of the larger size peak in fig. 1a. In addition, the morphology and arrangement of the paravesicles coincide with those of the original description of *G. catenatum* cysts. In contrast, in the smaller cysts found in the sediments, the paravesicles are larger, and there are fewer rows of them on the paracingulum. The vertical distribution and maximum concentrations of the two types in the sediments are also distinct.

The concentration of *G. catenatum* cysts (38-59 µm) diminished with depth in the sediments, with maximum values (20-500 cysts ml^{-1}) in the upper 8 cm; while the *G. catenatum*-like cysts (20-38 µm) had the opposite profile, and reached their maximum concentration (5000 cysts ml^{-1}) at 12 cm depth.

The two kinds of microreticulate cysts referred to here are known from the literature. Some descriptions correspond exact-

ly with the original description of *G. catenatum* cysts, eg. those of Blackburn et al (2) and Hallegraeff et al (3) from Tasmanian sediments. But from the same region, Bolch and Hallegraeff (4) have also mentioned a similar but smaller cyst (17-22 µm) which they called *Gymnodinium* sp 1 which germinated into "small (17 µm) diameter two-cell chains resembling *G. catenatum*". Their description suggests they may have been cells of *Gyrodinium impudicum*, recently described by Fraga et al (5), with morphological features very similar to those of *G. catenatum*. Finally, the cysts described by Nehring (6) and Ellegaard et al (7) from German and Danish coastal sediments respectively, and the fossil cyst described by Dale (8) all have diameters less than 40 µm, as well as microreticulation and paracingular patterns like those of the *G. catenatum*-like cysts (20-30µm) from the Galician-Portuguese shelf.

Attempts to germinate the smaller *G. catenatum* like cysts from the Galician-Portuguese sediments have so far failed, so that we do not yet know whether they are a second form of *G. catenatum* cyst, or whether they belong to another species of dinoflagellate. Ellegaard et al. (7) describe vegetative cell produced from a cyst very similar to those I have called *G. catenatum*- like, and they call them *G. catenatum* despite the smaller cell size and the absence of chains. They suggest that there is a *G. catenatum* "species complex". Whatever the case, it is clearly important that we learn more about this "complex" before we define the cyst distribution of *G. catenatum*.

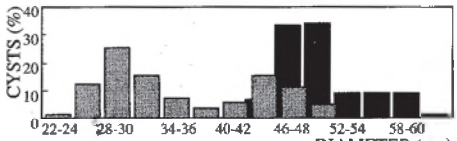

Figure 2. Size distribution of cysts, microreticulate cysts from sediment. *G. catenatum* cysts produced in cultures.

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◆ Brazil

14 South Americans trained on HAB

The IOC-FURG-DANIDA Regional Training Course: Taxonomy and Biology of Harmful Marine Microalgae was held at the Fundaçao Universidade do Rio Grande (FURG), Brazil between 4-14 March, 1997. It was the result of a joint effort between the IOC-DANIDA Science and Communication Centre on Harmful Algae in Copenhagen (SCCHA) and FURG.

Rio Grande, in south Brazil, was founded by the Portuguese in 1737 and presently has about 200,000 inhabitants. It is the major port of the province Rio Grande do Sul. The city lies on the shore of a channel connecting the Lagoa dos Patos (250 km long) to the Atlantic Ocean. Its economy is tightly bound to both marine and estuarine environments. The University of Rio Grande was founded in 1969 and several graduate studies in the humanities and scientific departments are dedicated to coastal-related subjects. The department of oceanography is oriented towards "the understanding of biotic structural components and functional aspects of marsh, estuarine and marine ecosystems".

The group of participants was composed of 14 advanced students and professionals from Latin American countries: Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Mexico, Peru and Uruguay. Each participant presented a description of research and management issues related to harmful algae in their countries.

The classes of Drs. Ojvind Moestrup and Jacob Larsen from the SCCHA, Botanical Institute, University of Copenhagen, on the taxonomy of Dinoflagellates, Prymnesiophytes and Raphydophytes were complemented by various contributions on other groups: Diatoms by Dr. Marta Ferrario (Universidad Nacional de La Plata, Argentina), *Prorocentrum* sp. and *Alexandrium* sp. by Lic. Rutt Askelman (INIDEP, Argentina; long-term collaborator of Dr. E. Balech), Cyanobacteria by Dr. Joao Sarkis Yunes (FURG) and the identification of dinoflagellate cysts by Dr. Kazumi Matsuoka (Nagasaki University, Japan).

Description and study of several species was carried with microscopic observation (transmission and fluorescence) of living or fixed material from culture collections or field samples, and supported with graphic material, such as TEM micrographs, which for instance, were necessary for descriptions of the genus *Pseudonitzschia*.

A day was dedicated to sampling of sediments for cyst determination, water sampling for quantitative and systematic studies on toxic microalgae, and for the establishment of algal cultures. Moreover, fixed field samples brought by the different participants were studied in order to facilitate the identification of species and favour descriptions.

In addition to theoretical lectures on taxonomy, technical sessions were performed on the identification of dinoflagellates (Dr. Jacob Larsen), quantitative methods of microalgae (Dr. Clarisse Oderbrecht, FURG), cyst preparation (Dr. Kazumi Matsuoka) and culture techniques (Dr. Virginia García, FURG).

Taxonomy training was complemented with presentations on the ecology of algal blooms (Dr. Clarisse Oderbrecht), on harmful algal blooms in South America (Lic. Silvia Méndez, INP, Uruguay), ciguatera and a general overview on microalgal toxins (Dr. Jorge Diogène, IOC-IEO Science and Communication Centre on Harmful Algae, Vigo, Spain).

Training courses in taxonomy respond to the demand clearly defined in the HAB Programme of the IOC in order to form scientists and improve their identification skills. Taxonomy constitutes a key-stone in the study of every HAB event. An immediate goal of such training courses should be that the participants become reference scientists in their own countries to assure the identification of harmful microalgal species.

Technical support was brought by FURG, the Brazilian Navy that collaborated during the field-trip, Zeiss-Germany that lent 15 microscopes and the IOC-DANIDA Science and Communication Centre on Harmful Algae, Copenhagen, that provided scientific publications to the participants.

Clarisse Oderbrecht, FURG

◆ Book review:

Patterns in the Ocean: Ocean Processes and Marine Population Dynamics

"The Sea Maid's Music: An Aid to Pattern Recognition", the title to the first section of the Introduction, boded well to Andrew Bakun's *Patterns in the Ocean – Ocean Processes and Marine Population Dynamics*, a book, like music, with several aims and recurring themes.

A preliminary glance at the eclectic-looking Table of Contents suggested that what was coming might complement Jumars (1993), Kaufman (1993) and Cushing (1995) in different ways.

The author has a double specialisation, physical oceanographer and fisheries scientist. This, coupled with a penchant for music and sitting on Pacific cliff tops watching the sea patterns change and decay, inspired him to write a book combining Fisheries, Oceanography, Climatology and Time series, with an eye to timely issues like carbon sequestering, global climate change and the "crisis" in fisheries science.

Jumars' book explained many topical issues concerning biological oceanographic processes in ways understandable to physical oceanographers: Bakun explains many basic physical oceanographic processes for biological oceanographers (like me). One muse he uses has been to banish all the 10 equations in the book, to a four-page appendix, which I hope to cherish evermore as a valuable reference.

So what has this to do with harmful algae? Although only 1% of the book is devoted directly to harmful algae (chain-forming dinoflagellates off the Galician rías), the indirect interest for HAN readers is that many phytoplankton species must be constantly selected to use fine-scale migrations to exploit ambient patterns of water movement to maintain suitable positions for their life stages over time, just as many fish (larvae and adults) and zooplankton do. The swimming speeds of organisms doing this will naturally limit the different scales that can be exploited, as Bakun discusses graphically for the life stages of different pelagic fish in Chapter 10.

In Chapter 6, "Thickening the Soup", Bakun introduces some of the myriad interfaces present in the ocean, at which organisms tend to concentrate, with the possibility of pushing concentrations over grazing thresholds for, say, fish larvae. He is clearly at ease here, exploiting his mastery of oceanographic processes combined with that of current fisheries issues, while keeping the "patterns" and the physical-biological interactions clearly in mind over 10^{-3} m to 10^{6} m scales.

Chapter 7, "Transport and retention: getting home", develops a recurring theme, that what happens to most pelagic larvae doesn't matter to recruitment: what does matter is what happens to the tiny percentage of survivors. He emphasises that the survivors frequently have been retained in physical oceanographic structures. Included amongst such stationary and moving retentive structures, he explains Taylor columns over shallow banks, and shelf-sea fronts, which tend to produce offshore-moving water at mid depths but shoreward moving water near both the surface and the bottom. Vertical shears, associated with practically all retentive structures are no doubt sometimes exploited for evolutionarily controlled position keeping and migration. They are also found, notably, in geostrophic currents associated with upwelling on eastern ocean boundaries in mid latitudes, where equatorward surface currents are countered by poleward flows at shallow depths. Onshore Stokes drift, produced in shallow and bottom layers by onshore waves "feeling" the bottom, may also provide shear structures, particularly off reefs subject to Trade Winds or swell, potentially exploitable for position keeping by plankton. A section, "Surface wind drift", includes much speculation on the complexities of Ekman transport. Apart for the *mean* transport, 90° to the right (N. hemisphere) of the wind, but more downwind (10° to 30°) at the surface, Ekman transport-induced circulation and the resultant structures are still too poorly understood quantitatively, even to allow speculation on the behaviour favoured by evolutionary pressures to exploit them, to which particular stocks organisms might be subject. One still has the right to guess, however, that such evolutionary pressure is likely to have honed certain populations to exploit some of the recurring patterns.

Bakun also cites authors reporting that slicks in surface convergences associated with underlying internal waves, move shorewards at 25 cm s^{-1}. He claims that neuston could thus "surf shorewards" at about this velocity. I wonder whether the phase velocity of a surface-film compression wave, with a slick at the moving zone of maximum compression, may not have been erroneously interpreted as surfing-type movement of the surface film itself. Shoreward movement of the slick might be produced, however, if there were also onshore wind stress, or shoreward movement of a surface layer thicker than the film, or both combined.

Showing how the intensity of oceanographic structures, such as upwelling zones, as well as phytoplankton productivity and feeding of fish larvae might vary in relation to turbulence, prepares the reader to associate recently perceived (Cushing 1995 and refs) similarity between peaks and troughs in landings of related fish species in different parts of the world with long-distance connections in climatic variability.

The dramatic nature of the cross-Pacific connections associated with the El Niño Southern Oscillation (ENSO) are now well known, but Bakun explains how Kelvin "waves" slowly propagating eastwards along the equator or polewards (equatorwards) on eastern (western) ocean boundaries provides a plausible mechanism for tele-connections delayed by a year or two, as do "slugs" of water in oceans, not mentioned by Bakun, but invoked by Cushing. One problem of perceiving this variety of possible tele-connection mechanisms, with variable time delays, is how to demonstrate from meteorological and other records, which mechanisms are acting, and to what extent. Much of the evidence in the literature unfortunately comes to resemble that in Bakun's Figure 13.9 (showing relationships between annual mean wind stress or atmospheric pressure at 16 localities from 1945 to 1990 around the Pacific and the Atlantic.

It is hard to "unravel the determinant mechanisms from happenstance" when functional relationships are, alas, frequently inferred from similar comparisons of selected and rather poorly correlated time series.

Although the importance of zooplankton food for fish stocks has been known at least since the early years of the century (see Hardy 1959 for the importance of *Calanus* to North Sea herring), Bakun's book has not shown much prowess in considering how plankton species composition determines the nature of the function *f*(physics, fish). This lacuna has unfortunately infected much of the fisheries and modelling milieux in recent decades. Fortunately, the pendulum is swinging back, as illustrated by the large European programmes within GLOBEC, ICOS (Investigation of *Calanus finmarchicus* migrations between Oceanic and Shelf seas off north-west Europe) and TASC (Trans-Atlantic Study of *Calanus finmarchicus*).

Since data on plankton variability are usually insufficient to estimate its role in any particular fishery at a given time, fishery workers have to fall back on trying to determine the linear part of *f*(physics, fish). This is even though they know that such relationships are, overall, likely to be:

1. non linear;
2. offset by an unknown and perhaps variable time;
3. subject to natural (e.g. volcanic and astronomic) and anthropic (e.g. pollution and fishing) forcing factors;
4. subject to the changes in gene frequencies in the fish species concerned or in their food, predators, parasites, pathogens or competing species (essentially unmonitorable for the foreseeable future).

Apart from short-term predictions, such as those of stocks and productivity modelled year by year from abundances of O-group larvae, perhaps we may have to face up to a scenario that the best available management policy is to prepare fishing fleets to cope with the unpredictable, to establish the limits of variability percentile probabilities, and to foster underfishing in general. Then, there should then nearly always be enough.

The kind of correlative research being undertaken in fisheries, climatology and many other branches of science with a view to improving forecasting, can rarely be justified as leading to much basic understanding of how things work. Fundamental knowledge, including of course that of how firstly processes and secondly correlative relationships work, can come only from understanding quantitatively the nitty-gritty mechanisms at work, including the passive properties and the reactive properties of the materials (here meant to include the organisms) that serve as their medium. Some understanding of form and function, of attractors, and of chaos, will also no doubt help.

One thing to emerge from this book, as from others on fisheries and other sets of processes taking place in turbulent continua is that even though there may be much understanding of the systems concerned at both scientific and intuitive levels, just as in modern weather forecasting, it becomes impossible to predict further ahead than a few times the timescale of the dominant structures or processes. In fisheries, a major time scale is that from egg to catchable size.

Although the science of harmful algal blooms (HABs)is still an infant compared to fisheries science, HABs and fishes live in the same environments, so similar restrictions on predictability are likely to exist for both.

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◆ Finland

Fish kill by *Prymnesium*

An extensive fish kill occurred in the oligohaline and meromictic coastal lake Vargsundet (110 ha, two major basins) in Cland, SW Finland in late June and early July 1997, in connection with a mass development of *Prymnesium* sp. The cell concentrations exceeded 20.000 cells per.ml in the uppermost 4-5 m layer but few *Prymnesium* cells occurred in deeper strata. Several tonnes of fish were killed. Hundreds of gulls and terns removed much of the smaller fishes. Several white-tailed eagles ate of the dying and dead fishes. The fish mortality was almost total in the more shallow, southern basin of Lake Vargsundet, whereas some fish escaped to deeper layers in the stratified, northern basin (max depth about 32 m, but anoxic conditions below 15 m). The birds appeared healthy.

The bloom of *Prymnesium (*presumably *P. parvum)* developed during warm and very sunny conditions. The surface water temperature occasionally exceeded 24°C. The bloom persisted from late June to late July (at least). The bloom was present and still ichtyotoxic on July 26, 1997. All fish species known to occur in the lake died. Also freshwater crayfishes (*Astacus astacus*) died. The conditions of Lake Vargsundet are rather unusual, with a salinity of about 2 per mille but with both freshwater crayfish and a brackish (marine) algal bloom. *Prymnesium* is now and then observed in the plankton of Finnish coastal waters but there is only one similar earlier fish kill episode from Finland, in June 1990, in a smaller basin with about 6 per mille salinity.

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◆ Greece

Blooms in Thermaikos Bay

In December 1996, a red tide predominated by the photosynthetic ciliate *Mesodinium rubrum* was recorded in the inner Thermaikos Bay (North Aegean Sea, Greece. In this virtually monospecific red tide, *Mesodinium rubrum* reached abundance levels of $14.5x10^6$ cells.l^{-1} and a chl a concentration value of 487 mg.m^3 was measured. This has been the last incident in a series of intense phytoplankton blooms that occurred in the inner bay the past few years. These blooms were dominated mostly by dinoflagellates: such examples are the red tide incidents in May 1996 (by *P. minimum,* 10×10^6 cells.l^{-1}), September 1994 (by an unidentified Gymnodinioid species, P. *cf. dentatum* and *P. rostratum,* 17.7×10^6 , 3.1×10^6 and 1.3×10^6 cells.l^{-1} respectively), April 1994 (by *P. micans,* $37. \times 10^6$ cells.l^{-1}) and October 1993 by *P. cf. dentatum,* 210×10^6 cells.l^{-1}). Intense blooms of diatoms or coccolithophorids have also been observed in the bay: for example, in August 1996 a bloom dominated by the coccolithophorid *Pleurochrysis carterae* was recorded and an abundance of up to 72×10^6 cells.l^{-1} was measured. A bloom of *Mesodinium rubrum* was also recorded in December 1985. These red tides occurred during periods of calm weather, lasted a few days and were attributed to the eutrophication of the inner part of the bay due to the high nutrient inputs of the domestic and industrial wastewater of the city of Thessaloniki. However, none of the red tide incidents recorded so far was studied in detail throughout its development and no firm conclusions can thus be drawn concerning their origin and the factors that triggered them, affected their development and led to their decline.

The occurrence of several potentially toxic/harmful phytoplanktonic species has already been reported in Thermaikos Bay (1, 2). Examples of such species are the dinoflagellates *Alexandrium catenella, Ceratium fusus, Dinophysis acuminata, D. acuta, D. caudata, D. fortii, D. tripos, Gymnodinium breve, G. catenatum, Gyrodinium aureolum, Noctiluca scintillans* and *Prorocentrum minimum,* the diatoms *Pseudo-nitzschia delicatissima* and *P. seriata* and the haptophyte *Prymnesium parvum.* However, these species have not dominated any major bloom recorded so far nor have they been reported to have reached abundance levels high enough to cause fish mortality or shellfish toxification.

Mussel cultures spread along the northern and western coasts of the bay with a production of 10,000 and 4,000 tonnes in 1995 respectively. According to the EEC Directive 91/492/EEC, bivalves from designated areas of culture should be examined for PSP and DSP toxins and also the waters should be monitored for toxic phytoplankton. In compliance with this directive, all samples examined so far by the Institute of Food Hygiene in Thessaloniki using mouse bioassay had PSP toxins below the tolerance levels i.e. <80 µg/100g meat (3). No human poisoning by algal toxins after consumption of shellfish harvested in the bay is known so far. The consequences of the blooms seem to have been restricted to small scale fish deaths due to oxygen depletion and occasionally they prevented fishing activities in the bay. However, the areas of mussel culture are not currently being monitored for toxic phytoplankton.

The phytoplankton of Thermaikos Bay has been monitored by the Department of Botany, School of Biology, Aristotle University of Thessaloniki for the past 7 years. The monitoring work was partially supported by the Greek Ministry of Environment, Physical Planning

◆ Scotland

Early reports of PSP

The first recorded instance of paralytic poisoning in Scotland arose from the consumption of mussels taken from the Firth of Forth at Leith in 1827 (Combe, 1828).

It took a further one hundred and thirty years before another episode was brought to the attention of the medical authorities. Gemmill & Manderson (1960) reported the first outbreak of neurotoxic or "paralytic" mussel poisoning ever recorded in Glasgow.

On June 20th 1958 three men employed by a Glasgow engineering works ate mussels left in a lorry which had previously carried a load of timber. The mussels had been attached to logs of Oregon pine taken from storage in the Junction Dock, Grangemouth in the Firth of Forth. The three men exhibited typical symptoms of PSP to a varying extent, however all eventually recovered.

Water samples from Junction Dock were examined for plankton content at the Marine Station, Millport and found to contain numerous flagellates dominated by *Euglenoids, Peridinians* and *Gonyaulax* including a small species of *gonyaulax* possibly *Gonyaulax tamarensis (Alexandrium tamarense).* The latter is one of the organisms most commonly associated with PSP in the northern hemisphere.

Gemmill & Manderson observed that in all nine previous European outbreaks and the latest reported, the incriminated mussels were invariably taken from waterways which were essentially enclosed, stagnant or polluted.

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and Public Works and the Ministry of Agriculture. A new plankton bloom monitoring program has been initiated in 1996 in Thermaikos Bay within the framework of the Long-term Program for Pollution Monitoring and Research in the Mediterranean (MED POL)-Phase 11. This monitoring program has been undertaken by the Department of Botany, School of Biology, Aristotle University of Thessaloniki under the co-ordination of FAO (GFCM) and MED UNIT. Special attention is to be given to potentially toxic phytoplankton species.

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Corrigendum to IOC-HAB Manual

Cover:

The *Alexandrium* cell illustration is optically reversed; the correct illustration appears on p. 293, Fig.15.

Cover page:

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List of contributors (p.xi):

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Chapter 3. Culture Methods.

Figure legend 3.2. (p. 48) ; item D, Latex tubing should be 1/4 inch (6.35mm) Table 3.2 . (p.57); K_2CRO_4 concentration (first column, last line) should be 0.0194 [This error is also in the original Guillard and Hargraves 1993 paper].

Chapter 5. Post-column derivatization HPLC methods for Paralytic Shellfish Poisons.

The specific toxicity values for dcGTX2 and dcGTX3 are erroneous.

p.83, line 16 from below should read: For the calculation of toxicity from HPLC chromatograms, the following values of specific toxicity (MU/micromole) were determined: GTX1 (2468), GTX2 (892), GTX3 (1584), GTX4 (1803), GTX5 (160), dcGTX2 (382), dcGTX3 (935), C1 (15), C2 (239), C3 (33), C4 (143), STX (2483), neoSTX (2295) and dcSTX (1274).

Chapter 10 A. *In vitro* biochemical and cellular assays.

p.188, last line: competitive binding assay
p.191, line 3 from below: 0.89 ± 0.07 nM
p.192, figure legend 10.5: competitive binding curves
p.200, line 16 from below: 0.65 micrometer Duropore membrane (not mm).
p.200, line 8 from below: volume-200 microlitre (not mL), amount of radioactivity-50 microCi (not mCi)
p.200, line 7 from below: 50 microCi / (39 microCi / nmol); STX concentration= 6.40 microM (not mM)
p.200, line 4 from below: 3 microL [^{3}H] STX (not mL)
p.201. The Table on the top of the page should read:

		conc.of standard	conc.in assay
15 microL	100 microM STX + 235 microL HEPES/NaCl buffer	6.00×10^{-6}	1.0×10^{-6}
50 microL	6.00×10^{-6} solution + 450 microL buffer	6.00×10^{-7}	1.0×10^{-7}
50 microL	6.00×10^{-7} solution + 450 microL buffer	6.00×10^{-8}	1.0×10^{-8}
25 microL	6.00×10^{-7} solution + 475 microL buffer	3.00×10^{-8}	5.0×10^{-9}
50 microL	3.00×10^{-8} solution + 200 microL buffer	6.00×10^{-9}	1.0×10^{-9}
50 microL	6.00×10^{-9} solution + 450 microL buffer	6.00×10^{-10}	1.0×10^{-10}
Reference		Buffer only	0

p.201, line 2 in text below Table: 100 microL aliquots (not mL)
p.201, line 6 from below: 35 microL [^{3}H] STX (not mL)
p.201, line 5 from below: 35 microL STX standard (not mL)
p.201, line 4 from below: 135 microL synaptosome preparation (not mL)
p.202, line 2: 200 microL of ice-cold HEPES buffer (not mL)
Note that ALL OTHER "milli" (m)-designations in Chapter 10 are correct.

Chapter 15. Taxonomy of Harmful Dinoflagellates

p.289. The illustrations in Figures 15.10 i-j depict *Gyrodinium impudicum* (not *Gymnodinium catenatum*) . A correct illustration for *Gymnodinium catenatum* appears in Fig. 15.5 (p. 287).

◆ Modelling

Modelling the kinetics of PSP toxin uptake and release in mussels

The Galician rías (N.W.Spain) are the second world producer of mussels but mussel farming is being adversely affected by blooms of toxic algae, since the mussels accumulate phycotoxins leading to closure of the market so as to avoid human intoxications. This is especially critical in the case of the PSP producer *Gymnodinium catenatum* (Graham) because blooms of this species are recurrent along the Galician coast. The possibility of predicting the duration of closure periods can contribute to optimize the resource management and minimize the economic losses due to market bans. By means of modelling the PSP accumulation kinetics in mussels, it is possible to obtain an estimate how much toxin the mussels would accumulate and how long it would take to reach the market closure threshold. Modelling the PSP release kinetics it would be possible to predict how long mussel toxicity remains above that closure threshold. Up to now, few studies have attempted such modelling (Silvert and Subba Rao (1), with domoic acid ; Blanco et al. (2), with DSP; Silvert and Cembella (3), with PSP), only the first of them included in the model an environmental factor (temperature). As a first approximation to build such a model we have considered that the PSP toxins are organic compounds accumulated by mussels when they ingest the toxic algae and that their posterior processing in mussels are similar to that of the remaining organic matter ingested. Therefore, our simplest model includes: filtration rate, assimilation efficiency and depuration rate and some measure of the toxin concentration in the plankton that in its most useful expression would be pg STXeq/cell. As the seston concentrations in the Galician Rías are usually below the threshold of pseudofaeces production of 5 mg l^{-1} (4) the filtration and ingestion rate can be considered equivalent.

We applied four models sequentially (Fig 1) to the data of toxic cells and environmental variables obtained weekly, from 1993 to 1995, by the Centro para o Control da Calidade do Medio Mariño. We started with a very simple one-compartment model and modified or made more complex the aspects that would lead to better fitting to the toxicity data obtained by the Consellería de Sanidade and the Centro para o Control da Calidade do Medio Mariño using the mouse bioassay.

Filtration rate and assimilation efficiency were considered constant and the values were taken from a study by Navarro and his colleagues (5) carried out in the ría de Arousa. The concentration of toxins in the plankton was estimated from the number of *Gymnodinium catenatum* cells assuming a constant toxin content per cell of 40 pg STXeq (6). Depuration rate applied was 0.15d^{-1} (7).

When the one-compartment model was considered, a delay in the toxin maxima and underestimates of toxicity could be observed in most of the events (Fig 2). The degree of underestimation varies depending on the sampling point and the toxic episode. These inadequate predictions may derive from incorrect values of parameters as the filtration rate and/or assimilation rate and/or the depuration rate but also may originate from incorrect estimates of the concentration of toxins. The values of the first set of parameters needed to match the observed toxicity levels would be unrealistic. Therefore, the variable most likely affecting the model output is toxin concentration in the plankton. This has two components: the cell concentration and the toxin content per cell of *Gymnodinium catenatum*. The first component was measured in this study but it is subjected to counting and sampling errors. The toxin content per cell will be discussed more completely below. The percentage of variance of the data explained by this simple model was 45%.

A delay can be observed in fall of 1993 in all sampling points except Vigo. Also, in the first bloom studied (spring of 1993), in both Muros and Pontevedra, the delay was observed. In both Vigo and Arousa, in this same bloom, the general discrepancies were too high to discern any process from the error. In the fall of 1994 the delay was not observed. The optimization of the model fitting, by allowing the delay to vary, gave an esti-

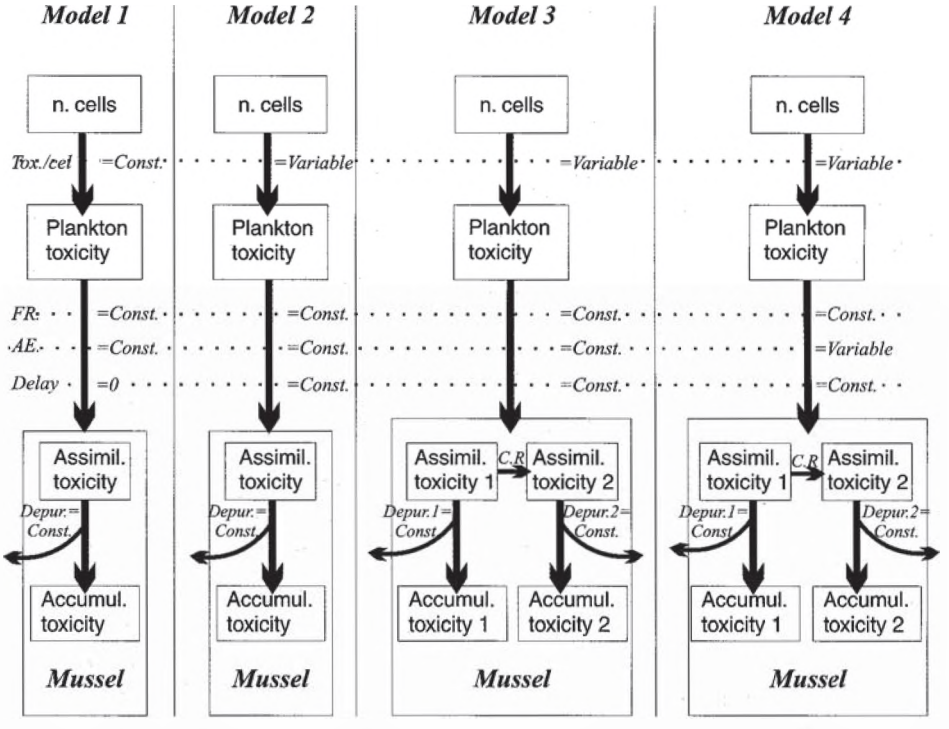

Fig. 1: Four models sequentially applied to the data of toxic cells.

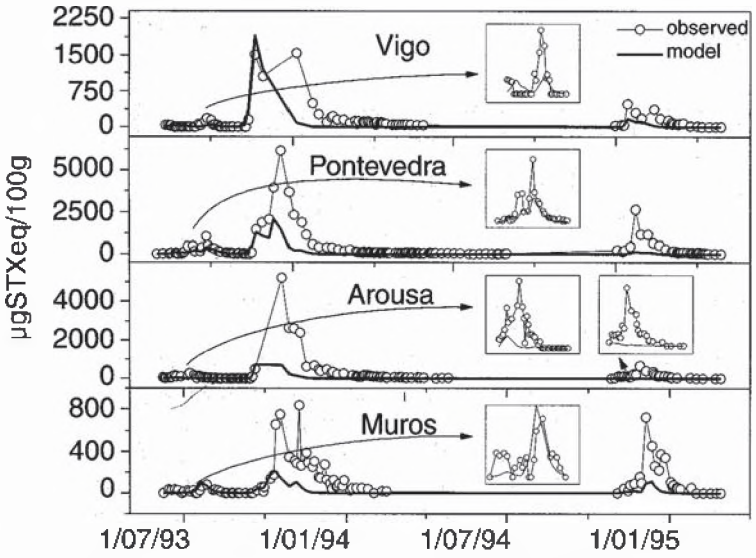

Fig. 2: Fitting of a one compartment model with constant toxin content per cell in the four Rías.

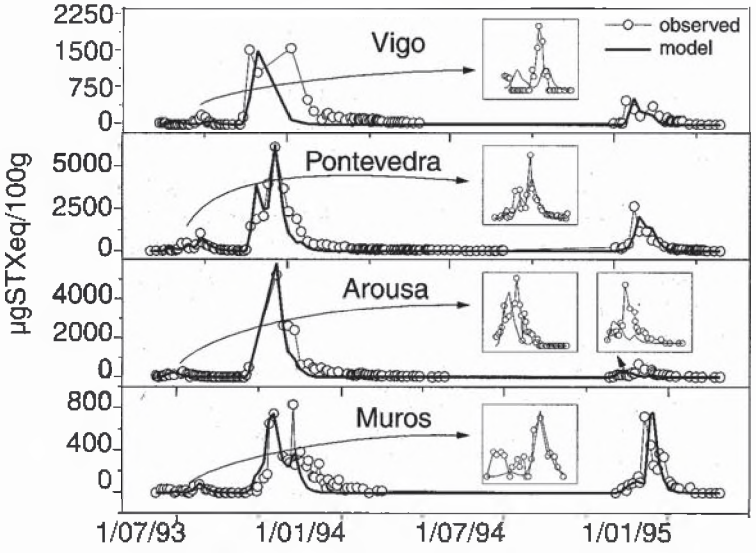

Fig. 3: One compartment model with variable toxin content per cell fitting in the four Rías.

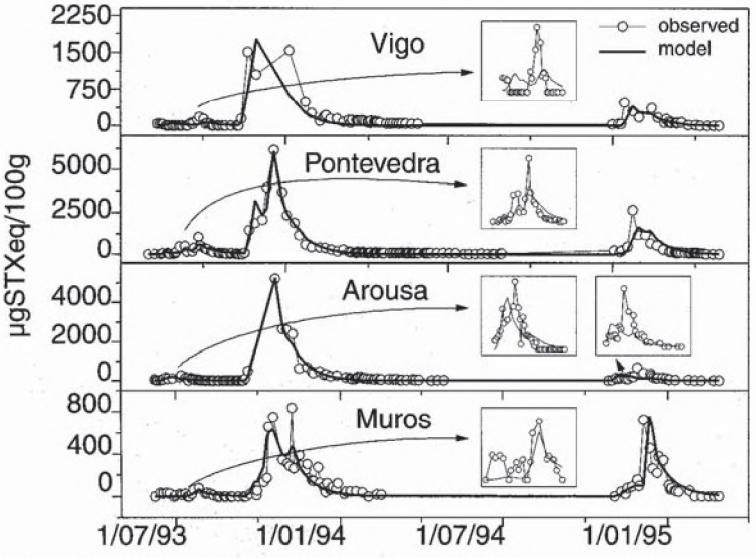

Fig. 4: Two compartment model fitting in the four Rías.

mation of 4 days for that delay. We can not discard that a real delay exists but we think that it might be an artifact due to sampling or counting errors – mainly because of the heterogeneity in the *Gymnodinium catenatum* distribution – or to errors derived from the interpolation of the plankton data. We included the delay in the model and optimized again the fitting but this time varying the toxin content per cell of *Gymnodinium catenatum* in each sampling point and each toxic episode.

Once optimized, the model fitted the data fairly well. The variance explained 80% of the deviation (Fig 3) but two obvious deviations were still present: a) some toxic values did correspond to samples in which a very small number of *Gymnodinium catenatum* cells were detected, and b) the initial portion of each detoxication process coincides in the model and in the actual data, but in the final part of the detoxication period the observed toxicity values are higher than the expected ones. The first discrepancy can be attributed to heterogeneity in the *Gymnodinium catenatum* distribution and thus sampling which is not representative. In order to avoid the second discrepancy we introduced a two-compartment model in which compartment 1 has a depuration process faster than compartment 2 and a toxin flow from compartment 1 to compartment 2. We optimized again the model fitting with a fixed depuration rate for compartment 1 ($0.21d^{-1}$ (7)) and a varying depuration rate of compartment 2 (the change rate between compartments and toxin content per cell of *Gymnodinium catenatum,* to each sampling point and each toxic episode). This change did not produce obvious differences in the expected toxin level during the early detoxication of each episode, mainly because most of the toxins were in the first compartment, but it produced a significant alteration of the detoxication in the middle-final portion, increasing substantially its fitting. So, this optimization gave a better fitting of the model to most of the toxic events and accounted for the 89% of variation in PSP data (fig 4).

The inclusion in the model of a new variable, the toxic quality, has been found to be relevant in laboratory studies (8). This coefficient was defined by analogy with seston quality for nutrition (5) as toxic content per unit volume of particulate matter. The volume of particulate matter was obtained from light transmission data by linear regression. The toxic Quality (Q_{tox}) was included in

the two-compartment model and a new optimization in the same conditions as the previous one led to a similar fitting and as expected to a positive effect of the toxic quality on assimilation efficiency.

We checked by multiple regression the effect of salinity, estimated growth rate of *Gymnodinium catenatum* (day^{-1}), and nitrate concentration on the estimated toxin content per cell of *Gymnodinium catenatum* (to each toxic episode and each sampling point). The three variables had a significant effect and in the case of nitrate the relation was found to be parabolic. By means of this regression a 80.7% of the variance was explained. Taking into account all this data, and the fact that no other variable in the model could affect so substantially the output, we think that the toxin estimations are correct and make further experiments on this subject indispensable.

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7) Blanco et al. in preparation.
8) Moroño et al. in preparation

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◆ South Africa

New regional emergence of cyanobacterial toxicosis

Although blooms of cyanobacteria are common in South Africa, animal deaths due to hepatotoxins were, prior to 1994, confined to the central and northern provinces (1). This pattern changed during the last weeks of 1993 when the first stock losses, attributable to cyanobacterial toxicosis, occurred in the south-western Cape (2). Subsequently, and until December 1996, 7 stock and domestic animal deaths were recorded in this region and along the southern Cape coast (Fig 1). During the same period a single incident of stock loss was reported from the Northern province. Together, these losses represented a financial loss of 1.1 million SA Rands (1 USD = R4.50).

The first south-western Cape poisonings occurred between December 1993 and March 1994 in the Malmesbury-Darling area (Fig 1). In both cases the stock losses, 3 cattle plus 5 exhibiting photosensitivity, and 29 sheep, respectively, occurred in camps where animal deaths with identical clinical signs had previously been observed (2). These earlier, deaths, however, had not been linked to toxins produced by the cyanobacteria. *Nodularia spumigena* was found in the drinking water supplies in both incidents. No analyses for the presence of nodularin were performed as appropriate methodology was not available locally at the time. These two incidents have been described in detail elsewhere (2).

During March 1994, a bloom of *Nodularia spumigena* in Zeekoevlei, a shallow lake near Cape Town (Fig 1) resulted in the death of a bull terrier bitch. HPLC analysis (3) of algal material collected from where the dog drank contained a nodularin concentration of 3479 g g^{-1} freeze-dried (f-d) material. This incident, described fully elsewhere (1), was interesting in that this was a short-lived appearance of *Nodularia* in a lake which has been dominated by *Microcystis aeruginosa* forma *flos-aquae* for the past five decades.

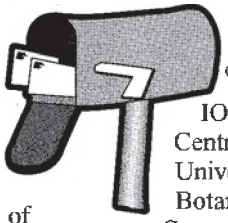
In May of the same year, a bloom of *Microcystis aeruginosa* forma *aeruginosa* near the town of Paarl (Fig 1) resulted in the death of 11 sheep and induced-photosensitivity in a further 20.

The cyanobacterial hepatotoxin, microcystin-LR, was detected in the algal bloom at a concentration of 1340 g g^{-1} f-d weight, and a second, unidentified, microcystin variant at 585 g g^{-1} f-d weight. The case history of this incident is detailed in Van Halderen *et al* (2).

After a lull of more than two years, a *Microcystis aeruginosa* forma *aeruginosa* bloom in the George district (Fig 1) resulted in the deaths of three calves and photosensitivity in a further 30. Analysis of lyophilized material from this bloom revealed the presence of the microcystins-LR and -YA at a combined concentration of 1720 g g^{-1} f-d weight.

The George case was followed by a massive stock loss of 290 in-milk dairy cows on a farm in the Kareedouw district (Fig 1). A further 70 animals presented with acute photosensitivity and

Identification Service



The IOC Science and Communication Centre on Harmful Algae, Copenhagen, and the Asian Natural Environmental Science Center offers an identification service. If you have problems with identification of harmful algal species, the Centres may assist you. You should send samples to one of the two addresses given below. It should be noted that this assistance is not given for routine identification/ monitoring of samples, but applies to particulary difficult species, or to species that require special techniques e.g. electron microscopy, for identification.

IOC Science and Communication Centre on Harmful Algae,
Univeristy of Copenhagen,
Botanical Institute,
Øster Farimagsgade 2D,
DK-1353 Copenhagen K., Denmark

Asian Environmental Science Center,
The University of Tokyo,
Yayoi 1-1-1,
Bunkyo-ku, Tokyo 113,
Japan.

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had to be slaughtered. Although the clinical signs and symptoms were consistent with acute hepatotoxicosis, the water supply was flushed out soon after the incident and no toxins could be detected at the scene of the incident. Microscopic examination of the rumen contents of two of the dead animals showed the presence of filaments of *Anabaena*, and a mat of *Oscillatoria* filaments was found growing on the walls of the cement-lined reservoir from which the animals had drunk. HPLC analysis of rumen and bile revealed a hydrophobic, microcystin-like component possessing a UV_{238} spectrum closely-typical of the microcystins. The results of further tests on the rumen contents, performed by Professor Geoff Codd (University of Dundee, Scotland), and using ELISA immunoassay and protein phosphatase inhibition, were consistent with the presence of microcystins.

During December 1996 another incident was reported, again from the Malmesbury district. Here three 8-month old lambs died after drinking water containing *Microcystis aeruginosa* forma *aeruginosa*, and containing the microcystins -LR, -YR and LY at a total concentration of 1890 **g g^{-1} f-d** weight. No clinical signs or symptoms were evident prior to the deaths. Post-mortem of the affected animals showed severe lung congestion and oedema, widespread endo- and epicardial haemhorrages, severe liver damage with yellow colouration, and moderate swelling of the kidneys. There were also widespread haemhorrages in the skeletal muscles. The histopathological examination showed pannecrosis of the liver, and acute nephrosis of the kidneys.

During the period encompassed by these incidents, a case of a death of goats was reported from the Northern Province (Fig. 1). Eighteen four-month old goats died after drinking from a cement-lined trough near the town of Alldays. As was the case at Kareedouw, the causative organism was found to be *Oscillatoria*. Post-mortem examination revealed swollen and congested livers, congestion of the kidneys with some nephrosis, and oedema of the lungs. The results of the histopathological examination were consistent with the effects of acute cyanobacterial poisoning. HPLC analysis of a water sample from this incident contained 71g^{-1} of an unidentified, hydrophobic microcystin.

Although cyanobacterial blooms have long been common in South Africa, the incidents reported on here represent the emergence of related poisonings in the southern and south-western regions of the country. They also indicate the appearance of *Oscillatoria*-related incidents in a country where *Microcystis* and *Anabaena* are the commonly-expected dominants of cyanobacterial blooms. The incidents involving *Oscillatoria* differ from those typical of *Microcystis* or *Anabaena* in that a lesser amount of cyanobacterial biomass appears to be involved, and that the hydrophobic toxins produced appear to have a toxicity greater than equivalent amounts of the more hydrophilic, commonly-encountered microcystins.

The cases reported on here are the first from this region, although others may previously have been incorrectly-diagnosed or the cyanobacterial-link undetected. A heightened awareness of the problem is now emerging amongst the veterinary and stock farming communities, this leading to improved sample collection and incident investigations. The growth of algal mats on the walls of drinking troughs and dams, as opposed to the readily-observed, buoyant scums of algae, indicates a requirement for increased vigilance regarding water supplies if stock losses are to be avoided.

References

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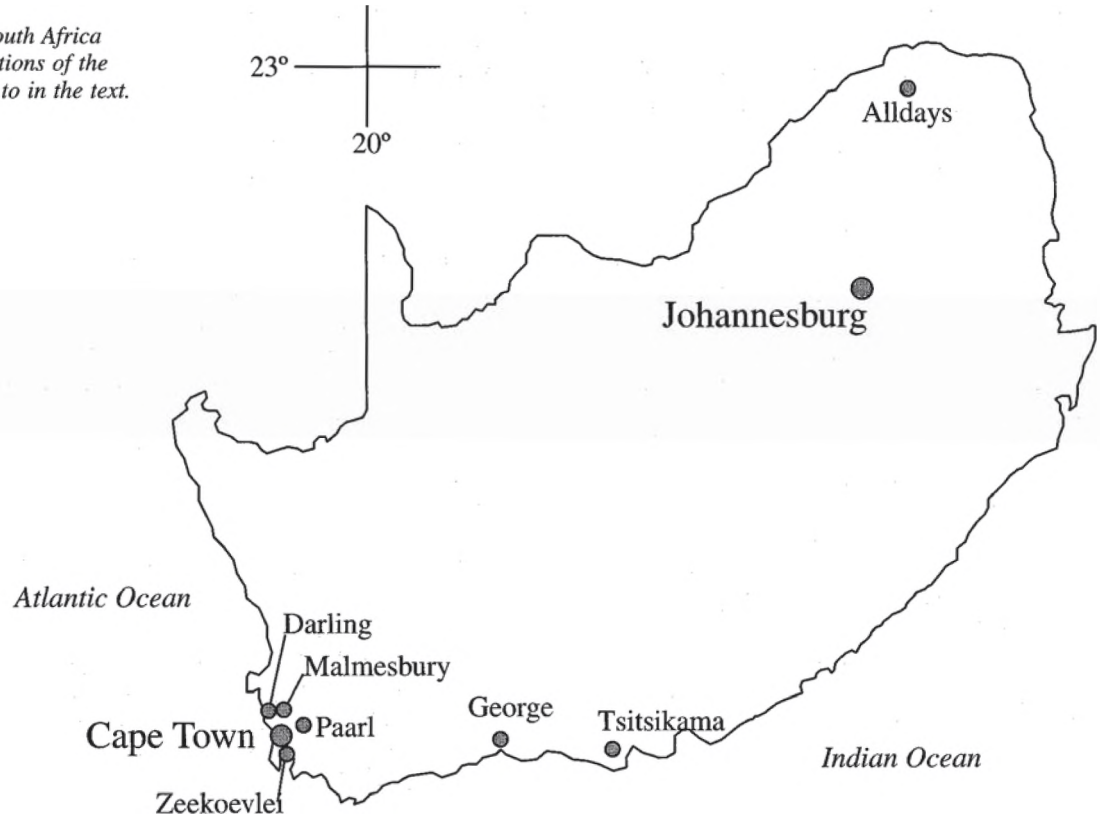

Figure 1: Map of South Africa illustrating the locations of the poisonings referred to in the text.

This special section is sponsored by the Organizing Committee of the VIII International Conference on Harmful Algae

HIGHLIGHTS OF VIII INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

The *VIII International Conference on Harmful Algae* was held in the Cultural Centre "Caixavigo" from 25 to 29 June 1997 in Vigo, Spain. 470 scientists and 41 accompanying persons from 58 countries attended this event. The conference was hosted by Instituto Español de Oceanografía, Ministerio de Sanidad y Consumo, and Xunta de Galicia. It has only been possible to bring you these reports thanks to the enthusiastic collaboration of those people who agreed to chair the scientific sessions and round tables and to act as rapporteurs.

A considerable number of people obtained grants to attend: 45 scientists from developing countries/economies in transition (supported by IOC, SCOR, UNEP, and the organizers); 16 young Europeans (Federation of European Microbiological Societies); 6 US doctorands (US-National Science Foundation) and 16 Spanish doctorands (Spanish Universities and the organizers).

There were 10 keynote and 62 oral communications, all presented in plenary sessions; 2 poster sessions where 332 posters were exhibited; six round tables on the most relevant or controversial topics identified by the organizing committee, and a special session about the "Standard, Measuring and Testing Programme" of the European Union.

Before the conference, the abstracts of all accepted communications (403) were edited and organized as a database. They were available to the International Scientific Community since the preceding May through the web site of IOC at internet: http://www.unesco.org:80/ioc/oslr/habcon7.htm.

Here we provide synopses of the scientific sessions and round tables held during the conference. The proceedings of this event will be co-published by the Galician Government and IOC. About 2000 copies will be printed by the end of 1997. Libraries from developing countries will have free copies distributed through the IOC Science and Communication Centres on Harmful Algae in Copenhagen and Vigo.

Beatriz Reguera, Instituto Español de Oceanografía.

SCIENTIFIC SESSIONS

Opening session

Chair: Marta Estrada, Spain

The opening session of the VIII Conference on Harmful Algae was devoted to the opening address by Ramón Margalef (Spain) and the contribution of Colin Reynolds (U.K.) and Ted Smayda (USA). Both lectures considered aspects of harmful algal events within a general ecological framework. Margalef remarked on the diversity of dinoflagellates and the complexity of their adaptive responses to hydrodynamic and chemical constraints. In this context, accumulation of toxic compounds might be interpreted as a result of continued chemical synthesis under conditions leading to limitation of cell division. The role of toxins could be compared with that of many secondary metabolites in flowering plants.

The second lecture, by Colin Reynolds and Ted Smayda reviewed the conceptual representation (also known as Margalef's mandala) of phytoplankton life-forms as "adaptation syndromes" responding to two main selective factors: nutrient supply, as related to growth rate, and water turbulence. In this representation, red tide sequences follow a path comparable to that of a typical phytoplankton succession, but displaced towards situations with higher stratification. Reynolds reaffirmed the conclusion that life-form and function are better predictors of fitness than phylogenetic affinities and pointed out that the ability to predict the exact composition of assemblages remains subject to a strongly stochastic element.

Population dynamics of harmful algae – physical forcing mechanisms – case studies

Chairs: Patrick Gentien, France
Kaisa Kononen, Finland
José I. Carreto, Argentina

Mainstream biological oceanography has been mainly concerned with biomass production in open waters, and formed the background to Victor Smetacek's (Germany) keynote talk, which looked at the potential for cross-fertilization of ideas between "normal" phytoplankton ecology and HAB ecology. Even though considerable accomplishments have been achieved in the understanding of speciation between diatoms and dinoflagellates due to conditions of new or of regenerated production, it is now recognized that so-called exceptional blooms, which tend to be harmful or a nuisance from the human point of view, do not fit in the above scheme. Knowledge of factors determining species or even group dominance in the open ocean is still vague. A major impediment is our lack of understanding of factors shaping succession.

The study of harmful algae blooms stemmed from the need to understand toxic events. Conceptual "species of interest" models are developing and take into account, not only the regulation of growth by inorganic subtrates, but all the processes the particular species is involved in during its whole life cycle. A large amount of the detailed physiological knowledge commonly used in biomass models, although necessary, has been shown to be insufficient to answer the question: "Why does this species bloom and why is it toxic?"

The life cycle of the species of interest has to be considered, starting from the inoculation and wintering processes, to the selection by substrate and competition, selective grazing pressure, behaviour at the cell and population levels in the physico-chemical field, and mortality factors. Cysts beds due to sexual reproduction are of primary importance, in that they determine the inoculation zone. *Alexandrium*, for instance, spends most of the year as an overwintering cyst, deposited on the sea-floor and transported as sediment particles. The processes affecting cyst resuspension will determine, when growth conditions become favorable, the success of the population. Hans Eilertsen (Norway) and Tim Wyatt contribution showed clearly the rôle of tidal kinetic energy phased on the lunar cycle in determining the success of a bloom event.

Results of a tentative more classical model based on competition between species for nutrients, presented by Keith Davidson (U.K.) were not shown to be as successful. It was clearly stated that simulation of the appearance of harmful algal blooms by applying models which are just sufficient to simulate monoculture data of the particular species of interest is unlikely. Mixotrophy, and grazing pressure have been presented as determining processes which have to be considered.

Compared to previous symposia, there has been an increasing number of contributions related to meso-scale oceanographic processes and controls of harmful algae blooms. Although the most detrimental effects of toxic blooms are often detected in seafood cultures in coastal or estuarine areas, the dynamics of bloom development and concentration is tied to off-shore or coastal oceanic processes rather than local estuarine conditions. Different presentations have shown that, in many cases, populations originate offshore where they are often confined in physical structures like fronts and are transported, episodically, inshore. Close relations have been shown between meso-scale circulation and bloom appearance and extension in Argentina (José Carreto et al.) South Africa (Grant Pitcher et al.), Bay of Biscay, France (Patrick Gentien et al), Celtic Sea, Ireland (Terry MacMahon et al.), Galicia, Spain (Francisco G. Figueiras), Portugal (Teresa Moita et al.), and Gulf of Mexico (Parsons et al., USA.). Mechanisms of onshore/offshore transport of HAB populations need to be included for an understanding of HAB events in coastal and estuarine regions. An interesting finding was the observation that the growth of the domoic acid producing diatom *Pseudo-nitzschia* was affected by neither N/P ratio nor salinity, but by small-scale turbulence. These findings give a lead towards the establishment of predictive models, provided that the pertinent ecophysiological processes already known are estimated in terms of their relative importance to population development and set into the real world, which is, in this case, the ocean at meso- and small scales.

Uptake and elimination of toxins by shellfish

Chair: Sandra Shumway, USA

In the plenary lecture (Bricelj and Shumway), Monica Bricelj (Canada) presented an in-depth overview of the kinetics of PSP toxin transfer in bivalve molluscs. Based on a suite of factors contributing to their detoxification kinetics, bivalves were broadly classified into two groups: **slow detoxifiers** (e.g., *Saxidomus giganteus, Spisula solidissima, Placopecten magellanicus* and *Mya arenaria*) and **fast detoxifiers** *(Mytilus edulis, M. galloprovincialis)*.

It was noted that a bi-phasic, two-compartment model can describe detoxification kinetics in some species. Biotransformation of toxins was shown to be most pronounced in species capable of enzymatic decarbamoylation. The use of bivalve toxicity to predict the timing and source of toxic blooms was also discussed. The modelling of aspect of toxin kinetics was further discussed by Bill Silvert et al (Canada) and Angeles Moroño et al (Spain). Bill Silvert presented a dynamic model of uptake, detoxification, and biotransformation that simulates the development of toxicity in different functional compartments. The model takes into account the effects of increasing body size, addresses the changes in toxicity caused by biotransformation and can be used to test hypotheses regarding pathways and detoxification processes. Moroño showed that DSP and PSP detoxification kinetics in mussels (*M. galloprovincialis)* can be described by two and one compartment models respectively, and that the effect of environmental conditions on those kinetics cannot be estimated independently from the model structure. While real progress has been made in realistically modelling toxin kinetics in bivalve molluscs, much work remains to be done if these models are to be available for general applications.

The presentation of Fletcher et al. (New Zealand) dealt with detoxification of Pacific oysters, and emphasized the interactions of intrinsic and extrinsic factors and the detoxification process in bivalve molluscs. In the final oral paper (Vasconcelos et al., Portugal) the focus was shifted to the toxic cyanobacteria and a proposal for a monitoring programme in the Tamega River (Portugal) was discussed.

The oral presentations concerning shellfish toxicity were supplemented by a large number of poster presentations. Topics covered included: accumulation and depuration of major toxins (PSP, DSP, ASP, brevetoxins) and others (microcystins), and seasonal changes in toxicity in some geographical regions not previously described (e.g. Cuba, Morocco).

Application of cellular and molecular probes in taxonomy and genetics of harmful algae

Chair: Donald M. Anderson, USA

This session began with a review by Chris Scholin and Don Anderson (USA) of recent progress in applying antibody and DNA probes to the identification and quantification of HAB species and then proceeded to direct demonstrations of developments and applications of these technologies. A number of poster contributions also related to the theme of this session. The review highlighted the **tremendous expansion in the number of HAB species for which there are now molecular probes of various types**. At least one probe-type, and often several, is available for nearly every one of the key HAB species or genera. **However**, the review pointed out that only a few actual field applications had thus far been attempted. Clearly, probe technology developed using unialgal laboratory cultures is not easily transferred to complex field assemblage, and significant work remains to make these tools useful in field settings.

A related application of molecular technologies was demonstrated in several studies of genetic diversity that relied on comparisons of rRNA sequences, RFLP or RAPD analyses of those sequences, and lectin or antibody binding to the cell surfaces of strains of individual HAB species. Populations of *Gymnodinium*

catenatum ((Chris Bolch et al., Australia), *Gambierdiscus toxicus* (Chinain et al., French Polynesia), *Microcystis aeruginosa* (Victoria López-Rodas et al., Spain) and other species exhibit considerable diversity over small spatial and temporal scales when examined in this manner. There is considerable genetic variability within blooms or regional populations of HAB species that is not evident using traditional methods for enumeration or identification. Progress was also reported on procedures to count or separate HAB species from co-occurring organisms (Louis Peperzak et al., The Netherlands). Flow cytometry applications were described by Sako et al. (Japan) and Nicole Poulton and Don Anderson (USA), and a novel method to separate *Alexandrium* cells from samples using antibodies and magnets was presented by Angeles Aguilera et al. (Spain). Here again, direct applications to field populations were on a trial basis at best, with no sustained usage in major studies for any of these techniques.

In the discussion that followed, Chris Bolch was asked whether genetic analysis of *G. catenatum* had shed any light on the long-standing hypothesis that ballast water discharges had introduced that species to Tasmania. The response was that no foreign strains had yet been identified that were genetically identical to Tasmanian isolates, so the putative "source" population remains to be found. Interestingly, the isolates that most closely resembled Tasmanian populations were from mainland Australia. Another general comment was that despite the increased resolution obtainable with the molecular techniques, the "big picture" of HAB species bloom dynamics or ecology is still lacking. Perhaps time and resources devoted to probe development or to resolution of the genetic diversity within regional populations should be re-directed to field studies of organism ecology and dynamics, which still remain largely unknown for most HAB species. One response to this observation is that many of those involved in probe design or genetic analysis are also actively involved in field programs, and thus have strong motivations to develop and use the tools needed to quantify HAB species abundance, distribution and physiological rates on appropriate temporal and spatial scales. Conventional methods have proven to be too coarse and time-consuming to provide the coverage that is needed. Another response is that the increased resolution may be needed to explain variability in population abundance or toxicity caused by different genotypes with different levels of inherent toxicity or growth requirements – i.e., that variability reflects the dominance of different genetic strains rather than control by external environmental factors.

Overall, progress continues to be rapid in molecular approaches to HAB problems, and the potential remains high that the tools will soon be heavily used in autecological studies of HAB species. Priority research topics for the future include:

- development of new probes for HAB species for which none are presently available;
- development and marketing of kits and instruments that allow molecular probes to be used by skilled and non-skilled users alike;
- application of probe technologies in field studies investigating bloom dynamics and ecology;
- development of methods to obtain species-specific physiological measurements using molecular techniques;
- expand regional biogeographic studies of key HAB species to include globally distant populations.

Toxin detection methods

Chair: Donald H. Hannah, New Zealand

This session included one keynote address and six submitted papers. The keynote address was given by Michael Quilliam (Canada) on the use of LC-MS. He posed the question "Is LC-MS a universal method for biotoxin detection?", and then proceeded to show its efficacy for the main situations in ASP, DSP, NSP and PSP and also for ciguatera (CFP) and many of the compounds and toxins associated with these seafood poisonings. The method was shown to be very versatile, capable of detecting virtually all the relevant compounds, though not in one single analysis. The major negative problem with the technique is the capital cost for purchasing the equipment (more that US$250,000). Quilliam explained that in situations where there are a large number of samples LC-MS is a cost effective and profitable technique.

Greg Boyer et al. (USA) described the development and application of an electrochemical detector for PSP toxins in the HPLC analysis. The method used the multiple isocratic separations of Y.Oshima but replaced the post-column fluorescence reaction method with a sensitive and specific electrochemical technique. The development and application of the neuroblastoma cell based detection of PSP toxins was outlined by Joanne Jellett et al. (Canada). This technique has been applied at 3 levels, a yes/no kit, a semiquantitative kit and a fully quantitative kit. J. Jellet outlined the collaborative testing with AOAC that is currently planned.

Kevin James et al. (Ireland), presented HPLC, radioactive protein phosphatase and electrospray mass spectrometric methods to determine cyanobacterial toxins in Irish freshwaters. Mercedes Vieitez et al.(Spain) outlined the use of 4-methylumbelliferyl phosphate and fluorescein diphosphate as substrates for the protein phosphatase inhibition assay for DSP toxins; this method is sensitive, fast and simple to use and this presentation represents a further example of the utility of the method. Two poster presentations by Roberto della Loggia et al (Italy) and Yuzuru Shimizu et al. (USA), also discussed the use of fluorescence substrates for this assay.

Armando García et al. (Cuba), presented an innovative method, based on the survival of neuronal cells, as a comprehensive assay for ASP, DSP and PSP toxins. The method has potential to be an in vitro based alternative assay to the mouse bioassay. The comparision of, and utility of, the neuroblastoma cell assays and the radiolabelled competitive binding assay with the mouse bioassay for the detection of NSP toxins was presented by Penelope Truman et al. (New Zealand). Again the two in vitro assays show great promise as alternatives to the mouse biassays.

General Comments

The techniques presented, both in the oral and in the poster sessions, clearly indicate that there are a number of chemical and *in vitro* biological assays that could be suitable alternatives to the mouse bioassays. There is an urgent requirement to proceed with supervised inter-laboratory collaboration trials, especially for the neuroblastoma assay for PSP toxins and the fluorescence based protein phosphates assay for DSP toxins. There is, however, still a gap on the development of a non-animal based alternative to the mouse bioassay that will detect "unknown" toxins that may present a hazard to public health.

New toxins

Chair: Kevin J. James, Ireland

A reeview of new developments in seafood toxins was presented by Takeshi Yasumoto (Japan). Considerable progress has been made in elucidating the causes of a number of unexplained shellfish toxic incidents in Europe in recents years. Thus, a new toxin, KT-3, has been isolated from shellfish in Ireland and it is a polyether carboxylic acid with a novel spiroamine moiety. New reports of yessotoxins in Chile, New Zealand and Italy indicate their widespread occurrence but a new fluorimetric HPLC analysis, using DMEQ-TAD, should facilitate their determination and this reagent may also find application in the determination of pectenotoxins. Appreciation of the incidence of cyanophyte toxins in seafood was also highlighted with the implication of aplysiatoxin in *Gracilaria* poisoning in Hawaii and lyngbyatoxin A in turtle poisoning in Madagascar.

New additions to the group of fast acting marine toxins, represented by the spirolides, was discussed by Jeffrey Wright (Canada) and the cyclic imine in these compounds is apparently crucial to their pharmacological activity. An intriguing theory on the characteristic methylation sequence in dinoflagellate polyether compounds should prove useful in the investigation of the source of new toxins.

Shigeru Sato et al (Japan) made an important contribution to the debate on the possible role of bacteria in the production of PSP toxins. Thus, it was shown that naturally fluorescent compounds may be confused with some PSP toxins using HPLC determinations. The complexity of the PSP group of toxins was highlighted by H. Onodera et al. (Japan). Remarkably, six new PSP analogues have recently been isolated from a freshwater cyanobacterium.

Physiology and toxin production

Chair: Stephen Bates, Canada

Seven oral presentations and 42 posters described how cell growth and toxin production are affected by cell cycle and life history events; inorganic and organic nutrient status; turbulence; salinity; and light intensity, quality and photoperiod. In his keynote, Kevin Flynn (U.K.) discussed how modelling ("simulation") is a useful tool for focusing objectives and showing cell physiologists which experiments could next be carried out, provided that these models are constructed with a sound physiological basis. The model presented was flexible and could simulate the interactions of various light and nutrients conditions with respect to toxin production. Current models still suffer from a lack of information about cell cycle and life history events, as well as growth rate data; some of this knowledge was provided in this session. For example, the timing of toxin production in relation to the light:dark cycle is becoming better understood for *Amphidinium operculatum* (Leighfield and Van Dolah, USA) and *Alexandrium catenella* (Young and Chan, Hong Kong). The study of light:dark cycles is being exploited to identify genes involved in saxitoxin biosynthesis by *Alexandrium fundyense*, by use of "differential display" of mRNA patterns, a novel genetic approach (Taroncher-Oldenburg and Anderson, USA). Growth rates of *A. fundyense* and *Prorocentrum micans* can be determined by flow cytometric analysis of the diel DNA cycle (Peperzak et al., The Netherlans). In contrast to looking at the molecular level, some basic biology of cell division and life history was given for *Prorocentrum* spp. (Pan and Cembella, Canada), *Alexandrium lusitanicum* (Honsell et al.), and *A. minutum* (Probert et al.). The pattern of sexual reproduction was described for two *Pseudonitzschia* spp. (Davidovich and Bates, Ukraine). This type of basic biology may help to understand the timing of blooms and of toxin production, and is still missing from several other algal groups.

The effects of nutrients on toxin production by different groups of toxic algae was summarized by Edna Granèli et al (Sweden); no simple correlations or patterns were found. It is essential to distinguish between toxin per cell and the rate of toxin production, and to take into account differences in cell volume (Bustillos-Guzmán and Diogène) in such comparisons. Several posters added to the information available on nutrient effects, but basically supported past findings. For example, phosphorus limitation seems to enhance toxin production by *Chrysochromulina polylepis* (Johansson and Granèli, Sweden), *Nodularia spumigena* (Panosso et al., Brasil), and *Alexandrium catenella* (Young and Chan, Hong Kong). What implication could this have for shellfish poisoning by *A. tamarense* in Hiroshima Bay, Japan, for example, where a decreasing trend in phosphate concentration may have resulted in phosphorus becoming a limiting factor for phytoplankton growth (Yamamoto and Tarutani)? In contrast, phosphorus limitation was shown to decrease microcystin toxicity in *Microcystis aeruginosa* (Hesse and Kohl, Germany) and it had no effect on okadaic acid production by *Prorocentrum hoffmannianum* (Morton et al., USA). In the above two examples of cyanobacteria (*N. spumigena* and *M. aeruginosa*), it would be interesting to determine why phosphorus limitation gave contradictory responses. New tools were presented for detecting phosphorus-limited cells: alkaline phosphatase was quantified in individual cells by use of a novel insoluble fluorigenic substrate (González-Gil et al., Spain), and two proteins (one, an alkaline phosphatase) induced by phosphorus stress were identified on the cell surface of *Prorocentrum minimum* (Dyhrman and Palenik, USA).

The major emphasis in this session was on macronutrients; there was less information on the effects of organic matter and trace metals on algal growth and toxin production. The concentration of humic substances, by controlling the supply of selenium via chelation, was shown to be important for the growth of *Gymnodinium catenatum* (Doblin et al., Australia). Likewise, the complex physiological and ecological roles of humic and fulvic acids in relation to nuisance algal blooms was demonstrated (C.Heil et al., Australia), as was their contribution to the nitrogen (Legrand and Carlsson, Sweden) and phosphorus (Panosso et al.) requirements for growth. Gambieric acid-A was shown to be an endogenous growth enhancing factor for *Gambierdiscus toxicus* (Sakamoto et al., Japan). What appears to be lacking (except for the study of Panosso et al.) is an examination in the same study of the effects of organic matter on both growth and toxin production. Trace metal limitation may have an important effect, but this was addressed in only one poster, where it was shown that iron limitation increased the toxicity of *Microcystis aeruginosa* (Kiefer and Lyck, Norway).

Another area that requires more attention than was devoted to it at this session is the study of differences in physiology and toxin production when several clones of the same species are examined under the same conditions. Characteristic differences were found among three strains of *Microcystis aeruginosa* with respect to growth and physiology; only one of the three strains was toxic (Hesse and Kohl). A comprehensive study of 20 unitrichome strains of *Anabaena circinalis* under controlled culture conditions revealed that none of the strains from outside Australia produced PSP toxins, and 11 of the Australian strains were non-toxic, indicating genetic divergence among the strains (Negri et al, Australia). The question of toxic vs non-toxic strains is becoming in-

creasingly evident for *Pseudo-nitzschia* spp., as seen in this session as well as the session on "Taxonomy; application of advanced techniques in taxonomy" (Rhodes et al., New Zealand). Two isolates of *P. pungens* from Washington and California, USA, were shown to produce low quantities of domoic acid (Trainer et al.); this species has been reported to be non-toxic elsewhere, except for one case in New Zealand. A parallel, independent study also demonstrated low toxicity in the same California clone of *P. pungens* (separate poster presented by Doucette and Scholin, USA).

This session showed a trend, continuing from past conferences, for an increased emphasis on the physiology of toxin production by cyanobacteria (Coyle and Lawton; Rouhiainen et al.; Rapala et al.; Ljungblom; Kiefer and Lyck, Hesse and Kohl; Panosso et al.; Negri et al.). Other algal groups appeared to be represented in proportion to their involvement in toxic events. Research on the physiology of toxic algae has benefited from techniques and approaches borrowed from other scientific disciplines. The study of HABs is still in its infancy, but one would soon hope for the construction of paradigms to better explain toxin production and bloom dynamics in relation to cell physiology.

Monitoring and management

Chair: Einar Dahl, Norway

The keynote talk of this session, by Geoffrey Codd (U.K.) reviewed toxicity problems in freshwaters, including drinking water supplies. Codd made a plea that some barriers need to be dismantled, since research and policy development with respect to toxins and their impacts on human activities are currently compartmentalized between freshwater and marine systems. Yet some of the toxins share common modes of action, and PSP is found both sides of the barriers.

This session included a description and rationale of the Galician monitoring programme (Joaquín Mariño et al., Spain), and emphasized that with only a small additional cost of the basic sampling requirements, it is possible to provide valuable research data. Capital and running costs, and staff requirements were detailed.

Moves to legislate in favour of mandatory ballast water exchange may under some circunstances be counter-productive. This was the conclusion of Elspeth MacDonald (U.K.), who showed that exchange may increase the risks of introducing harmful algae, at least in the regional seas of northern Europe. Motile dinoflagellates were found in most of 120 vessels arriving in ballast to Scottish ports, and cysts in more than 80% of them. *Alexandrium, Dinophysis* and *Pseudo-nitzschia* were common components of the ballast flora, and the diversity and abundance of diatoms and dinoflagellates was increased following ballast water exchange.

The fish-killing *Cochlodinium polykrikoides* causes severe losses to aquaculture in Korea. Hak Gyoon Kim (Pusan, Korea) described the success of spraying blooms with clay as a mitigation strategy. Water with 2-4 g l^{-1} of clay in suspension can remove 70-90% of the *Cochlodinium* in the water column within 20 mn.

Domoic acid in offshore *Pseudo-nitzschia* populations along the U.S. Washington coast presents a potential threat to razor clam resources. John Wekell et al. (USA) showed data indicating that they could become toxic very rapidly, making monitoring more expensive due to the sampling frequency needed to protect consumers.

Pedro Burdaspal et al. (Spain) described procedures used to reduce PSP levels in canned *Acanthocardia tuberculata*, a bivalve showing almost chronic low levels of PSP toxicity on the Mediterranean coasts of Spain and Morocco (Taleb et al.). The test showed by Burdaspal, after one year's appplication of an aproved exception in European Directives, showed that it leads to acceptably low toxin levels. ASP toxins associated with *Pseudo-nitzschia* spp. are being detected in a growing number of countries. Although the monitoring of ASP toxins in the European Union is not mandatory, some countries (Portugal, Spain..) have already included its control in their national/regional programmes.

Interactions between harmful microalgae and other microorganisms

Chair: Yuzaburo Ishida, Japan

In this session twenty eight contributions on (1) inhibitory effects of algal toxins on protozoa ingestion, copepods grazing (also egg production and egg hatching success) and fish, (2) competitive effects of algal toxins between algal species, (3) bacterial effect on PSP toxin production of dinoflagellates, (4) regulatory effects of bacteria and viruses on algal growth, and (5) others, were presented by oral and poster.

The relationships between toxic microalgae (PSP, DSP and microcystins) and grazing activities of protozoa and copepods are variable, and grazing may be influenced by food quality irrespective of its toxin content (Jeff Turner et al, USA; Jayatissa et al, Sri Lanka). The ecological interest in growth competition between algal species was that the okadaic acid-producing algae played a rôle of growth inhibition in the benthic dinoflagellates community (Linda Sugg and Frances VanDolah, USA), and that the breakage of *Gymnodinium catenatum* cells by *Polykrikos kofoidii* led to the end of a bloom of the former and rapid decline in bivalve toxicity (M. Antonia Sampayo, Portugal). On so-called PSP-producing bacteria, further experiments for identification of toxin compounds will be necessary; the expression "bacteria associated with dinoflagellates" is ambiguous as to whether "the association" means in natural environments or culture tubes or cell attachment. Finally research on regulation of algal blooms by algicidal bacteria and viruses is of ecological and economical importance (Yoshinaga et al.; Nagai et al., Japan). It may be possible to apply these bacteria and viruses as bioremediators to supress red tides.

Toxicology, pharmacology and mechanisms of action of phycotoxins in mammals

Chair: Lars Edebo, Sweden

The keynote lecture was given by Eisaburo Sueoka from the well-known research group at Saitama Cancer Centre Research Institute in Japan. This group, to which Hirota Fujiki and Masami Suganuma also belong, has discovered the tumour-promoting activity of DST (diarrheic shellfish toxins) and other structurally analogous, aquatic microbial toxins, and related it to inhibition of protein phosphatases 1 and 2A. Accumulating data indicate that this inhibition is a general mechanism for tumor promotion. It was emphasized that tumor promotion is the enhanced development of tumors after tumor initiation has taken place e.g. after exposure to chemical carcinogens. Tumor promotion might be mediated by the cytokine tumor necrosis factor (TNF) which is produced endogenously in the human body by a number of inflam-

matory stimuli. Early response genes are involved in the activation mechanisms and cellular cytoskeleton proteins such as cytokeratins appear hyperphosphorylated. Thus, this kind of toxins may participate in complex interactions with inflammation in a multitude of reactions at the cell and organ level.

DST are also toxic to neurons leading to apoptotic cell death possibly by inhibition of protein phosphatase 2A but not by the involvement of the protein kinases A and C (M.Teresa Fernández et al., Spain). Ciguatoxins, which are also polyether toxins, show more apparent neurotoxicity. At the cellular level it is seen as an increased number of coated vesicles and swelling of nerve terminals due to water influx (Jordi Molgo et al., France). Brevetoxins are also polyether compounds. In 1996 they caused a major epizootic of West Indian manatees killing more than 150 animals each ca. 700 kg. In succumbed animals toxin had accumulated in phagocytic cells in brain, spleen, liver, and kidney apparently leading to apoptotic cell death (Daniel Baden et al., USA).

Accumulating data seem to indicate that although the different groups of polyether marine toxins show specific reaction patterns, they use some common pathways. Furthermore, more toxins are being discovered, particularly with DST-like mechanisms, which draws attention to the possible health hazards of chronic exposure to subacute toxic doses.

Algae events and their impacts

Chair: Leonardo Guzmán, Chile

This session included five oral papers which demonstrated the wide variety of ways in which harmful algae can interact with human affairs. Anne Marie Legrand (French Polynesia) gave a wide-ranging review of many aspects of ciguatera, including the large scale geographical distribution and local patterns of patchiness in reef areas, the chemical structure of the main toxins and their mode of action, and how they are transferred through the food chain to reach man. Clinical treatment has advanced, but it is still necessary to identify substances capable of inhibiting or reversing the actions of ciguatoxins. Methods available to detect low concentrations of ciguatoxins in fish muscle need to be validated, and monitoring systems better defined.

Attention was drawn to the potential threat posed directly to humans via drinking water supplies, and to the fisheries in Patos Lagoon, Brasil, by blooms of *Microcystis aeruginosa.* Joao Yunes described the ecology of this cyanophyte and the toxin concentrations of perhaps four different microcystins detected in the blooms and the scums.

Arturo Sierra-Beltrán summarized potential problems in the coastal waters of Baja California, Mexico. Mortalities of fish, shellfish, birds and mammals have been recorded there since 1991, and linked to PSP. Scallops from Bahía Concepción yielded DSP toxins, first noted in 1992. *Dinophysis* and *Prorocentrum* present year-round in the bay are suspected sources. A pelecan (*Pelecanus occidentalis*) mortality at the southern end of the Baja California peninsula was probably caused by *Pseudo-nitzschia.* Domoic acid and *Pseudonitzschia* frustules were found in the stomachs together with remains of the presumed vector, the mackerel *Scomber japonicum.*

The well known *fish killer, Chatonella marina,* was linked to a major mortality of caged southern bluefin tuna (*Thunnus maccoyii*) in South Australia. The event took place in April 1996, and 1700 t of tuna (75% of the stock) were lost, with an estimated first sale value over $ 50 million. Gustaf Hallegreff argued that the relatively low cell concentrations encountered could have beeen responsible for the pathological signs observed in the tuna gills, since this species has a uniquely large gill surface area relative to body weight.

In March-April 1996, there was a major manatee, *Trichechus manatus latirostris* amongst the barrier islands in the southwest part of the state (Landsberg et al., USA). The event was attributed to the exposure to *Gymnodinium breve* brought about by penetration of the algae into the inshore waters and their persistence there, and dispersal of the manatees from upstream refuges as the bay waters warmed up. Similar events,but with fewer deaths, were recorded in1963 and 1982.

Cell cycle – life history – behaviour

Chair: Malte Elbrächter, Germany

Knowledge of cell cycles and life histories as well as of the behaviour of Harmful Algae is essential for understanding and – in a second step – predicting Harmful Algal Blooms (HAB). The session described the difficulties in elucidating life cycles. It also demonstrated that the understanding of the mechanisms of HAB-formation has made progress since the last conference. Therefore prediction of HAB-events is now more feasible than before. Nicolai Davidovich (Ukraine) and Stephen Bates presented the "true" life cycle of the AST-producing diatom, *Pseudo-nitzschia.* Some years before a "life-cycle" had been presented for this genus which apparently showed a parasitic infection by a fungus. Howard Glasgow et al. (USA) showed that the fish-killing potential of *Pfiesteria* zoospores (dinoflagellate) depends on the nutrient load of the water column and – more importantly – on the earlier history of the life-cycle stages forming the fish-killing zoospores. Besides parasitic food uptake by attacking fish, *Pfiesteria* may also feed phagotrophically on plankton-organisms including other algae. *Pfiesteria* is even able to live by mixotrophy, using *kleptochloroplasts* of their food items. This and related studies are important steps towards better understanding and predicting the pattern of fish-kills caused by *Pfiesteria.*

Mixotrophy is apparently a common and important but so far mostly neglected nutritional strategy of harmful algae. Maria Faust (USA) demonstrated this for benthic toxic algae during the session and several poster tackled the same topic.

M. Grazia Giacobbe et al (Italy) analysed the cell division cycle of dinoflagellates belonging to the genera *Dinophysis* (DST-producer) and *Alexandrium* (PST-producer). These organisms multiply by binary cell division, but this cell division is syncronized in the day-night cycle so that all cells undergoing cell division do it at the same time. This behaviour is important as recently divide cells can be recognized even a few hours later. Therefore the population growth rate can be determined in natural populations. This is the starting point to differentiate between population increase due to growth or by physical accumulation of cells from a larger water body. Frances Van Dolah et al. (USA) investigated the biochemical mechanisms of this endogenous cell cycle, using the benthic dinoflagellate *Gambierdiscus toxicus,* causing ciguatera. These investigations may be the starting point for growth rate determinations in field populations of species showing no morphological criteria for cell division or for those species which do not divide synchronously.

In conclusion, the session covered very well the progress which is being made with respect to a better understanding of population dynamics and predicting the occurrence of HABs. Both provide essential information for efforts to mitigate the effects of Harmful Algae Blooms.

ROUND TABLE DISCUSSIONS

Emerging technologies for biological and biochemical assays of phycotoxins

Chair: Allan Cembella, Canada

Rapporteurs: Marion Gillman-Coughlan, Alan Bishop and Ireland.

Alternatives to the various mouse bioassays for phycotoxin detection and quantification were explored. Development and implementation of alternative methods was acknowledged as a high priority for seafood regulatory agencies, the aquaculture and harvest fishery industry, and the protection of public health. A distinction was made between an assay, which yields a single toxin concentration or toxicity value, but no direct information on the specific toxin composition, and an analytical method (e.g., HPLC, coupled mass spectrometry, capillary electrophoresis, etc.), whereby the toxin spectrum is resolved, and total toxicity is derived from specific toxicity values of the individual components.

The participants recognised that currently available *in vitro* biological and biochemical methods may supplement or partially replace the whole animal bioassays conventionally used in phycotoxin monitoring programs. In principle, *in vitro* assays could be configured to yield at least semi-quantitative measures of toxin concentration or toxicity in a simple, rapid, and sensitive format. In a kit configuration, such assays could be employed routinely for high-volume screening of putatively toxic seafood extracts on board ship, in rudimentary dockside laboratories, and at aquaculture installations, as well as in well-equipped regulatory laboratories responsible for toxin monitoring. Nevertheless, regardless of the high level of technical sophistication, sensitivity, low cost and ease of use of several in vitro assays for phycotoxins, few such methods are in widespread distribution as commercial products, and acceptance as official standardised methods (by AOAC, U.S. Food and Drug Agency, EU committees, etc.) is even rarer. The panel discussion addressed the reasons for this apparent contradiction between technical facility and implementation:

1) technical performance limitations of in vitro assays?;
2) high cost of development versus low rate of economic return?;
3) conservatism by regulatory agencies?;
4) lack of properly controlled inter-calibration trials?;
5) poor availability of standards and reference materials?; and
6) need for advanced training in the proper use of these methods?

Since the Panel participants demonstrated a wide spectrum of expertise in alternative assay approaches for various groups of phycotoxins (PSP, DSP, ASP, NSP, ciguatera toxins, microcystins), and they have been instrumental in the technical development and/or application of these methods, their comments focused primarily on the technical performance aspects – rather than on regulatory acceptance.

In accordance with the scheme outlined in the Manual on Harmful Marine Microalgae (IOC-UNESCO, 1995), four basic categories of in vitro biological and biochemical methods were defined:

1) immunoassays;
2) enzyme-inhibition assays;
3) cellular-based ("cytotoxicity") assays and
4) receptor-based assays.

These were divided further into structural versus functional assays – in the former type (e.g., immunoassays), toxin detection is contingent upon the molecular configuration of the analyte (toxin), but this may not be directly correlated with the toxicity towards target cells. Some participants noted that initial enthusiasm for immunoassays (especially, enzyme-linked immunosorbent assays [ELISA]) has begun to decline in favour of functional assays (e.g., receptor-binding or cytotoxicity assays), whereby the toxic effect is exerted directly upon the target system, e.g. ion channel or membrane. This is a particular weakness of immunoassays applied to samples containing a complex mixtures of toxins with a wide range of specific toxicities, using antibodies with highly specific cross-reactivity. It was suggested that the problem of limited cross-reactivity could be partially overcome by employing an antibody "cocktail". In any case, sensitivity levels are more than adequate for regulatory purposes (usually <nM detection limits) but some *in vitro* methods are plagued with false positive responses, matrix effects, or the failure to detect certain key toxin analogues. There was also concern expressed regarding the mats, poor stability of reagents and other perishable supplies provided in kit foralthough efforts are being made to alleviate this critical limitation.

It was acknowledged that proper application of in vitro diagnostic methods for phycotoxins will require further knowledge regarding the acute and chronic toxicity of specific toxins towards mammalian systems (e.g., non-phosphatase inhibitory DSP toxins). An additional challenge would be to optimise clean-up techniques to reduce interfering matrix effects and/or to simplify the complex toxin mixtures in samples via chemical conversion, and thereby determine "maximum potential toxicity".

There was no unanimity regarding the ideal approach for *in vitro* assay development and implementation, but a consensus emerged that *in vitro* methods will play an ever increasing rôle in phycotoxin monitoring programs and that the inherent technical problems are not insurmountable.

New issues in taxonomy

Chair: Øjvind Moestrup, Denmark

Three topics were raised:
1. Documentation for the identity of potentially toxic species of phytoplankton
2. The *Heterosigma akashiwo/carterae* issue
3. The *Prorocentrum/Exuviaella* issue

1. Identification of potentially toxic algae is becoming increasingly more difficult to the use of sophisticated techniques which allow for a better definition of the species but at the same time for a narrower species concept. It also results in the erection of many new species. This development is not recognized by non-taxonomists. Thus *Microcystis aeruginosa* is still the most commonly used name in papers dealing with *Microcystis*, yet taxonomists know that this

species is actually very difficult to identify. Similarly the name *Pseudo-nitzschia seriata* keeps being reported worldwide (including in the tropics), although is a species restricted to cold water in the Northern Hemisphere. The many incorrect identifications creeping into the literature give rise to misunderstandings, wrong ideas about biogeography and toxicity, etc, and participants discussed how this situation can be ameliorated. The following suggestions were brought forward and generally adopted.

Scientific papers should always contain photographic evidence for the identity of the organisms used, or must refer to a previously published photographic illustration. Drawings should always be accompanied by photographic evidence.

The whole cell should be illustrated (important e.g. for *Pseudo-nitzchia* species). If possible, permanent slides should be prepared, which are available for future confirmation, and material should be deposited at a place where it is generally available.

2. Taylor (1992) argued that the correct name for *H. akashiwo* is *H. carterae*, based on Hulburt's description of the organism, the first description of the species (as *Olisthodiscus carterae*). Unfortunately Hulburt's description is invalid (no type), as pointed out by Throndsen (1996). The name change suggested by Taylor is therefore not required. However, the author citation given by Throndsen needs to be modified, since the genus *Heterosigma* was not validly erected by Hada (no type species). It was validated by Sournia and the correct name for the organism which has had such a confused taxonomic history is therefore *Heterosigma akashiwo* (Hada) Hada ex Sournia, or since the code allows deletion of the name preceeding "ex", *Heterosigma akashiwo* (Hada) Sournia. Let us all hope that this remains the last change.

3. The identity of *Prorocentrum lima*, a species described originally by Ehrenberg (as *Cryptomonas lima*), is uncertain. At the same time there are indications from ultrastructure, toxicity studies and ecology that the genus *Prorocentrum* as presently understood comprises more that one genus. McLachlan *et al.* (1996) suggested splitting the genus and use of the name *Exuviaella* for the species related to *lima*, i.e. most if not all the toxic non-planktonic species known at the moment. This would result in renaming of *P. lima* as *Exuviaella lima*. Malte Elbrächter (Germany) spoke strongly in favour of rejecting the name *Exuviaella* as the identity of the type species of *Exuviaella*, E. *marina* is uncertain. Elbrächter recommended that the name *Exuviaella* be rejected and that all species be retained within the genus *Prorocentrum* until more data is available, including studies on more species. This recommendation was accepted after a long discussion. Elbrächter will present his arguments for rejecting *Exuviaella* separately.

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Re-evaluating DSP

Organizer: Takeshi Yasumoto, Japan
Chair: Donald Hannah, New Zealand
Rapporteur: Jim Sim, New Zealand

This session continued discussion begun in other fora including a round table at the VIIth Harmful Algae Conference in Sendai, Japan.

Takeshi Yasumoto (Japan) gave an introduction to and a summary of the toxicology of yessotoxin (YTX). Perspectives of the DSP issues were given by Tore Aune (Norway), Z. Amzil (France), Aurelia Tubaro (Italy) and Donald Hannah (New Zealand). Eisaburo Sueoka (Japan) gave a brief summary of the tumour promoting potential of DSP toxins. Sources of YTX contaminated material were described by Patricia Ciminiello (Italy) and Lincoln MacKenzie (New Zealand).

During the discussion, issues raised included:

- the genotoxicity of okadaic acid
- restricted or no access to the mouse bioassay
- the use of risk assessment methods to determine acceptable levels of DSP and associated toxins in seafoods
- methods to extract DSP toxins
- alternative methods to the mouse bioassay to detect DSP toxins
- what, if any, levels are acceptable for those compounds not regarded as DSP toxins

1. Consensus was reached that YTX should not be regarded as a DSP toxin;
2. There was widespread agreement, with some dissenting views, that Pectenotoxins (PTX) should not be regulated at the same level as DSP toxins;
3. Agreement, with some dissension, was reached that the protein phosphatase methods would be acceptable alternatives to the mouse bioassays for DSP toxins;
4. Discussion also focused on how screening for DSP toxins fits in with screening for other shellfish poisoning toxins, showing methods are required to cover all types of shellfish poisoning in order to provide acceptable levels of food safety;
5. Establishment of appropriate levels for the non-DSP toxins (such as YTX, PTX and gymnodimine, etc.) requires robust oral toxicological studies that determine dose-response curves, the not observable adverse effect levels (NOAEL) and the lowest adverse effect level (LOAEL).

Bacterial production of toxins

Chair: Susan Gallacher, U.K.

Discussion focused on bacterial production of Paralytic Shellfish Poisons (PSP). The chair defined this area as consisting of three issues:

1. Are bacteria capable of autonomous production of PSP and, if so, how does this influence dinoflagellate toxicity?
2. Do bacteria influence dinoflagellate toxicity in some manner, not necessary related to autonomous toxin production?
3. Do bacteria influence shellfish toxicity?

The following issues were raised during discussion:

a) The location of bacteria in relation to the dinoflagellate cell. Some parties claimed that bacteria can be seen in *Alexandrium* cells, whereas others disagreed, stating that such observations may be explained by artifacts in the sampling process. A study by Jane Lewis (U.K.) and Susana Franca (Portugal) designed specifically to answer these questions, will commence soon this year, and will be the first comprehensive study of its kind.

b) PSP is produced by "axenic" cultures of dinoflagellates in Yuz-

aburo Ishida's laboratory (Japan). The panel pointed out that at least 95% of marine bacteria are non-culturable by standard laboratory procedures, hence demonstrations of the bacteria-free status of dinoflagellates by routine culture methods are not sufficient. It was noted that epifluorescence microscopy was also used, but the panel considered that the best proof of "axenic" dinoflagellate cultures would be based on molecular techniques.

c) Although evidence for the autonomous production of PSP by bacteria is available using techniques such as the receptor binding and mouse neuroblastoma assays, as well as HPLC and selective ion monitoring CE-MS, several participants agreed that definitive tandem MS data was still required. Greg Doucette (USA), Susan Gallacher (U.K.) and Elizabeth Maas (New Zealand) plan to colaborate on a study aimed at generating sufficient quantities of bacterial cell extracts for such analysis. Mike Quilliam (Canada) has agreed to perform the tandem MS experiments and provide a critical assessment of the results. The panel is hopeful that such data will be available before the next conference in Tasmania in the year 2000.

Georgina Hold (U.K.) said that shellfish can be made toxic to low levels after exposure to PSP- producing bacteria, as measured by the mouse neuroblastoma assay. Concern was raised about the use of this technique and the panel explained that very thorough controls were used which negated any possible false results due to the sample matrix. However, it was agreed that future work should concentrate on attempts to increase shellfish toxicity to levels allowing HPLC and mouse bioassay data to be obtained.

Allan Cembella (Canada) raised the issue that in his experience the toxin content of shellfish could be fully explained by the toxin content of the dinoflagellates to which they were exposed. The panel raised the question of shellfish toxicity in the absence of any known PSP-producing dinoflagellate cells. There was some disagreement over the validity of this point.

Some information was presented to demonstrate how molecular biology could advance our understanding of bacterial PSP production. Arthur Hosie summarized his efforts to produce mutants of PSP-producing bacteria to identify genes which could be involved in toxin synthesis. This approach complimentes the work by Gaspar Taroncher-Oldenburg (USA) on differential display in dinoflagellates. A. Hosie explained that once genes were identified, probes could be developed which would be used for various purposes, including examining dinoflagellates for similar genes, and the detection of toxin producing organisms in the environment.

The issue of proof of toxin production by bacteria was again raised. It was pointed out that a genetic approach provides an alternative definitive way to address this question. For example, if genes involved in toxin biosynthesis can be identified, one long term aim would be to express these in non-toxic bacterial species and determine if this is sufficient to mediate toxin production. Molecular techniques also have the potential to be used to investigate the regulation of toxin production in response to environmental stimuli.

It is accepted that cyanobacteria produce PSP, and Geoffrey Codd (U.K.), indicated that the question of autochtonous toxin production by cyanobacteria, or symbiotic bacteria, was also becoming an issue in this field.

The point was raised that attention should be focussed on demonstrating a bacterial contribution to PSP toxicity in the natural environment. Linda Medlin (Germany) described how the development of gene probes to toxic bacteria could be used to determine if they are present during periods of shellfish toxicity.

Future research areas identified were:

1. To elucidate the influence of bacteria on dinoflagellate toxicity with the help of molecular approaches;
2. To develop gene probes to bacteria of interest and enhance our understanding of bacteria-dinoflagellate interactions;
3. To identify genes involved in toxin synthesis and to develop probes.

Cyanobacteria

Chair: Ian Falconer, Australia
Rapporteurs: Karina Sivonen, Finland; Susan Blackburn, Australia

An introduction to current problems with toxic cyanobacteria in drinking and recreational waters was given by Ian Falconer (Australia) and focussed on the following topics:

1. Ecological issues
2. Toxicological issues
3. Remedial issues
4. Safety of drinking and recreational waters.

1-2. Ecology and Toxicity Issues

Cyanobacterial blooms are a long standing problem, and pre-date intensive agriculture and high populations. They are aggravated by nutrient inputs such as superphosphate fertilizers. But in the Baltic (K. Kononen), while nutrient input is important near the coasts, the heaviest blooms occur in the least polluted areas. They are a natural occurrence, and are hugely variable due to hydrodynamic effects. Many factors influence the growth of bloom forming species (C. Reynolds). Not all cyanobacteria are equal and the scum-forming species are the most problematic. Temperature and water stratification effects are very significant in temperate regions. In Australia (S. Blackburn), *Anabaena circinalis* is less toxic in the tropics and more toxic in the temperate region and forms winter blooms in Tasmania and summer blooms in Queensland. Are the same factors the trigger?

So far, there are no clear answers as to the reasons for toxin development, which occurs in quantity equal to chlorophyll, and thus cannot be an accident C.Reynolds). Toxins may be a means of communication between cells in the population-intrapopulation signalling. The high level of variation in toxin content may reflect signalling changes.

Cylindrospermopsis is a new large-scale toxic hazard in water reservoirs, and is less obvious because it forms dense layers at 3 m or more depth, with no surface scums. The toxin causes kidney and liver injuries.

Dense blooms of *Aphanizomenon* occurred in Lake Kinneret (Sea of Galilea) in 1994 which contained cylindrospermopsin (Sukenik). Due to depth of the lake below sea-level, the 30 atm pressure charge in the water supply pipeline broke the cells and freed toxin into the water. This was a new occurrence and the ecology of the bloom has not been studied.

Of 75 cyanobacterial isolates screened for toxicity, 25% were found to be toxic, and 5 were highly toxic; they are used for fertilization of agricultural crops in the Nile Delta (Yanni). Falconer commented that cyanobacteria are also harvested for human food (*Spirulina* + *Aphanizomenon*) which may be a health risk.

PSP has been reported in *Cylindrospermopsis* in Brazil, and

cylindrospermopsin in *Aphanizomenon.* According to G.Codd this may be an issue of species identity; molecular taxonomy is required as well as morphology.

Increased blooms in the Gulf of Riga (M.Balode) may be related to increased ambient temperatures from climate change. *Microcystis* blooms are associated with hot summers. Fish kills, livestock illness and human skin irritation have been associated with *Mycrocystis* blooms in Lake Victoria, Kenya.

In Portugal (V. Vasconcelos), 90% of the human population drink surface water, and for 3-5 months of the year (summer) all of the rivers are full of cyanobacteria. There are also small shallow reservoirs surrounded by agricultural land, including pig production. Some reservoirs have year round blooms, even with very low nutrient concentrations in the water.

A seawater channel was cut in Western Australia at $ 80 million cost to increase salinity and decrease nutrients in an estuary subject to repeated toxic *Nodularia* blooms. The blooms now occur in the river mouths to the estuary, and dinoflagellates are appearing in the estuary itself (Falconer, Blackburn)

3. Remedial Issues

Remediation of a lake which was repeatedly subject to toxic blooms from the 1980's, one of the most eutrophic in Sweden, was carried out by restoration by top-down and bottom up processes. Farming was moved away from the lake shore. 430 tons of grazing fish were removed, and predatory fish returned. This has resulted in an increase in macrophytes, *Daphnia*, clearing of turbidity and cessation of algal blooms for 3.5 years.

Aeration at depth to destratify lakes and reservoirs is commonly used, but not always successfully, depending on the depth and other characteristics of the reservoir. No information is available on surface sprays. Water supply authorities often use copper sulphate to clear algal blooms. This cause releasing of toxins into the water.

4. Safety of Drinking and Recreational Waters

Drinking water needs to be demonstrably safe for human consumption. Recent toxic blooms in England, Canada, and Australia have lead to concern over toxins in top water. WHO established a task force on setting a safety guideline value for microcystins, which is likely to be 1µg/l of microcystin-LR or equivalent. The main unresolved issue is tumour promotion. Epidemiological studies in China indicate a risk, but there are also uncertainties over exposure routes. Liver tumour studies in rats suggest that the WHO guideline of 1 µg/l is likely to be safe (E.Sueoka).

Ciguatera

Chair: Richard Lewis, Australia
Rapporteurs: Michael Holmes, Singapore;
Jorge Diogéne, Spain

Richard Lewis introduced ciguatera with the definition "ciguatera is a disease caused by sodium channel activator toxins that accumulate in the flesh and viscera of fish to levels that harm humans". Areas for discussion were:

Epidemiology: identifying human threats from fish
Toxinology: characterising the mode of action and chemical nature of toxins involved
Origin: identifying the organism(s) responsible
Detection: development of a cost-effective and reliable assay

The global distribution of the disease and its variation between Oceans were summarised. The South Pacific Health and Epidemiological Information Services (SPEHIS) data base was described and a comment made that comparable data was not available for other ciguatera-endemic regions.

At least four Caribbean ciguatoxins (C-CTXs) are now identified. The C-CTXs have similarities to the known Pacific Ocean-CTXs (P-CTXs) both structurally and pharmacologically (John-Paul Vernoux, France). However, C-CTXs are sensitive to alkaline media, unlike P-CTXs. While most C-CTXs are slow-acting toxins (like the P-CTXs), there are also a number of fast-acting CTXs that induce deaths of mice of in about 10.0 min.

Maitotoxin (MTX) is the major toxin produced by the benthic dinoflagellate *Gambierdiscus toxicus*. MTX is not found in the muscle (flesh) of fish, only in the gut contents and viscera of grazing fish and therefore is unlikely to be involved in ciguatera (Anne Marie Legrand, French Polynesia). Thus ciguatera appears to be caused only by CTXs, which have been isolated from fish as well as from wild and some cultured *G. toxicus*. Approximately 20 CTX analogs have been isolated from toxic fish of the Pacific, but many of these have not been structurally characterised; CTXs and brevetoxins (PbTxs) bind to site 5 of the Na^+ channel. CTX causes Na^+ channels to open at resting membrane potentials when they would normally be closed; this causes membrane depolarisation, spontaneous action potential and finally inhibition of action potentials (Evelyne Benoit, France). High ciguatoxin concentrations may block Na^+ and K^+ channels. Associated with the increased sodium influx and the spontaneous action potentials, CTXs cause an influx of water resulting in cell swelling, especially at nerve terminals.

Since ciguatera appears to be caused only by CTXs, its origin is probably restricted to *Gambierdiscus toxicus*. M. Holmes and R. Lewis showed that CTX production by *G. toxicus* is strain dependent. This was confirmed by T.Yasumoto's and A.M. Legrand's groups. Another factor may be environmental and Sperr and Doucette suggest that high N:P media ratios induce CTX production in most strains of *G. toxicus*.

A lot of benthic dinoflagellates produce toxins but the accumulation of these toxins in fish to cause human poisoning is not proved for any species except *G. toxicus*. Yasumoto's group have shown that *O. siamensis* produces palytoxin which may be implicated in fish poisonings such as clupeotoxism. However, palytoxin poisoning is distinct from ciguatera, and causes elevated serum enzyme levels not seen in ciguatera victims. Okadaic acid and analogs are produced by a number of benthic species of *Prorocentrum* (*Exuviaella*) and OA has been reported from the flesh of fish. However, its detection in fish at concentrations sufficient to poison people needs verification.

Recently, Maria Faust described a new sand-dwelling species of *Gambierdiscus*, *G. belizeanus* from the Caribbean and the Indian Ocean; however, its rôle in ciguatera is unknown. While the link between *G. toxicus* and ciguatera has been confirmed in the Pacific, the origin of the C-CTXs has yet to be identified.

Immunodetection of CTXs was discussed (Serge Pauillac, French Polynesia). CTXs are lipophilic, non-immunogenic low molecular weight compounds, so that a hapten-protein conjugate must be used for immunisation. The CTX are also difficult to acquire; only a few mg have ever been isolated to purity. By using the JKLM ring of CTX coupled to a protein carrier, a CTX-specific antibody had recently been obtained. Miniaturised assays indicate that it may be possible to detect about a 0.3 pM of CTX using the JKLM antibody.

Future events

IOC-NorFa Nordic-Baltic Course on the Taxonomy and Biology of Harmful Microalgae. Vortsjärv Limnological Station, Estonia. 15-23 August 1998. The objective of the course is to improve the taxonomic skills of the participants in order to enable them to make reliable identification of phytoplankton species causative of harmful algal events in the Baltic region. The Course will cover also ecology and toxin chemistry particularly of blue-green algae. Seats are available for participants from the Baltic countries, Northwest Russia and the Nordic countries. Course lecturers will include: Dr. G. Cronberg, University of Lund, Dr. K. Kononen, Finnish Institute of Marine Research, Dr. J. Larsen, IOC Science and Communication Centre on Harmful Algae, Professor Ø. Moestrup, University of Copenhagen, Denmark; Dr. K. Sivonen, Finnish Institute of Marine Research. Deadline for applications: 1 April 1998. Application forms can be obtained from the address below and from the WWW at http://www.unesco.org:80/ioc/oslr/oslr.htm. Contact: IOC Science and Communication Centre on Harmful Algae, Copenhagen. (See below).

6th Canadian Workshop on Harmful Algae, St. Andrews, New Brunswick, Canada, 27-29 May, 1998. Contact: Jennifer L. Martin, Fisheries and Oceans Canada, Biological Station, St. Andrews, N.B. E0G 2X0, Canada.
E-mail MartinJL@mar. dfo-mpo.gc.ca,
Fax (1) 506 529 5862, Tel (1) 506 529 5921.

IOC Danida Training Course on the Taxonomy and Biology of Harmful Marine Microalgae, July 1998. This two weeks course will focus on identification and preparation techniques supplemented by lectures on different aspects of the biology of harmful algae. Teaching staff will include: Dr Yasuwo Fukuyo (Univ. of Tokyo), Prof. Øjvind Moestrup (Univ. of Copenhagen), Dr Jacob Larsen (Univ. of Copenhagen/IOC Centre), Organized by the University of Copenhagen, and IOC Science and Communication Centre on Harmful Algae, Copenhagen. For application forms, contact: the IOC Science and Communication Centre on Harmful Algae, Botanical Institute, Ø. Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; fax: +45 33 13 44 47. Deadline 1. April 1998.

2nd European Phycological Congress (EPC2). Montecatini Terme, Italy. September 20-26, 1999. Prof. Francesco Cinelli, Dipartimento di Scienze dell'Uomo e dell'Ambiente, Universita di Pisa, Via A. Volta, 6, I-56126, Pisa, Italy.
Tel: 39-50-23054. Fax: 39-50-49694.
E-mail:cinelli@discat.unipi.it
Web page: http://www.area.fi.cnr.it/icaa.htm

Sixth International Conference on Modern and Fossil Dinoflagellates "DINO 6". Trondheim, Norway, 7-12 June 1998. Contact: NTNU Museum of Natural History and Archaeology, Attn. Morten Smelror, N-7004 Trondheim, Norway. Tel. +47 73 5921 47, Fax. 47 73 59 22 23, E-mail morten.smelror@vm.ntnu.no. More info at http://www.ntnu.no/vmuseet/dino6

4th International Toxic Cyanobacteria Symposium, 27 September-1 October, 1998
Duke University Marine Laboratory, Beaufort, North Carolina, USA. Symposium Co-Chairs: Hans W. Paerl & Wayne W. Carmichael. Themes: Toxin physiology and regulation; Detection and characterization of toxic Cyanobacteria, toxins and other harmful substances; Novel toxins, mechanisms, organisms, and toxic episodes; The functional roles of toxins from the cyanobacteria's perspective; From cultures to blooms: Clarifying harmful bloom dynamics in the laboratory and field; Ecology and biotic interactions of of harmful bloom cyanobacteria; Environmental monitoring and risk assessments of cyanotoxins, taste and odor causing compounds; Human alteration and manipulation of toxic cyanobacteria, including watershed management for bloom control and removal methods for toxins in water supplies; Manifestations and consequences of toxic cyanobacteria on environmental quality and human health: WHO guidelines for cyanotoxins (e.g. microcystins) in drinking and bathing waters;
Contact: Dr. Hans W. Paerl, Co-Chair, 4th ITCS, UNC-CH Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557 USA, Hans_Paerl@UNC.EDU

For a more comprehensive overview of up-coming activities and events you should visit the IOC International Marine Meeting List at the IOC homepage at http://www.unesco.org/ioc/infserv/meets.htm. The list of meetings related to the International Year of the Ocean might also be of interest http://www.unesco.org/ioc/iyo/conferences.htm

Literature for developing country libraries

Limited copies of the following titles are available from IOC's HAB Centre in Denmark, to libraries of marine science institutions *in developing countries only*:

- The Genus Alexandrium Halim (Dinoflagellata). **E. Balech, 1994.**
- Algae. An Introduction to Phycology. **C. van den Hoek et al., 1995.**
- Identifying Marine Phytoplankton. **C. Thomas et al. (eds.), 1997.**
- Proceedings of the International Symposium on Ciguatera and Marine Natural products, Hawaii, 8-10 August 1994. Hokama et al (eds.) 1995.
- Proceedings of the First International Congress on Toxic Cyanobacteria, Denmark, 20-24 August 1995, and, Proceedings of the First Maj and Tor Nessling Foundation Symposium on 'Recent developments in Cyanobacterial Research', Finland 16-18 August 1995. Moestrup et al (eds.) Phycologia. Vol.35 No.6. 1996

Applications must be submitted and signed by the responsible librarian, and should indicate why the requested title is of specific interest to your institution and its ongoing research or teaching. No requests from individuals will be honoured. Address applications to:

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IOC publications

- Summary Report of the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms, Vigo 30 june - 2 July 1997. Available in English, French, Spanish.
- Amnesic Shellfish Poisoning (ASP), Volume 1, IOC *Manuals and Guides*, No. 31.

These publications are free of charge and available from the IOC Secretariat (Paris)

- Manual on Harmful Marine Microalgae, IOC Manual and Guides No. 33.
- Harmful and Toxic Algal Blooms. Proceedings of the Seventh International Conference on Toxic Phytoplankton, Sendai, Japan, 12-16 July 1995.

The Manual and the Sendai Proceedings are free of charge but a fee of US$ 30 will be charged for handling. A cheque issued for the IOC SCCHA, University of Copenhagen, should accompany all orders. Only prepaid orders will be accepted. Excepted from the handling fee are marine science libraries and scientists in developing countries which can apply for delivery free of charge.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms *No. 17*

◆ South Africa

Low oxygen, rock lobster strandings and PSP

The latter months of the summer of 1997 were characterised by a massive bloom of the dinoflagellate *Ceratium furca* on the West Coast of South Africa. Inshore accumulations of the bloom were first observed in January and by April cell concentrations, in some instances, exceeded 20 million cells l^{-1}. Although dominated by the dinoflagellate *Ceratium furca* both *Alexandrium catenella* and *Dinophysis acuminata,* responsible for Paralytic and Diarrhetic Shellfish Poisoning respectively, were present as minor components of the bloom. Ex-

(Cont'd on p. 3)

◆ India

Another outbreak of PSP in India

An outbreak of paralytic shellfish poisoning occured in three villages, Vizhinjam, Poovar, and Karumkulum near Thiruvananthapuram, the capital of Kerala State in the south west coast of India. Seven persons died and over 500 people were hospitalised following consumption of mussels, *Perna indica* during the first week of September, 1997. The affected persons showed typical clinical symptoms such as tingling sensation in the facial area, numbness of limbs, vomiting etc. The UNESCO Mircen for Marine Biotechnology, Dept. of Fishery Microbiology, College of Fisheries, Mangalore carried out analysis of water and shellfish samples. The mussels had very high levels of PSP (levels exceeding 10,000 Mouse Units/100g). At the time of the outbreak, water had high counts of cyanobacteria.

While the exact source of the toxin has not yet been identified, this outbreak has created a scare among fish consumers in the Kerala State. The sale of shellfish has been banned and this is said to have rendered about 1000 families jobless. The toxin levels are being monitored by the College of Fisheries, Mangalore. The Vizhinjam Centre of Central Marine Fisheries Research Institute (CMFRI) has been providing the samples at regular intervals for the analysis. During the four weeks of monitoring the level of toxins in shellfish has dropped to less than 500 MU, but it is still not safe for human consumption. Further studies on the toxin and dinoflagellates in the region are under way. This is the second confirmed outbreak of PSP in India. The earlier outbreak was in the northern part of Kerala State, in a place called Kumble about 50 km South of Mangalore (Karunasagar et al., Curr. Sci; 53, 247, 1984). Recent observation of *Gymnodinium catenatum* in the waters close to this area (Godhe et al. HAB News 15, 1; 1996) and observation of cysts of toxic dinoflagellates in the sediment close to this area (Godhe et al., Abst. VIII International Conf. Harmful Algae, Vigo 1997, p88) suggest that this area is prone to shellfish intoxication and this calls for regular monitoring in this coast for toxins, dinoflagellates and cysts.

Indrani Karunasagar, Biju Joseph, K.K. Philipose, and Iddya Karunasagar, Department of Fishery Microbiology, College of Fisheries, Mangalore – 575 002, and *Central Marine Fisheries Research Institute, Vizhinjam, Kerala.*

◆ New Zealand

Gymnodinium sp. linked to fish kills

In the Southern Hemisphere, as seasonal warming progressed in the 1997-98 period, several major events occurred in the New Zealand region. In early January 1998 mass mortalities of sea lion pups were reported in the Auckland Islands area to the south of South Island (Fig. 1). Also reported from mid-January to early February, were sporadic fish kills along the Kaikoura and Wairarapa coasts on the central east coast of New Zealand, and shellfish toxicity at Great Barrier Island on the northeast coast of North Island.

Off the Wairarapa coast tuna, striped marlin, broad bill swordfish, sea urchins, starfish, and abalone were reported killed along the coastline between Castle Point and Palliser Bay. In early February 1998 there were reports of human respiratory symptoms in several areas off the coast. A group of school children, residents, swimmers, and beachgoers at Riversdale, Castle Point and Mataikona, complained of a dry cough, sore throats, burning nasal passages, and eye and skin irritations. From late January to early February in the region, nearly 200 people were reported to have suffered from these symptoms. Most people recovered on leaving the affected area and no lasting effect was noted. At the time, a non-thecate dinoflagellate, *Gymnodinium* cf. *mikimotoi* was identified by the National Institute of Water and Atmospheric Research (NIWA) from water samples in the region. The presence of these respiratory symptoms in humans coincided with the monospecific build-up of *G.* cf. *mikimotoi* (up to 1.17×10^5 cells l^{-1}) in the same areas.

In mid-February, "aerosol" effects on humans were reported in several coastal areas in Hawke Bay. At the time reasonably high concentrations of *G.* cf. *miki-*

(Cont'd on p. 4)

Funding for the Harmful Algae News is provided by the Coastal Ocean Program

◆ USA ECOHAB

Major study on Alexandrium

Harmful algal blooms, commonly called "red tides" or HABs, are a serious economic and public health problem throughout the world. In the U.S., the most serious HAB problem is paralytic shellfish poisoning (PSP), a potentially fatal neurological disorder caused by human ingestion of shellfish that accumulate toxins as they feed on dinoflagellates of the genus *Alexandrium*. These organisms cause human illness and death due to PSP, repeated shellfish harvest quarantines, and the mortality of fish and marine mammals. This phenomenon, which affects thousands of miles of U.S. coastline and numerous fisheries resources, has expanded dramatically in the last two decades, especially in the Gulf of Maine. As part of the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) research program, scientists are embarking on a six-year study that will address several fundamental issues regarding *Alexandrium* blooms in the Gulf of Maine 'The Ecology and Oceanography of Toxic *Alexandrium* Blooms in the Gulf of Maine; ECOHAB-GOM': 1) the source of the *Alexandrium* cells that appear in the fresh water plumes in the western Maine coastal current (WMCC); 2) *Alexandrium* cell distribution and dynamics in the eastern Maine coastal current (EMCC), and 3) linkages among blooms in the WMCC, the EMCC and on Georges Bank. Utilizing a combination of numerical modeling, hydrographic, chemical, and biological measurements, moored and drifting current measurements, and satellite imagery, ECOHAB-GOM will characterize the structure, variability and autecology of the major *Alexandrium* habitats in the Gulf of Maine.

In the western Gulf, *Alexandrium* blooms and patterns of PSP have been linked to a coastal current or plume of low salinity river outflow (the WMCC). One major project goal is to investigate the area implicated as the major "source region" for the toxic cells that populate that coastal current. ECOHAB-GOM will elucidate the biological, chemical, and physical processes that control bloom initiation and development, the delivery of cells from that source region into the WMCC, and the manner in which late-season, localized blooms are retained there to re-seed future blooms with cysts. The second major set of objectives is to characterize the linkage between toxic blooms and the EMCC, to investigate the role of tidal mixing, frontal systems, and upwelling/ downwelling in *Alexandrium* dynamics, and to define the linkage between EMCC *Alexandrium* populations and those in both the WMCC (downstream) and the Bay of Fundy (upstream). Another project element will examine toxicity on Georges Bank, a rich shellfish resource that is a downstream delivery site for toxic cells from both coastal currents.

A unique set of "process" studies will focus on discrete blooms or patches of cells and quantify such parameters as in *situ* growth rates and grazing losses of *Alexandrium*, the nutritional physiology, vertical migration behavior and transport of this species, the partitioning of toxins within the food web, and the extent to which mixotrophy supplements photosynthesis. A hierarchy of coupled physical-biological models will be used together with ECOHAB-GOM data for investigation of: 1) detailed structure within each habitat; 2) interconnections among habitats; and 3) the role of the large Gulf-scale circulation in the long-term maintenance of *Alexandrium* populations in the region. Advances in our understanding of the underlying mechanisms controlling the population dynamics of *Alexandrium* will be distilled into simple predictive models of PSP toxicity which can be driven by a relatively small number of observable parameters. ECOHAB-GOM is thus a combined modeling/observational program, utilizing the most current and innovative technologies in an approach commensurate with the multiple scales and oceanographic complexity of PSP phenomena in the Gulf of Maine.

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◆ Spain

Iberoamerican cooperation on HAB Training

The IOC, the Institute for Iberoamerican Cooperation (ICI), and the Spanish Institute of Oceanography (IEO) organized from 29 June to 16 July 1997 a broad course on toxic phytoplankton with special emphasis on the detection and quantification of marine phycotoxins (bioassays, *in vivo* and *in vitro*, chemical, immuno-enzymatic and biochemical techniques) implemented in research and monitoring programmes.

Participants were from Argentina, Brasil, Chile, Colombia, Cuba, Guatemala, Mexico, Peru and Madagascar. In addition to staff from the Instituto Español de Oceanografía, The Laboratorio de Sanidad Exterior (European Community Reference Laboratory on Marine Biotoxins), and Conselleria de Pesca, Marisqueo e Acuicultura (Xunta de Galicia), teaching was complemented by invited speakers from the Universita di Trieste (Italy), Tohoku University (Japan), Osaka Prefectural Institute of Public Health (Japan) and Iatron Laboratories (Japan). To complement the training, participants were also invited to attend the VIII International Conference on Harmful Algae that preceded the course. Technical assistance for the course was provided by Gilson-Pacisa (Madrid, Spain) and Upstate Biotechnology Research (Lake Placid, USA)

Since 1996, annual courses on toxic phytoplankton have been held at the IEO, Vigo. These courses are given in Spanish. Priority is given to applicants with responsibilities for research, monitoring, toxin control and other management tasks. Although most students have basic knowledge of English, classes in Spanish facilitate communication and favour scientific exchange, especially during a 15-20 day course.

Jorge Diogene, IOC-IEO Science and Communication Centre on Harmful Algae, IEO, Vigo, Spain

(Cont'd from p. 1 "Low oxygen, rock lobster strandings and PSP")

haustion of nutrients and eventual decay of the bloom inshore lead to oxygen depletion throughout the water column. As a consequence a stranding of more than 1500 tons of the rock lobster, *Jasus lalandii,* took place in April at Elands Bay on the West Coast, as lobsters concentrated in the shallow surf zone in an attempt to escape bottom oxygen concentrations which declined to below detectable limits (Figure). During the ensuing weeks, several further strandings were observed, all south of Elands Bay, and the total loss was eventually estimated to be 2000 tons of lobster. These strandings are the largest ever recorded and have raised concern about the future of stocks in this area. To provide perspective, the total allowable catch for the entire coastline is currently 1700 tons. Of particular concern was that almost all of the stranded animals were under the legal size limit.

Standings of rock lobster as a consequence of low oxygen are common on the West Coast and are most frequent in the vicinity of Elands Bay, one of the richer lobster grounds. This stranding was, however, unprecedented in terms of the magnitude of the losses which were estimated at 50 million US$. The timing, over a weekend, and magnitude of the stranding, combined with inadequate control and considerable confusion on whether lobsters could be harvested by the public, resulted in a chaotic situation which secured considerable media attention. Although some 200 tons of beached lobsters were saved through the co-operative efforts of Sea Fisheries, the Industry, the Police and the Defence Force, most of the animals that came ashore died before they could be rescued and were buried on the beach. Many were harvested and sold illegally and about 300 tons were ground up and turned into fish meal or tailed and packed for commercial retail. The authorities were opposed to any concessions allowing people to gather the lobsters for sale, not only because this would have contravened the law and established policy, but because of the danger of food poisoning arising from consumption of spoiled lobsters.

Because rock lobster are relatively slow growing and long-lived, the fishery in this region is likely to be slow in recovery, unless extensive migration occurs into the region. Trap and dive surveys conducted soon after the major stranding indicated dramatic reductions in the number of lobsters on the conventional fishing grounds in the Elands Bay region. Of the initial strandings of lobsters, 60-70% consisted of females, which normally comprise only 45 % of the total lobster population. This is thought to have occurred because females are typically restricted to shallower waters. The lobster population remaining in the area now appears to have a disproportionately large fraction of male lobsters – in recent trap samples less than 3% of the captured lobsters were female.

The economic implication of this catastrophe for those living in the Elands Bay region is uncertain. The rock lobster fishery forms the mainstay of the fishing industry in this area which in turn forms an indispensable part of the economic sector of the region. Local fishermen claim that the money they earn during the rock lobster season sees them through the remaining half of the year, when they are either unemployed or work part-time as potato diggers. The rock lobster industry is already beleaguered by slow lobster growth rates that have led to declining total allowable catches – these strandings are likely to contribute seriously to the adversity of the fishing community.

Although *A. catenella* generally comprised a small component of the blooms on the West Coast it was present in sufficiently high concentrations to render shellfish toxic and in March Sea Fisheries placed a ban on the collection of wild shellfish as PSP toxins exceeded the harvestable limit of 80 µg $100g^{-1}$ mussel tissue. Despite this banning several incidents of PSP were reported during April and May. On the 30 April a woman died after she and her partner had eaten a meal of white mussels, *Donax serra* and on the 9 May a second woman died from PSP after a meal of black mussels, *Mytilus galloprovincialis.* Toxin concentrations from mussel samples collected on the West Coast varied considerably with a highest concentration of 2600 µg toxin $100g^{-1}$ mussel tissue being recorded during May. On the 13 May four Taiwanese sailors were admitted to hospital following a meal of both raw and cooked mussels. On admittance the condition of all the men was described as ataxic, but one man was in a critical condition in that he was breathing intermittently and was placed on an artificial ventilator. This was the first case in South Africa in which a patient suffering from PSP has been recovered in this way. These represent the first fatalities on the South African coast as a consequence of PSP since 1958. The ban on the collection of shellfish was only lifted in August, 5 months after the initial banning. The above cases of PSP highlight the difficulties of communicating a restriction on the collection of shellfish in a country in which many languages are spoken and in which there is a high level of illiteracy. The need to police and enforce these bannings was therefore emphasized.



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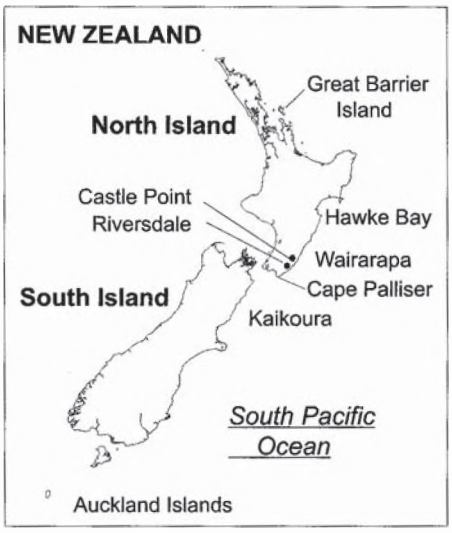
(Cont'd from p. 1 "Gymnodinium sp. linked to fish kills")

motoi were also recorded by Cawthron Institute from water samples in the Hawke Bay area. At this point in March further outbreaks of respiratory illness were reported in the Wellington Harbour, and again the culprit is suggested to be *G.* cf. *mikimotoi.*

This is the first time that a build-up of *G.* cf. *mikimotoi* has been recorded off the east coast of North Island, and the first time that the presence of this species has been implicated in outbreaks of respiratory symptoms in New Zealand. Previously, an aerosol episode on humans on the northeast coast of North Island, New Zealand, was related to a different, but closely related species, *G.* cf. *breve* [1]. In South Africa in the summer of 1995-96, the presence of *G.* cf. *mikimotoi* was also linked to an aerosol effect on humans, where beachgoers and seaside residents were reported to have suffered from respiratory symptoms [2].

To date, tests on the cause of the sea lion deaths are not conclusive and are on-going. However, samples collected from Kaikoura by scientists at NIWA were analysed and tested positive for algal toxin by the Environmental Science Research (ESR). Sea urchins and starfish tested were shown to contain a microalgal toxin that is similar to gymnodimine. Gymnodimine has previously been established as being produced by *G.* cf. *mikimotoi* [3]. The detection of this lipid-soluble toxin suggests that this dinoflagellate species might also be present off Kaikoura at the time of fish kills. No toxin, however, was detected in the guts of dead abalone or in striped marlin from the Wairarapa coast, and at this point the cause of death for both these species remains a mystery.

Fig.1. Sampling locations around New Zealand between mid-January and early February 1998.



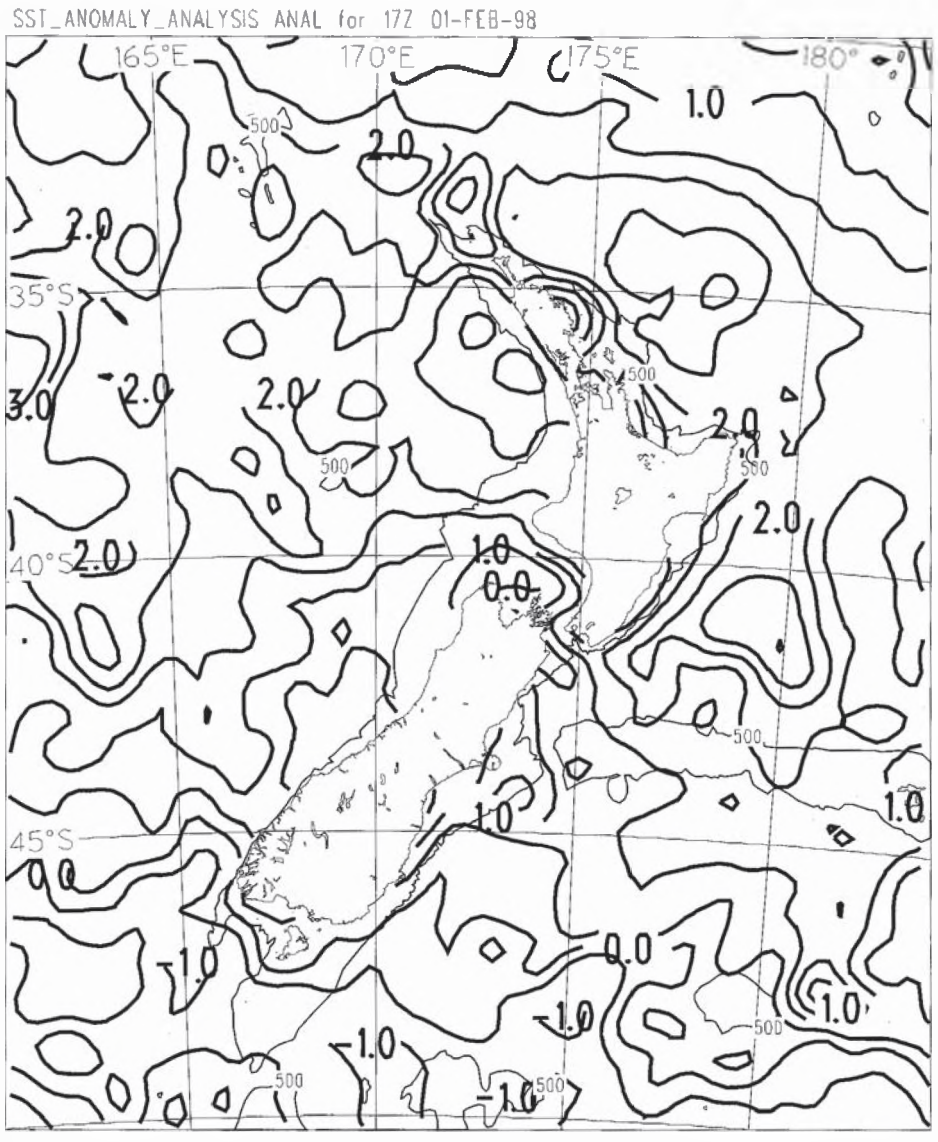


Fig. 2. Sea surface temperature (SST) anomaly in the New Zealand region during February 1998 (compared to the base period 1993 to 1997 inclusive). The contour interval is 0.5°C.

During the summer of 1997-98 *G.* cf. *mikimotoi* was a very widespread species on the east coast of New Zealand. Over this period large scale changes in oceanographic conditions in the region have been observed, and the widespread occurrence of this species has been suggested to be downstream effect of weather changes associated with the *El Nino* pattern.

High spatial resolution (1 km) sea surface temperature (SST) analyses derived from locally received NOAA Advanced Very High Resolution (AVHRR) data reveal that in January 1998 SST reversed the cooling effects of *El Nino* as experienced in the spring months (September to December 1997) around the entire New Zealand coastal area. In Hawke Bay the anomaly changed from approx. -1°C in December to +1°C in January. A similar change occurred along the south Wairarapa coast. During February this warming increased still further with the SST anomaly reaching +2.5°C (Fig. 2). This strong positive anomaly is opposite to what was expected. Past *El Nino's* have been accompanied by a cold anomaly (of the order of 1°C) in the New Zealand region.

Results from a research voyage off the east coast of North Island on NIWA's research vessel *Tangaroa* in the same period have shown that warmer subtropical water was found further to the south than usual, associated with strong southward current direction. This is consistent with observations of the SST from the AVHRR satellite images taken between mid January to mid February

1998. During this period the warming of nearshore waters by intrusions of warm oceanic waters, and the entrainment of nutrient-rich waters by upwelling have also been observed at several locations along the east coast. Research into the cause of these patterns and their relationship with the presence of toxic algae, is on-going.

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◆ France

New observations of raphidophyceae

During a routine sampling program on the impact of thermal perturbation from the Penly nuclear power plant in the western Channel (50° 00' N; 01° 11'E), conducted on 10 April 1996, significant numbers of *Chattonella*-like cells were observed for the first time in the coastal waters of northern France [1]. The phytoplankton community ($1{:}10^6$ cells l^{-1}) was dominated by the diatoms *Asterionella glacialis* and *Rhizosolenia delicatula* ($8{:}10^5$ cells l^{-1} and 1.10 cells l^{-1} respectively). Dinoflagellates (including *Scrippsiella trochoidea* and *Gymnodinium* sp.) were recorded at low densities ($3{:}10^3$ cells l^{-1}). Among nanoflagellates, presence of the cryptophyte *Plagioselmis sp.* was noted, while the silicoflagellate *Dictyocha speculum* reached concentrations of 1.5 10^{-4} cells l^{-1}.

Water temperature and salinity during the study were 8.7±1.5°C and 34.1±0.1 respectively, corresponding to the typical spring conditions for the area. Chlorophyll *a* concentration was 7.54±3.99 µg l^{-1}, and nutrient concentrations were measured as: nitrate + nitrite (13.77±0.67 µM), ammonia (0.14±0.09 µM), phosphate (0.51±0.05 µM) and silicate (2.07±0.71 µM).

Counts of cells in Lugol-fixed samples, using an inverted microscope, indicated *Chattonella* abundance of 1.7 10^4 cells per litre. The cells were intact and well preserved except for loss of flagella (Fig. A), although some cells did show remnants of subapical flagella. No other raphidophycean-like cells were present in the sample, and since the beginning of the survey of coastal waters around French power plants in 1976, raphidophytes in general had never been observed.

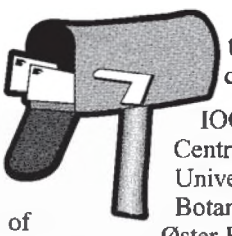
Living material is usually a requisite for reliable identification of raphidophytes, but the general aspect of the cell, the absence of a cell wall and the abundant peripherally located chloroplasts strongly indicate a raphidophycean species. Confusion with globular, fragile , uniflagellate naked stages of *Dictyocha* is unlikely, particularly as cells of this silicoflagellate were at the armoured stage at the time of sampling.

The cells of this raphidophyte are clearly distinct in shape and organization from another raphidophycean known to bloom in French coastal waters, *Heterosigma akashiwo* [2,3], illustrated at the comparable magnification in Figure B (from Lugol-fixed cultured material). Sporadic appearances of a third raphidophyte have also been previously reported in France, *Fibrocapsa japonica* [4, 3], but presence of rod-shaped, posteriorly located mucocysts which are ejected as long threads in dying cells (Fig. C) clearly distinguish this species from the genus *Chattonella* as a whole.

The general shape of the cells, the absence of mucocysts and the small size of this *Chattonella* (mean length 25 µm; mean width 12 µm) indicate that these could be *C. minima*, one of the smallest species of the genus and only known thus far from Japan [5]. The cells observed here were somewhat smaller than the dimensions given for the Japanese isolates (20-45 µm long, 20-30 µm wide). *C. minima* is sometimes considered to be an ecotype of *C. marina*, an ichtyotoxic species recently recorded, along with *C. antiqua*, in Dutch coastal waters [6].

In order to confirm the identity of *C. minima*, live samples taken from the same period (16 April 1997) the following year. No raphidophyceans were found, while the general pattern of the phytoplankton community was very different from 1996, with total number of cells reduced by half ($5{:}10^5$ cells l^{-1}) in which *Rhizosolenia delicatula* and *Chaetoceros curvisetum* were dominant ($3{:}10^{-5}$ and $6{:}10^{-4}$ cells l^{-1} respectively). Abundant colonies of *Phaeocystis glo-*

Identification Service



The IOC Science and Communication Centre on Harmful Algae, Copenhagen, and the Asian Natural Environmental Science Center offers an identification service. If you have problems with identification of harmful algal species, the Centres may assist you. You should send samples to one of the two addresses given below. It should be noted that this assistance is not given for routine identification/ monitoring of samples, but applies to particulary difficult species, or to species that require special techniques e.g. electron microscopy, for identification.

IOC Science and Communication Centre on Harmful Algae,
Univeristy of Copenhagen,
Botanical Institute,
Øster Farimagsgade 2D,
DK-1353 Copenhagen K., Denmark

Asian Environmental Science Center,
The University of Tokyo,
Att.: Dr. Y. Fukuyo, Yayoi 1-1-1,
Bunkyo-ku, Tokyo 113,
Japan.

bosa and *Pseudo-nitzschia* spp, not recorded the previous year, were also observed. Changes in temperature (10.7±0.9° C) and in nutrient concentration (6.54±0.94 µM nitrite + nitrate) could explain the absence of *C. minima* in 1997.

Chattonella species in general have so far never been observed by the phytoplankton monitoring network REPHY of IFREMER. *C. subsalsa* [5], a distinctive brackish organism from saline, eutrophic canals in southern France, is the only other recorded species of the genus in this country and it is known so far from its type locality only [7].

Of the 4 raphidophytes reported in France, only *Heterosigma akashiwo*, which is widely distributed along the French coasts, has been associated with fish kills, in 1994 [2]. Due to the toxic potential of most marine raphidophycean species, further problems related to their blooms might be expected in the future.

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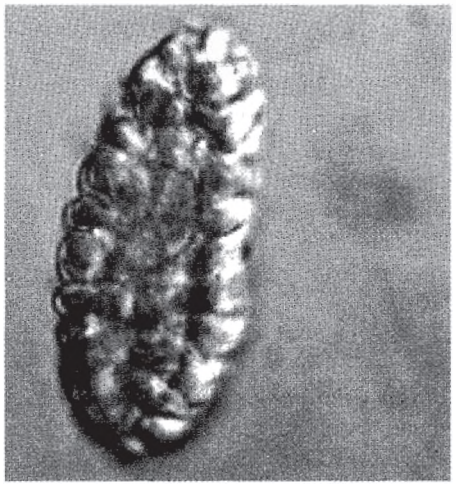
Figs. (A) Chattonella *cf.* minima *from Penly, Lugol-fixed. (B)* Heterosigma akashiwo *culture (RAP-1, Caen Culture Collection), Lugol-fixed cell. (C)* Fibrocapsa japonica *showing posterior ejected mucocysts, wild material from Calvados, Normandy (courtesy of J. Fresnel, Caen). Pictures taken with Nomarski interference contrast, (A) (B) obj. x 100 and (C) obj. x 40.*

(A)



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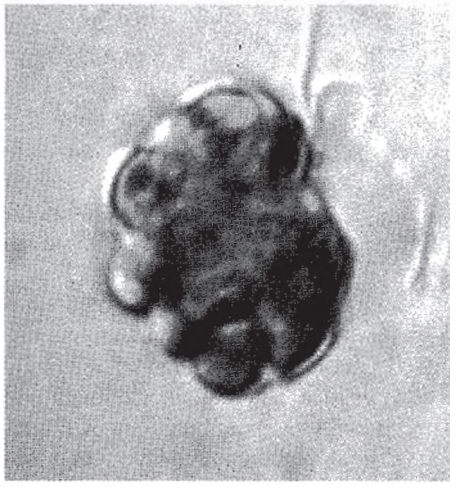

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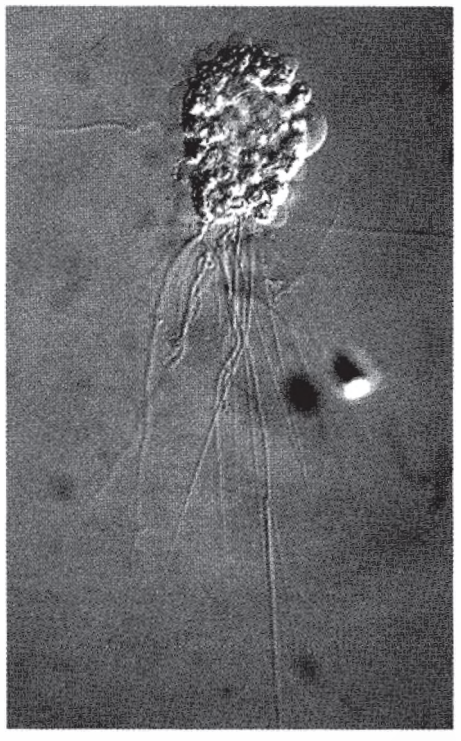

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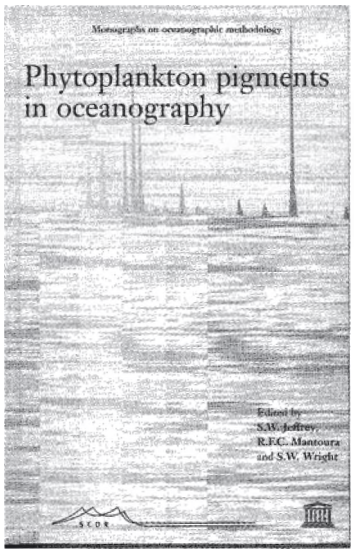

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http://www.unesco.org/ioc/isisdb/html/habd.htm

◆ France

Recurrent observations of a *Gymnodinium breve*-like species

Gymnodinium breve or *Gymnodinium breve*-like cells [1] have been observed every year since 1994, along the western Brittany coast (French Atlantic coast). This species is observed usually from July to September with occasional observations in May 1995 and October 1997. In any case, cells counts from Ushant Island to Concarneau never exceeded 1500 cells l^{-1} and no toxic event was reported.

Some morphological features were conserved *i.e.* peripheral chloroplasts, the round nucleus located in the posterior left quadrant (Fig.1) and the dorsoventral flattening. However, considerable variations in other characteristics of cell morphology were noted. The apex shape varied greatly from a pointed process to a prominent overhanging carina. Cell dimensions varied from 20 to 55 µm in width and from 15 to 30 µm in length. Nevertheless, it seems there is only one species and the different morphotypes have been counted together.

The species was not further identified because light microscopy doesn't allow a more detailed morphological analysis which should include the length of the sulcal intrusion onto the epicone and the length of the apical groove. Moreover, scanning electronic microscopy was not used because of the low concentrations in field samples and the lack of cultures.

Due to the toxicity observed along USA East coast, this species is now monitored by the French monitoring network (REPHY). Since a similar species has also been reported in Galician waters [2], observations should be compared along the West and East Atlantic coast, in order to decide on the name species and its possible toxicity.

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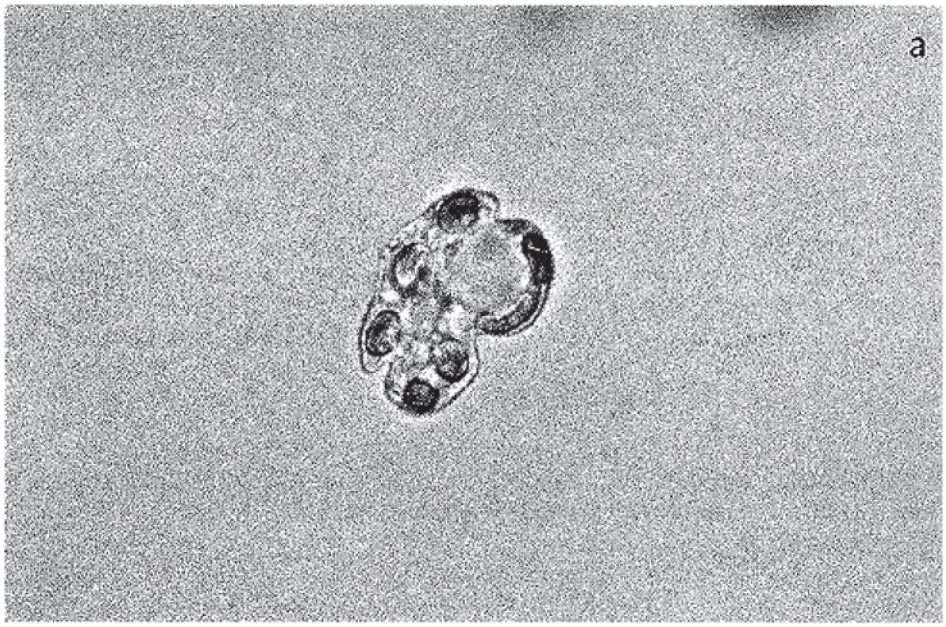
Fig. 1: Light micrograph (x 1000) of the Gymnodinium breve*-like species from Concarneau Bay.*



◆ ICES-IOC

A Global Survey on harmful algal events

The ICES-IOC Working Group on the Dynamics of Harmful Algal Blooms is presently carrying out a survey to acquire information about the oceanographic aspects of harmful algal events around the world.

The results will be collated in order to formulate a basic description of the occurrence and time development of harmful algae in different locations. By documenting the similarities and differences between occurrences for the same species, the important features of the physiology and environment can be identified. This information will be returned to the participants. We hope that global coverage can be schieved. The survey and its questionnaire is by no means complete. Everybody who feel that critical questions are missing, are welcome to identify them. A classification of the different types of events and scenarios would help greatly in the design of a science agenda for a potential international program on HABs. Broad representation across all harmful species is desirable in order to discover the processes that extend across species boundaries.

The questionnaire is available at http://www.unesco.org/ioc/oslr/survey.htm

Your reply will be highly appreciated.

The questionnaire can also be requested by mail to P. Gentien.

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Johns Hopkins University
USA

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◆ Netherlands – Germany

Fibrocapsa japonica and *Heterosigma* akashiwo: new observations

Since the early 1990s, potentially ichthyotoxic raphidophyte species like *Fibrocapsa japonica* (Fig. 1) and *Heterosigma akashiwo* (Fig. 3) have been observed in Dutch and French waters several times [1, 2, 3]. Although these species were reported to cause harmful effects to mariculture in Japanese [4] and French waters [7], there have been no such reports for other European waters so far. This note reports on results of routine sampling programmes in Dutch and in particular German coastal waters and focusses on *F. japonica* and in lesser detail on *H. akashiwo*.

Fibrocapsa japonica Field observations

In the summer of 1997, the species was found in almost all samples from the Dutch Algal Bloom Programme. In preserved samples, cell counts showed densities of up to 2 cells/cm³.

In Germany, *F. japonica* has been observed near Sylt (pers. comm. M. Elbrächter, Biologische Anstalt Helgoland). During a survey in the German Bight in July 1995 (RV Heincke), the species was observed alive on board, but not counted due to low densities. Also, since that summer, *F. japonica* has been found in phytoplankton samples from the Wadden Sea near the harbour of Büsum, on the west coast of Schleswig-Holstein [5, Fig. 2]. In 1996 and 1997, *F. japonica* was also listed in the messages of the German "Algenfrühwarnsystem" (AlgFES, Algae Early Warning System) for the German Wadden Sea [6].

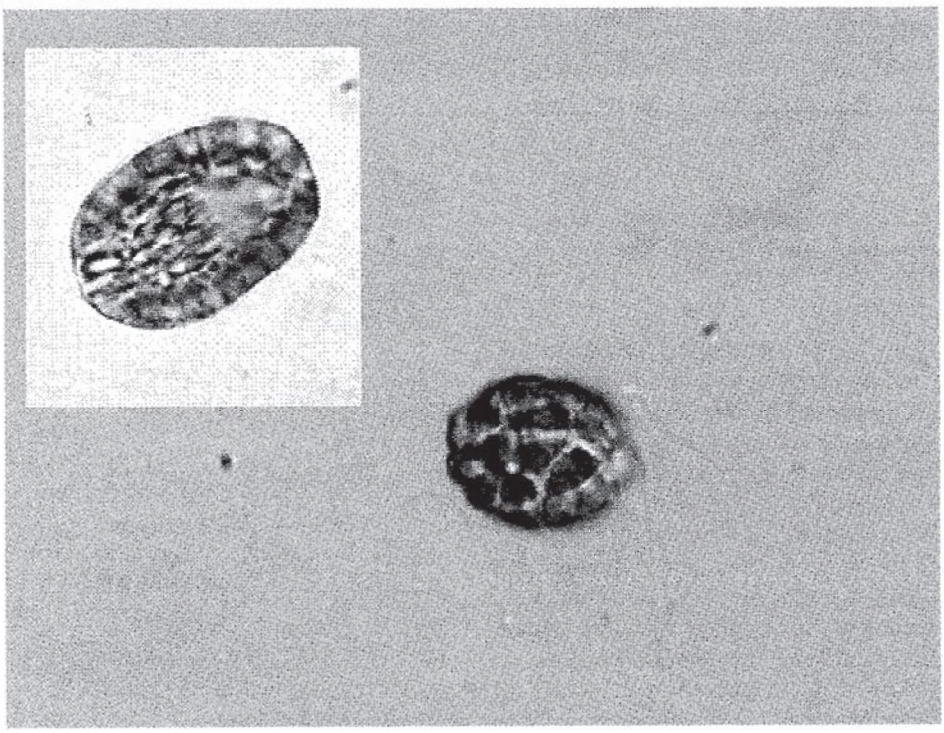
At Büsum harbour, phytoplankton samples are taken weekly. At this location, *F. japonica* concentrations increased from maximum numbers of 28 cells/cm³ in 1995 and 30 cells/cm³ in 1996 to 115 cells/cm³ in 1997 (counts by light microscopy in Lugol preserved samples). During the summer of 1997, *F. japonica* cells were also counted by flow cytometry (FacsVantage Cell Sorter, Becton Dickinson). Dotplots of the samples show a distinct group with very high red fluorescence and light scatter signals (Fig. 2). This cluster was positively identified as *F. japonica* by sorting the cells onto microscopic slides and subsequent light-microscopic examination. *F. japonica* was first detected in the flow cytometric samples mid-July, at an already high concentration (107 cells/cm³). The highest cell concentration of 327 cells/cm³ was recorded on July 24. After this date, concentrations decreased to about 5 cells/cm³.

In response to the bloom situation at Büsum Harbour at the end of July, a grid survey was carried out in Meldorf Bay off Büsum on July 31 (RV Südfall). Concentrations of *F. japonica* were very variable between stations, with peak numbers again near Büsum Harbour (Station 5, 128 cells/cm³), and decreased dramatically towards the inner bay stations, and also offshore (Fig. 2).

Method	Avg. Cells counted in 3 cm³	SD	CV (%)
LM	262	14	5.34 (n=5)
FCM	240	10	3.99 (n=5)

Tab. 1 Comparison of light microscopy (LM) and flow cytometry (FCM) counts of cultured *F. japonica*

Fig. 1 Fibrocapsa japonica*: Glutaraldehyde fixed, and live specimen (inset). Note the dramatic morphological change following fixation.*



Cultivating, counting and toxin identification

F. japonica from Büsum harbour was isolated and brought into culture in 1995 (FTZ culture collection). With this material, a comparison between light microscopic (LM) and flow cytometric (FCM) countings was performed to confirm the flow cytometry data. *F. japonica* undergoes a dramatical change when preservative is added: the cells clump together and usually cannot be counted reliably for calibration. We solved this problem by using a combined preservative (Formaldehyde, methanol free,

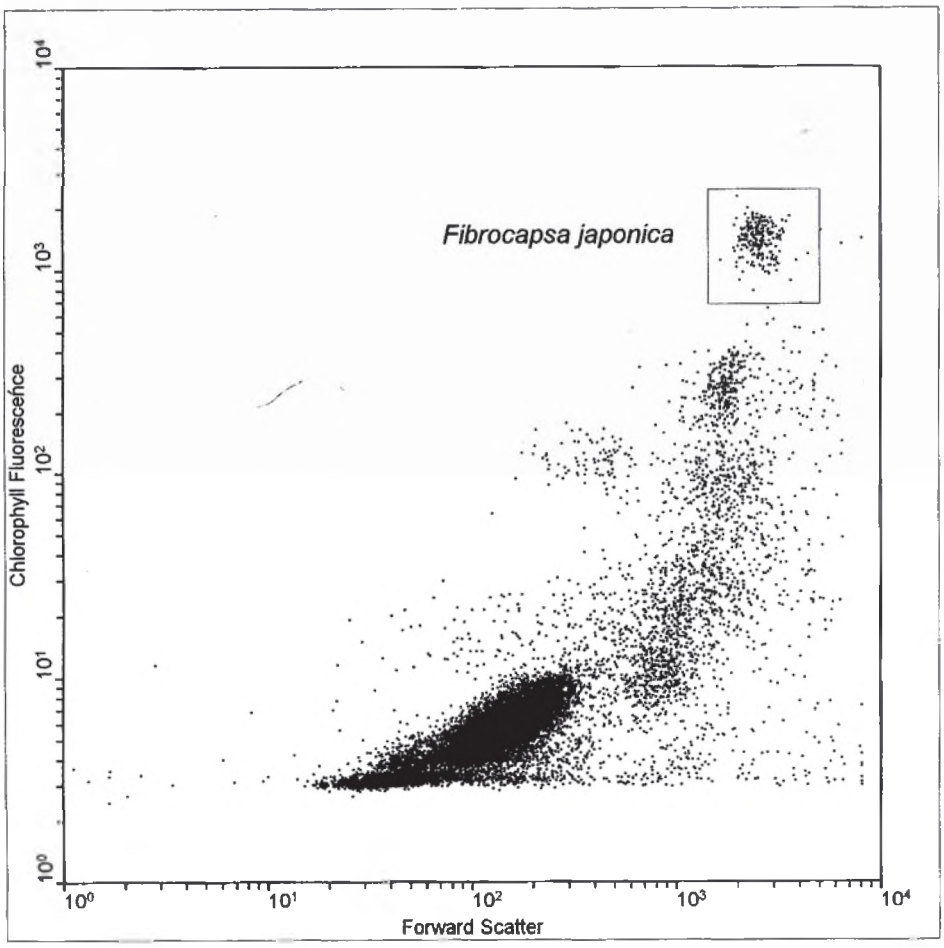


Fig. 2. Flow cytometric dotplot (forward light scatter vs. chlorophyll fluorescence), of a sample from Büsum Harbour (July 24, 1997). F. japonica *is represented by the uppermost cluster.*

10%, 1 cm^3 / 60 cm^3 fluid and Lugol (one drop of a Lugol solution, 100 g KI and 50 g KI_2 / 1 dm^3 with 100 cm^3 Acetic acid 96%)). Counts were made using 3 cm^3 culture material (5 replicates). The counts for LM were made using this preserved material, FCM counts were made using live material (Table 1). A t-test showed no significant differences between the two counting methods (1% significance level).

We strongly advise our preservation method for microscopic counting *F. japonica* cells.

From these unispecific cultures, toxic compounds were analyzed at the Terramare Institute at Wilhelmshaven. A new toxin was identified. This compound is structurally related to brevetoxin in being a cyclic polyether, and also contains a lactone moiety. However, it is more polar, and carries free OH groups. Preliminary tests indicate that this toxin interferes with signal transfer from nerve to muscle, presumably involving ion channels. The mode of action appears to be different from brevetoxin. In the mouse- diaphragm-test fibrocapsin shows higher activity than brevetoxin at the same concentration. The animal-test pointed out that fibrocapsin has a toxicity near tetrodotoxin. The detection limit of the toxin appeared to be at approximately 225 cells/cm^3 (with a toxicity test using luminescent bacteria). This threshold level was exceeded at Büsum Harbour during three observations in July 1997, but the water body rich in *F. japonica* was apparently diluted before higher, hazardous concentrations could emerge.

Conclusions

Concentrations of this potentially harmful flagellate along German coasts have reached higher levels than previously reported for this region. It appears that *F. japonica* has become more wide-spread in coastal areas of the North Sea. Under favourable conditions, i.e. warm and sunny weather, and a stable water column, it could well form harmful blooms in the coastal North Sea, as reported for Japanese waters.

Heterosigma akashiwo

Near the Dutch coast, the potentially toxic raphidophyte *Heterosigma akashiwo* (syn. *Heterosigma carterae*) was found for the first time in an algal bloom near Noordwijk on August 4, 1994, with cell numbers approx. 2.400 cells/ cm^3 [2]. In German waters, this species was observed in Büsum Harbour and Friedrichskoog in samples in the summer of 1997. Cell concentrations of this species are difficult to count, as in preserved samples the cells deteriorate very soon and therefore cannot be identified easily. However, several cells were observed and identified in living samples.

Video recordings of *H. akashiwo* from the French coast showed a very high resemblance to the specimens found in the Dutch North Sea in 1994 and German Wadden Sea in 1997.

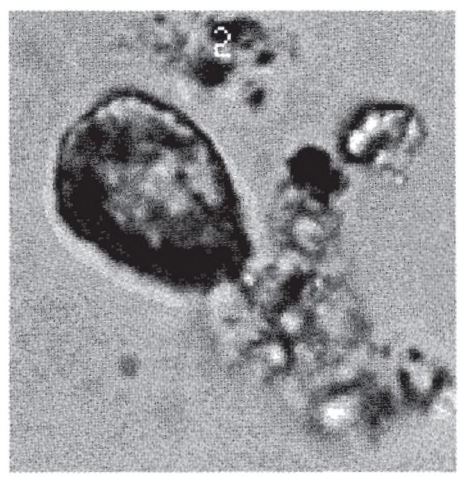
Due to the uncertainties in identifying *H. akashiwo* in preserved samples it is often overlooked in preserved material. Therefore, this species cannot be taken into account in biomonitoring programmes, in which only preserved samples are analysed. However, its presence has been demonstrated in the waters of the Netherlands and Germany.

Acknowledgments

Video tapes of *H. akashiwo* from the harbour of Camaret sur Mer in France (used for comparing Dutch and German video-images) were made by Werner Dietrich from Büsum (October 1997).

We like to thank Gerd Liebezeit and Michaela Nannen from Forschungszentrum Terramare at Wilhelmshaven and Hans Bigalke, Medizinische Hochschule, Hannover, for information on the toxins of *F. japonica*.

Fig. 3 Heterosigma akashiwo *(sample Friedrichskoog).*



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◆ Toxicology

Okadaic acid and carcinogenesis

Okadaic acid (OA) is one of the main toxins involved in Diarrhetic Shellfish Poisoning (DSP), a human seafood intoxication. This phenomenon of high morbidity covers a large geographical area and its expansion induces important problems for shellfish aquaculture and industries. In some countries, monitoring programs have been developed and the shellfish commercialisation is restricted and allowed only below definite levels of toxin content. However, DSP chronic exposure has been suggested to induce digestive tract tumor formation. It is also noteworthy that DSP occurs in several countries such as Japan, Chile, Norway, Holland and Spain, which are known to have high incidences of stomach cancer (Fujiki et al., 1987). In order to understand the possible implication of OA in carcinogenesis, it is important to study its effects at the different stages of cancer formation.

Carcinogenesis is a process consisting in three stages: initiation, promotion and progression. i) The initiation stage, irreversible, induces genetic damage to DNA. ii) Promotion is a reversible process. During this stage, the initiated cells are stimulated by tumor promoters, leading to a selective growth. iii) Progression is a irreversible phenomenon: the cells are more and more agressive and present increasing genetic abnormalities.

Genotoxicity, mutagenicity

Negative results were obtained when testing the mutagenicity of OA by the Ames test in *Salmonella typhimurium* TA100 and TA98 with or without a microsomal metabolic activation system. A similar negative result was also obtained with two other inhibitors of protein phosphatases 1 and 2A, microcystin LR and nodularin. Moreover, no any intitiation activity of OA was shown in experiments conducted on BALB/3T3 cells. Thereby, these tests have not brought any evidence to support the genotoxic activity of OA. However, other results suggest that OA does have a genotoxic capacity : increase of mutation frequences for some locus and induction of sister chromatide exchange in presence of bromodesoxyuridine.

In order to bring further data regarding the genotoxicity of OA, its capacity to form DNA adducts has been studied. An adduct is the product formed by the covalent binding of an electrophilic chemical compound to a nucleophilic site of cellular macromolecules such as proteins or DNA. The formation of DNA adducts is a necessary but not a sufficient step in chemical carcinogenesis. However, the data obtained to date provide unequivocal evidence that many, if not most, adducts have the potential to cause mutations either during DNA replication at the damaged site or during error-prone DNA repair. The ^{32}P post-labelling (Reddy & Randerath, 1986) is one of the techniques available to detect DNA adducts formation. This method presents a high sensitivity and can be used without prior knowledge of the adduct structures. Recent studies using this technique have shown that low doses of OA can produce DNA adducts during exposure of different cell types cultures or even zebrafish embryos (Fessard et al., 1996; Huynh et al., 1997). The way by which OA induces those adducts still remains unknown. Did it bind directly to DNA bases or is it via the formation of electrophilic metabolites? Did OA enhance the formation of a covalent binding between endogenous molecule(s) and DNA? These are subjects of further research to better understand the genotoxic capacity of OA.

It is worth noting that other toxins are also considered genotoxic. For example, nodularin, a PP1 and 2A inhibitor, has been found to have an initiating activity, this toxin being 400 times more potent than diethylnitrosamine. Moreover, some compounds, such as the ochratoxin A mycotoxin, providing negative results with mutagenicity tests, like the Ames or the Mutatox tests, have been finally classified as genotoxic for their ability to induce DNA adduct formation and have been proved to be involved in cancer outbreaks.

Tumor promotion

In 1987, Fujiki et al. have shown that an application of OA on mouse skin induces irritation and induction of ornithine decarboxylase activity, suggesting that this toxin can be a tumor promoter. In 1988, Suganuma et al. demonstrated this potentiality during a two stage carcinogenesis experiment in rodent. OA appeared to be a tumor promoter as potent as TPA for inducing papillomas and carcinomas. In 1992, experiments conducted on rats with OA added to drinking water after an initiation treatment with NMNG demonstrated that OA is also a tumor promoter for glandular stomach. This result points out that OA differs from tumor promoters like TPA or telecidine not only by its action pathway but also by the target organs. Finally, Yuasa et al. (1994) have shown that OA and its class of compounds administered orally can induce cell proliferation in the rodent gastrointestinal mucosa (oesophagus, stomach, guts) as well as in skin.

Two stage carcinogenesis experiments with rodents have demonstated that others protein phosphatases inhibitors are also potent tumor promoters : DTX-1 in skin and glandular stomach, calyculin A on mouse skin, microcystin LR and nodularin on rat liver.

Controversial results were obtained with similar two stage carcinogenesis experiments conducted in a cell system: some authors have described a promoter effect while others were not able to detect transformation. Moreover, OA has been shown to inhibit cell transformation induced by other agents.

Oncogenes and tumor suppressor genes

Inhibition of PP1 and 2A caused by OA type compounds leads to induction of oncogenes and inactivation of tumor supressor gene proteins. Both effects are correlated with tumor promotion and essentially with proliferation of initiated cells.

Oncogenes

Expression of several oncogenes, such as c-abl, c-fos, c-jun, jun B, jun D, is induced by OA in different cell types. The c-fos and c-jun mRNA accumulation is not only due to a transcription increase but also to the mRNAs stabilisation. Like TPA, OA induces c-jun protein phosphorylation which seems to stimulate its transcriptional activity. However, while the TPA induction of oncogenes is quick and temporary, the effect of OA is slower but lasts for a longer time or is biphasic.

Tumor suppressor genes

OA induces the phosphorylation of pRb and p53 proteins. It can also inhibit the dephosphorylation of p53. The phosphorylation of pRb decreases its binding to cellular oncoproteins and induces activation of the transcription factor E2F. The hyperphosphorylated p53 cannot form tetrameres and this disrupts the expression of down- regulating cell growth genes. This event can lead to the conversion of cells acquiring a transformed phenotype enhancing cell growth.

Apoptosis

Numerous toxic agents induce apoptosis (i.e. programmed cell death), specially in proliferating cells. This phenomenon can be important in carcinogenesis by retarding tumor growth, which is suggested to result from an imbalance between proliferation and apoptosis.

Specific modifications of apoptosis seem to be induced in cells treated by OA. However, if an internucleosomal DNA fragmentation typical of apoptosis can be obtained, it highly depends on the OA dose. At the same time, it has be shown that OA inhibits apoptosis induced by others compounds. In this case, it has been suggested that OA can inhibit the dephosphorylation of some proteins essential for apoptosis.

Gap junctions

Inhibition of gap junctions has been proposed as a mecanism by which initiated cells can isolate from surrounding normal cells and their regulation growth influences. A loss of these junctions can then play an important role in the carcinogenetic process, a lot of tumor promoters, like TPA, inducing a decrease of these intercellular communications (Budunova & Williams, 1994). However, no gap junctions inhibition has been observed in different cell types treated with OA, except for doses inducing morphological changes. Moreover, the gap junctions constituent protein, connexine 43, is not modified by OA, while its phosphorylation is induced by TPA.

Recent studies have pointed out that OA type tumor promoters, as well as TPA type tumor promoters, provoke in fact an induction of the Tumor Necrosis Factor α (TNF-μα) and it looks like that the tumor promotion capacity of this type of toxins, protein phosphatases 1 and 2A inhibitors, can act via this endogenous promoter (Fujiki & Suganuma, 1994). Then, it can be suggested that OA can be considered as a genotoxic compound forming (directly or indirectly) DNA adducts and that its promotion capacity is in fact due to the TNF-μα induction. In that case, we must be aware that the risks incured for human health, mainly during chronic intoxications, are under-estimated. Therefore, the toxins levels allowed for consumption should be reconsidered.

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◆ International

Saxitoxin regulations

The Organisation for the Prohibition of Chemical Weapons (OPCW) which is based in The Hague, Netherlands, is responsible for implementing the Chemical Weapons Convention (CWC). The Convention *inter alia* strictly controls the manufacture and use, as well as the transfers and re-use, worldwide of certain treaty-regulated ("Scheduled") chemicals. Saxitoxin is one such chemical, and it is included under Schedule 1. Chemicals under that Schedule may only be transferred between States Parties (to the CWC) and once transferred, cannot be re-transferred to a third country. Furthermore, the OPCW must be given at least 30 days notice prior to any such transfer. All this is without reference to a minimum amount which might escape these regulations.

The issue presented before the OPCW is the unintended consequence resulting from a strict application of these rules to, inter alia, the use of saxitoxin as a reference standard in test procedures for paralytic shellfish poisoning (PSP), including in relation to diagnostic test kits. The OPCW Secretariat is presently collecting information in order to assist the Organisation in making an educated and sensible decision on this issue. Towards this end, we would be very interested in receiving more information along the lines of the questions outlined below.

1) What are the advantages of diagnostic kits containing saxitoxin *vis-à-vis* other test methods, and how wide spread is their use today. What is your expectation in relation to the future demand for test kits containing the toxin as a reference standard.
2) Do you know of any companies which manufacture, and trade, in test/diagnostic kits for PSP poisoning containing saxitoxin; and also companies which purify and/or export saxitoxin itself for PSP monitoring or other (e.g. neuro-pharmacological research) purposes.
3) Have the (OPCW) regulations hindered, or even prevented, adequate and timely supplies of saxitoxin reaching those who requested it? If so, what were the consequences? More specifically, is this an issue from the supply perspective?
4) What are the ongoing research activities which require regular supplies of saxitoxin and have there been delays in getting timely supplies. If so what suggestions do you have for redressing this hurdle?
5) What would happen vis-à-vis research if the supply is reduced?
6) And finally, any names and contact details of those working on similar issues.

These are some of the issues which we feel need to be addressed further. Not only would we appreciate your thoughts on the above, but also your views on any other issue you feel we may have omitted or which may arise in the future.

We would be grateful for any advice and suggestions you can give us. Thank you in advance for your kind assistance. We are of course available for any questions you may have and look forward to hearing from you.

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◆ Ireland

Re-occurrence of Winter Toxicity

In HAN issue No 14 (page 2) we reported on the detection of toxicity of unknown aetiology in mussels from Killary Harbour, on the west coast of Ireland. The toxicity persisted from November 1995 until June 1996. At the time, no known toxins were detected in the shellfish and no known toxic phytoplankton species were identified in water samples. Mice injected with acetone extracts of mussel hepatopancreas gave a "PSP like" response with respiratory difficulties, spasms, paralysis and death within 20 – 90 minutes. Subsequent analysis has revealed the presence of at least one previously unknown toxin (Yasumoto 1997), which has been tentatively named spiramino acid. The toxin is known to cause marked necroses of parenchymal cells in peripheral portions of lobuli in the pancreas, focal necroses in the liver and/or swelling of hepatocytes when administed either orally or by interperitoneal injection to mice (Ito et al, 1997). Consumers of contaminated mussels suffered severe diarrhoea, nausea and vomiting.

In November 1997 we received reports of illness (severe vomiting and diarrhoea) in consumers of mussels from the Arranmore Island region of Donegal, northwest Ireland. Again no known toxic phytoplankton species were present in water samples examined microscopically and mouse bioassays gave identical reactions to those described above, with death times ranging from 35 – 60 minutes. PSP bioassays were negative and analyses carried out by Dr. Marisa Fernandez at the European Union (EU) Reference Laboratory on Marine Biotoxins (EU-RLMB) in Vigo, Spain showed that Okadaic acid, DTX1, DTX2, and their acyl derivatives, were undetectable. The clear similarities to the toxic event in Killary Harbour in 1995 suggested that the present toxicity was due to the re-occurrence of spiramino acid. This was confirmed by analysis af a sample of mussels by prof. Yasumoto in Sendai Japan Harvesting of shellfish from the affected area is currently prohibited.

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◆ New Zealand

Sea lion mass mortalities

A serious mass mortality event is currently sweeping through the two main breeding colonies of the New Zealand (Hooker's) sea lion (*Phocarctos hookeri*), with 30-40% of this year's pups already dead, and indications of increasing mortalities amongst adult females.

The New Zealand sea lion is found only in New Zealand waters and is one of the world's rarest seal species. In 1997, based on pup production estimates, the population was estimated at between 11,000 and 15,000 animals. Once distributed throughout New Zealand, from the tip of the North Island to Campbell Island, deep in the subantarctic, the species was taken to the brink of extinction by commercial sealers of the early nineteenth century. The major concern in recent times has been the impact of accidental catches in the trawl fishery for squid around the Auckland Islands, which is one of New Zealand's most important deepwater fisheries. For each of the past four years Ministers of Conservation and Fisheries have set a catch limit for sea lions. The squid fishery was closed early last year, because the sea lion catch limit was exceeded.

Over the past ten years, the Department of Conservation (DOC) has been monitoring the main breeding colonies of Dundas and Enderby Island, part of the Auckland Islands group located some 420km south of New Zealand's South Island. These two colonies (which collectively occupy an area roughly the size of six football pitches) account for some 95% of the sea lion breeding population. Annual pup production in recent years has been approximately 2,500 animals. Dundas Island is responsible for 80% of all pup production, but is surrounded by rocky reefs and is much harder to access than Enderby, where DOC has a research station.

DOC scientists arrived at Enderby Island in mid-January, and everything seemed to be normal. However, when weather conditions permitted a visit to Dundas on 26 January, the team discovered some 700 recently-dead pups. Symptoms included puffy eyes, ulcerated anuses and vaginas and lesions on the head. Dying pups often showed spasms and paralysis. Fewer adults than usual were on the beach, but at that time there were no signs that the older animals had been affected. Returning to Enderby, the team soon found that pups were beginning to die there also. In addition, adult females started showing signs of paralysis. Afflicted animals also had small raised lesions on the belly and neck. Post-mortems have shown pus-filled swellings around the salivary gland in the neck. The most recent estimate on the day of this posting is that 111 pups have died on Enderby, and a further 150 are unaccounted for. 20% of the adult females on the breeding beach are dead or afflicted with lesions or paralysis. The numbers of adult females on the beach are considerably lower than normal. An unknown number of females (and males) may have died at sea, and some pups are consequently starving to death.

Samples from autopsied pups and females and six pup carcasses were flown back to the mainland by helicopter on 29 January. Pathologists from Massey University Cetacean Investigation Centre in Palmerston North will begin detailed examinations of the samples on Monday 1 February.

Further updates will be posted with MARMAM (http://is.dal.ca/~whitelab/marmam.htm) on a weekly basis.

Any helpful advice or assistance will be warmly welcomed.

Mike Donoghue, Marine Protection, External Relations Division, Department of Conservation, mdonoghue@doc.govt.nz

◆ South Pacific

First regional HAB initiative

On 14 November 1997 a workshop on potentially harmful microalgae in the South Pacific was held as a specific session at the Conference on Marine Benthic Habitats & Their Living Resources, Noumea, New Caledonia. The conference addressed marine monitoring and management and its application to Pacific island countries. The conference was co-sponsored by the IOC and the South Pacific Applied Geoscience Commission (SOPAC). It was organized by the Moss Landing Marine Laboratories (US) and IFREMER (France).

The purpose of the session on harmful microalgae was to present an overview of the knowledge on the topic in the South Pacific region and, in general, the methods that can be employed to monitor and curtail the impacts of harmful algae on the human population. The session was chaired by Dr. Jorge Diogène (IOC) and Dr. Anne-Marie Legrand and Dr. Yasuwo Fukuyo acted as co-chairs.

The session had participants from Australia, Canada, Commonwealth of the Northern Marianas (CNMI), Cook Islands, Fiji, France (New Caledonia, French Polynesia, Metropolitan area), Guam, Hong Kong, Japan, Kenya, Kiribati, Marshall Islands, Mexico, New Zealand, Niue, Palau, Papua New Guinea, Samoa, Solomon Islands, Spain, Taiwan, Tonga, Tuvalu, United Kingdom, USA (including Hawaii) and Vanuatu.

The potential risk of ciguatera in relation to the presence of *Gambierdiscus toxicus* was discussed. In particular the question of the relationship between ecosystem disturbance (coral reefs) and densities of *G. toxicus* were raised. The effects of anthropogenic alteration of the benthic habitat (ship wrecks) or natural causes (hurricanes, recent volcano formation) in relation to the occurence of harmful algae were discussed. Other issues such as fluctuations of toxicity, tox-

in detection methods, and the relationship between *G. toxicus* densities and other organisms (sea cucumbers) were addressed.

The question was raised on whether ciguatera is the main problem regarding harmful marine microalgae in the South Pacific region, and/or if other syndromes should be considered. It was suggested that the real importance of ciguatera had to be defined in order to consider it a priority. Some epidemiological data on ciguatera cases in the South Pacific are available from the South Pacific Commission. These data were presented, and demonstrated the importance of ciguatera in the region.

Attendants were invited to contact the IOC, the IOC Science and Communication Centres (Dr. Diogene), and the IOC/WESTPAC-HAB (Dr. Fukuyo) for information, teaching material, and support. Attendants were also invited to contact their governments and encourage them to strengthen their collaboration with the IOC on this issue.

The workshop concluded by recognizing the importance of problems caused by microalgae in the South Pacific, and noted with concern the absence of coordination among scientists in the region.

It was recommended to list scientists, managers and institutions already implicated on harmful algae research and/or management and those potentially interested in the subject; to strengthen distribution of information on harmful microalgae to scientists, managers and institutions and stimulate their participation in training activities, research projects and working groups related with harmful algae; to gather experts (scientists, managers) from the South Pacific and promote discussion on harmful algae issues in the region; to invite other institutions, regional entities and countries in the South Pacific to joint efforts with IOC and cooperate in the development of the HAB Programme in the region.

The IOC is coordinating the actions to take and invites all interested to collaborate to fulfill the recommendations. Those interested please contact J. Diogène at the IOC-IEO Science and Communication Centre on Harmful Algae, Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Cabo Estay – Canido, APTDO. 1552, 36080 Vigo, Spain; fax: +34 86 – 49 23 51; e-mail: VIGOHAB@VI.IEO.ES, or the IOC Harmful Algal Bloom programme. IOC Secretariat, 1 rue Miollis, 75732 Paris cedex 15, France ; fax: + 33 1 45 68 58 12; e-mail: hab.ioc@unesco.org

◆ ICES

Small-scale physics and harmful algae

A theme session titled 'The Role of Small-scale Physical and Biological Processes in the Dynamics of Harmful Algal Blooms', was organized by P.Gentien and P.Donaghay at the Annual ICES Science Conference in Baltimore, 25 September – 3 October 1997.

Physical-biological interactions at small-scale have been identified as key processes in the understanding of HABs. This statement became obvious during the session due to development and application of new instrumentation, some of which was presented during the session, such as particle-size analyzers, syringe samplers, fast nutrient profilers and optical sensors.

Thin layers located in the pycnocline are sometimes subject to erratic movements and appear to be hydrodynamically confined. Mesoscale advection of these layers, which is still poorly understood, would allow a time-scaling of the populations of concern.

Conceptual models by which these thin-layers may be produced by shear have been presented. However, it appears that, at present, these models are not sufficient to fit the observations and, in particular, their duration and geographical extent. Chlorophyll concentrations observed can result from interactions of cell behaviour with physics, which lead the algae to concentrate in zones where growth is enhanced and/or mortality rates considerably reduced.

Different species may coexist in different layers on the same profile, being associated with specific environments, such as huge blooms of *Pseudonitzschia* in the bottom boundary layer.

Some experimental work has shown that the coordinates of the ecological space refer to the intensity of small-scale turbulence and predator concentration, dinoflagellates being clustered generally at low turbulence intensity. However, further experimental work demonstrates the large range of variation of the effect of turbulence on the growth rates of dinoflagellates: some are stimulated at high energy. Furthermore, it appears that the effects on cells depend on the time of exposure and the energy. Turbulence may also induce excess mortality in chain forming diatoms. So, generally, low turbulence can favour dinoflagellates versus diatoms, when considering biomass production models. However, when species-specific population dynamics are considered, it is necessary to determine the response of this species to small-scale turbulence.

It has been established that the coefficient of turbulent energy dissipation (å) does not really give much information about the immediate cell environment. A new technique, the holographic camera, allows 3D measurements of flow at the pertinent scale (10 µm). In the near future, this technique should permit direct measurements of turbulent strain on cells both in the lab and in the field and relate them to the turbulent energy dissipation coefficient.

An important challenge will be to relate processes operating at the scale of a cell to the effects on the population and its distribution in time and space.

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The local organizing committee.

◆ Philippines

International Conference on Molluscan Shellfish Safety

The Second International Conference on Molluscan Shellfish Safety (ICMSS) was successfully held on 17-21 November 1997 at Sarabia Manor Hotel in Iloilo City, Philippines. It was hosted by the University of Philippines, Marine Science Institute and the College of Home Economics at Diliman, and the Institute of Aquaculture and the Division of Biological Sciences of the University of the Philippines in the Visayas at Iloilo. The Conference was chaired by Rhodora V. Azanza of the Marine Science Institute, University of the Philippines who is also Vice-chair of the IOC Intergovernmental Panel on Harmful Algal Blooms, and the Coordinator of the ASEAN Canada Red Tide Network. Supporting agencies included the Department of Science and Technology (through its Philippine Council for Aquatic and Marine Research and Development and National Research Council of the Philippines), Agriculture (represented by its line-agency Bureau of Fisheries and Aquatic Resources) and Health of the Philippines and private agencies such as the Southeast Asian Fisheries Development Center and EASYCOM (Iloilo's first Internet service provider). The IOC provided financial support to participants coming from four developing countries.

The first ICMSS was held in Sydney, Australia on 13-17 November 1994. The second ICMSS provided a forum for scientists from many different disciplines, growers, and regulators. Discussions focused on the latest research, commercial practices and standards concerning the contamination, purification and overall safety of bivalves with respect to chemical, biological and microbiological contaminants. The Conference emphasized the problems and safety of shellfish in Asia.

This meeting was attended by more than 80 participants from 18 countries. The Philippines, Australia, Japan, New Zealand, Canada, the United Kingdom, China, Taiwan, the United States, Vietnam, Poland, Denmark, Indonesia, France, Mexico, the Netherlands and Spain; 37 papers and 1 video were presented, 17 of which were from Asia, 8 were from Europe, 7 were from US-Canada, 4 were from Australia-New Zealand, and 1 was from Latin America. These papers were classified into 9 sessions/groupings, namely: (1) Biology/Ecology of molluscan shellfish, (2) Microbiological/Biological contamination, (3) Paralytic Shellfish Poisoning (PSP): Causative organisms, (4) PSP: Toxicity and detection, (5) Toxic Algal Contaminants, (6) Biotoxin monitoring, (7) Harmful Algal Bloom (HAB) monitoring and management, (8) Shellfish safety, and (9) Management and regulation.

Simultaneous workshop sessions were held on the following subjects; i) molluscan shellfish safety: Problems and needs in developing countries; ii) Enhancement of Private Sector/Industry participation in molluscan shellfish safety programs; and iii) Molluscan shellfish safety: Research and development priority areas.

Papers presented during the Conference that will pass editorial requirements will be published in a special issue of the Journal of Shellfish Research.

In the local press ('Today', p.9, Dec. 9/97 issue), the call 'to speed up passage of shellfish code' for the Philippine government and lawmakers was highlighted. Both Drs. Rhodora Azanza and Sandra Shumway have emphasized the need for a concerted effort between government, the private sector and shellfish farmers to come up with a comprehensive shellfish sanitation code, during their interviews with the press.

Recommendations were made on specific actions to address problems and needs in developing countries. It was recommended to develop a directory of scientists and experts in all aspects of molluscan shellfish safety, to develop a mechanism for exchange of information concerning molluscan shellfish safety (inter-net and/or printed), and to develop a molluscan shellfish safety manual that establishes regionally harmonized rules and regulations compatible or consistent with international standards.

The need for enhancement of private sector/industry participation in molluscan shellfish safety programme was stressed. Given limited resources, it was recommended that education programmes should be directed first to the industry and then to the general public. Access to information through networking e.g. the IOC newsletter, etc. (Internet is not thought to be best route because industry may not be "wired" in everywhere).

The working session also debated research priority areas. One area that requires attention is the mitigation and treatment of the effects of harmful organisms. Regarding harmful algae, several actions were presented in relation to mitigation strategies. It was stressed that the discussion regarding their possible application should be extremely careful and the environmental impact should be of major concern. It was suggested to be better to learn to "accommodate" nature rather than alter it, especially in marine waters where water movement and transfer cannot be controlled. No large-

scale introduction of any species or substance should be attempted until the consequences are well know. Examples of mitigation techniques were i) use of clay, with concern on the effect of sedimentation on benthos and eutrophication, ii) alteration of phytoplankton assemblages, iii) introduction of parasites (e.g. to aleviate *Alexandrium* sp.) with concern for the specificity that has to be clearly defined.

Several other issues related to applied and fundamental research were raised. It was proposed that the effectiveness (UV)/ineffectiveness (ozone) of methods for treatment for PSP toxins in depuration plants should be clarified. The need for more studies on the movement, bio-transformation and bio-degradation of marine toxins through the food web was expressed. Such studies could provide a better understanding the effects of toxins on target organisms, binding and mechanisms of action of toxins and the possible role of detoxifying enzymes. Stress was put on the development of treatments and antidotes. It was stressed that good scientific data are necessary to establish regulations regarding shellfish safety.

An extended report including recommendations obtained in the workshops is located at the web site http://www.unesco.org/ioc/oslr/hab.htm and is also available upon request to the IOC (jorge.diogene@vi.ieo.es,hab.ioc@unesco.org,rhod@msi01.cs.upd.edu.ph)

The Third ICMSS will be chaired by Dr. Sandra Shumway of the Southampton College, Long Island University, New York, USA in the year 2000.

Dinoflagellates on cd-rom

Multimedia dinoflagellate identification

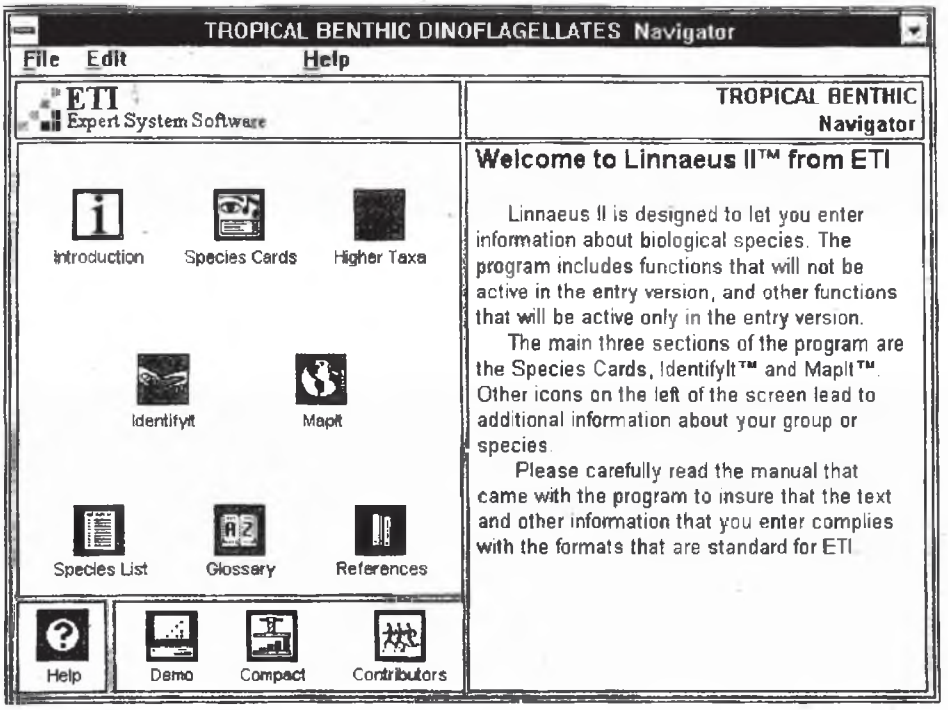
Dinoflagellates, distributed world-wide, are an important component of sea- and freshwater systems and are thought to be potential progenitors of toxins responsible for seafood poisoning. Identification of dinoflagellates is difficult and time-consuming. At present access to species information and identification is limited because the literature is scattered in a vast number of sources. However, electronic information systems, such as Linnaeus II, can provide computer-guided identification of these organisms.

Linnaeus II software was created by the Expert Center for Taxonomic Identification, ETI, of which UNESCO is a partner. This group is dedicated to improving the quantity, quality and accessibility of taxonomic information via interactive multimedia software on a global scale. In developing Linnaeus II, ETI has addressed the taxonomic need for a universal tool to create interactive identification keys, to document biodiversity, to enter and store species information in text and graphic format, and to easily exchange standardized datasets among specialists. Thus this information system can promote worldwide accessibility of species data via the electronic highway [1].

Dino-Linnaeus is a multi functional database containing species information on dinoflagellates from around the world, from the Caribbean Sea, to the SW Indian Ocean and to the North Pacific Ocean. This data file was compiled using Linnaeus II, a multimedia computer program that allows the specialist to enter, store and access species information, and to develop and use interactive identification keys on their own computer. Data can be stored in text, picture and video (movie) files.

At present access to dinoflagellate data is confined, but in using Linnaeus II we intend to develop taxonomic monographies and regional geographical data resources that ETI will compile and release on CD-ROM. Now interactive, multimedia taxonomic species information can be accessed easily and inexpensively, making this program an excellent modern teaching tool [1] for the identification of marine dinoflagellates.

Fig. 1. From the Navigator the user can access other sections of Dino-Linnaeus via clicking on the icons in the left field. A brief introduction to Linnaeus II is in the right field.



Linnaeus II

This software is a modern interactive research tool created by and for taxonomists and biodiversity researchers. Linnaeus II is a user-friendly program that can be used on a Macintosh or Windows PC. Data entry is simple using pull-down menus, keyboard commands and/or clicking on buttons. This program has three main components: A) **Taxonomic**

Databases, B) **Identification Key**, and C) **Geographical Information System (GIS)**. All sections are fully multimedia and accessible via the Navigator (Fig. 1).

Taxonomic databases

This section of Linnaeus II is made up of three main databases: *Species Cards* – designed to store species descriptions, taxonomic data and literature references in text format, along with pictures and movies; *Higher Taxa* – section designated to enter multimedia information on phyla, order, class, etc.; and *Species List* – section listing all species and higher taxa names entered into Dino-Linnaeus.

The *Species Cards* are made up of five data fields: a) *Description*, b) *Taxonomy*, c) *Synonyms*, d) *Literature*, and e) *Media Clips*. All fields in the *Species Cards* allow for unlimited data entry.

a. *Description* field contains the taxonomic description of the species in text form. The information in this field can be viewed in two modes, *Detail* and *Overview*. In *Detail* mode a large view screen allows access to the other information fields in the card, whereas in *Overview* mode a general picture of the species appears on the right with species description on the left, and no access to the other fields in the card (Fig. 2). The *Description* field is divided into user-defined sections for the specific group to retain uniformity in data entry.
b. *Taxonomy* field stores the most current taxonomic hierarchy for that particular species (phyla, class, order etc.). The taxa are also all linked to the *Higher Taxa* database; i.e. clicking on a name brings up the respective higher taxa description card.
c. *Synonyms* field stores all known synonyms and common names with author(s) and dates.
d. *Literature* field lists all relevant citations of the species cited in the *Species Card*. Citations are first entered into the *References* database with keywords/species names and then copied to the *Species Card* literature field. This linking eliminates entering repetitive citations.
e. *Media Clips* field stores all pictures, movies and sounds relating to the species. The first clip listed should be a general illustration of the species since this is the picture that will display in *Overview* mode in the *Description* field. An unlimited number of pictures and movies can be added following the designated format.

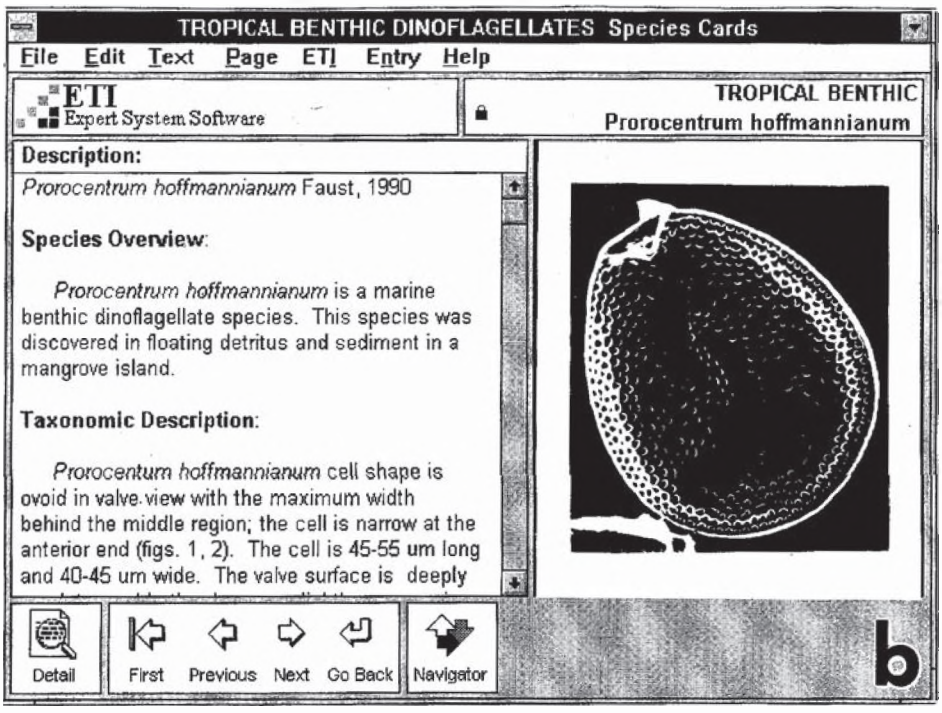


Fig. 2. Overview *mode where a general picture of the species is displayed, here* Prorocentrum hoffmannianum.

Higher Taxa cards are identical to the data fields found in the *Species Cards*, but without an *Overview* mode. Either card can be accessed indirectly via the *Species List*: clicking on a particular species or taxa name brings up the desired card.

The taxonomic databases also include three supporting databases: Introduction, Glossary and References. The *Introduction* database was designed to store general information on the taxonomic group or specific methodology. In Dino-Linnaeus the *Introduction* section contains three topics: >What is a Dinoflagellate?<, >Identifying Dinoflagellates<, and >Harmful Dinoflagellate Blooms<.

The *Glossary* database stores the definitions and illustrations for all scientific and technical terms (with synonyms). Most definitions in Dino-Linnaeus are taken from *Identifying Diatoms and Dinoflagellates* [2]. The *References* database contains all dinoflagellate literature cited in the program. The user can easily search through the *Glossary* and *References* sections through keyword fields. Moreover, all sections/databases in Linnaeus II are linked through the automatic hyperlinks, so cross-referencing is simple: clicking on an unknown word prompts a search in the *Glossary* section for the term.

Identification key: identifyit

IdentifyIt is a matrix-based key that allows multiple entry of characters and states to facilitate identification and diagnosis of an object (higher taxa or species. This section of Dino-Linnaeus is made up of structured databases of character states that can be linked to short text (A), long text (T), illustrations (P) and movies (M) to help define them.

IdentifyIt is a fast and effective system for species identification. The user can use any and all characters known about the specimen, even if it is damaged or incomplete. Any number of character states (even >unknown<) can be chosen, and based on the selections, the program sorts the object list, by percentage hits, and determines the probability of each object to best fit the character set. To verify the results of the computerized key the user can click on the object name with the highest percentage points and view the respective *Higher Taxa* or *Species Card*. The user can also compare a number of species with common character states by clicking on the *Compare* button [1]. *IdentifyIt* files in Dino-Linnaeus include toxic and non-toxic tropical benthic dinoflagellates.

Geographical information system: mapit

MapIt is a grid-based geographical distribution system designed to store biodiversity data at regional or global levels. With this system the specialist can enter, study and compare distribution patterns of one species or more, view overlay distributions and biodiversity ranges of different species for a particular area of the world. *MapIt* would provide geographical distribution maps of dinoflagellates in the newer version of Linnaeus II.

Dino-linnaeus networks/-eti partners

In using Linnaeus II we are committed to continue developing Dino-Linnaeus for data storage and identification of marine dinoflagellates. Such multimedia databases can cultivate and simplify world-wide cooperation and interaction among specialists to exchange scientific data. We are currently establishing regional and international dinoflagellate networks to continue building the Dino-Linnaeus database with different projects; e.g. tropical planktonic dinoflagellates, neritic athecate toxic dinoflagellates, etc. Dino-Linnaeus is a large undertaking that requires the expertise of many experienced taxonomists. All interested scientists should contact ETI to establish participation in the Linnaeus II program (e-mail:info@eti.bio.uva.nl). As ETI Partners members receive a free copy of the Linnaeus II software package. To ensure format and data entry uniformity contributors should send their data files to R.A. Gulledge or M.A. Faust for incorporation into Dino-Linnaeus. The networks then collaborate to compile modern species information and identification data sets. The completed data files are reviewed by two outside reviewers. And finally the completed systems are sent to ETI and are released on CD-ROMs which are free to the authors and available at cost to others. With ETI's FTP site (file transfer protocol) working on a project with international researchers has been simplified: storage and retrieval of data files is possible at the PC terminal [3].

Completing such a project would produce a highly useful and much needed computerized system to access dinoflagellate species data, to study dinoflagellate biodiversity, and monitor changes in species occurrence in the marine environment.

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The Fourth WESTPAC Scientific Symposium

The Fourth IOC – Western Pacific Subcommission (WESTPAC) International Scientific Symposium was hosted by the Ocean Research Institute of the University of Tokyo, the University of Ryukyus and the Ministry of Education, Science, Sports and Culture of Japan from February 2-7, 1998. The venue of the said Symposium was the Convention Center in Okinawa. "The Role of Ocean Sciences for Sustainable Development" served as the theme of the Symposium. There were 5 keynote speakers with talks concentrating on the achievements and plans of the Global Oceanographic Observing System (GOOS) program and the Northeast Atlantic GOOS project, the geology of Western Pacific Region and the coastal living resources of WESTPAC.

C. Summerhayes (Director of GOOS), in his keynote address entitled: "Implementing GOOS, the Global Ocean Observing System", said that the project is moving rapidly from planning into implementation through (i) pilot projects, (ii) technology demonstrator projects, (iii) incorporation or adaptation of existing national or international operational oceanographic systems, and (iv) development of purpose built systems. He also mentioned that scientists involved in the GOOS project plan to negotiate with the IOC HAB programme for possible collaboration as they will be doing with other existing programs involved in oceanographic/marine studies.

The different fields of study in oceanography such as Physics, Geology, Chemistry and Biology were covered by the oral and poster contributions from about 200 participants from the WESTPAC region and the rest of the world. Contributions on Harmful Algal Blooms (HABs) in the region were the following:

(1) "Harmful algal bloom management programs in Southeast Asia" by R. V. Azanza which is based on her review/visit of the member countries of the ASEAN region, in her capacity as the Coordinator of the ASEAN-Canada Red Tide Network in May, 1997. This paper presented a review of harmful algal bloom programs in Southeast Asia, especially those in line with the regional red tide/HAB alert and information networks. Likewise, the needs and concerns for improved monitoring, research and management of HABs for the region were highlighted in this paper;

(2) "Temporal and spatial distribution of sand-dwelling dinoflagellates in a tidal flat of Yashima Bay, the Seto Inland Sea, Japan" by S. Montani and L. Huang which is based on a 1-year quantitative study of benthic dinoflagellates in a tidal flat of the cited study area. Results of this study suggested that while temperature seems to play the most important role in the succession of these sand-dwelling dinoflagellates, nutrition status may be the deciding factor in their vertical distribution;

(3) "The establishment of a red tide monitoring center in Samar, Philippines" by J. T. Racuyal, R. C. Diocton and R. T. Severo, Jr. discussed the need to have more red tide monitoring centers scattered within the Philippine archipelago so that the government can have quick access to relevant information about red tide situations. Such data/knowledge can serve as basis by managers to draw up contingencies and minimize the effects of harmful algal blooms on fisheries and public health; and

(4) "Role of waves in initiating and triggering algal blooms" by R. P. Babaran and R. A. Espinosa espoused the idea that waves (generated by wind stress) may actually play a key role in the onset of algal blooms – a hypothesis which is not new in the field.

Dr. Yasuwo Fukuyo, chairman of the IOC WESTPAC Harmful Algal Bloom project reported on the achievements of the project which was primarily the training of monitoring and research personnel and the dissemination of reference materials for Southeast Asia, some of which were in collaboration with the ASEAN-Canada Red Tide Project.

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◆ Gulf of Mexico

New HAB experimental facility

New research activities are developing at the US Environmental Protection Agency's Environmental Research Laboratory, Gulf Ecology Division, in Gulf Breeze, Florida. With a commitment to assisting the national effort to resolve the increasing ecological, economic, and public health problems associated with harmful algal blooms (HABs), the Laboratory has recently established a new multi-disciplinary HAB research team composed of scientists with a variety of expertise that will be applied to this issue. We are in the process on developing a HAB Experimental Culture and Exposure Facility that will be the focal point for our studies on HAB nutrient ecophysiology, toxin production, diagnostic indicators, and effects of toxin exposures on aquatic organisms.We anticipate that the facility will be operational in early 1998.

We have recently incorporated a HAB page on the Gulf of Mexico Aquatic Mortality Response Network (GMNET), which you can visit at, http://pelican.gmpo.gov/gmnet/gmhome.html (follow the Items of Interest link to HABs). Our goal is to expand the GMNET aquatic mortality reporting activities to include information on HAB events in the five Gulf coast states. We think this database will be invaluable for examining causal relationships between aquatic mortalities and HABs, and we would like to hear about other existing or planned efforts. I would like to take this opportunity to invite all Gulf of Mexico colleagues to get involved with the new GMNET activities. Over the next several weeks, I will be contacting as many of you as I can to discuss our goals and their implementation. You would like to be added to the GMNET listserver or want more information on this or our other HAB activities, you can contact me at:

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◆ HAB ART

Algal sculptures

If you happen to be passing through Hiroshima, plan to visit Dr. Haruyoshi Takayama at the Hiroshima Fisheries Experimental Station for a rare treat indeed. Many of you may be familiar with Dr. Takayama's taxonomic papers and SEM's of dinoflagellates but few, we suspect, know of his other talent—carving.

Dr. Takayama began carving replicas of phytoplankton 12 years ago. His first object was a Raphidophyte, *Chattonella antiqua*, and the collection now numbers about 32 species and 35 pieces. The dinoflagellates are carved from pine, cherry, and other woods and all are done to scale. We are told that his wife scolds him regularly to not scatter scraps and thus he does some of his carvings during cruise time. The collection is kept at the laboratory in a small museum.

The 'organisms' are excellent teaching tools and make explanation of morphological characteristics a simple task, even for beginners. He uses these carvings during his training course on identification of red-tide organisms. It must be a real treat for his students when during his discussions of plates, apical pores, and other things seemingly foreign, he produces these models for examination. Exquisite only begins to describe them.

If you would like to see these sculptures on display at the 9th International Conference on Harmful Algae in Tasmania, contact Dr. Takayama and encourage him to bring them along!

Dr. Haruyoshi Takayama, Hiroshima Fisheries Experimental Station, Ondocho, Aki-gun, Hiroshima 737-12 Japan, Fax: 81 823 52 2683.

Sandra Shumway



Future events

6th Canadian Workshop on Harmful Marine Algae, St. Andrews, NB, Canada, May 27-29 1998. Topics of the workshop include; ecology and oceanography of harmful algal events, management and socioeconomic impacts of toxic and toxicological and epidemiological studies, models, and toxins. Contact: Jennifer Martin, Biological Station, St. Andrews NB Canada EOG 2X0; Phone: +506-529 5921; Fax: +506-529 5862; e-mail: martinjl@mar.dfo-mpo.gc.ca

IOC-Germany Training Course on Qualitative and Quantitative Determination of Algal Toxins, Friedrich-Schiller-University of Jena, Germany, 6 – 16 October 1998. The objective of the course is to improve the participants expertise on qualitative and quantitative determination of algal toxins in order to enable them to make reliable determination of algal toxins from phytoplankton species causative of harmful algal events. The course will contain a theoretical, and a practical part on toxin chemistry and toxicology. The practical part of the course consists of different analytical methods for determination of PSP, DSP, ASP, TTX as well as blue-green toxins. Priority will be given to younger scientists. Lectures at the course will include: Dr. Bernd Lucas and colleagues from Friedrich-Schiller-University of Jena, Dr. Malte Elbrächter, Biologische Anstalt Helgoland and a guest lecturer. Application forms can be obtained from the Harmful Algal Bloom Programme Office, IOC, 1, rue Miollis, 75732 Paris Cedex 15, France, fax: +33 1 45 68 58 12, e-mail: hab.ioc@unesco.org. Deadline for applications is 1 July 1998.

9TH INTERNATIONAL CONFERENCE ON HARMFUL ALGAL BLOOMS, 7-11 February 2000, Tasmania, Australia. The conference will provide a broad forum for phycologists, microbiologists toxicologists, physiologists, molecular biologists, aquatic ecologists and managers to address and exchange research findings and perspectives concerning all aspects of toxic and harmful algae, including freshwater cyanobacteria and ciguatera (fish poisoning). Harmful algae and their toxins pose a growing global problem for human health, aquaculture, fisheries, seafood trade, tourism and the aquatic environment at a time when human reliance on coastal zones for food, recreation and commerce is expanding. Contributions are invited which address the following topics: * Taxonomy of harmful algae, using conventional as well as sophisticated molecular and immunological approaches * Population dynamics of harmful algal blooms, including coupling of physical and biological processes, and interactions with zooplankton, viruses and bacteria * Ecophysiological, chemical and pharmacological aspects of algal toxins, with a focus on new analytical methods and novel toxins or toxic episodes * Monitoring and management of harmful algal blooms as they relate to eutrophication, aquaculture, public health and shipping (ballast water problem). Options for prevention, control and mitigation will be especially emphasized. Contact: HAB 2000 Conference Secretariat, Conference Design Pty Ltd, PO Box 342, Sandy Bay Tasmania 7006, AUSTRALIA, Tel: +61 3 62243773, Fax: + 61 3 62 243774, E-mail: mail @cdesign.com.au, or: Gustaaf M. Hallegraeff, School of Plant Science, University of Tasmania, GPO Box 252-55, Hobart, Tasmania 7001, AUSTRALIA, Tel: +61 3 62 262623, Fax: +61-3-62 262698, E-mail: Hallegraeff@utas.edu.au (Gustaaf Hallegraeff), Web Page: http://www.utas.edu.au/docs/plant_science/P.S._Homepage.html.

For a more comprehensive overview of up-coming activities and events you should visit the IOC International Marine Meeting List at the IOC homepage at http://www.unesco.org/ioc/infserv/meets.htm. The list of meetings related to the International Year of the Ocean might also be of interest http://www.unesco.org/ioc/iyo/conferences.htm

Literature for developing country libraries

Limited copies of the following titles are available from the IOC Science and Communication Centre on Harmful Algae, Denmark(see address below). to libraries of marine science institutions *in developing countries only*:

- The Genus Alexandrium Halim (Dinoflagellata). **E. Balech, 1994.**
- Algae. An Introduction to Phycology. **C. van den Hoek et al., 1995.**
- Identifying Marine Phytoplankton. **C. Thomas et al. (eds.), 1997.**
- Proceedings of the International Symposium on Ciguatera and Marine Natural Products, Hawaii, 8-10 August 1994. Hokama et al (eds.) 1995.
- Proceedings of the First International Congress on Toxic Cyanobacteria, Denmark, 20-24 August 1995, and, Proceedings of the First Maj and Tor Nessling Foundation Symposium on 'Recent developments in Cyanobacterial Research', Finland 16-18 August 1995 Moestrup et al (eds.) Phycologia. Vol.35 No.6, 1996
- Photography through the microscope. Kodak. **J.G. Delly, 1997.**

Applications must be submitted and signed by the responsible librarian, and should indicate why the requested title is of specific interest to your institution and its ongoing research or teaching. No requests from individuals will be honoured.

IOC publications

- Summary Report of the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms, Vigo 30 June - 2 July 1997. Available in English, French, Spanish.
- Amnesic Shellfish Poisoning (ASP), Volume 1, IOC *Manuals and Guides*, No. 31.

These publications are free of charge and available from the IOC Secretariat. 1, Rue Miollis, 75732 Paris, Cedex 15 France, Fax: +33 1456858, e-mail: hab.ioc@unesco.org

- Manual on Harmful Marine Microalgae, IOC Manual and Guides No. 33.
- Harmful and Toxic Algal Blooms. Proceedings of the Seventh International Conference on Toxic Phytoplankton, Sendai, Japan, 12-16 July 1995.

The Manual and the Sendai Proceedings are free of charge but a fee of US$ 30 will be charged for handling. A cheque issued for the IOC SCCHA, University of Copenhagen (see address belew), should accompany all orders. Only prepaid orders will be accepted. Excepted from the handling fee are marine science libraries and scientists in developing countries which can apply for delivery free of charge.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

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◆ China and Hong Kong

An unusual year for the occurrence of harmful algae

The Year 1998 has been a special HAB year for mainland China and Hong Kong. There was a huge toxic algal bloom which covered almost all Hong Kong and nearby south China waters in March and April, caused by *Gyrodinium aureolum*(?), followed by some smaller HAB events caused by *Alexandrium* sp. and *Cocholodinium polykrikoides* at the same time. They are thought to be the most devastating HABs in the history of Hong Kong, affecting about two-thirds of the fish farms and causing losses estimated at HK$350 million (about US$ 42 million). In early May, another algal bloom occurred in Hau Hoi Wan. The causative species was *Gyrodinium instriatum*. A bloom of *Ceratium furca* occurred in Port Shelter in September. The most recent red tide was caused by *Mesodinium rubrum* in early November. Seven beaches were closed to the public due to the red tide. A new HAB record was created in mainland China in September 1998, when *Ceratium furca* cov-

(Cont'd on p. 3)



Hong Kong fish farmers are cleaning fish farms and surrounding waters (article by Yang and Hodgkiss page 4)

◆ Mediterranean

A new PSP-like toxin in *Alexandrium andersonii* (Dinophyceae)

During an investigation on dinoflagellate cyst production in the Gulf of Naples (Tyrrhenian Sea, Mediterranean Sea), spherical, smooth-walled cysts, which germinated into *Alexandrium andersonii* Balech, were recorded in July-September (1). *A. andersonii* is a small-sized species, which differs from *A. minutum* in only a few minor morphological characters: the peculiar shape of

(Cont'd on p. 3)

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◆ Hong Kong

A *Gymnodinium mikimotoi* occurrence spring 1998



The first reports of *Gymnodinium mikimotoi* came from an upwelling area off the coast of Xiamen China just south of Taiwan in an area referred to as "Nanao". Near the northern border of Canton (Guangdong Province) between the coastal cities of Swatow and Chichyang is a group of islands where upwelling from the deep waters of the Bashi Channel brings nutrients to the fishing grounds of this area. According to the Chinese Press, *G. mikimotoi* was first reported in this area in late February of this year. The next report of this species was on 19 March when a chocolate brown to reddish brown bloom was reported by the Agriculture and Fisheries Dept. personnel (who identified *G. mikimotoi* as the dominant organism) from Crooked Island (Fig. 1, area 1) in the northeastern sector of Hong Kong and three other nearby sites. By 18 March, *Gymnodinium mikimotoi* had reached densities of 210,000 cells per litre in Port Shelter. *Scrippsiella trochoidea* reached 81,000 cells/L and *Gymnodinium sanguineum* had reached 290,000 cells/L. By the following week (24 March) *G. mikimotoi* at Port Shelter had declined to 34,000 cells/L and *Scrippsiella trochoidea* to 40,000 cells/L. On 27 March the bloom of *G. mikimotoi* had progressed to two new sites in the Sai Kung Peninsula (Fig. 1, area 2). On 1 April, a diatom pulse occurred at Port Shelter with *Pseudonitzschia and Chaetoceros* species forming the dominant genera. A week later *Noctiluca*, the giant dinoflagellate, reached densities of 10,000 cells per litre and by 14 April *Gymnodinium mikimotoi* had returned to Port Shelter and reached densities of 270,000 cells/L with a standard deviation of 43,000 cells/L for three replicate counts.

By 12 April, the Hong Kong press had reported that the worst red tides this decade had wiped out hundreds of thousands of fish (1,500 tonnes of fish stocks, the equivalent of half the entire amount produced in Hong Kong waters last year and more than all the chickens killed in Hong Kong as a result of the Chicken Flu virus which struck Hong Kong in November, 1997. Fish in holding pens (mariculture cages) were killed when mucus produced by *Gymnodinium mikimotoi* coated their gills resulting in their asphyxiation. The government estimated breeders' losses at HK$80 million (US$10.3 million), but fish farmers claimed the figure was at least HK$250 million. About 1,000 of Hong Kong's 1,500 fish farms were devastated by the red tides. Red tides usually subside in a few days, but this one "has spread out like an infectious disease," said Hong Kong Fish Culture Association Chairman Wong Yung-kan.

According to Dominic Lau (Hong Kong, April 15, Reuters) – Hong Kong's health chief, Margaret Chan, director of health, told reporters that anybody who decided to eat the sea fish should remove the gills and innards. Considered a delicacy by some, these parts could contain toxins if affected by the algae according to Margaret Chan. Hong Kong leader, Tung Chee-hwa visited some of the fish farms on outlying Lamma Island on Wednesday (April 15) to see the damage. Christopher Cheung of the Hong Kong Fisherman's Association said, "The government does not know the whole picture," he said. "Fish are continuing to die and losses are going up every minute. I think the whole industry will collapse because waters inside and outside of Hong Kong are covered by this algae."

By 10 April, the *Gymnodinium mikimotoi* had reached Lamma and Lantau islands. The surface waters at Mo Tat Wan on Lamma Island (fig. 1, area 3) were sampled on 15 April, 1998 by Manna Wan. *Gymnodinium mikimotoi* had reached densities of 102,000 cells/L and *Prorocentrum sigmoides* (8,300 cells/L) and *Protoperidinium* species (4,500 cells/L) were rare by comparison. Government scientists reported *Gymnodinium mikimotoi* densities in the millions of cells per litre but we did not observe such high numbers in our weekly surface water samples from Lamma Island and Port Shelter. By 15 April, many of the Lamma Island fish farmers had moved their enclosures from Sok Kwu Wan to the deeper more open waters of Mo Tat Wan. By 21 April, strong winds had begun to disperse the bloom and fish farmers began moving their fish enclosures back to Sok Kwu Wan.

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ered 3,000 square kilometres in Bohai Bay; it persisted longer than any previous bloom.

The year has also been an unusual year in terms of the seasonal distribution of toxic and harmful algal species in Hong Kong waters. In regular years, the most toxic algae, especially unarmored dinoflagellates, are abundant in spring, mostly from March to May, and are scarce in summer. But this year, toxic or harmful species were present almost throughout the year in Junk Bay, in south east Hong Kong. The number of toxic or harmful species in summer was even higher than in spring. These included *Alexandrium catenella, Alexandrium* sp., *Chattonella antiqua, Ch. marina, Chrysochromulina* sp., *Gonyaulax polygramma, Gymnodinium catenatum, G. breve, G. sanguineum, Gyrodinium instriatum* and *Phaeocystis pouchetii.*

There are 'rich resources' of harmful algae in Hong Kong waters and, so far, most of the known harmful algal species have been found here. Hong Kong has been, and is constantly facing the threat of HABs, since the causative species are there and can bloom whenever there are suitable environmental conditions. But, why has this year been the record year for HABs in both mainland China and Hong Kong, and why were toxic species so abundant this summer?

Statistics from the Hong Kong Observatory show that 830.2 mm of rain fell between January and May, 213.8 mm more than the seasonal norm and 205.1 mm more than in the same period last year. This heavy rain increased the input of nutrients into the sea and, together with the effect of water temperature changes, HABs could be easily formed. These events could well have been connected with the effects of El Nino this year.

Toxic algae new to Hong Kong

Gambierdiscus toxicus

Ciguatera is one of the most common forms of seafood poisoning in Hong Kong. About 100 people were reported to be sick after being poisoned by coral fish last year. So far this year, at least 3 ciguatera poisoning events have been reported and 72 people were involved. The majority of marine fish consumed in Hong Kong are imported from overseas and. according to government figures, 76 percent of fish eaten last year was imported. Most of the locally produced fish is cage cultured and so it was considered that the poisoned fishes came from other countries.

The origin of the ciguatoxin is believed to be *Gambierdicus toxicus*, a tropical dinoflagellate growing in association with corals and seaweeds. This species is widely distributed in tropical waters, but has not previously been reported from Hong Kong waters. In the summer of last year and again this year, *Gambierdiscus toxicus* was found in Junk Bay east of the Kowloon Peninsula.

Phaeocystis pouchetii

In November and December 1997, there was a huge *Phaeocystis pouchetii* bloom in the waters of north Guangdong Province and south Fujian Province, China. It killed thousands of caged cultured fish in the area. During the bloom, there was a thick layer of foam; the diameter of the gelatinous colonies was as large as 2 to 3 cm. The water looked like "dead water" when the blooming took place. *Phaeocystis pouchetii* is a cosmopolitan species. Blooms caused by this species were frequently reported in cold water areas such as north Europe, but blooms in subtropical waters have rarely been documented. The species is a newly identified one in Hong Kong waters. It was isolated from Junk Bay in September 1998, and is now in culture.

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the 6th precingular plate (6") and the anterior sulcal (S.a.) platelet (2).

A. andersonii has been described from the coastal waters of the eastern United States and, to our knowledge, the finding of this species in the Mediterranean Sea represents the first report outside its type locality. This species was reported as non-toxic (2, 3); however, our preliminary investigation carried out on culture material from the Gulf of Naples has revealed the presence of PSP-like toxins.

A. andersonii was grown in K medium without silicates, at a temperature of about 20 °C, and a photon flux rate of 100 mmol photons m^{-1} s^{-1}. Late exponentially growing cells were concentrated with a tangential flow filtration system, and centrifuged to obtain a pellet. The pellet was resuspended in an equal volume of 0.5 N acetic acid and subjected to three successive 20s sonifications. Supernatant obtained after centrifugation (3000 g, 20 min) was injected introperitoneally into CD-1 Swiss male mice (19-20 g). The injection produced visual signs resembling the effect of paralytic shellfish poisoning (PSP) produced by some *Alexandrium* species. Death occurred a few minutes after injection. HPLC fluorometric analysis, based on alkaline oxidation of the PSP toxins to fluorescent derivatives, was carried out to detect the presence of dinoflagellate PSP toxins. Three different isocratic conditions were adopted for the toxins, based on their basicity (4), using authentic PSP toxins as references. However, the retention times of the standard disagreed with those of toxin(s) present in our sample, thus suggesting the occurrence of a new PSP toxin in this Mediterranean strain of *A. andersonii*.

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◆ Hong Kong

Massive fish killing by *Gyrodinium* sp.

In March through April 1998, a massive red tide occurred in south China, including Hong Kong, coastal waters. This red tide covered a huge area (Fig. 1). It was reported that the red tide started from Nanao, which is located in the Shenzhen Special Economic Region of China, the other side of Mirs Bay. It showed up in Hong Kong waters on 18 March at Crooked Island. It then travelled southward to Grass Island, the Portshelter area at Saikung and Tung Lung Chau, reaching, by the beginning of April, the south and southwestern waters of Potoi Islands, Lamma Island, Cheung Chau Island, Soko Islands, and Mainland China's Wanshan Islands and Guishan Island, from where it continued up to the mouth of the Pearl River. This red tide event infected a large area and lasted for more than one month. By 20 April, it was reported to be declining and was predicted to have no further momentum to distribute on a large scale.

The deleterious effect and economic loss

Wherever the red tide passed it killed fishes, leaving scenes of fish farms as shown in the photo on the front page. The fish kills were claimed to show no discrimination, including, as claimed by the farmers, coral-trouts, coral cods, grouper, giant grouper, yellow grouper, brown spotted grouper, mud grouper, tiger grouper, mangrove snapper, red snapper, russell's snapper, sea-perches, Japanese sea-perch, seabream, black seabream, gold-lined sea bream, head grunt, eel, red pargo, yellow croaker, purple amberjack, and pampano. The red tide killed not only various kinds of caged fishes, it was also reported to affect nearly all kinds of natural coral fishes and killed some of them. Some fishes were claimed to have disappeared from the area they used to inhabit; butterfly fish for instance.

By 17 April, about one month from the start of the bloom, the red tide had invaded nearly all corners of the coastal waters of Hong Kong, including 22 of the 26 fish farms and 5 bathing beaches. In Hong Kong alone, 1,260 fishery households were affected, 2,500 tonnes of fishes were killed, and a direct economic loss of 250 million HK$ was estimated by the fish farmers. In Mainland China's Guangdong waters, it was reported that by 17 April, this red tide had killed more than 260 tonnes of fishes, causing a direct economic loss of about 40 million Yuan RMB.

Changes in meterological and hydrochemical parameters

Normally, during the winter period (October – March), Hong Kong coastal waters are influenced by two water masses (Williamson, 1970), the North China Coastal Water (Taiwan Current) which is characterised by moderate temperatures ranging from 19-23°C and salinities from 31-33 ppt, and the Kuroshio Current which originates in the Pacific and enters from the Luzon Straits and is characterized by high temperature (26-29°C) and high salinity water (34.4-35 ppt). Due to the El Nino event, this year's weather was characterized by a higher than normal air temperature and a higher volume of rainfall. From February 1998, wet, cloudy and foggy weather brought in by maritime airstreams was followed by a cold front and storms, and then an irregular short period of sunny days. These weather types formed a circle and were recircled several times. From February, Port-Shelter water started to become green to dark and an increasing trend of algal growth was recognized. Entering March, the weather was mainly cloudy with strong easterly winds affecting the coastal areas on the first two days. Winds moderated on 3 March before a band of squally and thundering showers accompanying a cold front swept across Hong Kong on the morning of 4 March. It became fine and clear the next day but clouds returned in the evening. Easterly winds freshened during the day on 6 March. Due to the maritime airstream, fog formed on 7 and 8 March. The next day it was very warm and the weather was cloudy with some showers. On 10 March, a cold front accompanied by rain passed across the coast of Guangdong. A northerly wind became strong offshore in the evening and moderated gradually the next day. And, on 12 March, it became cooler and temperature dropped to 12.4 degrees, the lowest in the month. On 17 March, the day on which red tide was reported from Nan-



Fig. 1
A direct loss of 250 million HK$ was estimated by Hong Kong fish farmers

ao it became foggy, and this lasted for several days. On 18 March when the red tide was reported first from Crooked Island fish farms, red tide could also be recognized in situ at our sampling station located in Port-Shelter. Laboratory counts showed a concentration of the fish killing species at about $2x10^5$ cells/L. A fast increase in sea surface water temperature was also recognized at this time, which is earlier than the rise in air temperature. The sea surface water temperature kept rising until the mid of May while the air temperature started to drop on 20 March. This may have indicated the influence of warm Pacific water brought by the Kuroshio Current. We believe that it was this current that triggered the algal bloom. Moreover, both the Kuroshio Current and the Taiwan Current during this time helped form a northeast to southwest coastal water flow that distributed the red tide over such a wide range.

The occurrence of the red tide event this year coincided with records of similar red tide occurrences in the past, as described by Wong (1989): "in September, 5-10 days before the bloom there were usually tropical depressions with strong wind and heavy rain followed by warm sunny weather for a few days. In December, the weather pattern usually involved a cold front with strong wind followed by some rain and then a fine sunny period".

Causative species

The causative species has been studied morphologically using light and scanning electron microscopy. It superficially resembles *Gyrodinium aureolum* from the eastern coast of America and northern European coastal waters, and *Gymnodinium mikimotoi* from Japanese waters. However, it is different from them in certain aspects. A detailed description of the species is under preparation.

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Announcement

Proceedings of the 8th International Conference on Harmful Algae is now available. It is published jointly by the Xunta de Galicia (Spain) and the Intergovernmental Oceanographic Commission, IOC.

The advantage of the joint effort is that this 630 page publication is available at a very low price. To libraries and laboratories in developing countries it is free of charge. For all others there is a 30 USD handling fee.

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◆ USA

A toxic bloom of *Pseudonitzschia Australis* in Monterey Bay, California

These days just about everything that goes awry is blamed on El Niño. Should we add a toxic bloom of *Pseudo-nitzschia australis* with associated sea lion illness and mortality to the list? Good question.

I've had lots of reporters ask me if I think this is so, and since I could not give a definitive answer¼ the question quickly became something like, "Are sea lions suffering because irresponsible farmers are polluting our waters?" My response in this case: "No comment – we need more data." Well, that was good enough for the local papers, but as the National Ocean Conference got underway in Monterey, California, with both the President and Vice President in attendance, television crews scurried about for environmental news that would make headlines. Let's see, toxic algae killing marine mammals in a National Marine Sanctuary – not bad. So when the television crews came I spoke of increased awareness, of the fact not all blooms are necessarily linked to human activities, and the challenge being to sort through all the possibilities to understand what REALLY drives these events. After all, you've got your chemistry and physics, your bottom-up and top-down controls, and so on. "Interesting," said the media, "forget chemistry and physics for now, but how about global warming – did the sea lions die because of global warning? Is that what made those toxic cells go crazy...?"

No doubt that Monterey Bay did experienced about a 2-3 week bloom of *Pseudo-nitzschia australis* in the latter half of May that reached its peak in one sample at 200,000 cells per litre. Concentrations of domoic acid thus far appear to rise and fall in concert with those cells (G. Doucette et al., unpubl. obsv.). It seems that *P. australis* may have been at the right place at the right time. A small storm passed through the Monterey Bay region dumping rain and churning the waters that had been supporting a mixed assemblage of diatoms and dinoflagellates, including toxigenic Alexandrium catenella. The storm passed, the sun shone bright and the rivers and streams continued to pump fresh water with nutrients into the near shore environment. Within a few days the concentration of *P. australis* rose sharply, but the cells were restricted to the immediate near shore region of the Bay. The nearly mono specific bloom increased in size covering a larger portion of the Bay, but nevertheless remained close to shore. On the water one could observe slicks of cells – dense patches – that smelled quite strongly. Little upwelling was observed during this time (F. Chavez et al., pers. comm.), suggesting that source of nutrients fuelling the bloom was of terrestrial origin. The latter possibility remains an active topic of study at present.

With the *P. australis* bloom in full swing, anchovies helped themselves to what must have been welcome feast. Indeed, the stomachs of anchovies collected during the peak of the bloom are packed with *P. australis* frustules (M. Silver and P. Miller, unpubl. obsv.), and as might be expected the fish were toxic too (50 –250 ppm domoic acid for whole fish; Susan Luscatoff. pers. comm.). At about the same time, there were reports of sea lions suffering from symptoms that included seizures, disorientation and unresponsiveness to human presence, even death. It is now clear that at least some of those animals suffered from domoic acid poisoning (F. Van Dolah, G. Doucette, V. Trainer, F. Gulland, and many others were involved in this work – I'm sure we'll hear lots more about this in the near future). As far as I know, this is the first documented case of marine mammal mortality linked to domoic acid. What also makes this event unique is that we were able to track the evolution of the bloom in real-time using species-specific DNA probes in both whole cell and cell homogenate formats, and the response of the cells towards the probes can now be viewed in light of measured amounts of toxin as well as prevailing nutrient regimes, etc. More detailed information about the latter is forthcoming. For now, suffice it to say that the probes allowed us to see the early stages of the bloom that might otherwise have escaped detection, and by knowing what was growing in the water we were able to offer a possible explanation for the rash of sea lion illnesses.

Although we may never be able to say exactly what drove the *P. australis* bloom, what we can say is that domoic acid remains a potential threat to humans and wild life and can not be ignored, El Niño or not.

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GET IT NOW!



See page 5

◆ USA

Domoic acid poisoning afflicts sea lions and birds

This past May, approximately 72 sea lions suffering from gran mal seizures, disorientation, diarrhea, and vomiting, were treated at The Marine Mammal Center in Sausalito, California. Of those treated, about 50% have died. The exact number of dead animals that have washed ashore remains unknown. The strandings began in mid May 1998 in San Luis Obispo, located on the central Californian coast, but seizing sea lions have since been found on several beaches to the north in Santa Cruz and Monterey counties. In addition, dead sea birds have been observed in Monterey Bay and the Gulf of the Farrallones. Unlike California sea lions and northern fur seals that have been washing ashore emaciated as a result of El Nino, the animals which stranded in this event were in good physical condition with normal blubber depth.

Initial autopsies of the dead sea lions turned up no obvious cause of death although lesions were noted in brain tissues. Lesions, along with other symptoms exhibited by the sea lions, indicated that these animal illnesses and deaths may have been related to a recent bloom of the diatom *Pseudo-nitzschia australis* which produces domoic acid. *Pseudonitzschia spp.*, were found in high concentrations (up to 200,000 cells per litre) in Monterey Bay, and was also present in the guts of anchovies, a primary prey of sea lions.

After completing several blood, tissue, and other biological tests, investigators now believe domoic acid to be the probable direct cause for the event and not El Nino, chemical human pollutants or an infectious disease. Post-mortem examinations of these animals revealed lesions in particular sections of the brain that are consistent with known effects of domoic acid. The National Oceanic and Atmospheric Administration's (NOAA) Coastal Ocean Program helped to direct the scientific response to the domoic outbreak. Biotoxin analyses were performed by both NOAA's National Marine Fisheries Service (NMFS) Northwest Fisheries Science Center and the National Ocean Service (NOS) Charleston Lab resulting in four confirmed positive results for domoic acid. The NOS Charleston Lab stated that although a positive test result for domoic acid is a sure indication of exposure to the substance, a negative finding is not necessarily indicative that an animal was not exposed. This statement is based on findings in experimental exposures of domoic acid in mice, where the toxin metabolizes in about four hours.

The Marine Biotoxin group from the /Northwest Fisheries Science Center, the Channel Islands National Marine Sanctuary, and California Fish and Game have been conducting research cruises since June 5. Researchers are focusing in the areas of reported sea lion and bird mortalities from Monterey Bay to Point Conception, CA, to trace the algal bloom temporal and spatial progression. *Pseudo-nitzschia* has been observed along the coast, particularly south of Point Conception during the first two weeks of June. Another research cruise by Dr. Barbara Prezelin (UCSB), was underway at the end of June and will continue to collect samples for additional analyses.

Researchers will be sampling for algal cells, toxins (both domoic acid and saxitoxin), nutrients, chlorophyll, salinity, temperature, and trawling for planktivorous fish, the primary prey item of pinnepeds. Information exchange and coordination between in-place state and Federal monitoring programs for water quality, shellfish, and fish is on-going. Real-time remotely sensed chlorophyll distributions are also being sought to further document spatial distributions of the dense aggregations of *Pseudonitzschia* spp. through time.

(The Monterey Bay Aquarium Research Institute and the National Marine Fisheries Service Northwest Fisheries Science Center contributed to this article)

Fish kills on Florida's east coast may be linked to *Cryptoperidiniopsis*

The Florida Department of Environmental Protection (FDEP) is continuing to investigate a fish disease event on Florida's east coast. Diseased mullet were first reported to the FDFP Fish Kill Hotline on March 2, 1998. Anglers have reported 33 species of fish with lesions, with the majority being mullet. The range of affected fish is from the Ponce de Leon Inlet near New Smyrna Beach to North Palm Beach, but the majority of the reports have been from the St. Lucie estuary. Many potential factors in the fish disease event are under investigation by numerous federal, state, and local agencies including: overall water quality, pesticides, heavy meters, toxic dinoflagellates, fresh water inflow from Lake Okeechobee, and disease agents such as bacteria and fungi. FDEP is investigating the potential that several factors are interrelated.

Cryptoperidiniopsis, a dinoflagellate, first found in June 1997 in the St. Johns River near Jacksonville, FL by FDEP researchers, has been found at five sites in the St. Lucie estuary (Roosevelt Bridge, Jaycee Park on the Stuart Causeway, Bird Island, northern Hobe Sound, and South Point. This dinoflagellate, a type of microscopic algae, is a possible factor in the on-going fish disease event because it was also found in areas in Maryland where fish with lesions were found last summer. All samples arc being screened for known and new species of dinoflagellates. FDEP has also found other small dinoflagellates that may play a role in this fish disease event. Each of these species is being identified and will be tested for potential toxicity.

The FDEP laboratory in St. Petersburg has received over 400 fish representing 22 species from the east coast. The severity and types of lesions are being characterized and documented. Selected specimens are further analyzed to look for microscopic changes in the fish tissue. Specimens of mullet and jacks with lesions have been sent to Texas A&M University for analysis of pesticides and heavy metals. Samples of other species with lesions, unaffected species, and healthy specimens of affected species were sent for analysis the week of May 18. Results of these analyses will be made available when they are completed. Water and sediment arc being tested for pesticides and heavy metals at the FDEP Central Laboratory in Tallahassee, and for bioactive compound testing at NOAA's National Ocean Service Charleston Lab. In addition, NOAA's Coastal Ocean Program is providing funding for identifying *Pfiesteria*-like organisms. Historical water

quality data collected by several agencies are being analyzed by researchers and compared with current information.

Bacteria isolated from the fish have primarily been those types normally found in fresh water. Fungi were found in the ulcers in mullet collected in early March and are believed to be saltwater species. These bacteria are primarily opportunistic pathogens. This means they arc not a problem for fish except under unusual conditions or stress. Bacteria isolated from fish kidneys appeared to be in the process of being excreted by the fish. The only organisms that appeared to be involved in the actual formation of mullet ulcers were the fungi. No one type of bacteria has so far been implicated in causing the ulcers observed in the different species of fish, although many of the bacteria isolated may be contributing to the problem. Towards the end of March, and in both April and May, other bacteria indicative of terrestrial runoff were also found in the fish and water samples. Normal freshwater bacteria however, predominated in the samples. Samples are still being analyzed.

During the week of April 20 fish populations in the lower Indian River Lagoon and the St. Lucie River were sampled. Established FDEP Fisheries Independent Monitoring (FIM) sampling protocols were used to obtain a statistically valid "snapshot" of the fish populations. Each of 5,825 fish caught in the 41 hauls of a 600 ft. seine was identified to species, measured, and examined for any external damage, abnormalities or lesions. Overall, 97% of the fish caught were healthy and only 45 fish had lesions (0.77%). In the St. Lucie River area, 925 fish were caught and 39 fish had lesions (4 22%). Any fish showing abnormalities were transported to St. Petersburg for further analysis. Additionally, the number and variety of fish caught in the Indian River sites were similar to those collected in sampling previous to the fish disease event. This type of sampling has not been conducted in the St. Lucie River so numbers are not available for comparison.

(The Florida Department of Environmental Protection contributed to this article)

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◆ South Africa

Brown tides on the west coast

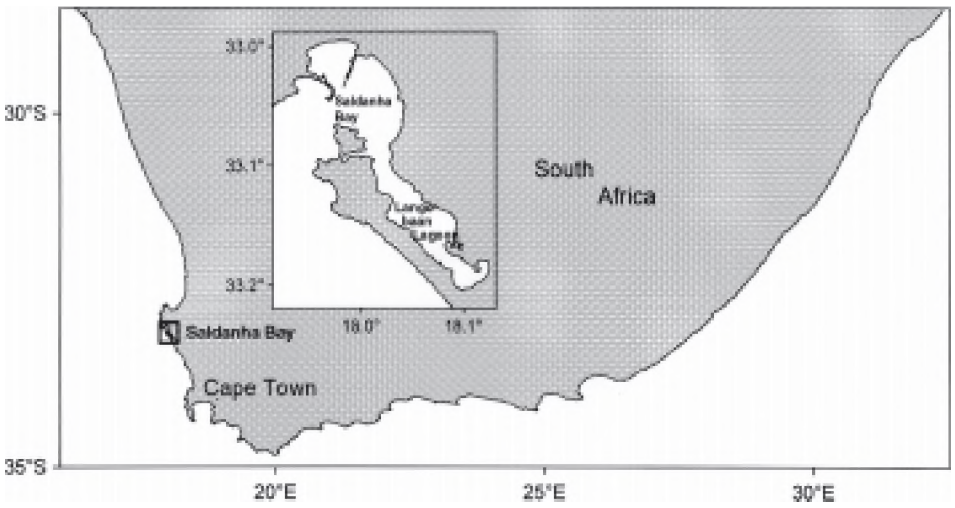
Saldanha Bay is a semi-enclosed embayment which is linked to the Benguela upwelling system on its western side and feeds Langebaan lagoon, a shallow tidal body of water, situated at the southern end of the Bay (Figure l). Both systems are suitable for mariculture, not only because they offer the protected waters often necessary for cultivation, but their strong link to the highly productive west coast upwelling system ensures a food environment and temperature regime optimal for the farming of shellfish.

During the latter months of the summer of 1997, oyster farmers in Saldanha Bay complained of discoloured water and reduced oyster growth rates, and inspection of water samples revealed the presence of many minute (2-3 mg), coccoid, non-motile cells. Again during the latter months of the summer of 1998 similar complaints were received. On this occasion queries and complaints were also received from the public and from conservation groups as the entire Saldanha Bay and Langebaan Lagoon turned a very unusual and distinct golden brown colour. The same non-motile coccoid cells were again evident on examination of water samples.

In further examining the 1998 event it was difficult to establish exactly when the bloom manifest itself within the Bay and Lagoon. Although the bloom was first reported on the 11 February 1998 it is thought that the bloom may have been present for some time before this date. Oyster harvest figures from the farm *Seafarms* clearly depicted the impact of this bloom on oyster growth (Figure 2). The oyster farm operates by continually sorting and harvesting oysters greater than 50 g mass. For the period November 1996 – May 1998, two periods of reduced harvest in addition to the seasonally induced low in winter were evident. During both years the harvest was lowest during February and March when the bloom was present in the Bay. In 1998 when the bloom was particularly noticeable the oyster harvest and sales were extraordinarily low. In addition to the impact on oyster growers, mussel farmers were also negatively affected. Instead of obtaining typical yields in excess of 70 kg per rope for that time of the year, the yield of market size mussels in March 1998 dropped to about 20 kg per rope. Mussels also developed an uncharacteristic ridge on the shell during the period of the bloom indicating growth arrest during this period. Both oyster and mussel growth rates appeared to recover following the very sudden disappearance of the bloom on 18 March 1998.

At the level of the light microscope identification of the small coccoid cells so dominant in water samples was diffi-

Figure l: Saldanha Bay a semi-enclosed embayment on the west coast of South Africa, linked to the Benguela upwelling system on its western side and feeding Langebaan lagoon at its southern end.



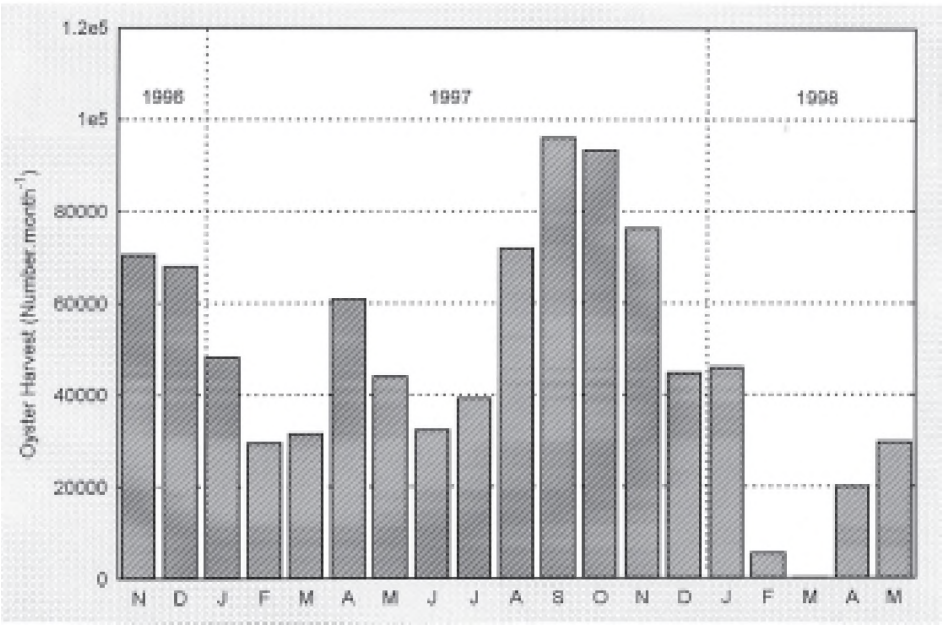
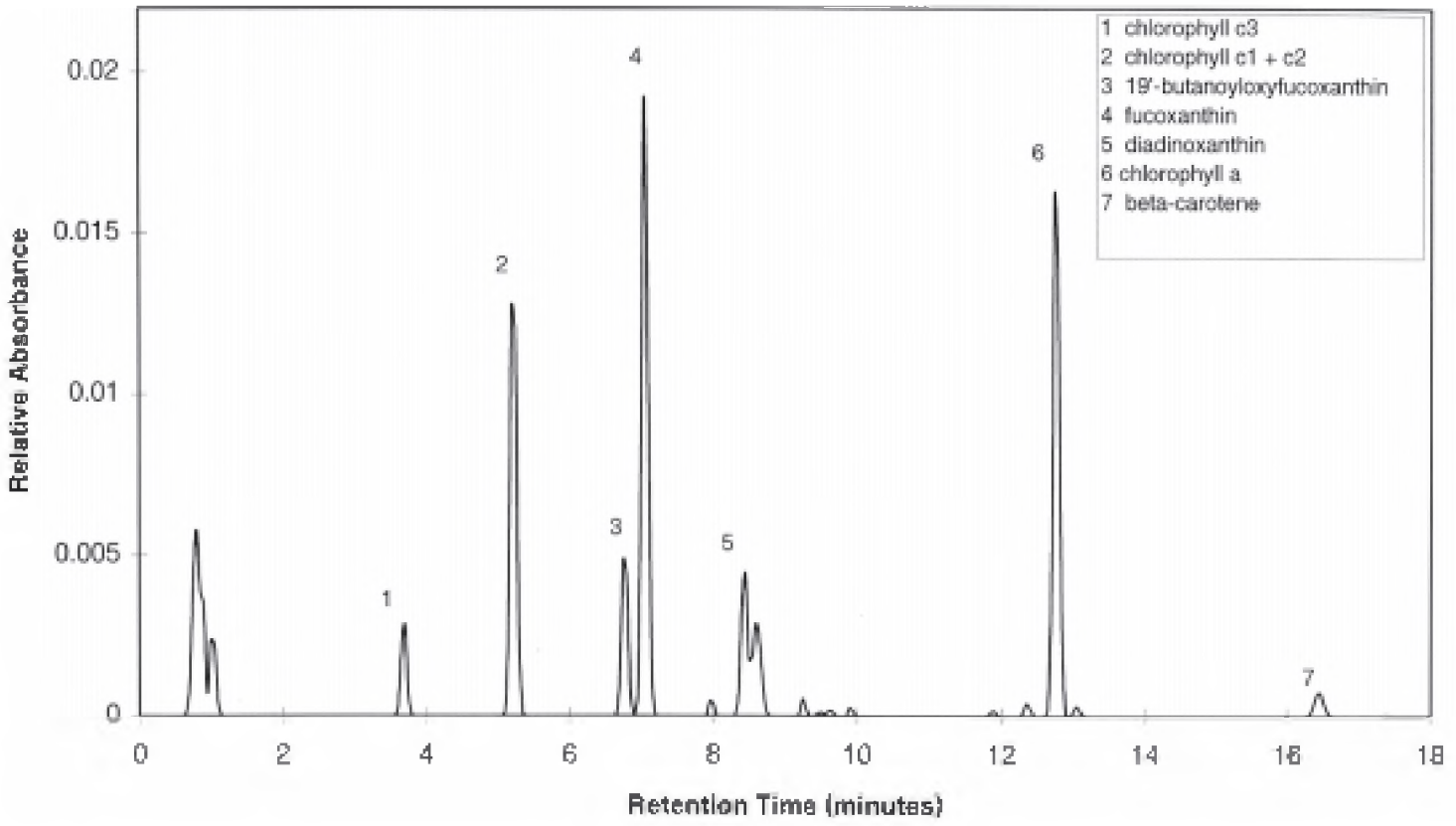


Figure 2: Oyster harvest figures from the farm Seafarms *for the period November 1996 May 1998.*

cult and further studies of this organism using transmission electron microscopy are ongoing (Figure 4[4]). Surface water samples collected during March 1998 from several localities within the Bay and Lagoon indicated that concentrations of the coccoid picoplankton ranged from 1.2-3.1x109cells.l-1 and corresponding chlorophyll *a* concentrations from 21.3-41.0 mg.m-3. Although these small non-motile cells dominated the samples a species of *Chrysochromulina* was also common and in the only sample in which it was counted a concentration of 67x106 cells.l-1 was recorded (Figure 4[1-3]).

Although the exact identity of the dominant picoplankton has yet to be established observations during this event were very similar to those during the so-called "brown tide" blooms, which have impacted coastal embayments along the mid-Atlantic coast of the United States since the mid-1980s. These blooms were attributed to the Chrysophyte *Aureococcus anophagefferens,* which was found to inhibit bivalve grazing, including scallops and mussels, as a result of the production of toxic or at least inhibitory substances on the cell surface. *A. anophagefferens* and a few other Chrysophytes such as *Pelagococcus subviridis* are characterised by various pigments including certain chlorophyll *c* pigments, 19'-butanoyloxyfucoxanthin, fu-

Figure 3: Chromatogram of a Saldanha Bay sample collected during March 1998.



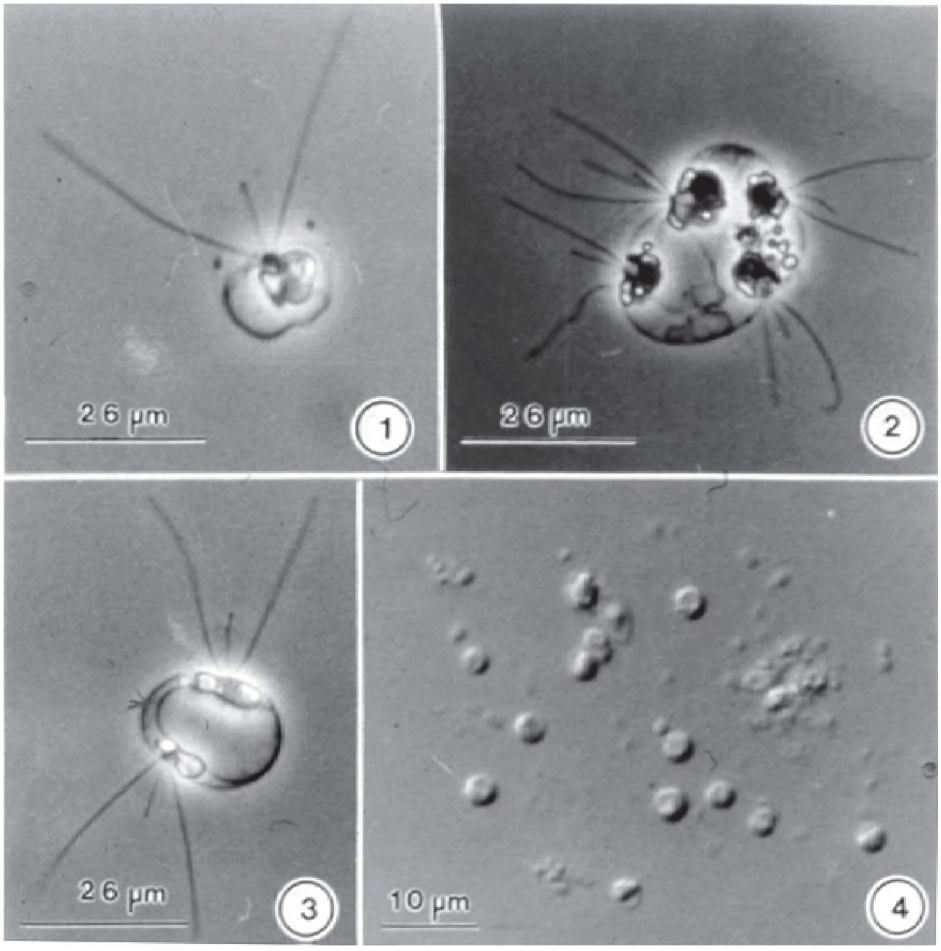


Figure 4: Light micrographs of (1-3) a Chrysochromulina *species and (4) the small, coccoid, non-motile cells which together dominated the phytoplankton of Saldanha Bay and Langebaan Lagoon during February and March 1998.*

coxanthin, diadinoxanthin, chlorophyll *a* and ß-carotene. Analysis of Saldanha Bay samples by means of High Performance Liquid Chromatography revealed the presence of the same pigments including 19'-butanoyloxyfucoxanthin which distinguishes *A. anophagefferens and P. subviridis* from most other Chrysophytes which lack this pigment (Figure 3).

As was the case in the embayments of the United States, the reduced shellfish production in Saldanha Bay was most likely attributable to the small coccoid chrysophyte which dominated the phytoplankton. However, the possibility of the *Chrysochromulina* species, which was also a conspicuous component of the phytoplankton, impacting negatively on the shellfish cannot be discounted, as certain species of this genus are known to produce toxins. Few sites are suitable for mariculture on the South African coast and although present mariculture concerns in Saldanha Bay are relatively small, there is considerable potential for expansion of shellfish culture. The entire mussel industry is for example located in Saldanha Bay where the mussel *Mytilus galloprovincialis* is cultivated on ropes suspended from rafts, and although various explanations and causal mechanisms for these brown tides have yet to be explored their recurrence may well threaten the future development of shellfish culture within this region.

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FIRST ANNOUNCEMENT



JUNE 19-24, 2000

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◆ South Africa

Saving a wetland and a marine mussel resource impacted by cyanobacterial hepatotoxins

In the first such incident recorded in South Africa, microcystins produced in a coastal wetland accumulated in black mussels, Choromytilus meridionalis, in the intertidal zone at the point of discharge of the wetland to the sea. This necessitated a four-month ban on harvesting, during which time a bold initiative was launched to eradicate the responsible cyanobacterium.

During late 1997, a partial collapse of the sago pondweed (Potamogeton pectinatus) community in the Wildevoelvlei wetland, situated on the west coast of the Cape Peninsula, south of Cape Town (see Map), resulted in a dense bloom of Microcystis aeruginosa. Shortly after the onset of the bloom, the hepatotoxins microcystin-YR and -LR were detected in freeze-dried algal extracts, and subsequently in the tissue of mussels collected from the reef adjacent to the wetland outflow to the sea (Figure 2). An immediate ban was placed on the collection of shellfish by a community relying on this resource for both food and income.

The Wildevoelvlei wetland, a shallow (mean depth = 1 m), 25 ha system comprised of two interconnected basins, receives between 4 and 7 megalitres of treated wastewater effluent per day and, as a consequence, is perennially-hypertrophic. The wetland is further threatened by the uncontrolled inflows of three large urban runoff carriers. As a consequence of this regulation, the wetland has lost both its seasonal character, and the beneficial effects of intermittent tidal ingress.

In order to eradicate the bloom of M. aeruginosa, an initiative, proposed by the author, was launched to temporarily enhance the salinity of the eastern basin of wetland through the addition of coarse rock salt. Alternative options to pump seawater were neither practicable nor feasible. Experience gained from other coastal lakes and estuaries in the region indicated that M. aeruginosa has low salinity tolerance, while the other, desirable components of the wetland biota could withstand an elevation of salinity levels to between 7 and 10 ppt for a short period.

After lowering the water level of the wetland, 600 tonnes of salt was added to approximately

50 000 m^3 of water in the eastern basin (see Map). The salt was applied, in two 300 tonne batches, seven days apart, as a solid using aerial application from a purpose-built pallette transported by a helicopter, and in dissolved form via one of the clarifiers in the adjacent wastewater treatment plant. For the duration of the operation, effluent outflows from the wastewater treatment plant to the wetland were curtailed.

Salinities rose to 3.5 ppt after the first application, and to 8 ppt after the second. There was an immediate decline of the M. aeruginosa bloom, with the concentration of chlorophyll-a falling from 700 to 150 microgrammes per litre during the first week. Thereafter the cyanobacterium was replaced by a bloom of Kirchneriella sp., followed by a clear water phase (chlorophyll-a less than 5 microgrammes per litre), dominated by chlorophytes and diatoms, together with the zooplankter Daphnia longispina. Salinities started to decline after 14 days, i.e. once it became necessary to once again release effluent to the wetland. The ambient salinity of the system had returned to zero by end-May.

By the end of April complete depuration of the mussels had occurred, such that the restrictions on harvesting could be safely be lifted by end-May.

The bold use of artificial salinity enhancement on this scale demonstrated the value of this environmentally-sensitive technique for use in coastal lakes and estuaries where site-specific characteristics and logistics allow. The short-term elevation of the ambient salinity of this, previously-estuarine, system resulted in the rapid and total eradication of the toxin-producing cyanobacterium, and the concomitant alleviation of the risks to human and animal health in both the freshwater and marine environments. The application further highlighted the intrinsic value of retaining estuarine character and tidal interaction in coastal lake systems which have been modified through catchment development.

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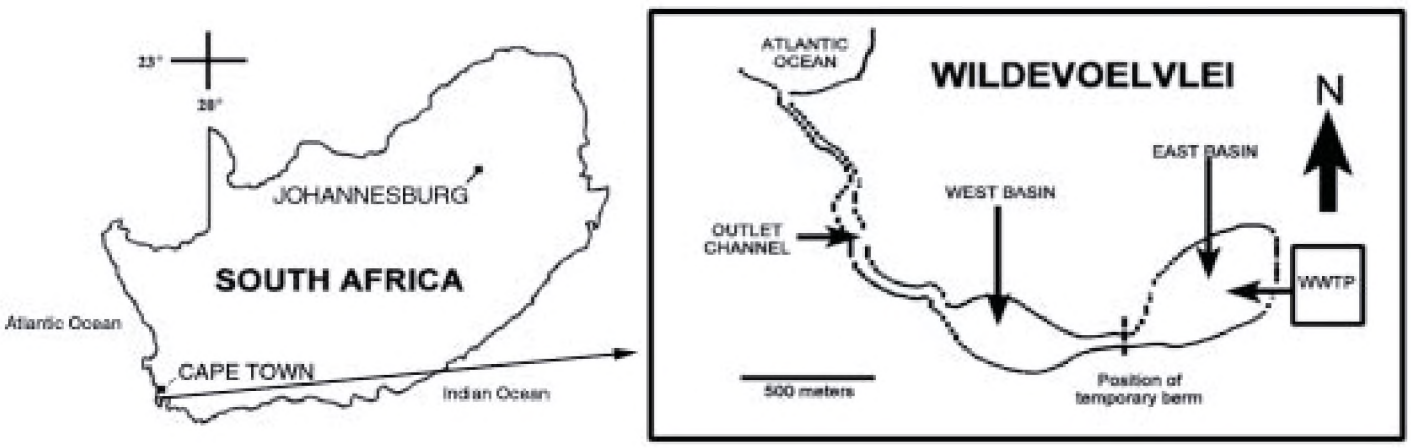
Figure 1:
Map showing the location of Wildevoelvlei in relation to South Africa, together with an insert showing the essential features of the wetland.



INTERGOVERNMENTAL OCEANOGRAPHIC COMMISSION
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SCOR
SCIENTIFIC COMMITTEE ON OCEANIC RESEARCH

IOC and SCOR launches new science programme on HAB

From 13th to 17th of October 1998, 37 scientists including phytoplankton ecologists, physical oceanographers, algae-physiologists and marine chemists met at Havreholm, Denmark, to formulate the outline of a new international science programme on the ecology and oceanography of harmful algal blooms,- GEOHAB

The initiative for this new science programme is taken jointly by the IOC and the Scientific Committee on Oceanic Research, SCOR.

The ecological, economic and health problems caused by harmful marine microalgae are expanding. The HAB phenomena have many causes, both natural and anthropogenic, but what can be done about them in a practical sense? The answers, of course, require scientific investigation, and fortunately the community of scientists working on HAB problems has grown in size and skill in parallel with the expansion of the problem. To date, however, there is little effective international coordination of these efforts, at least in the realm of scientific research. What is clearly needed is a coordinated international scientific program on HABs that incorporates the full participation of numerous countries. The rate and extent of progress will depend in large part on how effectively the skills and energies

The participants at the SCOR-IOC GEOHAB Workshop, Havreholm, Denmark, October 1998. (The HAB research community is getting younger and younger.....)



of the scientific community can be targeted on key problems. In particular, there is a need to understand the population dynamics of the toxic species, leading to predictive capabilities and management strategies, and, to the extent possible, mitigation plans. This requires a thorough understanding of the ecology and oceanography of harmful algal blooms.

Consequently, SCOR and the IOC have taken joint initiative for a new international science programme on the global ecology and oceanography of harmful algal blooms – GEOHAB. Along with several agencies in the US and in Europe, SCOR and IOC agreed that the first step was to bring together key researchers to agree on common objectives for this coordinated approach. The goal of the workshop was thus to develop consensus on the objectives and to draft a Science Plan for the proposed programme for approval by IOC and SCOR. Recently, the governing bodies of both the IOC and SCOR have endorsed GEOHAB as a new joint programme.

The focus of GEOHAB is squarely on ecology and oceanography. Beyond that, it is up to the participants to define the programme. The draft Science Plan is in essence a request to proceed, which is expected to lead to a much broader consultation to identify the major features of an international programme. The main purpose of the Havreholm Workshop was thus to set in motion a much larger and inclusive process aimed at developing an Implementation Plan as soon as possible. The Organizing Committee of the Havreholm Workshop was Don Anderson, John Cullen (Chair), Patrick Gentien, Edna Graneli, Kaisa Kononen, Tom Osborn, Adriana Zingone, Henrik Enevoldsen (IOC), Elizabeth Gross (SCOR), Kaisa Kononen (Nessling Foundation), Kevin Sellner (US-NOAA), Bjorn Sundby (SCOR), Phil Taylor (US-NSF), M. Carr (US-NASA).

The development of GEOHAB will be report in this newsletter including annoncements of comming activities.

The Report from the Havreholm SCOR-IOC GEOHAB Workshop is available at

http://www.phys.ocean.dal.ca/~jhurst/SCOR/GEOHAB/GEOHAB.html

Additional information can be required from etiher the IOC at fax: +45 33 13 44 47, E-mail: hab@bot.ku.dk, or the SCOR Secretariet at fax: 1-410-516-4019, e-mail: scor@jhu.edu

◆ International

The fourth international conference on toxic cyanobacteria, Beaufort, North Carolina, 27 September-1 October 1998

Whether the maritime community of Beaufort, North Carolina, at times beset with problems with *Pfiesteria piscicida*, the attendant media interest, and hurricanes, was aware of the Fourth International Conference on Toxic Cyanobacteria in their midst, I am not sure. If so, they may have wondered, justifiably, "why us?" and "what next"? The reasons included the facts that Beaufort was an excellent location for a scientific conference and that the necessary experience and ability in the fields of cyanobacterial toxicology and ecophysiology were on hand (W.W. Carmichael, H.W. Paerl and colleagues) to attract and organise an international forum in these developing multi- and interdisciplinary fields. About 157 participants attended from 27 countries and about 50 oral papers and 65 posters were presented.

The Beaufort Conference differed from a series of recent triennial international meetings on cyanobacterial toxins due to its US location and to a broadening of interest and participation in relevant environmental, applied and management issues, in addition to advances in the basic science of the subject. Previous international meetings on cyanobacterial toxins over the past decade have been held in Europe and Australia. This has reflected a relatively high level of interest and action among environmental agencies, health authorities and water utilities/companies in the occurrence, properties and impact of toxic cyanobacterial blooms and the toxins in European and Australian environments and resources. The holding of the latest meeting in the USA has coincided with a noticeable increase (to this European participant) in the recognition and need for management of toxic cyanobacterial blooms and toxins in North America, taking into account the long-established core of US research expertise in the field. This was evidenced, for example, by the financial support and high level of interest of the US Environmental Protection Agency at the Conference, in addition to the US National Oceanic and Atmospheric Administration.

Presentations were organized in the following sessions:

- Chemistry, Detection, New Toxins
- Cyanobacterial Toxin Regulation, Production, Degradation and New Approaches to the Taxonomy of Toxigenic Cyanobacteria
- Ecology, Toxic Waterbloom Formation and Biotic Interactions
- Toxicology, Health and Economic Consequences of Toxic Cyanobacteria
- Cyanobacterial Toxin Risk Factors, Occurrence and Management of Toxic Waterblooms.

A selection of developments, particularly since the previous international cyanobacterial toxins conference (Bornholm, Denmark, 1995) includes:

1, increased applications of molecular and computer-based methods to cyanobacterial taxonomy to complement the traditional phenotypic and environmental approaches (e.g. J. Komarek; B.A. Whitton; C.J.S. Bolch and S.I. Blackburn; J. Lehtimäki *et al.*, F. Pomati *et al.*, M. Gugger *et al.*, D. Schatz *et al.*, B.A. Neilan).

2, increased attention to the structure and activities of numerous cyanobacterial peptides in addition to microcystins and nodularins (e.g. R.E. Moore; K. Sivonen *et al.*; A. Forchert *et al.*; K-I. Harada *et al.*).

3, the analysis, fate and management of cylindrospermopsins, produced by *Cylindrospermopsis raciborski* and *Aphanizomenon ovalisporum* (e.g. G. Shaw; R. Chiswell *et al.*; R.L.G. Norris *et al.*; M.J. Smith *et al.*; A.K. Törökné *et al.*).

4, the toxicological significance of cyanobacterial picoplankton. Toxicity associated with cyanobacterial picoplankton (e.g. *Synechocystis*, *Synechococcus*) is occasionally reported and the need to determine whether strains and mass populations of these common members

of the phytoplankton have the capacity to produce toxins exists (e.g. L. Blaha *et al.*; B. Oudra *et al.*), not least because these small cells can pass through some conventional water filtration systems.

5, advances in cyanobacterial detection and analytical methods. Although physico-chemical methods continue to be applied and occupy a central role, immunoassays, mainly for microcystins (and nodularins) are being used increasingly. These include ELISA using antibodies to microcystin-LR (e.g. W.W. Carmichael *et al.*; K.A. Beattie *et al.*; E.Y. Hirooka *et al.*) and CIPPIA, a microcystin immunoassay coupled to protein phosphatase inhibition assay (J.S. Metcalf *et al.*; S.G. Bell *et al.*).

6, advances in the basis and mechanisms of toxin production. The genetic and enzymic basis of microcystin production continues to be identified: a large gene cluster has been identified and cloned in *Microcystis* which is characteristic of microbial non-ribosomal cyclic peptide synthetases (D. Tillett *et al.*) and is involved in microcystin production in *Microcystis* strain PCC 7806 (E. Dittmann *et al.*; T. Börner *et al.*). A cyclic peptide synthetase operon has also been cloned from an *Anabaena* strain and characterization of the peptide product is in progress (L. Rouhiainen *et al.*).

7, identification and implementation of quality control procedures and standards for cyanobacterial dietary supplements. Although cyanobacteria have been used as traditional foods in ancient societies (e.g. China, Mexico, North Africa), the recent industrial scale of cyanobacterial biomass production for human dietary supplements, and increasing awareness of the occurrence and health significance of cyanobacterial toxins, have focussed attention on the need for policy development and procedures to ensure that cyanobacterial toxins, if present, are within acceptable limits. C. Drapeau and F.B. Gutermuth, and D.J. Gilroy and F.S. Chu presented posters on these needs and the actions now being established. The latter authors detailed the background to the establishment of (perhaps the world's first?) regulatory standard for a "safe level" for microcystins in cyanobacterial products, namely 1 microgram per gram dry wt, introduced by the Oregon Health Division in 1997.

8, policy development for the protection of health. One of the most obvious developments since Bornholm '95 was the increase in interest which has been given at inter- and multinational level to the development of policies for the recognition and management of circumstances leading to the presentation of unacceptable health risks by cyanobacterial toxins. Previous national initiatives e.g. in the United Kingdom, Australia, Germany and the USA are now complemented by a World Health Organization development. The work of the WHO in developing "guidelines" for the protection of health has been extended to cyanobacterial toxins. The derivation in 1997 of a WHO (provisional) Guideline Value of 1 microgram of microcystin-LR equivalents per litre for drinking water was discussed (I.R. Falconer) and an outline presented by J. Bartram of a forthcoming WHO book on cyanobacterial toxins in water, to which a number of the Conference participants had contributed.

At times in the Conference, I was reminded of the films, which everyone must have seen, on the construction of an igloo. The blocks of packed and neatly-sawn snow are built up by the igloo-maker and the structure begins to rise and narrow to completion. As indicated at this Conference, blocks, the "wheres", "whens", "whats" and "hows" of cyanobacterial toxins understanding, are being identified and fitted. Many spaces between the adjacent blocks still need to be filled, an exercise of vital importance to turn an igloo from a circle of snow blocks into a strong, usable cohesive structure. The key element which is needed to hold the igloo together, and, if fashioned from a slab of ice, which can provide some light to the interior, is the uppermost, central "key" block. This latter can be considered to be the "why" of the cyanobacterial toxins "igloo" and a suitable block has yet to be found. There may be more than one reason for cyanobacterial toxin production and candidates (such as deterrence against grazers and intracellular signalling messengers) continue to promote speculation and experiment. However, understanding of the reason(s) for cyanobacterial toxin production remains inadequate and must surely remain as a major requirement in the subject. For the present, Wayne Carmichael and Hans Paerl and their colleagues are thanked for a most useful and enjoyable Conference.

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Announcement

APEC-IOC Symposium on management and mitigation of HAB's Philippines, May 1999

The purpose of this Symposium is to facilitate the management and mitigation of the impacts of HABs. The symposium is a cooperative effort of several organizations, including APEC (Asia Pacific Economic Cooperation) and the IOC, which have recognized the need for focused programs on the management of HABs. It is intended that the symposium will provide managers with training and exposure to current methods and strategies used throughout the world for managing HABs and their diverse impacts. It is also intended that the symposium will provide the basis for compatible regulatory procedures.

The geographic emphasis of the symposium are in the Asia Pacific region, in recognition of the significant impact HABs have had in that region and the importance of fish and shellfish for food and for commerce throughout the region.

Distinct from the many scientific conferences on HABs the Conference will focus attention primarily on exploring practical, currently available options for their management and mitigation. It is hoped that there will emerge from the symposium a consensus with a solid technical and practical basis that will provide for policy makers a spectrum of management options applicable to the needs of diverse economies and cultures, faced with an array of HAB problems and societal constraints. In addition to the proceedings, it is intended that the symposium will produce a brief guidance document, outlining these management options, that will assist the various economies in developing or improving their own HAB programs. Practical training workshops and demonstrations will be held before, after, and throughout the symposium.

For more information please contact: Dr. Sherwood Hall, FDA HFS-426, 200 C Street SW, Washington, DC 20204, E-mail: SHall@bangate.fda.gov

International Society for the Study of Harmful Algae
I S S H A

The increasing public and scientific awareness of the destructive effects of harmful algae, coupled with reports of increasing frequency, intensity and geographical distribution (now worldwide) of these events, led to the formation of the International Society for the Study of Harmful Algae (ISSHA), an international non-governmental, nonpolitical and not-for-profit organization. The realization of the need for the creation of ISSHA arose through a series of international conferences on harmful algae that began in 1974 with the First International Conference on Toxic Dinoflagellate Blooms.

The initiative for ISSHA was given further impetus by the request of the Intergovernmental Oceanographic Commission (IOC) of UNESCO for an international programme on harmful algae. The formation of the Society was first addressed at the Sixth International Conference on Toxic Marine Phytoplankton, October 1993, in Nantes, France. The Society was formally established at the Seventh International Conference on Harmful Algae, Vigo, Spain, June 1997 by the election of officers (President, Secretary, and Treasurer) who are charged with the responsibility for coordinating the initial activities of ISSHA.

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Secretary: Dr. Allan Cembella,
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E-mail hab@bot.ku.dk.

Requests for membership should be directed to the Treasurer, all other inquiries to the Secretary.

The Society will seek affiliation to the ICSU family of organizations as constituent part of the International Union of Biological Sciences (IUBS) through the International Association of Biological Oceanography (IABO). Observer status will be sought from the Intergovernmental Oceanographic Commission (IOC) of UNESCO.

The Society shall; a) promote the study of harmful algae, including their occurrence, related oceanographic factors, ecophysiology, taxonomy & systematics, genetics, toxin chemistry, toxicology, management and mitigation; b) collect, evaluate and disseminate information on harmful algae; c) promote harmful algal research, training and extension for fostering the related management and mitigation; d) develop material for the promotion of public awareness of the social, economic and ecological effects caused by harmful algae; e) arrange and co-sponsor national, regional and international conferences, seminars, symposia and working group meetings; organize lectures and courses; publish scientific and popular articles; f) support and implement research projects and programmes; g) promote consultation and cooperation with other appropriate organizations, public and private; h) take any other action deemed appropriate to further the objectives of the Society.

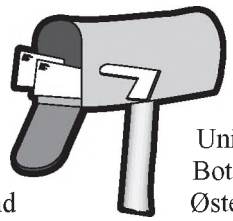
The Society is registered in Copenhagen, Denmark, an has at the moment a membership of 230 scientists worldwide. The membership of the society compromises (i) honorary members: such members will be elected by the Council, on the recommendation on the Executive Committee, in recognition of their outstanding services to the furthering of the objectives of the Society; (ii) institutional members: such members will be from government organizations, academic and research institutions, science academic, professional societies, public and private industries, and bilateral and international organizations; (iii) individual members: such members will be from individuals interested in promoting the aims and objectives of the Society.

The Society has revenue derived from annual contributions paid by members. Institutional members will pay an annual subscription of not less than 40 US dollars or its equivalent in Danish Crowns/EURO. Individual members will pay an annual subscription of not less than 20 US dollars or its equivalent in Danish Crowns/EURO. The Society has generously received a significant contribution from the Organizers of the 7th International Conference on Toxic Phytoplankton, which was held in 1995 in Sendai, Japan.

The 1999 activities of the Society will be announced in the next issue of this newsletter.

Identification Service

The IOC Science and Communication Centre on Harmful Algae, Copenhagen, and the Asian Natural Environmental Science Center offers an identification service. If you have problems with identification of harmful algal species, the Centres may assist you. You should send samples to one of the two addresses given below. It should be noted that this assistance is not given for routine identification/monitoring of samples, but applies to particulary difficult species, or to species that require special techniques e.g. electron microscopy, for identification.



IOC Science and Communication
Centre on Harmful Algae,
Univeristy of Copenhagen,
Botanical Institute,
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DK-1353 Copenhagen K., Denmark

Asian Environmental Science Center,
The University of Tokyo,
Att.: Dr. Y. Fukuyo, Yayoi 1-1-1,
Bunkyo-ku, Tokyo 113, Japan.

◆ New Zealand

Mortality among marine life in Wellington Harbour

Wellington City is the capital of New Zealand and surrounds a shallow natural harbour located at the southern tip of the North Island of New Zealand about 410 16' S; 1740 50' E. The harbour has a surface area of about 85 km2, a maximum depth of 31 m, and is, on average, 14 m deep with a volume of about 1,400 m3. Freshwater input into the harbour is relatively small (daily maximum 180 X 106 m3; minimum 2.6 X 106 m3) from a catchment of 630 km2.Residence time of water in the harbour is about 9 days and is replaced by clean oceanic water from Cook Strait.

During the last week of February, 1998 a *Gymnodinium mikimotoi* bloom of unprecedented intensity developed within the harbour, and at its height in early March, cell densities reached 30 million per litre. Dead and dying marine fish including seaward migrating freshwater eels were first noted during the first week of March, 1998.Mortalities were being actively harvested by sea gulls with no apparent effects on the scavengers. Fish and some shellfish continued to be washed up along the harbour beaches over the next two weeks. Concurrently, seawater entrapped in the more sheltered eastern bays and in the inner Harbour became straw coloured, and a build-up of yellow-green deposits on the surface of the sub-tidal sediments began to accumulate.

On Tuesday 24 March, video footage of the phenomenon was taken between 1.0 m and 10.0 m. The video revealed that marine life at all levels of complexity and representing all trophic levels, with the possible exception of macroalgae, had been affected, and that both invertebrates and vertebrates had been dying for at least two weeks, as estimated from the state of decomposition shown by animal carcasses. Dead and dying animals were not being scavenged or predated, and this indicated an absence of predators. Also, there were no signs of bioturbation, indicating that animals living beneath the sediment surface were also inactive or dead. This was confirmed during April, when sediment samples from the harbour basin showed that species richness, abundance, and biomass of the benthic biota was down to about 10% of previous baseline levels. The zooplankton and pelagic fish fauna recovered quite rapidly within one month, primary through infusion of new Cook Strait water

I lead a small team of staff and graduate students at the Victoria University Marine laboratory who are committed to evaluating the present post-bloom status of the intertidal and benthic biota. We will be concentrating on the benthic biota of the harbour basin (depths > 10 m) which is a low energy environment with sediment organic carbon levels of 6% or more. About 90% of the sediments lie in the silt/clay fractions. We are fortunate in that our previous work provides a reasonably good quantitative baseline for the pre-bloom biota, and specific stations are re-locatable by differential GPS. These have already been sampled with a vanVeen grab. It appears that only the larger, deep burrowing crabs, maldanid and eunicid polychaetes have survived – the previously dominant smaller crustaceans such as amphipods, the echinoderms, bivalves, echiurids, flounders and epibenthic fish are all mostly absent.

We intend to monitor the recovery of the harbour biota over time, perhaps for 3 or 4 years so that cycles of dominance can be seen. We will be using the usual shannon-weiner indices of diversity and even-ness, indices of similarity, and ABC k-dominance statistical methods to back up our qualitative observations and species identifications. Our present opinion is that although the migrant species may be relatively unaffected, and that the intertidal biota may recover quite rapidly, the benthos may take some years to return to pre-bloom community structure. It will be interesting to see what will occur in the absence of bioturbation and nutrient recycling by the benthos.

Dr R.G. Wear, Associate Professor, School of Biological Science, NZ

REVIEW

By Roger Bradburry, Bureau of Resources Sciences, Canberra, Australia

The Ecology and Oceanography of Harmful Algal Blooms

A special issue of *Limnology and Oceanography*

(Volume 42, Number 5, part 2, 1997) D M Anderson & D J Garrison (eds)

I had, in my formative years, a professor who liked his whiskey neat and his science pure. This was in the years before such barbarisms, such mongrels, as whiskey & cola or environmental science, so Bill"s greatest worries were that some barman might reflexively dilute his drink and that someone, somewhere might find his science useful.

I am not, of course, talking about someone finding his science useful in the scientific sense: that some other scientist, some peer, might use his science to advance his own, to help that peer add another brick to science"s edifice. Bill fully embraced the cooperative, collegial ethos of science. What he was afraid of, rather, was that some outsider, some lay person, might find his science useful for solving a non-scientific problem, might

dilute his science with some utilitarian concern of the workaday world.

As a result, Bill had a broad and fertile scientific career, as he moved from field to field pursued by the phantom of utilitarianism. He began as an experimental physiologist with elegant work on the excretory system of the liver fluke, *Fasciola hepatica*, not because this might help sheep farmers, but because parasites offer superb scientific challenges in understanding adaptation in the ultimate habitat, another organism. However such basic knowledge helps design parasite control strategies. He worked for a while on the behaviour of alpheid shrimp, the ones that click with their nippers to defend their territories, and became fascinated with the underwater soundscape, until the navy, with its more covert interests, offered to fund his research. Crab taxonomy came next, but this led to an interest in numerical taxonomy, and from that flowed work on the classification and ordination of benthic communities, just as coastal development accelerated, and his techniques were in demand for Environmental Impact Statement for every coastal project in the nation.

Had he not retired, I think Bill would have moved to algal ecology next. Now there is a safe scientific backwater, a place where one could take the time to think and dream and understand.

Or so I thought until the special issue (Volume 42, Number 5, Part 2, 1997) of *Limnology and Oceanography* landed on my desk. It is entitled "The ecology and oceanography of harmful algal blooms" and it is an issue that just reeks of utility. Indeed the whole field of harmful algal blooms or HABs, as they say in the journal, has the feel of a subject on a roll ("unstoppable momentum"). It has the indicators of some science that has suddenly found itself useful. It has found a market for its product. For starters, it has gentrified itself, tried to move its brand upmarket, as it were, by some clever rebranding, draping itself in a sonorous sobriquet instead of the simpler 'red tide". HABs sound so much more serious and expensive, so much more bankers" pinstripe, than the blue denim of red tides. It has changed its advertising to reflect this new positioning: HABs are much more complex than previously thought, and hence their science that much more expensive to buy. It has diversified its product line to saturate the market: there are more sorts of HABs than you can point a stick at, each in urgent need of work.

Thus HAB research is going the way of climate research and other environmental crises that make a special claim on the public purse beyond the apparent merit of their science. Because such rent-seeking is completely intertwined with the science, when we review the science, we must also review the special pleading, and the interaction between the two. Funds, it seems in the world of crisis, follow fear, just as surely as in biology, form follows function. So we must ask questions about the merit of the science, and also whether the privileged access to the funds has resulted in a commensurate rise in the quality of the science.

So what do I see when I look at HAB research from this perspective, using the L&O issue as the primary evidence?

Firstly, I see a science that is Newtonian rather than Darwinian. One might say proudly or even muscularly Newtonian. The papers of Franks and Donaghay & Osborn are a case in point. Here blooms are treated as if they are the result of the working through of a Newtonian dynamics applied to some particles or fluids. Much is made, in several papers of the first section of the issue, of environmental forcings setting up a bloom. In this reading of the science, it seems to me to be in thrall to physics rather than biology. Perhaps the fact that the organisms involved are so small makes them seem as if they are more like inanimate particles than living things. Perhaps the need for a lot of hardware in studying blooms encourages the development of physical rather than biological metaphors in their analysis. Perhaps the physical oceanographers just claimed the territory first.

Now this is a pity, because Newtonian approaches are particularly unsuitable for these sort of phenomena, even if we set aside any general disquiet about the misfit of Newton and biology. Think instead about some of the defining characteristics of blooms: they are outbreaks, singularities, discontinuities; they are complex, heterogeneous; they have histories. Newtonian approaches are ill-equipped to deal with such phenomena. Classic Newtonian systems are cyclic not singular, continuous not discontinuous, simple not complex, ideal not historic.

So I looked hard amongst the physics for Mr Darwin, searching for the wildflowers amongst the thistles and weeds. And I only found his shadow, or where he might have been. The physiology and biochemistry, molecular biology and genetics, such as the papers by Plumley and Sellner, were strong and good. Like many cell biologists, they tacitly acknowledge Mr D in their talk of adaptation and so on, but they don"t really think about it too deeply, being content to leave such stuff to the ecologists. They utter their Darwinisms as a catechism, a necessary cost of being part of modern biology, while they get on with their essentially Linnaean work of describing the taxonomy of the cell. They do not ask the terrible 'why" question that only Darwin answers.

Darwin was missing too in the ecology papers, and thus they are really natural history papers, because we cannot imagine an ecology uninformed by evolution. Those papers captured the natural history of blooms with great force and erudition, and the classification of blooms that emerges from the issue will be one of its enduring benefits.

Despite these strengths, the issue had a retro, back-to-the-sixties flavour for me, as I looked in vain for words like 'complexity", 'emergence", or 'novelty" but stumbled instead over words like "forcing" and "control". It reminded me of all the trees sacrificed for now-forgotten IGBP publications, of how we biologists forgot about Darwin and tried to ape physics, and how we thought that we could understand ecological phenomena with more instrumentation, more environmental measurements, and more and more boxes of punch cards.

The sixties was the high point of that ecological detour called trophodynamics, where things like carbon and energy flows were seen to be the key to ecology because they were commensurate with the state variables of physics and chemistry, and were accountable with the instruments of those disciplines. My problem is that the ecology of HABs presented in this issue reeks of this sort of trophodynamics.

So I returned to one of my ecological heroes of that era, L B Slobodkin, who

also happened to like plankton and even worked on red tides, as a few of the authors in the issue acknowledge. I reinforced my doubts about trophodynamics by rereading his elegant put-down, as fresh and relevant today as it was in 1969. The problem, according to Slobodkin in a review of a book by Harold Morowitz, is that trophodynamics leads us to commit Schrödinger"s Fundamental Biological Error:

"Essentially, Schrödinger said that animals consume food because they are thermodynamically open systems which are producing positive entropy and must consume an equal amount of negative entropy to maintain their steady state. In a sense this is equivalent to saying that animals must eat to avoid starving to death. The fallacy arises from the fact that for, say, a normal rabbit population, being eaten is a much more important source of death than starvation.

Part of the food eaten by rabbits is required to maintain the rabbits against thermodynamic decay, but a large part is to keep one jump ahead of the foxes and dogs. The energetic requirements of a population are therefore partially predictable from the thermodynamic properties of the animals but the residual part is predictable only if we know the precise ecological situation of the rabbit population. The actual energy flow patterns in nature are neither simple and direct consequences of thermodynamic theory, nor are they directly predictable from any extant evolutionary theory."

Slobodkin reminded me that HABs are, at base, ecological phenomena. And, all ecological phenomena are, at base, contemporary expressions of ongoing evolutionary dynamics, as Hutchinson, that other planktologist, reminded us. We cannot hope to understand them without using entities that are evolutionarily coercive-species, individuals, genes. Having now done the natural history of HABs at all sorts of levels, the task is now to inject some Darwin and start to look seriously at real population dynamics. That might even appeal to the Bills of the world.

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◆ Spain

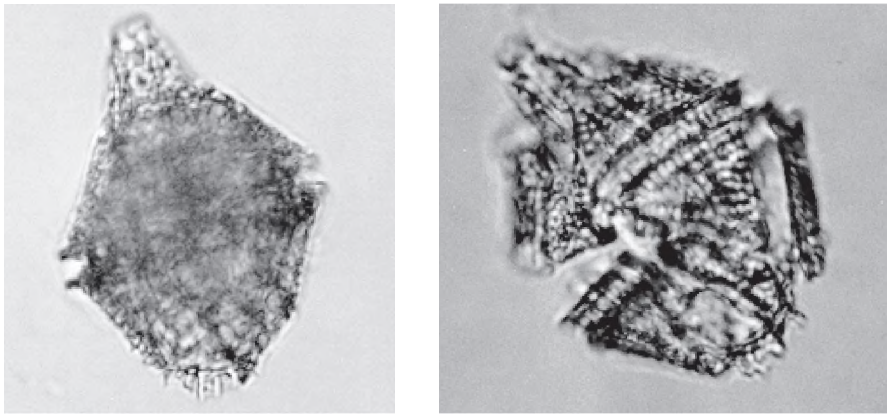
Bloom of *Gonyaulax polygramma* in the Galician Rías

In November 1997, a bloom of *Gonyaulax polygramma* Stein occurred on the Galician coast (NW Spain) and caused red coloration especially in the Rías de Pontevedra Vigo and Arousa. The Centro de Control da Calidade do Medio Mariño carries out a monitoring program in the Galician Rías (1) and samples at 40 coastal stations weekly. *Gonyaulax polygramma* cells were first detected in net samples during southerly winds on 20th October at almost all stations. This was a period of downwelling with an influx of oceanic water of 35.2 PSU and 17 ºC at 15 meters (Fig. 2). In the following two weeks the same situation was maintained with a slight rise in cell numbers at stations in the Ría de Pontevedra, but always below 8700 cells l^{-1}. The maximum concentrations were detected in mid-November. The highest cell concentration (767,800 cells l^{-1}) of *G. polygramma* was detected on 17th November at a station in Ría de Pontevedra. The same week, a concentration of 233,160 cells l^{-1} was observed in Ría de Vigo. However, maximum cell concentrations may have been much higher since very intense red patches were observed at several points. The same oceanographic conditions were maintained throughout the bloom.

In spite of the problematic identification of the *Gonyaulax* (2), the cells observed showed the typical sculpture with longitudinal ridges and small regular alveoli, elongated cell with tapered epitheca and hypotheca, epitheca convex to angular, hypotheca symmetrical, rounded or truncate, may have a variable number of short antapical spines, girdle descending, displaced about 1.5 widths. These characteristics are in accord with revised taxonomic descriptions (3, 4, 5). Some naked forms were seen, probably due to elimination of thecae before cell division (6).

There are several reports of *G. polygramma* in Galicia waters (7), but a visible bloom of this species had not previously been reported. Blooms of this species have been observed elsewhere, and have sometimes had serious consequences such as wild fish mortality in Cape Town, South Africa (8); mass mortality of fish and shellfish in Japan (9); mass mortality of marine organisms in Venezuela (10), in this last case associated with the presence of *Alexandrium tamarense* and *Gymnodinium catenatum*. There was no evidence of low oxygen levels during the event reported here. The only noxious effect of this bloom was the social alarm due to the red coloration of the sea. It was reported in press and Galician television and caused special alarm in the shellfish harvesting sector.

Fig. 1.
Light microscope view of a cell of this bloom with (left) and without (right) cytoplasm.



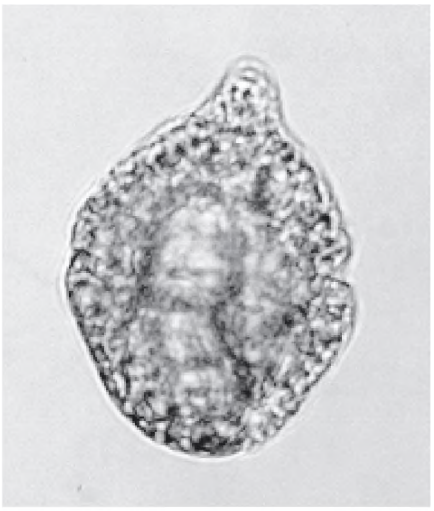


Fig. 2
Light microscope view of a naked form (without thecae).

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10) La Barbera-Sanchez, A., and Ferraz-Reyes, E. 1993. In: *Toxic Phytoplankton Blooms in the Sea*. Smayda, T.J., and Shimizu, Y. (eds), pp 281-285.

Yolanda Pazos, Centro de Control da Calidade do Medio Mariño, Vilaxoan, E-36611 Vilagarcía de Arousa, Spain

Future events

July 1999

IOC Danida Training Course on the Taxonomy and Biology of Harmful Marine Microalgae, July 1999. This two weeks course will focus on identification and preparation techniques supplemented by lectures on different aspects of the biology of harmful algae. Teaching staff will include: Dr Yasuwo Fukuyo (Univ. of Tokyo), Prof. Øjvind Moestrup (Univ. of Copenhagen), Dr Jacob Larsen (Univ. of Copenhagen/IOC Centre), Organized by the University of Copenhagen, and IOC Science and Communication Centre on Harmful Algae, Copenhagen. For application forms, contact: the IOC Science and Communication Centre on Harmful Algae, Botanical Institute, Ø. Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; fax: +45 33 13 44 47. Email: hab@bot.ku.dk. Deadline 1. April 1999.

February 2000

9TH INTERNATIONAL CONFERENCE ON HARMFUL ALGAL BLOOMS, 7-11 February 2000, Tasmania, Australia. The conference will provide a broad forum for phycologists, microbiologists toxicologists, physiologists, molecular biologists, aquatic ecologists and managers to address and exchange research findings and perspectives concerning all aspects of toxic and harmful algae, including freshwater cyanobacteria and ciguatera (fish poisoning). Harmful algae and their toxins pose a growing global problem for human health, aquaculture, fisheries, seafood trade, tourism and the aquatic environment at a time when human reliance on coastal zones for food, recreation and commerce is expanding. Contributions are invited which address the following topics:

- Taxonomy of harmful algae, using conventional as well as sophisticated molecular and immunological approaches
- Population dynamics of harmful algal blooms, including coupling of physical and biological processes, and interactions with zooplankton, viruses and bacteria
- Ecophysiological, chemical and pharmacological aspects of algal toxins, with a focus on new analytical methods and novel toxins or toxic episodes
- Monitoring and management of harmful algal blooms as they relate to eutrophication, aquaculture, public health and shipping (ballast water problem). Options for prevention, control and mitigation will be especially emphasized.

Contact: HAB 2000 Conference Secretariat,
Conference Design Pty Ltd, PO Box 342,
Sandy Bay Tasmania 7006, AUSTRALIA,
Tel: +61 3 62243773, Fax: + 61 3 62 243774,
E-mail: mail @cdesign.com.au,
or: Gustaaf M. Hallegraeff,
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GPO Box 252-55, Hobart, Tasmania 7001, AUSTRALIA,
Tel: +61 3 62 262623,
Fax: +61-3-62 262698,
E-mail: Hallegraeff@utas.edu.au (Gustaaf Hallegraeff),
Web Page: http://www.utas.edu.au/docs/plant_science/P.S._Homepage.html.

For a more comprehensive overview of up-coming activities and events you should visit the IOC International Marine Meeting List at the IOC homepage at
http://www.unesco.org/ioc/infserv/meets.htm.
The list of meetings related to the International Year of the Ocean might also be of interest
http://www.unesco.org/ioc/iyo/conferences.htm

Literature for developing country libraries

Limited copies of the following titles are available from the IOC Science and Communication Centre on Harmful Algae, Denmark(see address below), to libraries of marine science institutions *in developing countries only*:

- The Genus Alexandrium Halim (Dinoflagellata). **E. Balech, 1994.**
- Algae. An Introduction to Phycology. **C. van den Hoek et al., 1995.**
- Identifying Marine Phytoplankton. **C. Thomas et al. (eds.), 1997.**
- Proceedings of the International Symposium on Ciguatera and Marine Natural Products, Hawaii, 8-10 August 1994. **Hokama et al (eds.) 1995.**
- Proceedings of the First International Congress on Toxic Cyanobacteria, Denmark, 20-24 August 1995, and, Proceedings of the First Maj and Tor Nessling Foundation Symposium on 'Recent developments in Cyanobacterial Research', Finland 16-18 August 1995. **Moestrup et al** (eds.) Phycologia, Vol.35 No.6, **1996**
- Photography through the microscope. Kodak. **J.G. Delly, 1997.**

Applications must be submitted and signed by the responsible librarian, and should indicate why the requested title is of specific interest to your institution and its ongoing research or teaching. No requests from individuals will be honoured.

IOC publications

- Summary Report of the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms, Vigo 30 June - 2 July 1997. Available in English, French, Spanish.
- Amnesic Shellfish Poisoning (ASP), Volume 1, IOC *Manuals and Guides*, No. 31.

These publications are free of charge and available from the IOC Secretariat.
1, Rue Miollis, 75732 Paris, Cedex 15
France, Fax: +33 1456858, e-mail:
hab.ioc@unesco.org

- Manual on Harmful Marine Microalgae, IOC Manual and Guides No. 33.

The Manual is free of charge but a fee of US$ 30 will be charged for handling.

A cheque issued for the IOC SCCHA, University of Copenhagen (see address below), should accompany all orders. Only prepaid orders will be accepted. Excepted from the handling fee are marine science libraries and scientists in developing countries which can apply for delivery free of charge.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 19

A new "diablillo parasite" in the toxic Dinoflagellate *Alexandrium catenella* as a possibility to control harmful algal blooms

A red-tide of the paralytic shellfish poisoning producing dinoflagellate *Alexandrium catenella* was detected in the harbor of Tarragona (Catalonia, Spain) in June 1998, when monitoring noxious phytoplankton. During the microscopic enumeration of phytoplankton cells present in water samples, collected in the decline phase of the bloom, several unknown round cysts were present (Fig. 1) distinct from those of *Alexandrium* [1]. Water samples, maintained in culture flasks, showed that these cysts infected *A. catenella* cells, which subsequently released hundreds of flagellated

(Cont'd on p. 2)

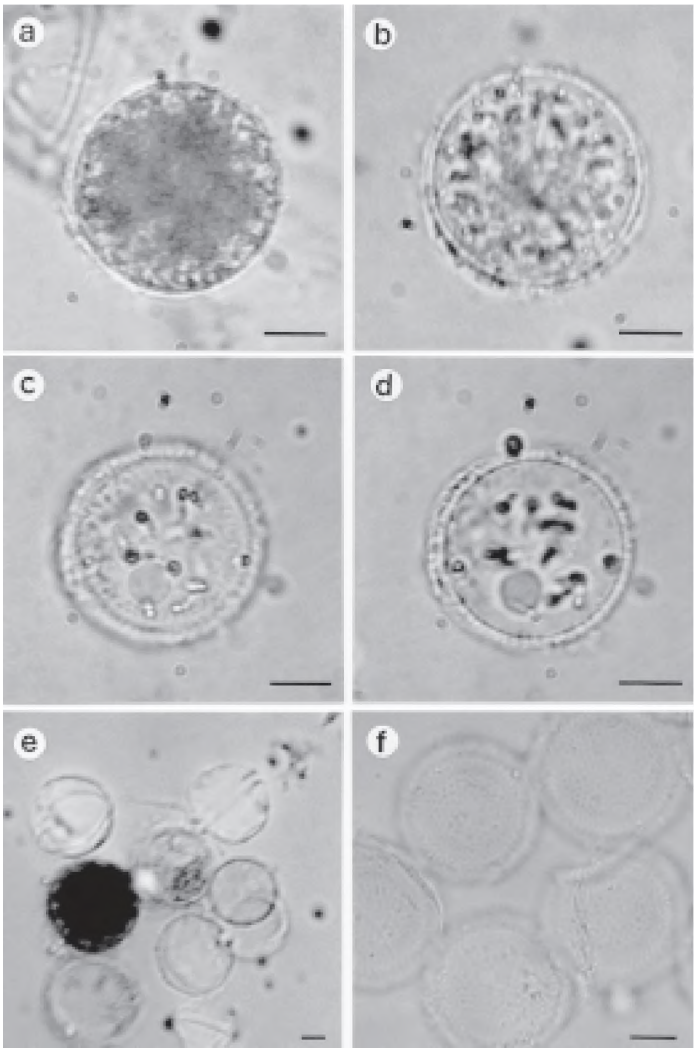

Fig. 1. *Ligh microscopy of "diablillo parasite" in living samples infecting* A. catenella. ***a****, View of a dark parasite cyst full of quiet swarmers.* ***b****, View of a parasite cyst during germination, containing inside about half of the total number of swarmers formed in this cyst; swarmers in very active movement.* ***c****,* ***d****, Two photos of the same parasite cyst in the final process of germination, containing about a dozen swarmers; a drop remains inside.* ***e****, Various parasite cysts and empty thecae of the dinoflagellate; one full dark cyst and 3 empty translucent cysts are apparent.* ***f****, Cluster of empty parasite cysts. Scale bars represents 5 μm.*

◆ New Zealand

A new species of *Gymnodinium* that caused the 1998 summer human respiratory syndrome and decimation of marine life in Wellington Harbour, New Zealand

Immediately after a series of fish and marine fauna kill episodes and outbreaks of human respiratory illness being reported off Wairarapa coast and Hawke Bay on the North Island east coast (Fig. 1) (Harmful Algae News 17: 1-5), Wellington Harbour experienced a severe toxic outbreak that persisted from mid-February to April 1998. This was the first toxic outbreak ever to occur in the region and decimated almost all marine life (including seaweeds) in the harbour. During this unusual outbreak, eels and flounders were first noticed as the major harbour kills, and then spread across to kills of other pelagic fish (e.g., yellow-eyed mullet, barracouta, mackerel, leather jacket, stargazers, and kahawai) and marine invertebrates (e.g., sea slug, starfish, sea urchin, and abalone).

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cells (Fig. 1b,c,d). Newly formed flagellates then infected other *A. catenella* cells. These results indicate the parasitic nature of the unknown cysts, which were given the name "diablillo parasites" (a Spanish word related with devil). The "diablillo parasite" was analyzed to reveal its possible effectiveness as a biological tool for controlling toxic algal blooms. Besides the parasite itself twelve strains of *A. catenella* were isolated from field samples and cultured for this purpose.

The new parasite

The flagellated cells and cysts remained non fluorescent with blue light excitation in light microscopy, hence lack chlorophyll pigments. Scanning electron microscopy images show morphological details of the parasite (Fig. 2). The flagellates (Fig. 2a,b) are 3 µm long and 1.5 µm wide and possess two unequal flagella (of 8 µm and 2 µm length respectively). The long flagellum shows hairs over its full length, whereas the short one is naked. The cyst stage (Fig. 2c,d) is spherical and of variable diameter, depending on the size of the infected host cell (30-40 µm in *A. catenella*). Cysts show very small spiny protuberances on their surface and a variable number of pores (usually 2 to 7 pores, 2 µm of diameter) in their transparent walls. These pores are difficult to resolve with light microscopy. The wall of the cyst shows yellowish fluorecence when stained with calcofluour [2].

Life cycle of the parasite

During the reproductive cycle of the parasite, the swarmers infect the motile vegetative cells of *A. catenella*, but do not infect non-motile cells. They first attach to the cingulum of the dinoflagellate, which subsequently stops its movement some hours later, and degradation of the hosts chloroplast becomes apparent (shown as a clear area usually located at the center of the host cytoplasm). During the following hours the clear area increases in size until it occupies the entire host cytoplasm 24 hours after infection. The parasite cyst is totally formed within 48 hours after infection and the theca of the dinoflagellate is disjoined. In undisturbed cultures cysts remained joined to empty thecae of the dinoflagellates (Fig. 2c).

One infected host usually develops only one parasite cyst, but occasionally several small cysts (2-5 cysts) were observed inside a single dinoflagellate cell. These observations possibly suggest multiple infections (various swarmers infecting the same cell). The parasite cysts are transparent when the swarmers escape throughout the pores (Fig. 1C,D). The dark color of the full-grown cysts is due to the dark body of the swarmers. The exit of swarmers from the cysts occurs naturally, but can be enhanced experimentally by addition of freshwater (20% of volume). These enhancements of germination are probably related to osmotic changes affecting the disruption of the membrane occluding the pores. A few minutes (4-10 minutes) before their exit, the swarmers begin to

Table 1.- Species of phytoplankton infected experimentally with the "diablillo parasite". C, cultures; NP, natural population.

Species sensitive to infection
Alexandrium catenella C, NP.
Alexandrium minutum C, NP.
Alexandrium affine C.
Protoperidinium bipes NP.
Scrippsiella trochoidea C, NP.
Gymnodinium sanguineun C.
Prorocentrum micans NP.
Dinophysis sacculus NP.

Species resistant to infection:
Alexandrium taylori C.
Gyrodinium corsicum C.
Coscinodiscus cf. *radiatus* C.
Thalassiosira weissfloguii C.

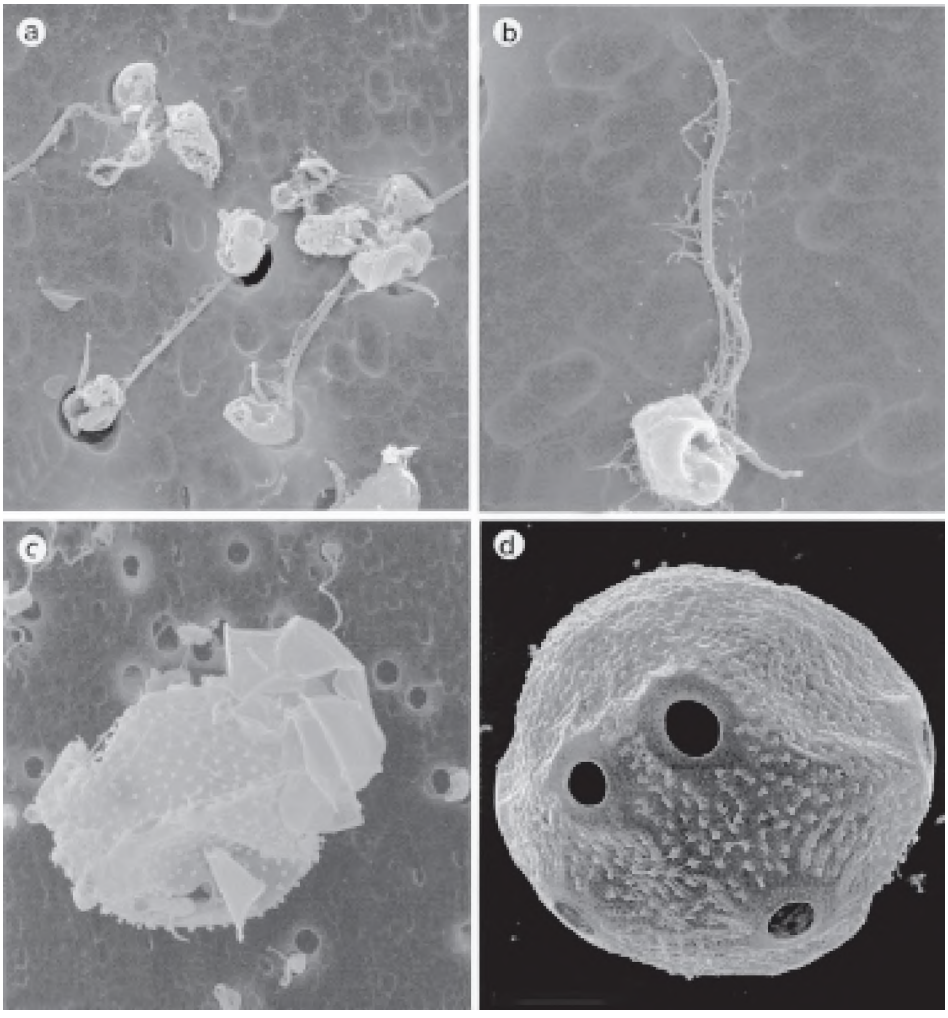

Fig. 2. *Scanning electron micrographs of the diablillo parasite infecting* ***A. catenella****, retained on polycarbonate filters.* ***a****, View of 8 swarmers. Scale bar 2 µm.* ***b****, View of a swarmer, showing long flagellum with hairs and short naked flagellum. Scale bar 2 µm.* ***c****, parasite cyst with protuberances on surface and the remaining empty theca of the infected dinoflagellate. Scale bar 10 µm.* ***d****, parasite cyst showing pores (3 in front view and 2 in side view) through which swarmers escapes from cyst. Scale bar 10 µm.*

move inside the parasite cyst and subsequently escape through the pores of the cyst wall. The escape of the swarmers from the parasite cyst lasts 3-7 minutes.

A droplet of unknown nature, about 8 µm diameter, remains inside the parasite cyst (Fig. 1c,d). Counts of swarmers escaped from a single parasite cyst showed numbers between 100 and 400. Occasionally some of the swarmers were unable to find the pores of the cyst wall and died in few minutes. Once outside the cysts swarmers moved with the long flagellum in front, and rotated along their axes. Swarmers accumulated near the surface of the culture vessels. They stopped their movement, and died few minutes later, when unable to infect a new host. The parasite cysts adhered easily to culture vessel surfaces, and also aggregated to form clusters of cysts (Fig.1f).

Use of the parasite as control agent of harmful algal blooms

Several organisms, including viruses, bacteria, dinoflagellates, euglenozoa, fungi and trematodes, have been described as dinoflagellate parasites [3]. However, until now the only described parasite of *Alexandrium* species is *Amoebophyra ceratii* [4]. *A. ceratii* showed low infective rates (maximum percentage of infection 32% in *A. catenella*), and its use as a biological control agent was considered unacceptable [5]. The parasite studied in the present work showed a much higher percentage of infection among the motile host population. Within seven days after inoculation of the parasite in dinoflagellate cultures, values of infection ranged from 92.2% to 100% in *A. catenella* and from 99.8% to 99.9% in *A. minutum*. The "diablillo parasite" also proved able to infect six more dinoflagellate species, but did not infect other phytoplankton groups (Table 1). These results show the selectivity of the parasite within the phytoplanktonic community. One particular case seems to be the dinoflagellate *A. taylori*, which remained resistant to infection after several attempts. This species, belonging to the subgenus *Gessnerium*, differs from the other *Alexandrium* species employed in the sense of daily formation of temporary cysts [6].

The capacities of this new "diablillo parasite", including the relatively rapid development (about 48 hours to maturity) of hundreds of new parasites per infected host, opens new perspectives for controlling blooms of harmful dinoflagellates in the natural environment. It can be expected that the effectiveness of this parasite in natural environments will require high cell densities of sensitive hosts, localized blooms, and little renovation of the water. Recurrent *Alexandrium* blooms commonly occur in semi-enclosed coastal water and these sites are thus ideal candidates. One feature of the "diablillo parasite" is that it only infected motile cells, and not the quiet cell stages of the hosts, present as temporary and resting cysts. This will present difficulties for the total destruction of the harmful algae populations in natural environments. However, this limitation of the infective potential is considered positive, since it prevents major changes in the species composition of the natural phytoplankton community. The possibility to culture the parasite on non-toxic and widespread dinoflagellate hosts (as *G. sanguineum* and *S. trochoidea*) could facilitate its use and protect against the introduction of toxic species in different geographic areas.

Research in progress

Cooperative research is in course with Dr. Malte Elbrächter and Dr. Michael Schweikert to reveal the taxonomical position of the parasite by transmission electron microscopy and with Ms. Beatríz Díez and Dr. Carles Pedrós-Alió to determine its affiliation by analysis of 18s ribosomal sequence.

Acknowledgements

J.M. Fortuño and Ll. Cros provided technical assistance with SEM and cultures, respectively.

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The pattern of human respiratory syndrome in Wellington Harbour tracked well with those reported on the open coast around Wairarapa and Hawke Bay. In Wellington Harbour, 87 people reported suffering from respiratory illness; beach-goers, swimmers, and wind-surfers all complained of a dry cough, a severe sore throat (like swallowing razor blades), running nose, and skin and eye irritations. Furthermore, hatchery workers and divers working for the National Institute of Water and Atmospheric Research (NIWA) complained among other symptoms also of severe headaches, and a facial sun-burnt sensation.

During the period from 16 February and 30 March 1998, brine shrimps and all phytoplankton cultures, kept at the NIWA's hatchery facilities, were the first to be affected. Cultures, maintained in filtered seawater which was sourced from the harbour during the outbreak, died in a matter of days. Subsequently, sea horses, juvenile and large turbot, and cockle spat cultured at the hatchery, also crashed.

The unprecedented bloom that wreaked havoc in Wellington Harbour was found to be dominated by an undescribed *Gymnodinium* sp. (cell concentration 33.3 million cells per litre). The morphological characters of this new species are more like the Japanese *Gymnodinium mikimotoi* than any other species. The cell is dorso-ventrally flattened and has very short expression of the apical groove on the ventral surface of the epicone (Fig. 2). A full description of this new species has been made by the author and is now in press (Phycologia 38(5)).

One of the notable features of the 1998 Wellington Harbour bloom was the build-up of extensive sea-foam. Apparently the dissolved organic substances released at the end of the bloom by both the new Wellington *Gymnodinium* sp. and marine organisms killed by toxins were the source of this sea-foam accumulation in the harbour. The foam persisted for several weeks in the semi-

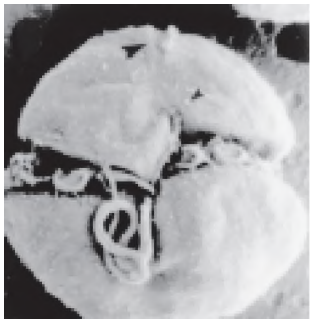

Fig. 2. Scanning electron micrograph of the new Wellington Harbour Gymnodinium *sp.*

enclosed harbour between March and early May 1998.

During this toxic outbreak, mussels growing on NIWA's hatchery rafts in Wellington Harbour were collected for toxicity tests. Mouse bioassay tests conducted by Environmental Sciences Research showed positive results; mice died very quickly in 4-6 minutes. At first it was thought that a fast-action, lipid-soluble toxin, similar to the gymnodimine that was isolated from the 1994 toxic shellfish outbreak around Foveaux Strait, New Zealand, was responsible for the mice kills. But, unlike gymnodimine, the Wellington toxin is extractable with ether. Further chemical analysis is currently being undertaken.

The Wellington Harbour toxin is stable in both alkaline and acidic conditions, but is not stable in weak acid. The instability of this toxin in weak acid makes it less likely to pose any human health risk when it is eaten. It is also thermally unstable as when heated to 100°C it lost most of its toxicity. But a certain level of toxicity was maintained, even though boiled for 30 minutes. This toxin is also characterised as being highly oxidisable, and therefore can be destroyed by ozonation. During the outbreak, ozone was used to get rid filtered seawater of toxins in NIWA's hatchery facilities before being used for cultures.

Several cultures of the new Wellington *Gymnodinium* sp. from the harbour were developed during the toxic episode. A wide range of marine organisms were tested with these newly developed *Gymnodinium* cultures. Brine shrimps, abalone larvae (Fig. 4), tintinnids, amphipods, barnacle nauplii, polychaete worms, lobster larvae (phyllosoma stage I and III), and turbot fish exposed to toxins produced by the new Wellington *Gymnodinium* sp. were all being killed

Toxins produced by *Gymnodinium* sp. also lysed cells of naked nanoflagellates and non-thecate dinoflagellates (including the Japanese *G. mikimotoi*, and the European *G.* cf. *mikimotoi*), and killed thecate dinoflagellates (including *Alexandrium minutum* and *Prorocentrum lima*) and diatoms.

The most notable of all is that seaweeds were killed when exposed to the toxins produced by this new Wellington Harbour *Gymnodinium* sp., particularly the common brown seaweed, *Macrocystis pyrifera* found in the harbour. In laboratory tests, when *Macrocystis pyrifera* was introduced to the culture of *Gymnodinium* sp., the soft, leafy part of this seaweed collapsed and fell apart in 24 to

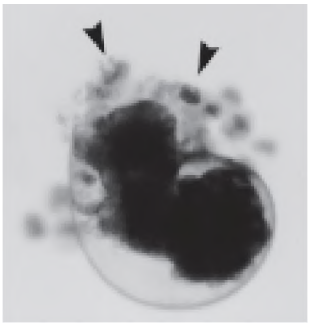

Fig. 3. Abalone larva was killed when introduced to the culture of the new, toxic, Wellington Harbour Gymnodinium *sp.; membrane surrounding the velum started to disintegrate, and cells (arrowheads) within the larva were being discharged from the dead animal.*

48 hours. This confirmed the earlier observations of dead *Macrocystis pyrifera* (up to 75% of seaweed cover) during the toxic outbreak in Wellington Harbour.

It is thus very clear that the new Wellington Harbour *Gymnodinium* sp. is extremely toxic to a wide range of marine invertebrates, vertebrates, microalgae and macroalgae. This is consistent with observations made in the harbour during the toxic outbreak. Impacts of this new *Gymnodinoium* sp. on marine life certainly are more severe than those caused by *G. mikimotoi* from Japan, *G. breve* from the Atlantic coast of the U.S.A., *G.* cf. *mikimotoi* from Western Europe, and *G. galatheanum* from the North Sea. In terms of impacts of airborne and waterborne toxins on humans, the Wellington Harbour *Gymnodinium* sp. toxin is pretty much like those of *G. breve* Davis from the Atlantic coast of the U.S.A. and a *Gymnodinium* sp. recently reported from South Africa.



Fig. 1. Locations where toxic outbreaks and respiratory syndromes (shaded) were reported on the east coast and southern part of North Island, New Zealand.

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◆ Chile

Fish kill in Chile associated with bloom of Gymnodinium sp.



Fig. 1a

The event

A large-scale fish kill developed in waters around Chiloë, Xth Region, Chile, during March and April 1999 (Fig. 1). The first kills reported were of abalone and salmon from marine farms near Quellon on 13 March, associated with patches of brown water ("marea café"). The coloured patches were sharply delimited. The following days air-borne observations showed, in a first phase, the presence of discrete patches accompanied by mortality of salmon in fish-farms on the eastern coast of the island of Chiloë. Kills of molluscs and sipunculids were also reported. The movement of the patches, northwards in the inland sea, appeared to follow the currents. The presence of coloured water in the northern sector of the island (Chacao Channel) as well as mortalities of worms, molluscs and sipunculids, were observed on 12 March. This phenomenon developed during a period when Neap Tides and unusually sunny weather combined. The period of Spring Tides from 27 March contributed to attenuate the blooms. Subsequently, the coloured water presented a more diffuse aspect, and fish kills were reported only when Spring Tides returned around the 9 April.

Examination of water samples revealed that a *Gymnodinium* sp. comprised 99.9% of the population, the density of which reached 8 to 9 million cells per litre in coloured patches. The alga is sensitive to light: in the presence of clouds and in the dark, it diffuses down into the water column, and the maximum cell concentration is found around 10 m. Sunshine favours its migration to the surface, where it forms discrete patches with an exponential vertical distribution.

Many factors suggest that this bloom was of oceanic origin: within a phytoplankton-monitoring program, no cell resembling this species had been observed in the area previously. The first cells counted (10 cells/ml) were in samples from oceanic coastal waters in late February (Austral summer). One possible cause of the event could be the current climate anomaly for the last 14 months in the area, with a drought and strong sunshine. It has resulted in temperatures, 15°C, 1.5°C higher than normal in the area. Furthermore, because of smaller than usual inputs of fresh-water run-off, oceanic waters (33 g/l) penetrate more easily bringing with them phytoplankton species normally found only in more oceanic coastal waters. The presence of *Gymnodinium* sp. appears strongly associated with the frontal structure of water masses in this area, and the arrival of a water mass of salinity 30 g/l is likely to have contributed to the disappearance of the alga within 24 h in the area of Quemchi.

Among the consequences of this bloom, it is important to mention a large mortality of salmon. It constitutes a severe loss for the farmers concerned. The relationship between the mortalities of marine animals and the presence of coloured water was first established in salmon-farms. The animals affected presented abundant mucus around their gills.

The species

The algal cells were very similar to the genus *Gymnodinium*, approximatively 30 µm wide, with a large nucleus (10 µm) located opposite the apical extremity. Numerous small chloroplasts were visible (Fig.2). A few small cells looking like chlorophytes (1-2 µm) were also observed in fresh samples.

After storage in culture of the Gymnodinium sp. samples from the coloured patches, smaller cells (10-15 µm) appeared. The large proved to be particularly fragile, and up till now, this has prevented their being transported live to Denmark for identification by J. Larsen.

Some of the properties of this species have been studied during the bloom, and they resemble those of other known species of ichthyotoxic *Gymnodinium*.

The haemolytic property: This allows one of the modes of action of the toxin (lysis) to be investigated, and it permits this toxicity to be related to a minimum concentration of algae. Freshly pre-

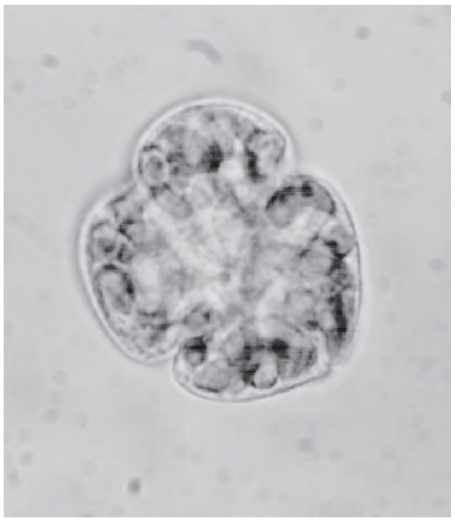

Fig. 2. Photo by M. Seguel, IFOP, Chile

served samples, taken from coloured water patches, as well as a culture grown in the laboratory in f/2 medium, were tested according to the usual protocol, on human red blood cells (RBCs). The results showed strong haemolytic activity in all cases, apparent after 30 minutes incubation at 18°C, with samples corresponding to a density of 4000 cells/ml. The test carried out on diluted samples showed that a concentration of 250 cells/ml showed a still non-negligible activity (18% haemolysis in an RBC suspension of 40 million/ml).

The allelopathic property: it reveals the presence of an ectocrine secreted by the algal cell, and may explain the almost monospecific nature of the blooms. Water from samples tested previously, from which the *Gymnodinium*

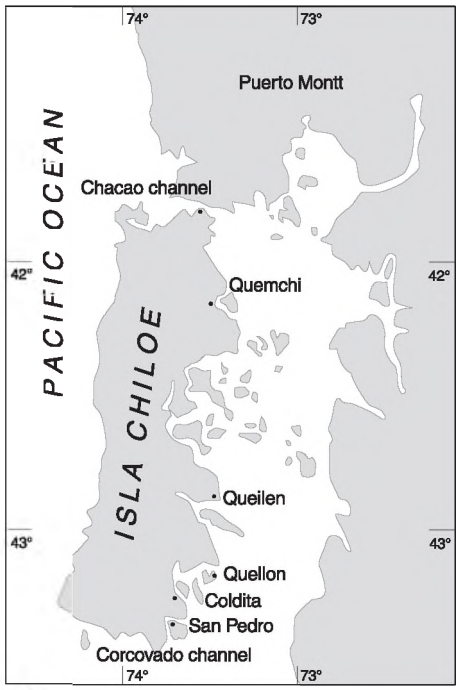

Fig. 1b

cells had been removed by gravity filtration through Whatman GF/F filters, were then sterilised by 0.22-µm filtration. In order to extract any allelopathic substances, an aliquot of water was passed through Sep-Pak C18 and Florisil (Waters) cartridges. After enrichment in f/2 medium, the fractionated samples were respectively seeded using two stocks of the alga isolated in this region of Chile. The results showed that development of diatoms was totally inhibited by water from the sample having contained *Gymnodinium* sp., but that growth of a culture of *Alexandrium catenella* was practically unaffected.

This phenomenon illustrates the importance of the climatic situation and the physical oceanographic conditions in the process of bloom dynamics. High temperatures and strong sunshine, "inoculation" currents and the stratification of water columns are probably some of the factors responsible in this case. The biology of the species in question completes the factors controlling bloom dynamics. In producing substances with properties allelopathic to other species, this alga limits their development, and itself develops profusely. The presence of either the same substances or of other biologically active products also derived from the metabolism of the alga present at high concentration, leads to the serious impacts observed on marine organisms sensitive to these substances.

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◆ International

APEC Red Tide/HAB Project Enters Final Year

In past newsletters, the APEC Red Tide/HAB Project has been described. This project represents an effort to coordinate monitoring and management of HABs within the 21 APEC (Asia Pacific Economic Cooperation) economies in order to facilitate a free flow of goods and services, and in particular, shellfish and fish products potentially contaminated with algal toxins. These efforts are similar in some ways to activities within the European Union, which has also been establishing import and export guidelines.

The Red Tide/HAB project structure is composed of a Program Steering Committee, which provides general oversight, and two Task Teams – a Task Team on Algal Biotoxin Regulations and a Task Team on Analytical Methods and Standards. The former is chaired by Sherwood Hall and the latter by Mike Quilliam. The project Overseer is Rod Forbes of Canada.

The latest round of APEC meetings took place during the conference on Harmful Algae Management and Mitigation (HAMM) which took place in Subic Bay, Philippines in May, 1999. One of the major activities of the Task Teams during the last year has been the organization and convening of this conference, which was co-sponsored by the IOC and received additional support from institutions in the Philippines, Chinese Taipei and the United States. The Task Teams have also be polling individual economies on their action limits for HAB toxins, and have been working towards establishing "performance criteria" for each of the algal toxins, rather than specifying a specific method which must be adhered to by all. The Analytical Methods and Standards Task Team is preparing summary tables of the many different methods that can be used for algal toxin detection and measurements, providing guidance on their suitability with respect to the proposed performance criteria. In addition, a proposal is being submitted to obtain external funding for a program which will address the needs for analytical standards as well as for an inter-calibration exercise involving laboratories within the APEC economies.

In a departure from the approach taken by the European Union, APEC does not feel it is feasible to have a single Regional Reference Laboratory as a central node in a network of National Reference Laboratories. Instead, two networks are envisioned both voluntary. The first is a Group of Authorities on Algal Biotoxin Regulations. This group would comprise individuals and institutions responsible for management of seafood product safety and trade. It would meet periodically, but would also communicate frequently over the Internet to insure that high level attention is given to issues related to the certification of seafood potentially affected by algal toxins. The second network, a Group of Experts on Analytical Methods, could assist in resolving disputes or provide advice to individual APEC economies in need of assistance. In the coming months, individuals and agencies who will become part of these two networks will be identified both by Task Team members and by individual APEC economies through formal invitations for nomination. This is one area where we hope that the project can influence activities and policies within the APEC economies long after it terminates at the end of this next year.

Another activity that will be undertaken during the intercession will be the establishment of an APEC Web page for this project. In addition to providing access to a number of the reports and general information that is generated, the Web page will also link to many sites throughout the world where useful pub-

lic education information is available on HABs. With the help of many in our HAB community, Georgina Lembeye compiled leaflets, brochures, fax sheets, and other sources of general information used in countries world wide to communicate the facts and dangers of HABs. This compilation is very useful, but it was felt that it would be easier to disseminate this type of information through a Web page which links to existing sites where this information is available.

Overall, the APEC Red Tide Project is proceeding well, and will finish its deliberations within a year. The growing restrictions on trade involving seafood products potentially contaminated with algal toxins underscores the importance of these types of activities.

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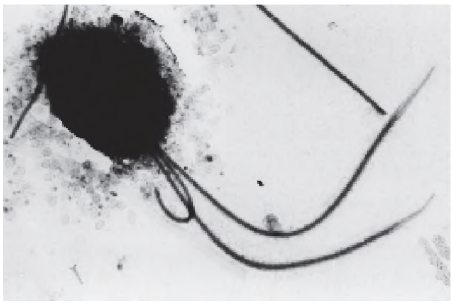
◆ Italy

Toxicity of *Prymnesium* in the shallow lake of Massaciuccoli (Migliarino-San Rossore- Massaciuccoli Regional Park -Pisa-Tuscany)

In January 1999 a *Prymnesium sp.* (presumably *P. patelliferum*) bloom occurred with 18000 cells/ml; it caused an extensive fish mortality in the lake of Massaciuccoli (1). This lake is connected to the sea, and has brackish waters with a range of Cl^- of 700-2000 mg/l, its average depth is 2,5 m, volume 11000 m^3, area 6,8 km^2. It is situated in a wetland zone below sea level (- 0,80 + 0,35 m). From 1930 the area around the lake has been reclaimed as agriculture land. Subsequently, the trophic conditions and anthropogenic influences in the area have led to increased eutrophication, and a progressive loss of submerged macrophytes in favour of microalgae growth. The increased amounts of phytoplankton revealed the presence of *Prymnesium sp.*, which caused fish mortality in 1972 (2) and in later years. Usually the blooms of *Prymnesium* have taken place during the winter period (December-March),preceding cyanobacterial blooms (*Lyngbya* spp., *Microcystis* spp.), when the water temperature is 7-8 °C. The *Prymnesium* toxins kill fishes and *Daphnia magna*, but not Copepoda such as *Cyclops*.

Mortality of Cladocera alters the trophic web, and throws the ecosystem out of balance (3). Toxicity of *Prymnesium* is demonstrated in the laboratory by bioassays using *Carassius auratus* (4) which die within 24 hours, and *Daphnia magna* which die within 4 days. Generally the toxicity occurs with concentrations of 10000 cells/ml. Electronic microscopy, in co-operation with the Department of Botany of Florence University has shown that the species is *Prymnesium patelliferum*.



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◆ Review

Cortes Atamirano, R.

LAS MAREAS ROJAS

This is a book written in Spanish about red tides that includes all of the harmful algae that cause or do not cause discoloration of sea water. The section on toxic events is confined mostly to the coastal region of Mexico and serves as a useful reference for other coastal regions. The book covers a range of aspects related to red tides, from the dynamics of formation to suggestions for prevention of the toxic effects of harmful episodes at the national and international level. The opening chapter briefly describes the dynamics of formation, maintenance and disappearance of red tides. Three chapters follow on experimental methods of monitoring and a description and summary of toxic episodes in Mexican and associated waters and causal species. A series of diagrams are included to aid identification. The next four chapters are dedicated to toxicology and start with a description of the most frequent toxins (PSP, DSP, NSP, ASP) and their effects. It then describes human symptoms contracted from ingestion of the four types of toxin. The next two chapters are dedicated to methodological aspects of toxin concentration determination by rat bio-assay and HPLC. The author then places everything into a series of research programmes for the prevention of the toxic effects of red tides at the National and International level and in the last chapter includes suggestions of how this may be achieved. Each chapter has a relevant bibliography section and to complement this there is a more extensive list of useful titles compiled as an appendix. A series of other appendices are included which relate to the diverse themes of the book and of most interest is the list of micro-algal species responsible for toxic red tides. In this list there

are series of keys, which allow the identification of various species, associated aspects such as toxin types, and effects on human health. This book is very useful to Spanish readers who work in the monitoring and management of coastal waters.

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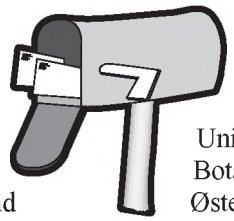
Identification Service

The IOC Science and Communication Centre on Harmful Algae, Copenhagen, and the Asian Natural Environmental Science Center offers an identification service. If you have problems with identification of harmful algal species, the Centres may assist you. You should send samples to one of the two addresses given below. It should be noted that this assistance is not given for routine identification/monitoring of samples, but applies to particulary difficult species, or to species that require special techniques e.g. electron microscopy, for identification.



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IOC-IEO-AECI III Course on Toxic Microlgae and Marine Phycotoxins

The third training course on toxic microlgae and marine phycotoxins organized by the IOC-IEO Science and Communication Centre on Harmful Algae was held from 1st to 17th of June 1999, at the Instituto Español de Oceanografía (IEO) in Vigo, Spain. This course is part of the Harmful Algal Bloom Programme of IOC and is funded by the IOC, the Agencia Española de Cooperación Internacional (AECI) and the IEO. Twelve scientists from different developing countries participated in the course (Argentina, Angola, Brazil, Colombia, Costa Rica, Cuba, Chile, Ecuador, Guatemala, Haiti, Mexico and Venezuela). Lecturers were given by personnel from the IEO, The Instituto de Investigaciones Mariñas (Consejo Superior de Investigaciones Científicas), The European Union Reference Laboratory on Marine Biotoxins, Centro de Control da Calidade do Medio Mariño (Xunta de Galicia), ANFACO-CECOPESCA, the Science and Communication Centre on Harmful Algae, plus other guest lecturers from three Spanish Universities (Oviedo, Vigo and Santiago).

Apart from a general introduction to harmful microalgae, the practical training part of the course was focussed on four different aspects of toxic phytoplankton: Toxin determination, Establishment of algal cultures (collections and large-scale), Taxonomy, and Benthic harmful microalgae. At a seminar all participants made a presentation describing HAB research and management related activities in their respective countries. Time was also available for the participants to work on their own plankton samples. The laboratory training was complemented with field sampling methodologies and visits to the local research laboratories working with HAB.

The general impression of the outcome of the course was very positive among both trainees and lecturers, and valuable contacts for future collaboration were established among the participants. This IOC training course is organized annually, and additional information can be requested from the IOC-IEO Science and Communication Centre on Harmful Algae at fax +34-986-492003 or e-mail: vigohab@vi.ieo.es.

For general information on IOC Training Courses on HAB please consult **http://ioc.unesco.org/hab/train.htm**

◆ China

Phaeocystis bloom in southeast China coastal water 1997

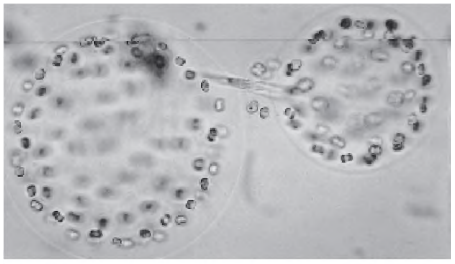

Fig.1. Phaeocystis globasa cells in gelatinous colonies

From beginning of October to late December 1997, one intensive bloom along the coast of Fujian and Guangdong provinces occurred. This huge bloom lasted about two months. The causative species was identified as *Phaeocystis globosa* Scherffel (Fig.1). The spherical colonies in nonmotile stage of cells are about 1.5 to 3mm in diameter. Cells are evenly distributed along the surface of the colony. The gelatinous colonies with or without lobes and distributive patterns of cells in the colony stage are two distinctive characteristics for separating *Phaeocystis pouchetii* and *Phaeocystis globosa*. According to this, the bloom species in southeast China coastal waters is not *Phaeocystis pouchetii* (Hariot) Lagerheim but *Phaeocystis globosa* Scherffel. This species is also recorded in the coastal waters of Hainan province and Hong Kong. The discoloration caused by this species during the late autumn and early winter was detected by SeaWiFS imagery (Fig.2). The bloom caused severe mortality of caged-fish. An estimated 60 000 tons of fish were killed. The direct loss was 66 million Chinese Yuan (about 8 million US dollars).

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Fig.2 SeaWiFS image of P. globasa bloom on 13th of November 1997 (arrows)

◆ Mexico

National HAB Training Course in Mexico. Monitoring Program Launched Early 1999

The first report of red tides impacting human health in Mexico was in the XIX century, and led to coughing and cutaneous rashes in people living near the Veracruz coast, as well as mass fish mortalities [1]. But previously, Brongersma-Sanders [2], had reported fish mortalities as "very frequent" since 1797 in the Gulf of Mexico. From that time on, there were sporadic reports until 1979, when the first documented casualty due to Paralytic Shellfish Poisoning (PSP) occurred near Mazatlan Bay, Sinaloa [3,4]. Since then, continuous observation of the area has been mantained [5], and later extended to Bacochibampo Bay farther north in Sonora State.

Regarding the presence and distribution of Marine Biotoxins in Mexico, until now we have positively identified events caused by saxitoxins (PSP), brevetoxins (NSP), domoic acid (ASP), ciguatoxins (CFP) and cyanobacteria hepatotoxins [6]. In addition, we are aware of the presence of okadaic acid and dinophysistoxin-1 (DSP) in shellfish, but without any directly related events so far [7].

This information was gathered in spite of the absence of a coordinated network for monitoring red-tide events. Furthermore, there was a lack of properly trained individuals to record the ecological and toxicological aspects of



these phenomena. Thus, it was decided that one of the main goals to was to train individuals to evaluate and manage red-tides. These efforts started during the First Training Course "Evaluation and

Prevention of Red Tide Effects in Mexico", held in Mazatlan, Sinaloa, in May 28-31, 1996. This course, organized by the Plankton Laboratory (Mazatlan Station-UNAM), and attended by nine trainees, was given by specialists from the Biotoxins Laboratory (CIBNOR), National Fisheries Institute (SEMARNAP), Invertebrates Laboratory (UNAM), and the Phytoplankton and Productivity Laboratory (ICMyL-UNAM), with broad experience in the biology, taxonomy and toxicology of harmful algae. Toxicological training ranged from mouse bioassays to HPLC methods for the determination of marine biotoxins. As a by-product of this course, a manual was prepared which was finally published in 1998 [8].

In April, 1997, the Central Laboratory of Chemistry and Aquatic Toxicology (INP-SEMARNAP), the Regional Fisheries Research Center and 5 Regional Institutes from the State of Veracruz, hosted the Second Training Course on Red Tides, with 25 trainees. The same year (March, 1997), the National Shellfish Sanitary Program held a Training Course in Acapulco, Guerrero, where the main subject was the mouse bioassay for PSP toxins; it was restricted to their own local personnel.

Recently, from 23-26 November, 1998, a joint venture of CIBNOR, UECYyM-SEP, ICMyL-UNAM and PROFEPA, led to the Third National Training Course on the Evaluation and Prevention of the Effects of Marine Biotoxins and Red Tides in Mexico. This course was held at CIBNOR premises in La Paz, B.C.S. There were twenty five attendants from eleven Mexican States. Each trainee has the opportunity to study samples of all species that produce red tides in Mexico (dinoflagellates, diatoms and cyanobacteria). Additionally, biology and ecology of the organisms, monitoring tools, remote sensing, toxicology and qualitative and quantitative determination of algal toxins were broadly discussed by participants.

Nowdays, the facilities of CETMARs and ITMARs, medium and high level educational institutes respectively, coordinated by Unit of Education, Science and Technology of the Sea-Education Ministry (UECyTM-SEP) which are spread all along the coasts of Mexico, and the Monitoring and Research Program developed by the Biotoxins Laboratory at CIBNOR, have merged efforts to foster a network for the permanent study of red-tides. The Education Ministry have provided the funds to run local monitoring activities in each locality, as well as the necessary equipment. The seven institutes on the Pacific coast received new microscopes, cell culturing equipment and toxin extraction equipment, and the rest of the institutions will be supplied with the necessary equipment within the next two years.

These goals would not have been accomplished without the sponsorship of IOC via scholarships to Roberto Cortes Altamirano (to attend the Training Course in Vigo, Spain) and Arturo P. Sierra-Beltran (to attend Training Courses in Jena, Germany and Helsingor, Denmark). We hope to link up with other initiatives in the IOCARIBE region in the near future, and to participate actively in the Harmful Algae Project in the Caribbean (ANCA). We are now organizing the First IOCARIBE Training Course on Harmful Algae to be carried out in November 1999 at CIBNOR premises in La Paz, B.C.S., Mexico.



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ICES- IOC Working Group on Harmful Algal Bloom Dynamics

The ICES- IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) was established in 1994. One aim of the Working Group is to outline the various physical, chemical and biological interactions associated to harmful algal blooms (HAB) and to define the main gaps in research.

An important task of WGHABD is to collect up-to-date information about HAB occurrences. This information maintained as a HAB database by the IOC Communication Center on HAB in Vigo, and summarized as decadal maps in the ICES web-page (http://www.ices.dk/status/status.htm).

The next meeting of the Working Group will be held in Barcelona, Spain, from 20 to 24 March 2000.

The IOC and ICES are encouraging scientists from outside the North Atlantic to join the Working Group. If you are interested you can get more information on the Working Group at http://ioc.unesco.org/hab/act6.htm or by contacting the Chair of the Working Group Dr. Kaisa Kononen at kaisa.kononen@nessling.fi The IOC has a limited number of grants for scientists from developing countries to participate. If you are interested you should contact Henrik Enevoldsen (hab.ioc@unesco.org) at the IOC Science and Communication Centre on Harmful Algae with a description of your background and interest in the activities of the Working Group.



Since August 1994 the ASEAN-Canada Cooperative Programme on Marine Science has sponsored the South East Asian Harmful Algae Bulletin, SEAHAB. Unfortunately funding for this has come to an end. While SEAHAB is investigating ways and means to continue, Harmful Algae News has agreed temporarily to include a section for SEAHAB.

If you are interested in supporting the survival of SEAHAB please contact the Editor, Dr. Rhodora V. Azanza, Ph.D., Marine Science Institute, CS, University of the Philippines, Diliman, Quezon City 1101, Philippines, Tel: (632) 9215967/9223959, Fax: (632) 9215967, Email: rhod@msi01.cs.upd.edu.ph

The articles which follows hereafter were submitted via SEAHAB.

◆ Philippines

Ciguatera Fish Poisoning: Occurrence and Research in the Philippines

Ciguatera Fish Poisoning is a newly known type of algal biotoxin poisoning in the Philippines. The presence of causative organisms such as Prorocentrum lima, Prorocentrum concavum and Ostreopsis siamensis was first observed by Fukuyo (pers. comm.) in Cebu and Iloilo in 1994. Azanza and Taylor also observed the presence of ciguatera causing organisms in the seaweeds, Jania spp. and Laurencia spp. collected from White Island, Camiguin, Philippines in 1995.

Recently, a "Ciguatera Fish Poisoning" event was reported in Romblon, Philippines in September 1997. There were 35 cases of fish poisoning from this date until February 1998 and out of these cases, 25 showed symptoms that fit very closely those of Ciguatera Fish Poisoning while 3 other cases showed symptoms of palytoxin poisoning. The remaining cases showed symptoms not related to the abovementioned poisonings. Samples of the vector fish Lethrinus sp. locally called "dugso" were sent to Dr. Richard Lewis of the University of Queensland, Australia and Dr. Fran Van Dolah of the US. National Marine Fisheries Services, Charleston for analysis. Dr. Allan Dionisio of the Philippine General Hospital and Dr. Teresa Castillo of the Philippines' Department of Health visited the site in February 1998 and conducted seminars on the detection of symptoms of ciguatera poisoning to local medical officers. Awareness on this kind of poisoning had increased especially in Cajidiosan, Romblon.

At present, the Bureau of Fisheries and Aquatic Resources and the University of the Philippines in the Visayas are conducting serial monitoring of fish and dinoflagellates from selected sites in Romblon in relation to ciguatera fish poisoning. Medical doctors in Romblon have been provided by Drs. Dionisio and Castillo with a questionnaire relevant to the epidemiology of this type of poisoning.

Rhodora V. Azanza & Lilibeth N. Miranda, Marine Science Institute, University of the Philippines

◆ Australia

IAEA Regional Training Workshop on Harmful Algae

The International Atomic Energy Agency (IAEA) sponsored a Regional Harmful Algal Blooms Training Workshop on Sedimentation and Algal Cysts Studies wich was held in Hobart/Sydney, Australia 5-10 July 1999 in line with its regional project on the "Application of Nuclear Techniques to address specific red tide (harmful algal blooms) concerns." This regional project was developed based on the National IAEA program of the Philippines with almost the same title. The training program was composed of two parts. The first part was on algae identification held at the Department of Plant Science, University of Tasmania. The second part was on field sampling and radiometric dating of sediments at the Environmental Radiochemistry Laboratory, Australian Nuclear Science and Technology Organization (ANSTO).

The workshop participants were familiarized with the techniques on Radiometric dating using Polonium-210, Radium-226 and Lead-210 by alpha and gamma spectrometry analysis.

There were 16 participants from China, Indonesia, Malaysia, Pakistan, the Philippines, Thailand and Vietnam. Dr. Gustaff Hallegraeff of the University of Tasmania and Dr. Hendrik Heijnis of the Environmental Radiochemistry group of the Environment Division, ANSTO, headed the team of lecturers. Dr. Heijnis is the IAEA/RCA/UNDP technical expert on the application of Nuclear Techniques to address specific red tide (harmful algal blooms) concerns, i.e. use of radio labeling for dating of sediments. Dr. G. Hallegraeff was in charge of the training on isolation and identification of algal cysts from the sediments.

Rhodora V. Azanza, Ph.D. & Lilibeth N. Miranda, Marine Science Institute, University of the Philippines, 1101 Diliman Quezon City

◆ International

First Conference on Harmful Algae Management and Mitigation



The first Conference on Harmful Algae Management & Mitigation (HAMM) was successfully chaired by Dr. Sherwood Hall of the United States Food and Drug Administration (USFDA) and hosted by the Marine Science Institute (MSI) of the University of the Philippines on 10-14 May 1999 at Crown Peak Gardens Hotel, Subic Bay, Philippines.

Dr. Rhodora V. Azanza of MSI and Mr. Robert Jara of the Department of Environment and Natural Resources served as the co-chairmen of the Local Organizing Committee. The Conference was sponsored and held under the auspices of the Asia-Pacific Economic Cooperation (APEC) and the Intergovernmental Oceanographic Commission (IOC), and co-sponsored by the Government of the Republic of the Philippines (University of the Philippines= Marine Science Institute, Department of Agriculture-Bureau of Fisheries and Aquatic Resources, Department of Science and Technology-Philippine Council for Aquatic and Marine Research and Development and the National Research Council of the Philippines), Environmental Protection Administration of Chinese-Taipei, National Taiwan University (Chinese-Taipei), Environmental Protection Agency of the United States of America and the Interstate Shellfish Sanitation Conference.

The conference aimed to facilitate the management and mitigation of the impacts of harmful algal blooms to enhance sustainable use of marine resources. The primary goal of this Conference was to share and develop mechanisms for minimizing the impact of harmful algae and their toxins on human health, commerce, fisheries resources, and the marine ecosystems throughout and beyond the Asia-Pacific region.

HAMM was attended by 104 participants representing the academe, industry, and policy-makers from 25 countries (Australia, Brazil, Bulgaria, Canada, China, Chinese-Taipei, Denmark, Japan, Malaysia, Mexico, New Zealand, Peru, the Philippines, Republic of Korea, Russia, Singapore, Slovenia, Spain, Sweden, Thailand, Uruguay, USA, Venezuela and Vietnam). There were about 69 papers presented. The papers were categorized into six different themes, namely: (a) Organisms, Toxins, Mechanisms, (b) Economic and Social Impacts, (c) Management Options, (d) Mitigation Strategies, (e) Intergovernmental Cooperation, and (f) Management and Regulatory Compatibility.

Three workshops were held during the conference. These were a Toxin Workshop, a Plankton Workshop, and the Focus Group Work Sessions. The Toxin workshop consisted of specialized lectures on toxins and detection methods, detection method training and demonstrations and focus group discussions related to toxins and detection methods. The Plankton workshop included specialized lectures on plankton taxonomy and biology, training in taxonomy, sampling and observation and focus group discussions related to plankton. While the Focus Group Work Sessions was a discussion of issues and production of consensus text which will be assembled to form the Guidelines for HAMM.

The six Focus Group Sessions were: (a) Scope of the Problem and Impacts, (b) Monitoring Programme Structure and Design, (c) Management Support Tools, (d) Analytical Methods and Standard, (e) Environmental Control and Mitigation Strategies, and (f) Regulation and Trade.

Some of the important questions/ topics addressed during the sessions were: How does one decide when a management program is justified? Is risk assessment a useful tool for managing HABs? How much should an economy with no history of HAB problems be doing?

The discussions also focused on prudent forms of management given a lack of historical data; coordination of efforts between various government agencies; co-ordination among individual governments within biological region; industry involvement /certification programmes; the use of readily observable phenomena as indicators of HABs; effective training and education tools; incorporation of other monitoring programs; policies for the consumption of fish killed by HAB; risks to humans from direct exposure; how do current regulatory requirements impact on trade?. Major attention was also given to the cost-benfit of HAM monitoring: are regulations and effective monitoring programs a cost or good business practice? Is there value in considering the adoption of standardized or complimentary regulations among APEC economies, and how should this be done? How can importing economies improve confidence in the management programs and certification of product safety of an exporting economy? How can an exporting economy develop confidence that, if they are forthright in divulging information on shellfish toxicity in their shellfish products, importing economies will respond properly?

It is planned that there will be proceedings and potentially other products of the HAMM Conference addressing some of the above issues. Information on this will follow in coming issues of Harmful Algae News and at http://vm.cfsan.fda.gov/~frf/sfhamm.html. During the conference it was suggested and discussed if the HAMM Conference could be the first in a new series of conferences on HAB management and mitigation.

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◆ Vietnam

Trichodesmium erythraeum bloom

For many years scientists of the Institute of Oceanography, Nhatrang (ION), Vietnam, have been investigating the waters of Binhthuan province, Vietnam. There is a strong upwelling in the area in July-August every year, but the oceanographic conditions and the phytoplankton associations have never been studied in detail. Preliminary results indicate that diatoms, particularly *Proboscia alata*, are found in high densities during the upwelling period. Also dinoflagellates, e.g. *Noctiluca scintillans*, can sometimes be dominant.

Recently, Nguyen & Doan (1996) reported a bloom of *Trichodesmium erythraeum* in the Binhthuan waters. According to the experience of the local fishermen living in the coastal villages, this is apparently a recurrent phenomenon during the period March to July. In some cases, fish kills occur, but the reason for this is not clear. So far there has been no official reports of socio-economic problems relating to blooms of *T. erythraeum* or other phytoplankton organisms in Vietnam.

In November 1998, a cooperative research programme on harmful algal blooms, HABViet, was commenced between the IOC Science & Communication Centre on Harmful Algae, University of Copenhagen, Denmark, and the National Oceanographic Institute in Nhatrang, Vietnam. The project is sponsored by DANIDA and will survey the distribution of potentially harmful microalgea in Vietnamese waters. A sampling programme has been initiated and covers most of the Vietnamese coast (Fig. 1A) including the Binhthuan area (Fig.1B). In the samples from 26-28 March 1999, *T. erythraeum* was recorded again. The sea was calm and the bloom discoloured the water and formed long scattered bands (Fig. 2) extending about 8 km along the coast of Tuyphong, in the Binhthuan Province. The highest cell density was at northern end of the area, near Cau Island (Fig. 1B). The samples were collected at 10-15 metres depth, the salinity of the surface layer was about 32 ppt. and the temperature of the surface and bottom water (15 m) was and 28-29°C, and 26-27°C respectively. In the surface layer, diatoms and dinoflagellates were very few, but the composition of other phytoplankton species associated with the bloom has not been analysed in detail.

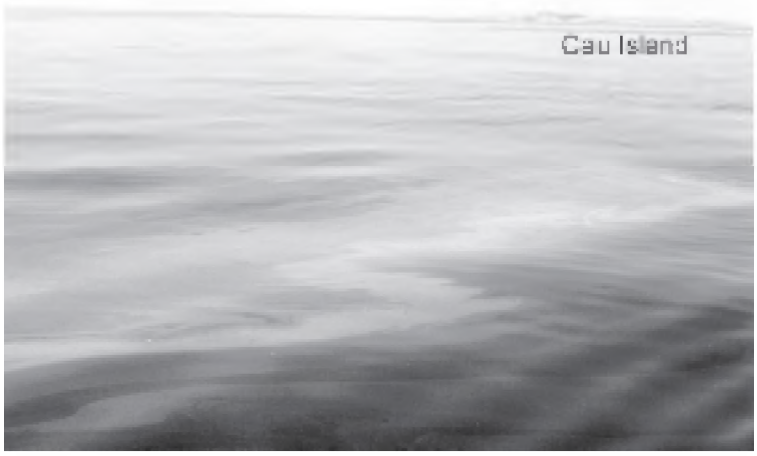

Figure 2. Grey tide of Trichodesmium erythraeum

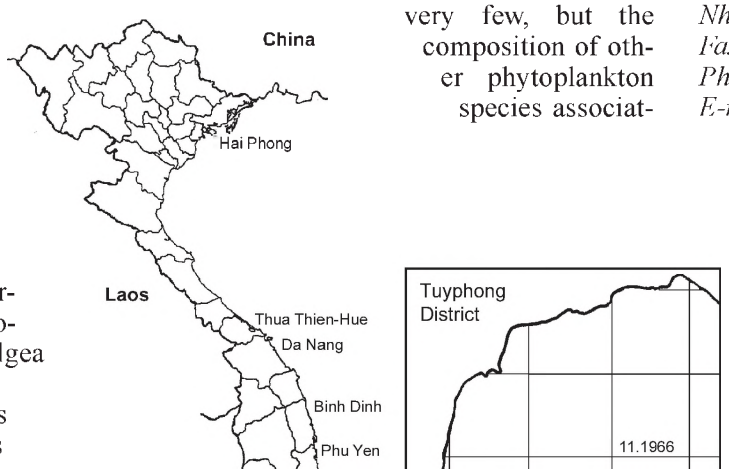

Figure 1. (A) Map of Vietnam indicating the coastal provinces where the HABViet Project has sampling sites. (B) Area affected by grey tide.

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HAB-DIR on the net again!

The International Directory of Experts In Harmful Algae and Their Effects on Fisheries and Public Health, in short HABDIR, is an international directory which lists research scientists, fisheries managers, public health officials, aquaculture professionals, physicians, and animal health specialists who are experienced in dealing with toxic and harmful algal event and their impact on fisheries and public health. The directory is intended to improve and accelerate international information exchange and rapid access to experts who can respond quickly to these outbreaks and thus help reduce the consequences to fisheries and public health.

The first edition of this directory was published in 1990 by Woods Hole Oceanographic Institution Sea Grant program. The second edition was a cooperative effort by the U.S. Department of Commerce, U.S. NOAA National Marine Fisheries Service, U.S. Northeast Fisheries Science Center, and the IOC. The Third edition which provide the basis or the WWW version was prepared by the IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Denmark.

After the introduction of the Directory to the WWW it can be currently updated and amended. The Directory is by no means comprehensive, but it is hoped that all users will help correct mistakes and encourage their colleagues to join the Directory.

http://ioc.unesco.org/hab/data1.htm#1

◆ International

Updated Questionnaire on Design of Harmful Algal Bloom Monitoring Systems

Harmful Algal Blooms (HAB) continues to be a problem to seafood safety and to local or regional economies. The IOC Harmful Algal Bloom Programme is endeavouring to take initiatives which help to provide a better basis for designing and implementing HAB monitoring and management systems.

A global survey was made in 1995 on how various countries had designed and implemented their HAB monitoring programmes. This first survey resulted in the publication of a report in the IOC Technical Series (No.44) summarizing the results. The report has been one of the most requested IOC publications ever since and is now out of stock. The full material from the survey is also available as a meta data base which is accessible via the IOC HAB homepage.

Almost five years have passed, many more countries or areas have now HAB monitoring systems, and many monitoring programmes have probably been modified. It has therefore been decided to update the IOC-ICES database on how harmful algae are monitored around the world.

Your assistance in up-dating, or submitting for the first time, information on HAB monitoring in your country, region, or area, is extremely valuable and the success of this initiative depends on the collaboration of national monitoring agencies.

The questionnaire can also be downloaded from http://ioc.unesco.org/hab/data2.htm#1 and we welcome as many electronic submissions of the questionnaire as possible.

Thank you in advance for you cooperation.

IOC Science and Communication Centre on Harmful Algae

Henrik Enevoldsen

Proceedings of the 7th and 8th International Conference on Harmful Algae

Are still available at a very low price. To libraries and laboratories in developing countries it is free of charge. For all others there is a 30 USD handling fee/copy.

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Future events

February 2000

HABTech 2000, A workshop on harmful algal bloom & marine biotoxin monitoring Cawthron Institute, Nelson, New Zealand, 3-5 February 2000. More details at: http://www.cawthron.org.nz and http://webnz.com/nelson Registration: People who wish to attend should register by post or E-mail at Info@cawthron.org.nz as soon as possible. If you wish to make personal contact with the organizers please e-mail, or fax (0064 3 546 9464) either: Jenny Ragg (info@cawthron.org.nz), Lesley Rhodes (Lesley@cawthron.org.nz), Lincoln Mackenzie (Lincoln @cawthron.org.nz).

February 2000

9TH INTERNATIONAL CONFERENCE ON HARMFUL ALGAL BLOOMS- "HAB2000", 7-11 FEBRUARY 2000, HOBART, TASMANIA, AUSTRALIA

The HAB2000 conference will provide a broad forum for phycologists, microbiologists, toxicologists, physiologists, molecular biologists, aquatic ecologists and managers to address and exchange research findings and perspectives concerning all aspects of toxic and harmful algae, including freshwater cyanobacteria and ciguatera fish poisoning. Harmful algae and their toxins can have serious implications for human health, aquaculture, fisheries, seafood trade, tourism and the aquatic environment. These phenomena pose a growing global problem at a time when human reliance on coastal zones for food, recreation and commerce is expanding. CONFERENCE SECRETARIAT: Conference Design Pty. Ltd., PO Box 342, Sandy Bay, Tasmania 7006, Australia, Telephone: intl +61 3 62243773; Fax: intl +61 3 62243774; e-mail: mail @cdesign.com.au CONFERENCE WEBSITE (including registration & hotel reservation forms): http://www.utas.edu.au/docs/plant_science/HAB2000/

June 2000

3RD INTERNATIONAL CONFERENCE ON MOLLUSCAN SHELLFISH SAFETY, Southampton College, Long Island University, New York, USA, 19-23 June 2000. The sessions will be dealing with shellfish biology and ecology; impacts of harmful and toxic algae; monitoring and management; health and sanitation; chemical and microbiological contamination and assessment; depuration technology; aquaculture and harvesting sites; quality assurance programmes and regulatory controls. Contact: ICMSS c/o Dr. Sandra E. Shumway, Natural Science Division, Southampton College, LIU, Southampton, NY, 11968 USA, Fax: +1 516 287 8419, E-mail: sshumway@southampton.liunet.edu

August 2000

IOC DANIDA TRAINING COURSE ON THE TAXONOMY AND BIOLOGY OF HARMFUL MARINE MICROALGAE

This two weeks training course will focus on identification and preparation techniques supplemented by lectures on different aspects of the biology of harmful algae. Teaching staff will include: Dr. Y. Fukuyo (Univ. of Tokyo), Prof. Ø. Moestrup (Univ. of Copenhagen), Dr. J. Larsen (Univ. of Copenhagen/IOC Centre). The Course is organized by the University of Copenhagen and the IOC Science and Communication Centre on Harmful Algae. The course has 10 seats free-of-charge for participants from developing countries, and up to 5 seats for self-paying participants. Application forms can be downloaded from the IOC HAB home-page at http://ioc.unesco.org/hab/courses.htm or by contacting the IOC Science and Communication Centre on Harmful Algae, Botanical Institute, Ø. Farimagsgade 2D, DK-1353 Copenhagen K, Denmark, Fax: + 45 33 13 44 47, E-mail: hab@bot.ku.dk. *DEADLINE 1 April 2000.*

For a more comprehensive overview of up-coming activities in marine science you should visit the IOC International Marine Meeting list at the IOC homepage at http://ioc.unesco.org/iocweb/IOCinfoservice/activities.htm

Literature for developing country libraries

Limited copies of the following titles are available from the IOC Science and Communication Centre on Harmful Algae, Denmark(see address below), to libraries of marine science institutions *in developing countries only*:

- The Genus Alexandrium Halim (Dinoflagellata). **E. Balech, 1994.**
- Algae. An Introduction to Phycology. **C. van den Hoek et al., 1995.**
- Identifying Marine Phytoplankton. **C. Thomas et al. (eds.), 1997.**
- Proceedings of the International Symposium on Ciguatera and Marine Natural Products, Hawaii, 8-10 August 1994. **Hokama et al (eds.) 1995.**
- Proceedings of the First International Congress on Toxic Cyanobacteria, Denmark, 20-24 August 1995, and, Proceedings of the First Maj and Tor Nessling Foundation Symposium on 'Recent developments in Cyanobacterial Research', Finland 16-18 August 1995. **Moestrup et al** (eds.) Phycologia, Vol.35 No.6, **1996**
- Photography through the microscope. Kodak. **J.G. Delly, 1997.**

Applications must be submitted and signed by the responsible librarian, and should indicate why the requested title is of specific interest to your institution and its ongoing research or teaching. No requests from individuals will be honoured.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms *No. 20*

IX Conference on Harmful Algae



Convenor's report

The 9th International Conference on Harmful Algal Blooms (HAB2000) was held in Hobart, Tasmania, Australia, from 7 to 11 February 2000. The conference venue was the Wrest Point Convention Centre located on the magnificent foreshores of the Derwent River estuary, and the conference period was blessed with glorious summer weather conditions. HAB2000 was attended by some 500 participants (481 full and 45 day registrations) from 47 countries. This was the first time this prestigious conference series was scheduled in the Southern Hemisphere, but this appeared not to have deterred strong participation from Europe (122 participants), USA (77) and South East Asia (84).

A total of 130 talks and 308 posters were presented, and where parallel sessions were scheduled the plenary introductory session included reviews covering the full range of topics. The scientific programme focussed on new algal bloom species (e.g. *Pfiesteria*, new gymnodinioids), new toxic phenomena (e.g. azaspiracid poisoning, Californian seal mortality), new regional bloom events (e.g. Scotland ASP outbreak), ecophysiology and bloom dynamics, cysts and sediments, eutrophication, novel toxins, ecophysiology of toxin production, exotoxins, foodchain

(Cont'd on p. 2)

◆ **Spain**

Harmful algae events in the Mediterranean: are they increasing?

The reported world-wide increase in harmful algal blooms (HABs) has also involved the NW Mediterranean, where there have been numerous dinoflagellate blooms, especially *Alexandrium* species, in the last 50 years (1). Three explanations for this increase have been suggested, i) increased study and knowledge, ii) stimulation due to anthropogenic eutrophication, and iii) increase in the geographical dispersion due to transport of cysts in ballast water or to movement of mollusc stocks from one area to another. Here we propose iv) a new explanation, which may be important for calm tideless seas, and very much *humanised* like the Mediterranean. This stems from the ability of dinoflagellates to colonize and proliferate in habitats newly created by human activity. These four points are discussed below.

Without underestimating the importance that mesoscale phenomena can have in the Mediterranean in relation to the onset of algal blooms, the experience of the monitoring of HAB shows that the Mediterranean problem is associated, in the majority of cases, with phenomena on a lesser scale. Also, in recent decades, there has been an elevated population growth in coastal areas and a clear trend towards the exploitation of the coastline for recreational purposes. Recreational use leads in most cases to a demand for calm waters, and causes a significant increase in nutrients and in confined areas (ports, breakwaters, semi-closed beaches). This creates new microhabitats with modified hydrodynamic regimes (higher water stability and water residence time). Both factors favour dinoflagellate growth.

The Mediterranean region, especially the north-western basin, supports a very high population (100 inhabitants/m of coastline in some areas). In addition, the seasonal population related to recreational use of the coastline increases total population by more than 50 % in some regions. There has also been a marked increase of confined waters, for e.g. to 40 harbours in 400 Km of coast in Catalonia. We hypothesize that use of the coastal zone (man sheltered areas) is an important factor in HABs expansion and recurrence in the Mediterranean. In the NW Mediterranean, *Alexandrium* proportionally causes most HABs.

Three species of *Alexandrium*, two well known (*A. minutum* and *A. catenella*) and one recently described (*A. taylori*), have

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(Cont'd from p. 1)

effects, population genetics, molecular probes, immunological methods, role of bacteria, and impacts on finfish and shellfish aquaculture operations. Being held in Australia, this was the first conference in this series which prominently covered problems caused by freshwater cyanobacterial blooms, including impacts of cyanotoxins on human health and aquatic food webs, and aspects of drinking water treatment (see separate report by Glenn Shaw). A public forum on "Algal blooms: impacts on health, environment and economy" was created to deliver the conference message to a wider public.

This forum coincided with a public art event entitled "Ecological Roulette", in which 30m large dinoflagellate images were projected on the outside of the Wrest Point building. Participants enjoyed mid-conference excursions to Tasmanian wilderness forests, wildlife park, and local shellfish and aquaculture operations. A highlight for many participants was a 35min theatre production "The Deadly Dinoflagellate", which portrayed in a powerful fashion the dire consequences of a Gym Nodinium ship ballast water introduction into pristine Tasmanian waters. The HAB2000 conference was dedicated to the life-time pioneering achievements of Prof. Max Taylor, who also presented the conference summary. The conference received extensive media coverage and made a significant contribution to wake up Australia from its "algal apathy".

Gustaaf M. Hallegraeff, University of Tasmania, GPO Box 252-55, Hobart, Tasmania 7001, AUSTRALIA.



Cyanobacterial presentations at HAB 2000

While the main focus of this conference series had previously been marine algae with dinoflagellate bloom presentations dominating, the organisers on this occasion were successful in incorporating a significant number of presentations and posters on freshwater cyanobacteria and their toxins. Parallel sessions tended to feature marine dinoflagellate presentations concurrently with cyanobacterial speakers where possible. It is hoped that this very successful conference is a model for the future incorporation of both toxic dinoflagellate and cyanobacteria research findings for future events.

Keynote speakers on toxic cyanobacteria were Professor Ian Falconer from the CRC for Water Quality and Treatment in Australia and Dr Gary Jones from the Australian Commonwealth Scientific and Industrial Research Organisation. Professor Falconer gave a presentation on toxic cyanobacterial blooms in Australia from a human and animal health perspective, and highlighted the large number of stock poisoning incidents that have been caused by cyanobacteria in Australia, which date back to the late 1800's. Of more recent concern was the bloom of Anabaena circinalis in the Darling River which stretched for 1000km, killed 10,000 livestock and required emergency water treatment facilities to be established to produce water safe for human consumption. Other aspects highlighted by Professor Falconer were the significant occurrence of toxic Microcystis aeruginosa and Cylindrospermopsis raciborskii blooms that regularly occur in water reservoirs and rivers in Australia, together with their health implications.

Dr Jones spoke on the Australian experience in cyanobacterial bloom ecology and management. In particular he highlighted the fact that Australian reservoirs tended to be stratified in the warmer months of the year and that at these times, Australian rivers tended to be very slow flowing or were comprised of a series of weir pools, all of which featured cyanobacterial blooms. The conditions for a large part of the year in water bodies in most of Australia favoured cyanobacterial dominance due to thermal stratification and short photic zone depths. Other aspects of Dr Jones' talk featured management strategies such as catchment management, and reservoir techniques such as destratification and biomanipulation.

A variety of speakers spoke on various aspects of toxic cyanobacteria. A number of sessions were organised, including: occurrence of toxic blooms; water treatment to remove cyanobacterial toxins; freshwater ecology of cyanobacteria; and the impact of cyanotoxins. In addition, a number of sessions featured presentations both on cyanobacteria and dinoflagellates. These included the ecophysiology of toxin production, control and management of blooms, population genetics and phylogeny, the use of molecular probes for detection of algae and immunological methods for toxin determinations. One interesting aspect that involved both cyanobacteria and dinoflagellates was a study on dissolved nitrogen release by the marine cyanobacterium Trichodesmium erythraeum and consequent uptake and bloom formation by marine dinoflagellates.

The marine cyanobacterium Lyngbya majuscula produces dense blooms in Southern Queensland marine waters, and has been responsible for human dermatological problems. Regional events featured included Cylindrospermopsis dominance in reservoirs, the effect of hydrological manipulation in reducing cyanobacterial blooms, Nodularia blooms in Finland, and the potential effects of global climate change on cyanobacterial abundance and toxin production. While microcystin producing cyanobacteria are still very important on a global scale, the presence of the hepatotoxin producing cyanobacterium, Cylindrospermopsis raciborskii is being recognised as significant in many countries around the world.

Some presentations dealt with methods to remove cyanobacterial toxins from drinking water such as ozonation, chlorination, bacterial degradation and photolytic degradation using UV/TiO2.

Toxicological issues discussed ranged from the toxicodynamics of microcystins ingested by dairy cattle and implications for milk contamination, to human health risk assessment of cylindrospermopsin, and an interesting evaluation of the effects of the lipopolysaccharide endotoxins in combination with microcystins. Ecological effects of cyanotoxins received considerable attention. such as the effects of toxins on aquatic organisms including fish, and uptake and metabolism by aquatic plants.

Population genetics and phylogeny of cyanobacteria are important areas of research. State of the art molecular techniques for species identification, and the identify toxin producing genes of cyanobacteria also received attention. So did rapid methods for analysis for cyanobacterial toxins and immunoassays for a number of toxins, including microcystins and saxitoxins. A novel and sensitive method for saxitoxins is the saxiphyllin assay, developed by Dr Llewellyn of the Australian Institute of Marine Science which involves the use of a naturally occurring saxitoxin binding protein.

Highlights of the conference from the cyanobacterial research perspective stemmed from the considerable number of participants interested in this field. Presentations covered a wide range of disciplines from taxonomy to health effects of the toxins. An integrated approach to the field was evident with various research groups interacting to evaluate environmental health aspects of cyanobacteria and to produce possible solutions to problems. These solutions arise from the understanding of the ecology of the organisms and toxin behaviour and lead to catchment management and water treatment protocols to reduce the effects of these organisms on man and the environment.

Glenn Shaw, National Research Centre for Environmental Toxicology, Queensland, Australia

A view from New Zealand

2nd February 2000, 100 Attendees met in Nelson, New Zealand for a pre-conference workshop, where the developers of the latest and greatest methods met to show their wares. Demonstrations were given of many of the techniques employed in algal/shellfish toxin research, covering all aspects from algal collection using nets, to remote telemetric sensing by tethered or roving buoys. Presentations from many countries showed off technologies including microscopy, nucleic acid hybridization, immunochemistry, bioassay applications, fluorimetry and more. The relatively small size of the workshop facilitated contact and exchange of information between attendees.

The vision for the 21st century "sea's" a self contained, remote early warning system, backed up by sophisticated laboratory analysis to ensure the safety of the consumer, and the health of the industry. For a report visit http://www.cawthron.org.nz/news_habtech2000_report.htm

7th February 2000, A brief hop across the ditch to Tasmania. About 500 Attendees met in Hobart, for the IX Harmful Algal Bloom Conference. My first thought was that it was great to travel such a short distance to an international meeting! Normally it takes a while for my time-clock to adjust to the time difference in travelling half way around the world to the Northern Hemisphere!! Previous meetings have concentrated on the harmful marine algae, and it was very noticeable that here the freshwater algae had surfaced to greet us. Indeed, as the conference opened, a bloom in one of the local reservoirs was making news headlines - just a coincidence Gustaaf tells us!

Concurrent sessions were required to accommodate the large number of papers presented, and while this must have been a logistical nightmare, in the main they were organized such that the overlapping of subjects from similar fields was minimized.

Much new data was presented in the oral papers, newly discovered HAB species (e.g. new *Pfiesteria* species), newly discovered toxins (e.g. Azaspiracid), new technologies for the detection of both (e.g. RNA probes, immuno-probes), and new understanding of the genetics of toxin synthesis (e.g. microcystin biosynthesis). The recognized distribution of harmful algae appears to be increasing, both as toxicities occur, and as more of the world community becomes aware of the issues (e.g. ASP in Scotland, *Gymnodinium* in Hong Kong). The introduction of freshwater cyanobacteria to this meeting was a good move, as many attendees' research involves only one, or other environment. There is much that we can learn from the techniques applied across both these fields. The death of cattle and sheep from PSP, for example. Where else but in Australia could these animals die from shellfish poisoning!! The chemistry and mechanism of action of the saxitoxins produced by the marine and freshwater algae are naturally exactly the same. It was again a difficult task to visit all 390 posters, and to take in the large amount of new information presented here. The meeting design was conducive to spending a large amount of time at the posters, but of course, as always much more time seems to be needed to view and digest these, and to catch up with colleagues and plan collaborations. Fortunately the latter was facilitated at an establishment called Irish Murphy's, and at the conference functions so well provided. Thanks Gustaaf and the organizing committee for a very well run conference. We appreciate your hard work. The beauty of the HAB series of conferences to me is the range of disciplines that the field encompasses, and the degree of cross-fertilization of ideas that this generates. There are few other conferences that I have attended where this happens to the same degree. This one again achieved this goal.

The conference is growing larger each time, and extrapolations produced by our after dinner speaker showed that by the time we travel to South Africa in 2004, we should have over 800 attendees representing every country in the world. I look forward to meeting many old friends, and new friends from landlocked countries in Florida, October 2002 for the Xth International Conference. Our turn for the jet-lag next time!!

The final question for attendees from the North, Did you check which way the water turned as it went down the plug hole??

Dr Ian Garthwaite, AgResearch Ruakura, Hamilton, New Zealand.

Reflections on the Hobart Symposium

The Ninth International Conference on Harmful Algal Blooms convened in Hobart during 7 - 11 February 2000 continued the tradition that has characterized these Conferences. Gustaaf Hallegraeff and his organizing committee are to be commended for their good efforts on our behalf. Attendees were once again exposed to new discoveries, techniques and insights; opportunities to communicate in formal and informal settings; to interact socially; to form new friendships, and to continue old ones; to plan collaborative research; to "talk shop", and to experience the novel, "red tide" theatre. The Conference also revealed both the remarkable esprit that continues to characterize the harmful algal bloom community, and its equally successful and diverse scientific, pedagogical and societal efforts. The multitude of workshops and training courses given to date; the establishment of the IOC Science and Communication Centres on Harmful Algae; IPHAB; national and regional programs of HAB research, the highly informative Harmful Algal News, and planned new initiatives such as GEOHAB, are some of the tangible results that have accompanied the ever-deepening scientific insights into harmful microalgae. To me, this collective spirit was vividly reflected by the great enthusiasm with which Yasuwo Fukuyo distributed his CD Discs from his booth in the Convention Center lobby, and in continuance of his great service to the HAB community.

Don Anderson's photographic journey depicting the development of the HAB initiative was enjoyable, but this "remembrance of things past" made me wonder whether we are now en route to a different future. I fear that we are in danger of compromising, if not losing, the historical conditions that have engendered the HAB community esprit and achievement. I wonder about this because the Conference revealed some worrisome features spawned by our remarkable growth as a subdiscipline: we are becoming too big and have outgrown the Conference format traditionally followed! The 130 oral presentations and 308 posters scheduled, and crammed into only four days of formal meetings (with concurrent sessions) were too concentrated a dose. I found that there was not enough time to hear and review adequately my many selections from among the nearly 450 presentations. The 100 minutes allotted for Tea and Poster Review Sessions equaled 3 minutes per poster. And the approximately 500 attendees, while delightful, made it difficult to find individuals with whom I wished to talk. I offer these personal inconveniences not as a criticism of the Conference organization, but as a reflection of the great attendance and scientific presentation pressures placed on the conveners confronted also with providing social and tourism venues expected by attendees. Do we lengthen future Conferences and/or reduce tourism options? How do we accommodate the growing demand for presentations? How do we prevent splintering into sub-groups compromising valuable interdisciplinary exchange? Is the quest for diversity, such as inclusion of freshwater cyanotoxicity issues, for example, compromising effective communication of pressing marine HAB issues? Karen Steidinger and her co-conveners in organizing the St. Petersburg meeting will probably face even greater attendance and presentation pressures. It seems that we can now safely say that the traditional HAB Conference format will not accommodate those expected pressures.

The Conference revealed some fissures that are accompanying the growing scientific maturity and size of the harmful algal bloom community. The issues of publication of Conference presentations and "what is a meaningful contribution" remain unresolved, made even more difficult by some sensitive issues embedded within this controversy. Scientific observations become information only after communication, usually through publication; thereafter, this information belongs to everyone: it is a principle that we must protect. Decisions as to what is, or is not worthwhile information deserving of publication can easily become a policy of censorship unless based on clear and acceptable criteria. While I do not believe that the proposed "new" criteria of manuscript selection were adequately defined at Hobart to warrant changes in publication of Symposium papers, this valid concern over publication should revive discussions as to the merit of starting a journal dealing with HAB issues. I support this option and the derivative publication opportunities which it presents.

The selection of future Conference sites continues to be an unstructured process, with need for a revamped approach to this important decision. For, once again, an element of arm-twisting seems to have been a factor. I am very pleased that Grant Pitcher will host the 2005 Conference in South Africa, but many attendees missed the proposal from Ireland, and there was confusion (and loss of vote) over the voting procedures to be followed. Convening a Conference is a formidable task, obviously, and proposers need to be encouraged, but clearly there is need to develop standard procedures of proposal and voting.

The still embarrassing and marginally successful efforts to organize ISSHA seven years and three conferences since Nantes (1993) are inexplicable. It was hoped that ISSHA would be off and running after Hobart, and take a lead role in helping to guide the HAB community through its continued growth and in coming to grips with the issues mentioned above. I was not encouraged by the society meeting, and now wonder whether a society is too soon, too structured, too manipulatable, or even needed to serve our collective needs. The esprit and achievement of the HAB community to date could easily be dampened by overly organized regulation of our diverse scientific, pedagogical and societal efforts. Should we revisit the need to have a society?

In summary, Gustaaf Hallegraeff and his co-organizers provided a great service in organizing a stimulating Conference: scientifically, socially, aesthetically, and in its revelation of a number of logistical, organizational issues which we, now forewarned, can more effectively deal with, modify and build upon to ensure continued success of future Conferences.

Ted Smayda, Graduate School of Oceanography, University of Rhode Island, U.S.A.



Marine Toxins

Many important presentations on algal toxins were given at the recent HAB conference in Hobart. Dr Burkholder gave the opening plenary address on the toxicity of *Pfiesteria piscicida* under different conditions. The type and nature of toxins produced by P. piscicida, and whether "never-toxic" strains exist in the wild require clarification.

Dr Lewis reviewed ciguatera, highlighting the significant milestones achieved over the last 50 years. These include:

- In the late 1950s, Banner established a program of multidisciplinary research that has led modern ciguatera research;
- Based on the hypothesis of Randall (1958), Yasumoto et al. in 1997 identified G. toxicus (subsequently confirmed) as the origin of Pacific CTXs;
- In 1981, extending earlier studies of Rayner in Hawaii, Lazdunski's group determined that CTX was a "site 5" VSSC activator;
- Palafox et al. in 1988, following its chance use in an undiagnosed severe case of ciguatera in the Marshall Islands, mannitol was introduced as a treatment for severe ciguatera;
- In 1989, following the pioneering work of Scheuer's group, Yasumoto's group determined the structure of Pacific CTX and its likely precursor from *G. toxicus*;
- In 1998, taking advantage of high field NMR techniques, the structure of Caribbean CTX was determined by Lewis et al.

Despite this progress, many important new milestones in ciguatera research remain to be achieved. These included:

- Determining the specific environmental and genetic factors that influence toxin production and the proliferation of *Gambierdiscus* species;
- Determining the origin of Caribbean and Indian Ocean ciguatoxins;
- Determining the structure of Indian Ocean ciguatoxin(s);
- Development of simple (oral) therapies for ciguatera;
- Development of rapid polyether synthesis;
- Development of a commercially available, validated, cost-effective screen (antibody and/or VSSC) for ciguatoxins in ciguateric fish;
- Development of improved analytical methods (LC/MS etc) to confirm and accurately quantify ciguatoxins in fish, humans, and dinoflagellates.

Achieving these new milestones represents a number of significant scientific challenges requiring well-funded multidisciplinary research, probably with an increasing emphasis on interlaboratory collaborations.

Professor Yasumoto led a session on ciguatera, describing an exciting new mass spectrometric (MS) method for the elucidation the primary structures of polyether toxins with microgram quantities of material. The strucutres of 20 ciguatoxins were defined using this method. The technique offers for the first time the prospect for elucidating the structures of all minor ciguatoxins, and will allow detailed studies of the biosynthesis and food chain transfer of ciguatoxins. Professor Yasumoto also described some collaborative work with Dr Legrand, describing liquid chromatography/MS methods for identifying new ciguatoxins.

Professor Shimizu reported studies that confirmed a dual mechanism of methyl introduction in brevetoxin biosynthesis. This unusual incorporation pattern may apply to the synthesis of polyketides by dinoflagellates in general.

A new approach towards the development of antibody based tests for ciguatoxins was reported by Dr Pauillac. High affinity antibodies were produced to fragments of ciguatoxin and are being evaluated in simple assay formats.

Kevin James reported on Azaspiracid Poisoning (AZP), an important new shellfish toxic syndrome in Europe. This class of toxin has caused human intoxications, following the consumption of Irish mussels (*M. edulis*).

Three toxins have been isolated and structurally elucidated and they contain a novel spiro ring assembly. The origin and pharmacology of Azaspiracids are under investigation.

The pharmacology yessotoxin, a marine toxins with uncertain human health impacts, was reported on by Dr. Botana. This group found that yessotoxin acts to increase the release of calcium from intracellular stores and to inhibit cAMP levels in resting lymphocytes. Thus yessotoxin may be an important new tool for the study of signal transduction mechanisms.

Dr Goto reported that pectenotoxin-2 and analogues were identified for the first time from Chile in two species of mussels, and in *Dinophysis norvegica* collected along Swedish coast. In the Baltic Sea, hydrolysis of *D. norvegica* extracts revealed dinophysistoxin-1 and okadaic acid. The Chilean mussels also contained high concentrations of yessotoxin (YTX) and 45-hysdroxy-YTX. These studies indicate the need to expand the list of toxins screened in certain locations.

Richard J. Lewis, University of Queensland

Tasmanian delights

Hobart, 7-11 February, 2000

As an exercise in solid geometry, one can calculate the maximum possible separation of three points on a spherical surface. It's easier than you think! A rough approximation has been achieved by **Hirofumi Goto** from Tokyo with colleagues in Chile and Sweden. This simply shows the "reach" of harmful algologists. We will continue to argue about whether HABs are spreading, but this example suggests habologists cannot spread out further without getting nearer again. Maybe habologists are spreading the HABs? Did anybody *en route* to Hobart check their shoes or their pockets for cysts? Even the Arctic is full of *Alexandrium*. Why, they've found phycotoxins in Antarctic lakes; would you credit that? Earlier worry that the relative remoteness of Hobart might limit attendance at the 9th HAB conference has proved unfounded. As **Max Taylor** said to me, somewhat bemused, as the first plenary began, "The whole family's here." Capo di capos! The family has grown too, this is I think the first time we have seen participants from Bangladesh, Hungary, Kuwait and Qatar. We also had a wedding; **Mark Poli** and **Sandra Flynn** tied the knot on Mount Nelson. An old quip maintains that Sydney,

Australia, is the most populous Irish city in the world. The press of Irish in the conference bar might have suggested Hobart is; their numerical participation, calculated as a national *per capita* quotient, normalized for distance travelled, could be a record. The Danes may have run them close; someone should do the sums. I don't know the population of Denmark. There was a good turnout from China, but then aren't there rather a lot of them?

My personal short list of highlights from the conference would include the recently discovered parasite *Parvilucifera*, especially the beautiful pictures shown by **Marie-Josèphe Chrétiennot-Dinet**, the notion of **Chris Scholin** that domoic acid can hide in the mud and kill later (it surely was not evolved to do that!), and the continuing saga of the *Alexandrium* dynasty. The morphospecies *A. tamarensis* occurs in environments as distinct as the Arctic (**K.C. Ho**) and the Gulf of Thailand (**Gires Usup**). Three *tamarensis* genotypes have been identified from Australia and New Zealand by **M.F. de Salas** who thinks they are endemic in the region. **Linda Medlin** showed that the *tamarensis* bloom encountered in the Orkneys in 1997 is "American", and considered by which Arctic sea route it might have entered the Atlantic from the Pacific. Did they come on the feet of capelin which made the same journey? The dispersal of *Gymnodinium* species with reticulate cysts was discussed by **Chris Bolch**; these are not found in high northern latitudes and their dispersal must have been by warmer routes. Tropical *tamarensis* strains would perhaps have accompanied them. At the Key Biscayne conference in this series (1978), Ramon Margalef introduced his famous mandala, a phase space in which phytoplankton strategies can be ordinated. **Ted Smayda** explored some of the ramifications of this mandala at the Vigo conference, and at Hobart had a closer look at the turbulence axis. Provocative stuff. **Yasuwo Fukuyo**, as usual, was dispensing free gifts, this time a magnificent CD.

A few new species have been added to the HAB cuisine, a *Nitzschia* from shrimp farms in Vietnam (**Yuichi Kotaki**) which produces domoic acid, a second *Pfiesteria* (**Howard Glasgow**), and some may need to be reduced among *Dinophysis* (**Beatriz Reguera**) and *Chattonella* (**Yoshihiko Sako**). There is also a *Gyro-/Gymnodinium* boundary dispute (**A.J. Haywood**), and a new *G. breve* imposter in New Zealand (**F. Hoe Chang**). A quick look at the Latin-name stakes, the guys that keep us in business (the grass roots of Habology); *Alexandrium* still seems to be our best loved genus (not counting the newcomer cyanophytes), as it always has been in this conference series, *Pseudo-nitzschia* is currently running second, close behind are *Dinophysis* and *Pfiesteria*, and the *catenatum's, mikimotoi's,* and other sundries are awaiting their chances.

On the toxin sideboard, we have a new dish, azaspiracids. Ask **Sandy Shumway** how to say it with an eastern US accent. These were first found in Irish mussels, later elsewhere in northern Europe, and **Kevin James** suspects a dinoflagellate source for them. We can anticipate that they will shortly turn up all over the planet (in time for the next conference, and in further illustration of the global spreading hypothesis). Their impact on the intestinal villi (**Emiko Ito**) sounds rather like that of *psilosis*, tropical sprue, (of obscure aetiology; is this a clue?). Our society magician, **Takeshi Yasumoto,** has introduced new toxin extraction procedures. There is growing attention to the functional rôles of toxins (too long classified as defensive chemicals) by **Allan Cembella** (polyethers), **Eden Rue,** and **Nicolas Ladizinski** (domoic acid). There were around seventy contributions on toxins, and reviews from experts would be very welcome, volunteers please. (Hey, you! Can you play music? Then come and help carry this piano). Still with the molecules, it has now been shown that DNA sequencing can be done with single *Dinophysis* cells, even those preserved in lugol (**Bente Edvarsen**) or methanol (**Angeles Aguilera**). This quantum leap will allow new questions to be asked about *Dinophysis* and other taxa which have not yet been brought into culture.

Neurological disorders, and especially symptoms like vomiting and diarrhoea *run* like refrains through the professional lives of habologists, but we rarely give a thought to the diagnostic problems such complaints pose the medical profession. Some conference posters highlighted these difficulties. **Susan Gerber** took us inside a Chicago restaurant where an amberjack dish gave diners ciguatera; she showed us how tracking credit card records can lead to better diagnoses. Physician performance itself was scrutinized with respect to ciguatera in Dade County, Florida, where the condition is endemic (**Donna Blythe**). Epidemiological data on *Pfiesteria* exposure is also now being brought into better focus (**L.M. Grattan**). Still on the medical front, there is an urgent need for toxicological studies of the effects of the *new* pecteno- and yessotoxins on human consumers.

At the Takamatsu conference in 1987, **Masaaki Kodama** reported experiments which he claimed indicated that *Alexandrium* toxins are synthesized by intracellular bacteria (reviving an earlier suggestion of Estela Silva). This continues to be a hot research topic, but remains unresolved. **Jane Lewis** now shows that bacteria (including intracellular ones) are associated with all life history stages of *Alexandrium*, but **Ya Hui Lu** says that toxicity is not enhanced by bacteria in culture, and **Michael Schweikert** failed to detect toxins in extracellular bacteria. **Masao Adachi** thinks bacteria may promote encystment, and suggests a symbiotic rôle for them; **R. Brinkmeyer** and **G. Gerdts** both noted a close association between bacteria and an *Alexandrium* bloom in the Orkneys. In contrast to these studies of *Alexandrium*, some bacterial strains may enhance domoic acid synthesis in *Pseudo-nitzschia* (**Susan Gallacher**). New techniques may be required to fully untangle the secrets of bacteria living on and in phytoplankton cells.

Algicidal bacteria, capable of killing *Coscinodiscus wailesii*, a pest of *Porhyra* culture, were described by Satoshi Nagai and Imai at the Vigo conference. Several studies presented in Hobart built on this theme, and we now know more about bacteria which can kill *Chattonella* (**Achiro Imai**), *Heterosigma* (**Hirotaka Kitaguchi**; **Noriko Kuroda**), *Gymnodinium breve* (**Xavier Mayali**), *G. catenatum* (**J.H. Skerratt**), *G. mikimotoi* (**Ikuo Yoshinaga**), and a variety of other algae. In several of these studies, it has been established that extracellular substances are involved in the lethal mechanism.

Mitigation is not a new idea, but this is the first time in this conference series that it has been served as a main course. Parasites and algicidal bacteria may eventually be used for biological control of HABs, but so far the general impression is that they are not very choosy about their victims, so their selective application will not be straightforward. *Parte beatum.* **Patrick Gentien** panicked at the thought that such delicious commodities as oysters might be secondary hosts to *Parvilucifera*. What would it do for their flavour? Even less

selective is an algal doomsday gadget already being marketed; if you want to kill *all* the algae, get in touch with **Hilaire Thomas**. But mitigation at present is mostly about mud, which can be modified to improve its flocculent action (**Sun Xiaoxia**). Throwing mud about (what mud does to the flavour of Coquille St. Jacques is another problem) is a technique with a promising future according to **Jack Rensel**, who gave an excellent overview of practical alternatives for saving aquaculture stock from algal threats. One of them, towing, saved a lot of money in China last year (**Lu Songhui**). Chemical mitigation procedures also offer some promise; two cysteine compounds tested experimentally reduce fish mortality due to *Gymnodinium* (**Ian Jenkinson**), and electrolytically generated NaOCl reduces dinoflagellate concentrations in laboratory cultures (**Hae Jin Jeong**).

Mechanisms which put the B in HAB were examined in more than twenty contributions. There was an intriguing display of how inoculating *Alexandrium* populations might adopt different distribution patterns under the influence of wind-controlled advection in a buoyant coastal plume (**Don Anderson**); but we shall have to wait longer to learn how the planozygotes find their way back to the cyst bed to close the life cycle. A prize to Don for coordinating his talk with three simultaneous movies. Advection inoculates coastal waters with offshore populations, *Gymnodinium breve* to Texas (**Tracy Villareal**) and Florida (**Gabriel Vargo**), *Gymnodinium* cf *aureolum* to Donegal Bay in Ireland (**Robin Raine**), *G. catenatum* to the Galician rías (**Ignacio Sordo**), *Alexandrium* to Maine (**David Townsend**), and perhaps *Pseudo-nitzschia* to Rio Grande shores in Brazil (**Clarice Odebrecht**). The new *Gymnodinium* from New Zealand catches rides on the East Auckland Current (**F. Hoe Chang**). Advection is implicit too in the putative link between *Dinophysis* and the buoyancy frequency (**Yolanda Pazos**). Conditions dependent on upwelling favour blooms of pennate diatoms including *Pseudo-nitzschia* in California and Oregon waters (**Vera Trainer**), and *Dinophysis* and other dinoflagellates in the southern Benguela region (**Grant Pitcher**) and off the Portuguese coast (**Teresa Moita**). In some species, cyst beds provide the basis for inoculation, and **Barrie Dale** stressed the need to learn more about the processes which control tranfers of cysts between the water column and the mud. Coastal engineering seems to have eliminated *Nodularia* blooms from an Australian estuary (**Wasele Hosja**), presumably by increasing flushing (see article by **Esther Garcés** in this issue for the opposite effect). Something analogous may have occurred in Alexandria Harbour in Egypt, where *Alexandrium* has abandoned the type locality (**A.A. Ishmael**). The harbour engineers' records might help to track this one down.

It is obvious that phytoplankton *stock* (advective and diffusive losses aside) is the difference between production and the yield to planktonic or benthic consumers, to parasites, to the sediments. Thus, as has been pointed out many times, correlations between stock levels and nutrient uptake rates are not to be expected. Then the correlation between eutrophication and the sedimentation rate of *Pseudo-nitzschia*, as in the Mississippi plume (**Michael Parsons**), can be interpreted as an increase in yield (to the sediments in this case), but not necessarily in planktonic stock levels. Increase in the latter must be due to a change in the balance between production and yield, the $(R - G)$ term of many models. But measuring the growth rates and loss rates of field populations is no doddle, as anyone who has tried will vouchsafe. This may be why no estimates of *in situ* growth rates were presented, and only one field study looked at phyto-/zooplankton relations (**David Rissik**). Interesting results obtained in "semi-natural" grazing experiments were reported by **Gregory Teegarden**. Modellers of algal population dynamics did not obtain a full meal from this conference.

HAB 2000 introduced several innovations. Parallel sessions – these have been resisted in past conferences in this series. Here they worked. This was due I think to excellent planning as well as timing - it was possible to sample both courses with a judicious choice from the menu. The combination of posters with *in situ* lunches – this was a balancing act, and for my part the cuisine won several times; perhaps people with more self-discipline saw rather more of the posters than myself? Art – as well as the **Gym Nodinium** theatre and a habological light show, we were regaled by a virtuoso performance from **Barrie Dale** who delivered his lines with the instinctive timing of music hall comedy. A mediaeval English monk, **William of Ockham**, was mistaken for a Greek. But his famous *Principle of Parsimony* (*Ockham's Razor*), variously rendered as "plurality is not to be posited without necessity", or "it is vain to do with more what can be done with fewer", can be found in Aristotle ("Nature operates in the shortest way possible", something like that), who was more reliably Greek. M.J. Dunbar said "to hell with parsimony...it is essential to abandon all subservience to the Razor of Ockham and to have the courage to construct outrageously untestable hypotheses" (1). This too happened in Hobart, and my prizes for outrageous (and essential) speculation go to **Chris Bolch** for his phylogenetical musings, and to **John Walsh** and **Karen Steidinger** for their inspired but quite unbelievable conjecture, that Saharan dust blown across the Atlantic Ocean promotes production on the west Florida shelf by cyanobacteria, and that the nitrogen these latter extract from the atmosphere is released to feed *Gymnodinium breve*. Far out! Do people still say that?

Reference:

1. Dunbar, M.J., 1980. Canadian J. Zool., **58**: 123-128

Timothy Wyatt, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas

Corrigendum for Harmful Algae News, no. 19

On P. 4, middle column, the first two sentences in the first paragragph, "The Wellington Harbour toxin is stable …..".
As it was written, these sentences do not make much sense. They should read: "The Wellington toxin is stable within the range from pH 3 to 9. The instablity of this toxin beyond pH 3 makes it less likely to pose any human health risk when it is eaten."

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Molecular genetics at HAB2000

The increasing use of molecular techniques in the HAB field was clearly evident at the Hobart HAB2000 meeting. Two entire sessions and more than 20 oral and 30 poster presentations, all of an exceptional standard, featured or incorporated molecular approaches to HAB organisms or blooms. The cross-fertilisation of methods and approaches between cyanobacteria bloom and marine HAB workers at the Hobart meeting was a particularly valuable experience for all. The meetings presentations covered an impressive range of topics, over which I admit to skating thinly in my summary of the main themes. The prominence of cyanobacteria blooms in Australian fresh and coastal waters ensured a strong representation of molecular studies in this area. Molecular characterisation of toxic species and populations, using a variety of gene sequences and approaches, featured in presentations of **Brett Neilan** and **Chris Saint**, the posters of **Julianne Dyble** and **Carolina Beltran**, and the study of bloom variation by **Judy Baker**. Of great interest to those of us struggling with toxin biosynthesis and genetics in marine HAB species, was the presentation by **Elke Dittmann** (**Thom Borner**) describing the structure, function, expression and *de-novo* biosynthesis of microcystin by the microcystin synthase enzyme complex of *Microcystis* spp.

Molecular methods are now becoming a useful tool to examine variation at the intraspecific and population level, showing a surprising level of population and individual variation in bloom-forming phytoplankton. **Christopher Bolch** kicked off the molecular sessions by presenting population genetic data and biogeographical hypotheses to explain the phylogeny and regional variation of the dinoflagellate *Gymnodinium catenatum*. A range of posters extended understanding of global rRNA gene variation among *Alexandrium* species **Leinonen-DuFresne**, **M.F. de Salas**, **Wendy Higman**, **Antonella Penna**, **Gires Usup**. **Martin Lange** demonstrated the huge individual variability and spatial structure of Orkney *Alexandrium* populations using amplified fragment length polymorphism (AFLP) fingerprinting techniques.

The use of ribosomal rRNA gene sequence data for HAB taxonomy and phylogeny featured strongly with the following reports filling significant gaps in rRNA phylogeny of dinoflagellates:

· **Alison Haywood**'s detailed study of the morphological/biochemical characteristics and rRNA sequence phylogeny of Gymnodinoid species including 2 new species.

· **Bente Edvardson** reporting rRNA sequences of species of *Dinophysis* spp.

· **Malte Elbrächter**'s studies to resolve benthic and planktonic *Prorocentrum* spp. and re-evaluate their position among the dinoflagaellate crown taxa (GPP complex *sensu* Saunders et al.).

Development and application of genetic probes from this increasing base of rRNA phylogenetic data continues apace as more sequences become available. Reports of new probes, new probe detection approaches, and most encouragingly, the incorporation of the DNA probe technology into HAB research and monitoring programs, were highlights of the meeting:

· New species and probes for *Pfiesteria* and *Pfiesteria*-like species by **Dave Oldach** and **Geraldo Vasta**

· Integrating flow cytometry and in-situ hybridisation with rRNA probes for field studies **Louis** Peperzak, **Rene Groben**, **Joachim Brenner**, **Yoshihiko Sato**)

· New electrochemical detection approaches for probes using PNAs from **Wayne Littaker**

· The use and integration of rRNA probes into New Zealand's routine plankton monitoring program by **John Tyrrell** and **Lesley Rhodes** and the development of autonomous in-situ monitoring systems by **Chris Scholin**.

Christopher J. S. Bolch, Centre for Coastal and Marine Sciences, Dunstaffnage Marine Laboratory, Oban, Scotland

A note on abstracts

Is it not time to give a little editorial attention to books of abstracts? For example, does an abstract really need to tell us that dinoflagellates have two flagella? Is it not rather obvious that toxins are pharmacologically active? And so on. Abstract books being the size they are these days, given too that they quite often get cited, even in the *primary* literature, pending the arrival of the proceedings, a good index is a help; it doesn't take too long with appropriate software. Abstracts which come to a sudden end because the page stopped, missing titles, things like that, rather sloppy. Non-native English speakers, too, deserve a little help in getting their 200-odd words into reasonable shape. *Ed*

Intergovernmental Panel on HAB Meets

The IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB) was formed in 1991 in order to identify adequate resources for a broad international programme to try to solve some of the problems caused by harmful algae. The Fifth Session of the Panel was held 22-24 November 1999 at UNESCO Headquarters in Paris. The Session was attended by representatives of: Canada, Finland, France, Georgia, Germany, Greece, Italy, Japan, Kuwait, Norway, Philippines, Portugal, Spain, Sweden, United Kingdom, United States of America, Uruguay, Korea (observer), Scientific Committee on Oceanic Research (SCOR), International Council for the Exploration of the Sea (ICES), International Society for the Study of Harmful Algae (ISSHA), and the International Union of Pure and Applied Chemistry (IUPAC). The Chair of the HAB Panel is Dr. Adriana Zingone (Italy),

During its meeting the Panel reviewed the actions completed by the IOC HAB Programme the last 3 years, and decided upon the workplan for the coming years.

Regional IOC-HAB Groups

The Panel reviewed the results and reports under the regional components of the HAB Programme, WESTPAC/HAB (Western Pacific), IOC/FANSA (South America), and IOCARIBE/ANCA (Caribbean), and noted with satisfaction the establishment of a working group on harmful algal blooms in the Caribbean and adjacent regions (IOCARIBE-ANCA)

The Panel recognized the importance of the work carried out by the regional project leaders.

Harmful Algae News

"Harmful Algae News" has been published since January 1992 and has more than 2000 subscribers. The Panel reappointed the Editor (Dr. Tim Wyatt), and recommended that special issues on selected topics is prepared, and that HAN should be issued more regularly, even if some issues would then have relatively few pages.

(Cont'd on p. 13)



























Some of the habologists at HAB2000:
1, 7, & 13 facets of food web dynamics; 3 & 6 various Mediterranean haptotypes and an introduction from the South Atlantic, and 9 three more from the Gulf of Thailand; in 11 a view of the conference bar reveals compatible strains from Munster and Connaught waters; 2, 4, 5, 8, & 10 distinctive (and distinguished) morphotypes from around the world, and 12 one example (from many sighted at the conference) of «North meets South» (remember Bermuda? - see HAN 15, p22)

(Cont'd from p. 1)

provoked new incidents in the region over the last decade, and are associated with areas modified by human activities. Their blooms are recurrent and frequently cause public health, economic, and political problems.

Alexandrium minutum is a widely distributed PSP organism. This is the toxic species most widespread in the Mediterranean basin and in the last decade, recurrent blooms have been described in various harbours and shellfish farms. Substantial economic losses are associated with this organism Since the record of Halim (2), blooms of this species have been only sporadically detected, with an evident increase of reports in the last decade in the Mediterranean (see table 1). On the Catalan coast, since 1995 when pilot HAB monitoring of harbours began, the number of harbours where *A. minutum* has been detected increased. This species appears along much of the Catalan Coast (1) but only proliferates massively in concrete harbours; recurrence is evident in at least one of them (Arenys Harbour).

Alexandrium catenella is widely distributed in cold temperate waters. In the Mediterranean basin, Margalef and Estrada (3) reported it as rare in oceanic waters. Since the first bloom detected in Barcelona harbour in 1996, there has been growing evidence of an increase in abundance and geographical range of *A. catenella* on the Catalan Coast (table 2). In late spring 1998, the first toxic event was described in the Mediterranean Sea, not only in some harbours but in open-sea waters. In 1999, a huge bloom of *A. catenella* was again observed (4).

Alexandrium taylori was described in 1994 from the Atlantic. The presence of intensely green-brown coloured water during summer months at some tourist beaches in the Balearic basin was known to the local population since the early 1980s, at La Fosca (Catalan coast), Cala Tarida, Pagera, Cala en Porter (Balearic Islands) (5) causing deterioration of water quality for recreational uses, and economic losses to the tourist industry. No explanation of the phenomenon was found until the cause of the water discoloration was described as a non-toxic bloom of *A. taylori* Balech. *A. taylori* does not appear to be mentioned in the Mediterranean prior to its identification at La Fosca (Catalan coast) (6); since then, it has been recorded mainly on beaches (Table 3 and ref. 7).

This species appears in several places in the NW Mediterranean (Costa Brava, Balearic Islands, Sicily) during summer, reaching concentrations 10^2 cells L^{-1} (8). In some places concentrations reach 10^5 cells L^{-1}, but dense (>10^6 cells L^{-1}) recurrent blooms in July and August occur only at certain beaches.

The maintainance of concentrations in excess of 10^6-10^5 cells L^{-1} for 6 weeks is rare during *Alexandrium* blooms (9). The blooms at La Fosca beach have been studied since 1995. *A. taylori* blooms are manifestations of large-scale proliferation in specific areas where the local environmental conditions and life history features interact to amplify numbers. The factors involved are moderate *in situ* growth (0.4 d^{-1}), low water renewal rates, i.e., weak flushing), formation of temporary cysts (80% of population daily) and their retention in the sediment acting as a reserve of the population (10). These species (*A. taylori*, *A. minutum*, *A. catenella*) are responsible for new, emergent problems and it can be deduced from the advance of the species range that new beaches and harbours will be affected in the near future.

Increased knowledge

In recent years (1997-99), areas with *A. taylori* blooms in Mediterranean coastal waters have certainly increased. This is the case in Eivissa Island where 3 more sites have been detected since 1997 (table 2). Also there has been an increase in dense blooms of *A. taylori*. In places where it was formerly found in low numbers, it now forms concentrations up to 10^6 cells l^{-1}. Also, since summer 1997, *A. taylori* blooms have occurred at St Pol (Costa Brava) where previously water discoloration was not detected. At present this species is limited to the northern Catalan Coast (Costa Brava). Future work should reveal whether this is due to local environmental conditions here and in the Balearic Islands, or is a prelude to range expansion.

Eutrophication

It does not seem that *Alexandrium* has been favoured by enrichment over other species, and in any case there is no evidence of near-shore enrichment in the northern part of Mediterranean basin in the last 30 years, since human population growth on the coast has been accompanied by better

Table 1. Areas of A. minutum *blooms (> 10^4 cells L^{-1}) in Mediterranean.*

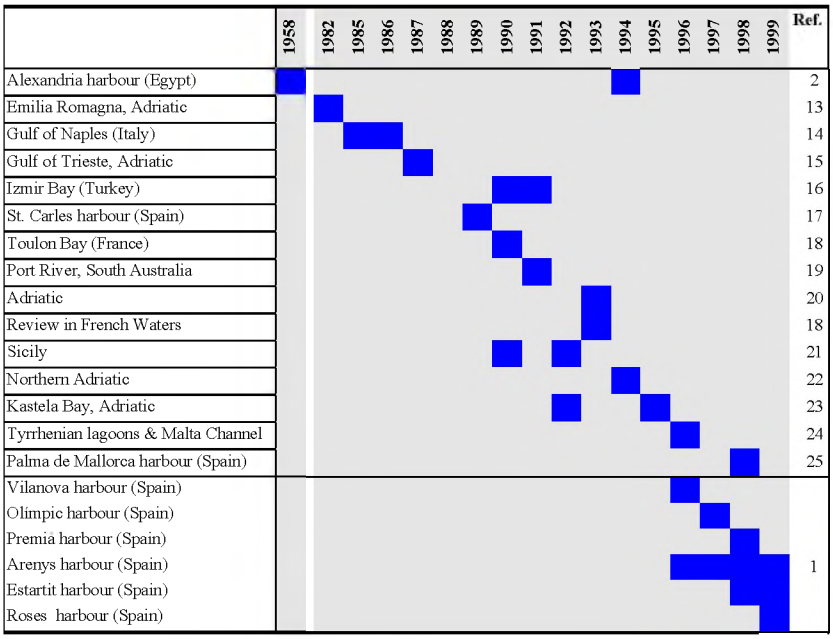


Table 2. Areas of A. catenella *blooms (> 10^4 cells L^{-1}) in Mediterranean.*

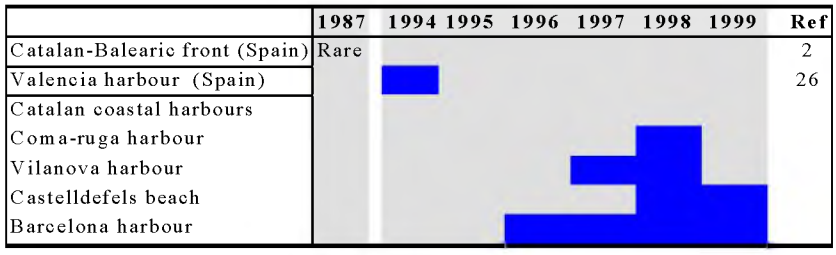
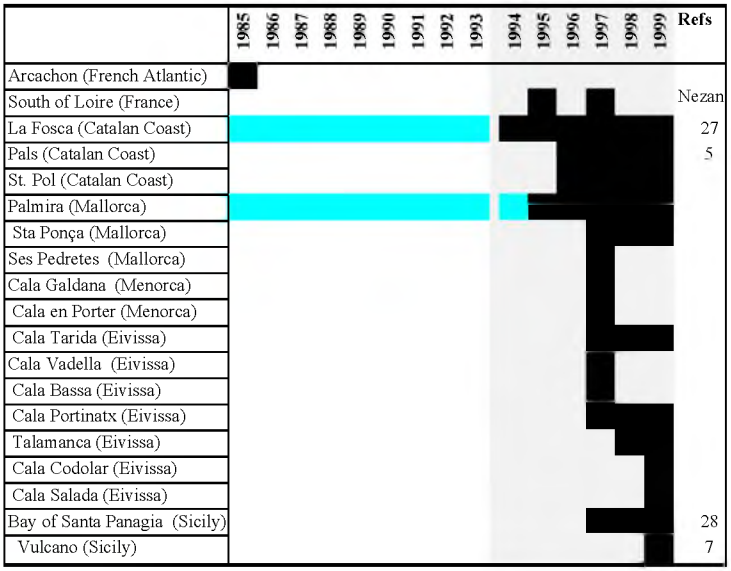


Table 3. Areas of A. taylori *blooms with up to 10^5 cells L^{-1} in Balearic basin.*



* this species is most probably under-estimated since it is easy to confuse it with other species of the sub-genus *Gessnerium*.

wastewater treatment. Nor do changes in nutrients ratios seem likely to have been involved (11).

Species introductions

The problem of introductions in ballast water or mollusc shipments has not yet been studied in the Mediterranean. The present distribution of *A. catenella,* known in the Mediterranean since 1987, may indicate a ballast water origin. A preliminary study of *Alexandrium* genetics (7) has established that Italian and Spanish strains of *A. taylori* are closely related, and very distinct from morphologically similar species such as *A. margalefi* recently found in the Tyrrhenian Sea.

Newly created habitats

The fourth aspect proposed here, the capacity of dinoflagellates to colonize and proliferate in man-made sheltered areas, has received scant attention. This aspect may be particularly relevant in regions like the Mediterranean Basin which are calm and have weak tides. Information on *Alexandrium* supports this view. Many *Alexandrium* species have a benthic resting which allows them to survive in sediments; then population development is possible in more locations. Reservoirs of resting cysts may be of great significance in sheltered waters. Two of the species analysed here, *A. minutum* and *A. catenella*, appear preferentially in harbours and in most cases recurrently. Thus confined waters may play an essential role to maintain high numbers of resting cysts, which would otherwise be dispersed, at specific sites. The behavioural strategies (swimming patterns, active migrations and self-regulated aggregation) of these species allow them to reach high levels of biomass in these particular localities.

What is certain is that *Alexandrium* blooms in Mediterranean coastal waters have increased in recent years, and that areas created or modified by human activities may be responsible for this increase. We must therefore examine the role of confined areas created or modified by human activity in the expansion and diversification of *Alexandrium* blooms, and establish the basis for integrated coastal zone management in coastal recreational waters (i.e. formulate new criteria for use of near-shore waters). Obvious questions that emerge are; How important are confined areas (proximity, number, area) for the expansion of some species? Are there causal links between mesoscale blooms and those in man-made sheltered areas? Could the adaptive potential of *Alexandrium* together with the existence of man-made sheltered areas lay a base for future speciation?

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◆ **Mexico**

Should Bahía Concepción, Mexico, be a model for Harmful Algal Blooms?

An analysis of the information of microalgal blooms in Mexico since 1970 shows that during the second half of the 1990s, records of Harmful Algal Blooms (HAB) have increased notably (Fig. 1). Of the total blooms recorded in Mexico, about 78% have occurred on the coasts of the Gulf of California. The dinoflagellate *Noctiluca scintillans* and the ciliate *Mesodinium rubrum* were the most recurrent species (1,2). Other microalgae involved in blooms, in order of importance, are dinoflagellates, diatoms, cyanophytes, silicoflagellates, and a haptophyte.

Most of these events were innocuous, though toxic species such has *Alexandrium catenella, Pyrodinium bahamense* var. *compressum*, *Gymnodinium catenatum, Gymnodinium breve,* and *Pseudo-nitzschia* spp. have been asociated with health, economic, and environmental issues in Baja California Sur (3,4,5), Guerrero (6), Oaxaca (7), Sinaloa (1,8), and Veracruz (9) (Fig. 2). Moreover, other potentially toxic dinoflagellates have been recorded along the coasts of Mexico; *Alexandrium pseudogonyaulax, A. minutum, Prorocentrum dentatum, P. lima, P. minimum, P. mexicanum*, *Dinophysis acuminata, D. hastata, D. rotundata, Gambierdiscus toxicus, G. yasumotoi, Pseudo-nitzschia australis,* and *P. delicatissima* (10, 11,12).

At times, the toxin concentrations exceeded the maximum level allowed for human consumption of clams, and human intoxication by paralytic shellfish toxins has occurred. Around 136 human poisoning cases have been recorded and 9 fatal cases have been confirmed. However, these are underestimates since many clinical diagnoses are inaccurate. Other adverse impacts are mortality of fish, birds, marine mammals, and economic losses in aquaculture, fisheries, and tourism (4,9). Not only paralytic toxins have been detected, but also diarrheic, amnesic, neurotoxic, and ciguatera toxins have also been confirmed in marine organisms (6,7,8,11,13,14). Despite these adverse impacts, Mexico does not have a permanent monitoring program and most studies have been limited to reporting and describing these events.

To contribute research under the objectives proposed by the Intergovernmental Oceanographic Commission, the Northwest Biological Research Center (CIBNOR) began, in 1993, to investigate the biological and hydrological processes that regulate the occurrence of harmful species in the large Bahia Concepcion, Gulf of California (Fig. 3). It supports extensive shellfish fisheries and tourist activity. PSP and DSP toxins in mollusk (3,14), toxic dinoflagellates (10), and high concentrations of dinoflagellate cysts in sediments (15) have been confirmed.

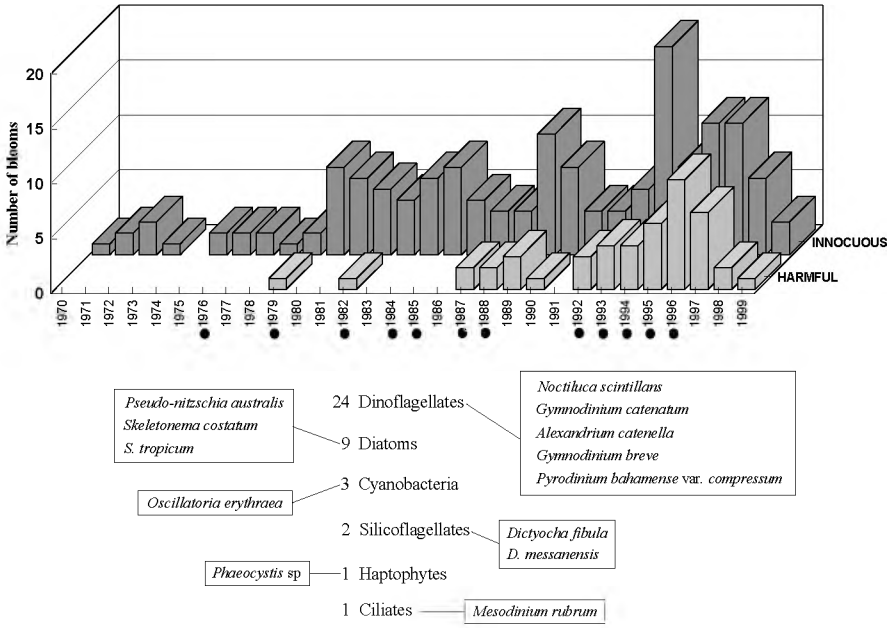

Fig. 1. Record of harmful and innocuous microalgae blooms in Mexico (1970-1999). Toxin activity was detected in the years indicated with black circles.

In this bay the assemblage of harmful microalgae is composed by *G. catenatum*, *A. catenella*, *A. pseudogonyaulax*, *A. minutum*, *P. minimum, P. dentatum,* and *D. acuminata. N. scintillans* is a recurrent species forming red tides, mainly in fall and winter. Other occasional blooms have been produced by *Gonyaulax verior*, *Dinophysis caudata*, *P. mexicanum,* and the cyanobateria *Oscillatoria erythraea.* PSP toxins in shellfish were found in Bahía Concepción, but no human poisoning has been reported though the toxin concentration has exceeded the allowable limits for human consumption of shellfish (3,10).

In winter, before harmful dinoflagellates appear, the water mass is characterized by a diatom-dominated community, and a well-mixed water column. The decline of the diatom population, increased temperatures, and the beginning of thermal stratification are present when dinoflagellate populations such as *Dinophysis* and *Alexandrium* increase. Thereafter, the increasing water temperature and the development of strong stratification are accompanied by *Ceratium* and *Prorocentrum*, and *Alexandrium* and *Gymnodinium* decline(3).

Our research is now attempting to define small-scale hydrobiological conditions that are involved in the presence of toxic dinoflagellates to describe the phytoplankton community before, during, and after the toxic dinoflagellate upsurge. To explain the dinoflagellate vertical distribution, and the influence of the hydrodynamic structure of the water column, we started intensive monitoring (since 1997). We also recently started to investigate the contribution of cysts to the population dynamics of dinoflagellates, including the beginnings and ends of blooms, survival during long periods, and recurrence of blooms. We will try too to



Fig. 2. Locations in Mexico where toxin activity have been detected. The species responsible for this toxicity are indicated for each area.

determine the influence of hydrodynamic conditions on the dinoflagellate life cycle, particularly the cyst phase. To do this we will characterize the cyst assemblages in marine sediments, define their distribution patterns, and determine the principal period and hydrodynamic conditions that promote cyst formation and germination under laboratory and natural conditions. Controlled laboratory experiments are under way to study exogenous (temperature) and endogenous factors (pigments, enzymatic activity, and toxins) inducing cyst germination in toxic species of *Alexandrium* spp. and *G. catenatum*. Bahia Concepcion is far from free of urban and industrial influences, so is a huge natural laboratory for HAB studies.

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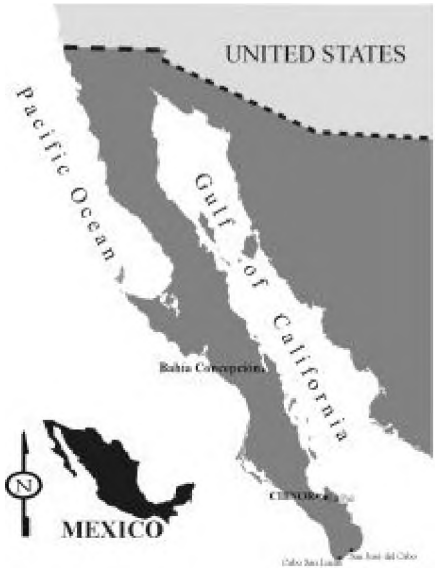

Fig. 3. Map of Baja California (Mexico) illustrating the location of Bahia Concepcion and the Northwest Biological Research Center (CIBNOR).

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IOC Manual on Harmful Marine Microalgae

The IOC Manual on Harmful Marine Microalgae (IOC Manuals and Guides No. 33) has proven to be one of the most successful products of the IOC HAB Programme, and is now out of stock. The Panel endorsed the preparation of a second revised and expanded edition of the IOC Manual on Harmful Marine Microalgae. The second edition of the Manual will have the same editorial team as the first edition: D. Anderson (USA), A. Cembella (Canada), G. Hallegraeff (Editor in Cheif), and H. Enevoldsen as Technical Editor.

Database on HAB events - HAE-DAT

The IOC-ICES Metadatabase on Harmful Algal Events, HAE-DAT, is now accessible via the IOC home-page. The Panel recommended it as a high priority to start establishing a mechanism for non-ICES countries to contribute to HAE-DAT. This will be done jointly by the IOC HAB Centres in Vigo and Copenhagen, WESTPAC/HAB, IOC/FANSA, and IOCARIBE/ANCA.

Training

Over the last years significant support has been provided by Denmark, Japan, and Spain for training and capacity building activities, and there has been cooperation on training activities with the Asian Pacific Economic Cooperation (APEC) and the Nordic Research Academy (NorFa). The Panel noted the difficulties in evaluating the impact of the significant training efforts under the IOC HAB Programme, and requested the Centres and the Secretariat to propose to the next session of the Panel a model for assessment of the IOC HAB Training Programme.

GEOHAB

The overall goal of the IOC-SCOR international research programme on the global ecology and oceanography of harmful algal blooms, GEOHAB is to develop the scientific knowledge needed in this field in order to increase the capability of mitigating the impacts of HABs. The Chair of the IOC-SCOR Scientific Steering Committee (SSC) for GEOHAB is Dr. Patrick Gentien (France).

The GEOHAB SSC and the SCOR and IOC Secretariats are working on the establishment of an International Project Office

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◆ Morocco

Pseudo-nitzschia pseudodelicatissima bloom in Atlantic Moroccan waters 1999

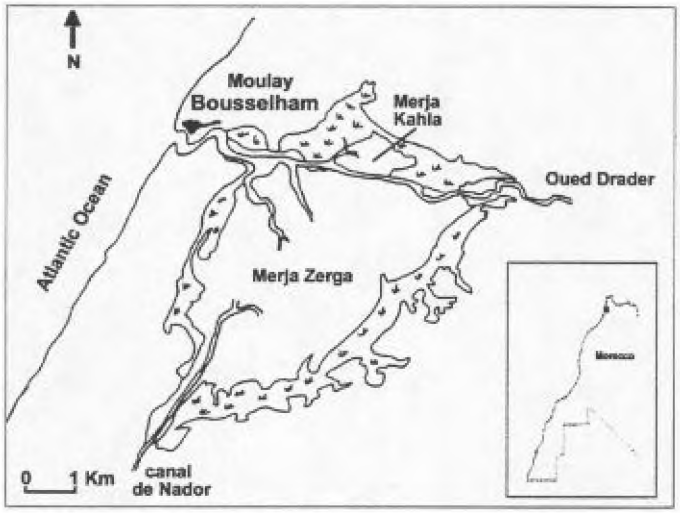
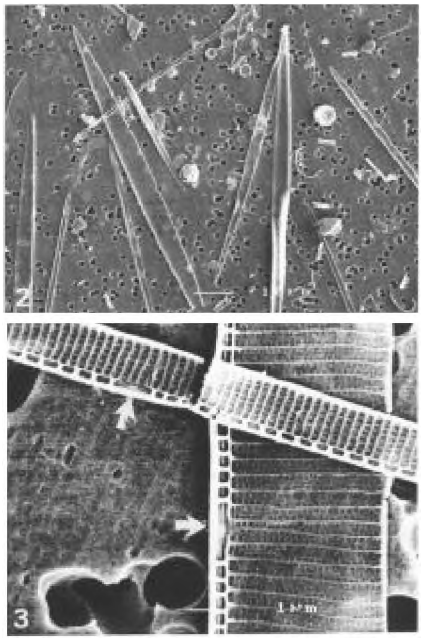


***Fig. 1**. Location of the Moulay Bousselham site*

Various harmful algal blooms (HAB) have been recorded since 1966 on the Mediterranean and Atlantic coasts of Morocco, including blooms causing fish mortalities [1] or human casualties due to PSP [2]. Whereas toxic phytoplankton may be a permanent phenomenon on the Mediterranean coast, HABs are sporadic on the Atlantic Moroccan coastline [2]. Causative species have not always been identified due to the absence of a coordinated network for monitoring red-tide events in Morocco.

In 1998 and 1999 we performed phytoplanktonic surveys on the Atlantic coast between Moulay Bousselham and Témara. On 18 April 1999 high concentrations of pennate diatoms of the genus *Pseudo-nitzschia* were observed in the lagoon of Moulay Bousselham. This lagoon (Merja Zerga) is a natural park area and the most important wintering site for migratory birds in Morocco. The phytoplankton community featured various dinoflagellates (including *Prorocentrum micans*, *Dinophysis acuminata*, *D. acuta*, *Ceratium furca* and *Protoperidinium diabolus*), the silicoflagellate *Dictyocha speculum* as well as centric diatoms (*Coscinodiscus concinnus*, *Rhizosolenia imbricata* and *Chaetoceros curvisetus*). Sampling was conducted at the mouth of the Merja Zerga lagoon (Fig. 1) which is known to receive substantial amounts of micropollutants such as organochlorates [3]. Water temperature and salinity during the study were 18 ± 1°C and 35.8 ± 0.1 respectively, corresponding to local spring conditions.

Scanning electron microscopy of acid-cleaned frustules was necessary for a critical identification of the species of *Pseudo-nitzschia*. In the 18 April 1999 sample, a total of five species of *Pseudo-nitzschia* belonging to two different size groups were formally identified (Fig. 2). Although exact cell densities of individual species are unknown, *P. pseudodelicatissima* was the most abundant species of *Pseudo-nitzschia*. It was readily recognized by its narrow linear frustules (transapical axis ca. 3 µm or less), single rows of square poroids, along with the presence of a central larger interspace (Fig. 3). The other species present were *P. fraudulenta* (larger frustules seen Figs 2 & 3), *P. pungens*, *P. subpacifica* and *P. delicatissima*.



***Figs 2 and 3**. Scanning electron microscopy of the* Pseudo-nitzschia *species. **2**. General view showing species belonging to two different size groups. **3**. Details of* P. pseudodelicatissima *(narrow frustule) and* P. fraudulenta *(large frustule); arrows indicate the central larger interspace.*

In this assemblage *P. pseudodelicatissima* is a potentially toxic species, some clones being domoic-acid producers. In Canada the presence of *P. pseudodelicatissima* in 1988 was correlated with high levels of domoic acid in mussels and clams [4] and very recently in France (1998) low levels of domoic acid detected in mussel tissues (Z. Amzil pers. com.) coincided with local blooms of *P. pseudodelicatissima* (C. Billard, unpubl. results). Other clones of this species are known to be non-toxic.

Four of the five species of *Pseudo-nitzschia* (including *P. pseudodelicatissima*) were still present at Moulay Bousselham in June 1999, while *P. fraudulenta* had disappeared. This is the first report documenting the presence of *Pseudo-nitzschia* species in North African waters. The high abundance of *P. pseudodelicatissima* in the lagoon of Moulay Bousselham had no harmful consequences. However it remains a potential threat and monitoring for domoic acid in the shellfish harvested in the area could be considered in the future.

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International Society for the Study of Harmful Algae -ISSHA-

The second General Assembly of ISSHA was held on 10 February 2000 during the Ninth International Conference on Harmful Algal Blooms, in Hobart, Tasmania. As current President of ISSHA Max Taylor presided over the Assembly meeting. He gave a brief history of ISSHA and outlined its affiliation with other organizations and the possible direction the Society could take in relation to other international bodies that deal with HABs.

The results of the election for the new Executive were announced and are as follows:

Vice-President (Taxonomy/Genetics/ Evolution): **Karen Steidinger**
Vice-President (Toxicology/Pharmacology/ Epidemiology): **Yasukatsu Oshima**
Vice-President (Ecology/Monitoring/ Management): **Allan Cembella**
Treasurer: **Henrik Enevoldsen**
Secretary: **Stephen Bates**

The election of a new President from among the three new Vice-presidents was postponed as Dr. Oshima was absent and would thus be unable to give a statement at the meeting. Second, there was considerable debate about the current Society Statues and related election procedures. During April it became clear that Yasukatsu Oshima and Allan Cembella have decided not to run for President, and therefore Karen Steidinger becomes the new President by default.

Secretary's and Treasurer's Report

The leaving secretary (Allan Cembella) reported on the initiatives carried including the establishment of a Society website (http://www.cbr.nrc.ca/issha/) and Society awards (see below). The Treasurer (Henrik Enevoldsen) presented the 1999 Financial Statement for ISSHA, and the very generous contribution of 10,000 US$ from the Organizing Committee of the Seventh International Conference on Toxic Phytoplankton (Sendai) was acknowledged.

Statutes debate

Some members pointed out that the Statutes had never been ratified by Society members; others stated that because ISSHA is a legally registered society in Denmark, the Statutes are legal. It was decided that the Statutes, which were originally mailed to members on 4 January 1999 and are also available on the ISSHA website, will be mailed to the membership for amendments. Members can send their proposed amendments to the Secretary, who will post them on the ISSHA website. A deadline of 6 months from time of receipt of the current Statutes was given to propose amendments. Amendments may be approved by postal ballot, providing two thirds of the membership support the amendment (Article XVII of the Statutes).

Student Membership

After a brief discussion, it was agreed that the Society is presently too small to warrant a student representative to sit on the Executive. Efforts should be made to encourage student membership in the Society. A related debate ensued about the Student and other Awards. Questions were raised about the following: How much membership participation is there in voting? Is there a bias for high tech labs and against good science? Do awards promote competition rather than co-operation? Some strongly felt that the Society should not provide such awards. Members of the past Executive explained the rationale behind the awards and the process for choosing them. A vote was then held and it was decided that the Society should provide Awards as planned by the Executive.

Future Initiatives of the Society

Max Taylor outlined the importance of membership initiatives and of input from members. A number of initiatives remain from the first General Assembly, including creation of a Society logo. No proposals have been received so far. Also the involvement of the Society in future conferences and meetings of the international HAB community need to be discussed. How much input and involvement should there be from the Society Executive or International Organizing Committee? Should the International Organizing Committee be maintained? How should the Proceedings be published?

Another issue which has been discussed among ISSHA members is the need for a specific HAB or Society Journal. Nevertheless, the discussion at the Assembly was very short as it was announced that an international journal for HABs is already planned, independent of the Society.

ISSHA Awards

The ISSHA awards mentioned above were presented during the HAB 2000 Conference dinner. The nominees for the awards were selected jointly by the ISSHA Executives and the HAB 2000 International Organizing Committee prior to the HAB 2000 Conference.

The Yasumoto Lifetime Achievement Award is given in recognition of long time outstanding contribution to the HAB field. It is named after Professor Takeshi Yasumoto in recognition of his unique contribution to HAB research. The Award was presented to Prof. F.J.R. Taylor, University of British Columbia, Canada, for an outstanding lifetime contribution to harmful algal research, including major achievements in the field of taxonomy, evolutionary biology, and marine ecology.

The ISSHA Award was given in recognition of recent scientific excellence in HAB research. The Award was presented to Dr. Chris Scholin, Monterey Bay Aquarium Research Institute,USA, for the development of molecular probes for harmful algal species, major advancements in biogeographical studies on the dispersion of key taxa (e.g., Alexandrium spp.), the "global spreading" hypothesis, and the recent characterization of the events leading to sea lion mortalities caused by domoic acid in California.

(Cont'd on p. 16)



Chris Scholin recieves the ISSHA Award 2000. The award winners received magnificent dinoflagellate wood carvings made by Dr. Haruyoshi Takayama.

(Cont'd from p. 15)

The ISSHA Student Awards are named after Dr. Maureen Keller. Maureen Keller died rather suddenly on Wednesday, November 17, from a highly aggressive form of cancer. She was active in a wide variety of scientific research projects related to harmful algae. For example, she was deeply involved in work on DMS production by Phaeocystis blooms in the Gulf of Maine and her key presentation on haptophytes at the NATO-Advanced Study Institute in Bermuda will be remembered by all participants. Maureen was also responsible for an important component of the US ECOHAB project on Alexandrium dynamics in the Gulf of Maine. In earlier work with Bob Guillard, Maureen made valuable contributions to the development of nutrient media for marine phytoplankton. In honour of Maureen's efforts in the field of harmful algal research, ISSHA has initiated the Keller Student Awards for best oral and poster presentations by students and post-doctoral fellows attending the international HAB conferences. These awards were presented for this first time at the HAB 2000 meeting to Kathi Lefebre (Univ. of California) for best oral presentation: "Outward excitotoxic effects and tissue distribution of domoic acid in a prominent vector species, the northern anchovy (Engraulis mordax)"; to Lizeth Botes (Univ. of Cape Town) for best poster: "An investigation of the identity and potential toxicity of Gymnodinioid species present in False Bay, South Africa"; and to David Seaborn (Old Dominion Univ, US), Torstein Tengs (Univ. of Maryland, US), and Judy Baker (Univ. of New England, AU) for posters with honorable mention.

All interested can find more about ISSHA and instructions about how to apply for ISSHA membership at the ISSHA web URL (http://www.cbr.nrc.ca/issha/).

Stephen Bates and Henrik Enevoldsen
Secretary Treasurer

(Cont'd from p. 13)

for GEOHAB. France has offered to host the Project Office at IFREMER, Centre Brest, and the USA has offered to investigate their possibilities for providing support.

The Panel focused its discussions on the strategy to provide an efficient mechanism for promoting GEOHAB at both the global, regional, and national levels. To this effect, the Panel made recommendations to Member States on how to build up GEOHAB.

Taxonomy

The Panel decided to continue the work with a reference list on potentially harmful algae, which is very needed in the international research and management community.

Toxins

The Panel noted with concern the potential incompatibility of regional and/or national regulations on aquatic biotoxins, and the associated potential impediments to trade with seafood products. The concern was in particular expressed with respect to compatibility of EU and APEC regulations. The Panel offered itself as a mechanism to strengthen interaction between the relevant expert groups which advice e.g. EU and APEC on biotoxin regulations.

Resource protection

The Panel established a Task Team to draft a document providing guidelines on emergency measures for harmful algal events. The Panel decided that the draft document should be reviewed by the IPHAB Members and the ICES-IOC Working Group on Harmful Algal Blooms Dynamics. The Panel also endorsed the potential publication by IOC jointly with APEC of a revised version of a major report on design of HAB monitoring strategies prepared by a consortium of international experts and originally published by Hong Kong in cooperation with APEC.

HAB monitoring and GOOS

The Global Ocean Observing System, GOOS, has included HABs among the issues to be considered in designing a global observation system that meet the needs of a wide range of end-users. It has also been acknowledged that HABs and their consequences on human health and mass mortality of natural and cultivated living resources are among the prominent indicators of changes in coastal waters. A global and long-term observing system of HABs would allow to detect general trends and distinguish them from locally driven phenomena. It would also offer a link between complementary data sets at high spatial and temporal resolution, which allows integrated data analysis and increases the reliability of models. It would constitute a mechanism for the intercalibration and optimisation of monitoring operations, world wide dissemination and safe long-term archival of data. The overall result is an improved capacity of predicting HABs over different time scales. It was recognized that existing HAB monitoring is not uniform neither in methodology or scope, and it was decided that an information document on the nature of HAB monitoring, including identification of HAB monitoring parameters, and examples of national HAB monitoring and management is prepared and presented to GOOS in order to facilitate the identification of HAB monitoring parameters suitable for inclusion in a long term global ocean observing system.

The full report and the detailed recommendations of the Panel can be found at: http://ioc.unesco.org/hab/act2.htm

Subscriptions

Subscriptions to HAN and change of address at: *http://ioc.unesco.org/hab/news.htm*, or send an e-mail clearly marked "Harmful Algae News" to Ms. F. Schiller at *f.schiller@unesco.org*

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms *No. 21*

Mitigation in HAB Research

At the very beginning of HAB research, there was no "**M**": we just investigated red tides, usually in panic, as they came along and caused havoc. Now there are three. We started **Monitoring**: taking samples and measurements on a more-or-less routine basis around certain coasts to detect incipient harmful blooms, and follow both the occurrence and, we hoped, the dynamics, of the algae which cause them. In order to get a better idea of where HABs come from and how they develop, this soon expanded to monitoring the occurrence of algae and ancillary data at sea from ships, aircraft and satellites. Later, we learnt to do **Management**: taking steps to prevent them doing harm. These steps include information campaigns, whether they be for the general public or for groups such as fish farmers, planning site-selection strategies, including those to design and relocate fish cages for HAB avoidance, early harvesting procedures, and so on. (Measures to control the introduction and transfer of marine organisms (ITMO) are not counted here). More recently, we have started **Mitigation**. In the field of HABs, the word "mitigation" is used in two distinct ways. Mitigation *sensu lato* is used to mean reduction of the effects of HABs by all means, by monitoring, management of all types, and information programmes for both public and professionals. Mitigation *sensu stricto* is taken here to mean reduction of harmful effects, ways of making blooms less harmful when they do occur, despite any monitoring or management we may (or may not) have done.

The successive rise in the three M's

To illustrate the successive rise in these three activities I perused the proceedings of the series of World Red Tide and HAB scientific conferences starting with that in Takamatsu, Japan. Figure 1 shows the results.

Monitoring: There were three papers on monitoring at Takamatsu (1987), one at Lund (1989), and a jump to 19 at Rhode Island (1991). That it has remained at this high level (12 to 24) since, may reflect a constancy in novel types of monitoring, since absolute levels of monitoring world-wide appear to be increasing markedly. **Management**: The first paper I counted on management, by Wong (Takamatsu [1]) described an action scheme implemented in Hong Kong. I could find none either at Lund (1989) or Rhode I. (1991), but after Nantes (1993) where there was one, the number of papers on management has increased steadily, reaching 13 at Hobart (2000) (Fig. 1). **Mitigation**: Although at

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◆ Republic of Korea

Inter-state symposium on harmful algal blooms and clays

An Inter-state symposium on "harmful algal blooms and clays" was held on 30 May 2000 at the National Fisheries Research and Development Institute (NFRDI) in Pusan, Korea, under the auspices of NFRDI and sponsored by the Ministry of Maritime Affairs and Fisheries of Korea. The symposium was attended by more than 100 participants from Korea, China, and USA. The objectives were to evaluate the mitigation capability of clays on harmful algae blooms and their impacts on marine ecosystems.

Eleven papers were presented in 3 sessions: (1) Properties and application of clays, (2) Mitigation capability and impacts of clays, (3) Evaluation of clay dispersal and new techniques for mitigation.

Interest focussed on the following issues; "Recent approaches on the

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The publication of Harmful Algae News is sponsored by the Botanical Institute, University of Copenhagen

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Nantes (1993), Yang and Albright presented a paper on mitigation of the harmful effects of the diatom *Chaetoceros* on salmon gills, they published elsewhere [2], and we had to wait until Vigo (1997) to see the first publication about mitigation in the HAB proceedings. It was by Hak Gyoon Kim, who presented strategies for mitigating the effects of *Cochlodinium* blooms in Korea by spreading clay and loess. At Hobart (2000), the number of abstracts on mitigation rose to ten, of which two were about cyanobacteria. The paper by Lu Songhui *et al.* [3], which referred to the moving of rafts as mitigation, I have counted under management.

Recent progress in Mitigation *sensu stricto*

Preparing the way: The sharp increase in the number of mitigation papers reflects growing interest in the subject, and its encouragement by HAB working groups and panels. In 1997, in "Turning back the harmful red tide", Anderson [4], (using the term "mitigation" *sensu lato*), pointed out that while HABs were already a serious and increasing problem in marine waters, scientists and funding agencies had been slow to investigate possible control strategies. Barely three years later, the tide is turning. For instance, we have now seen the "First Conference on Harmful Algae Management and Mitigation" at Subic Bay, Philippines, hosted by Rhodora Azanza and chaired by Sherwood Hall [5]. On the home page of The IOC Harmful Algal Bloom Programme [6], we see "The overall goal of the HAB Programme: To foster the effective management of, and scientific research on, harmful algal blooms in order to understand their causes, predict their occurrences, and *mitigate their effects*" (my italics).

Mitigation techniques relative to finfish survival: At the Hobart meeting, not including communications expressing intentions and future needs for mitigation, I counted ten oral papers on the results of mitigation work. However there were no posters. Why not? Did the lack of posters reflect the importance and novelty of the field, as considered by the Selection Committee? The mitigation techniques described fell into the following categories.

Clay was the big subtopic in mitigation. H.G. Kim [7] had started the topic rolling at the Vigo conference with the effects of montmorillonite and loess on *Cochlodinium polykrikoides* blooms. He found montmorillonite the more efficacious of the two, with 90% of the *Cochlodinium* removed after 60 minutes' exposure to a concentration of 2 g/L montmorillonite. No less than thirty types of clay were tested by Sengco *et al.* [8] for their capacity to flocculate and sediment different HAB species. With some combinations, removal efficiencies as high as 80%, for instance with *Pfiesteria*, could be achieved with clay concentrations as low as 30g/m^3, while 100% mortality of precipitated *Gymnodinium breve* was achieved with 50 g/m^3 of phosphatic clay. Surfactants, particularly polyaluminium chloride (PAC), which is approved for drinking water, tended to increase removal efficiency by nearly an order of magnitude. PAC also decreased the tendency for the nutrients NO_3, NO_2, NH_4, PO_4, and SiO_4 to diffuse out of the clay flocs, which could otherwise fuel HAB regrowth. Cell removal in some cases was increased by agitating the water, but reduced with increasing salinity and with increasing cell concentration. Yu Zhiming *et al.* [9] also presented the results of coagulation tests, with derived models, for different clays and algae, and investigated the effects of surfactants such as PAC, mixed metal layered hydroxide, and pH. Zhiming *et al.*, like Sengco *et al.*, found montmorillonite to be a good flocculator, but suggested kaolin is even better.

Sodium hypochlorite: A study was presented by H.R. Kim *et al.* [10] on the effect sodium hypochlorite NaOCl, generated by electrolysis *in situ*. It was found to reduce markedly the concentrations of various HAB dinoflagellates at an NaOCl concentration of ~0.4 g/m^3. On the other hand, copepods remained unaffected at concentrations up to 3 g/m^3. Under bright sunlight, half the NaOCl was converted to NaCl. So it was suggested that this technique could reduce concentrations of red tide dinoflagellates, while secondary effects on ciliates and copepods would be minimal. Treatment costs were estimated at ~US$70,000/km^2, mainly to set up the equipment.

Cysteine compounds: In Vigo, Jenkinson and Arzul [11] reported methods to measure both the rheotoxicity (toxic action due to rheological thickening of the water) and the cytotoxicity to fish of substances secreted by *Gymnodinium mikimotoi* and two other algae. They also showed how the rheological and cytological components contributed to total ichthyotoxicity. At Hobart the same authors [12] described how they used similar methods to investigate the cytotoxicity and rheotoxicity to fish of two fish-killing algae, *G. mikimotoi* from Brittany (predominantly rheotoxic) and *Gymnodinium* cf *maguelonense* from Tunisia (predominantly cytotoxic due to active oxygen radicals). They then tested three cysteine compounds added to the water on the different types of toxicity and fish-killing power. In both these harmful algae, they found that the cysteine compounds mitigate both types of toxicity. The estimated cost of treatment would be less than US$1/tonne of fish. Cysteine compounds, notably L-acetyl-N-cysteine, is widely used in medicine, and should not prove a risk to fish consumers or to the environment at the concentrations proposed.

Ultrasound: Thomas & Gerritsen [13] described a method of eliminating all algae, not just the harmful ones. It is called *Aquasonic*, and works by transmitting ultrasonic vibrations into the water. It is claimed to kill all algae in a volume of 50,000 m^3 with just a 15-Watt probe. This may take up to 2 weeks for the more refractory, filamentous algae. Already 2000 units are installed, mainly in Belgium and the Netherlands, primarily in water reservoirs and market gardens. The authors describe the device as "environmentally friendly", although some people might question this description of a technique that kills a whole trophic level. In its favour, however, its primary environmental impact stops when you switch the machine off. This device will probably be of most use in freshwater systems, or in those of limited extent. Particularly in view of their claimed ease of use and cheapness, quantitative investigations are required into the lethal and sublethal effects of ultrasound producers on the biota of water bodies.

Overview

For the first time, in this series of HAB meetings, there was an overview of mitigation techniques for fish farms, by Rensel [14]. He covered mitigation techniques using clays, and drew attention to the need for studies on the environmental effects of substances used for mitigation. Rensel's approach was very practical, and was also devoted to what I have called "management", particularly the design of net pens to minimise the effects of HABs, site selection, and the drawing up of contingency plans for when a HAB threatens and strikes. This presentation brought the same author's article in the IOC Manual [15] up to date, material which he is preparing for the second edition of the IOC Manual.

Cyanobacteria

For problems caused by cyanobacteria mitigation is already current, particularly in Australia. The mitigation itself can cause secondary problems, as explained by three groups of authors. Therefore, mitigation research is already at this second level. For drinking water, two presentations were made on the harmful effects that might arise from current mitigation techniques. Senogles *et al.* [16] told us that *Cylindrospermopsis raciborskii* is commonly found in drinking water in Queensland reservoirs. It produces a hepatotoxin, cylindrospermopsin, that is currently removed from the water by flocculation, sedimentation and filtration. Often, however, these techniques lyse the organisms. Methods of removal of the resulting dissolved cyanotoxin include oxidation by Cl_2 and ozone. UV treatment is also used, and is improved by adding TiO_2. Müller *et al.* [17] reported research which suggests that if drinking water containing lysed cells of *Microcystis* or *Cylindrospermopsis* is treated with chlorine, then the chlorine reacts with organic matter liberated by this lysis to form toxic lower chlorinated phenols. Höger *et al.* [18] reported that ozonation reduces the level of cyanobacterial toxins in water containing *Microcystis* or *Oscillatoria*, where toxicity is measured by protein phosphatase inhibition (PPI). They found that this worked up to a certain cell density (e.g. 10^5cells/ml for 1.0 mg O_3/L), above which toxicity actually increases due to lysis of cells and release of toxins. A residual level of 0.05 mgO_3/L was recommended in order to completely destroy this residual toxicity.

Possible techniques for the future

Effects of bacteria on harmful algae: Bacteria have been found to infect and kill harmful algae. For instance, Ishio *et al.* [19] reported at Takamatsu that the bacterium *Vibrio algoinfestans* killed at least 16 species of flagellate, although the harmful *Chattonella antiqua* manifests what appeared to be defensive behaviour. At Hobart, several communications mentioned algicidal bacteria. In particular, Mayali *et al.* found two strains lethal to *Gymnodinium breve* from Florida waters, and there were suggestions that it is promising as a future control agent.

Effects of parasites on harmful algae: Norén *et al.* [20] reported that a recently described parasite, *Parvilucifera infectans*, related to the oyster-killing parasite, *Perkinsus*, was found in a natural population of *Dinophysis*. It has also been found to infect other dinoflagellates, notably *Alexandrium*. The parasite has a high rate of lethal infection. The authors suggest that large-scale culture of this organism could be a useful adjunct to HAB mitigation, and would shift the plankton community towards non-toxic athecate dinoflagellates or other plankton groups.

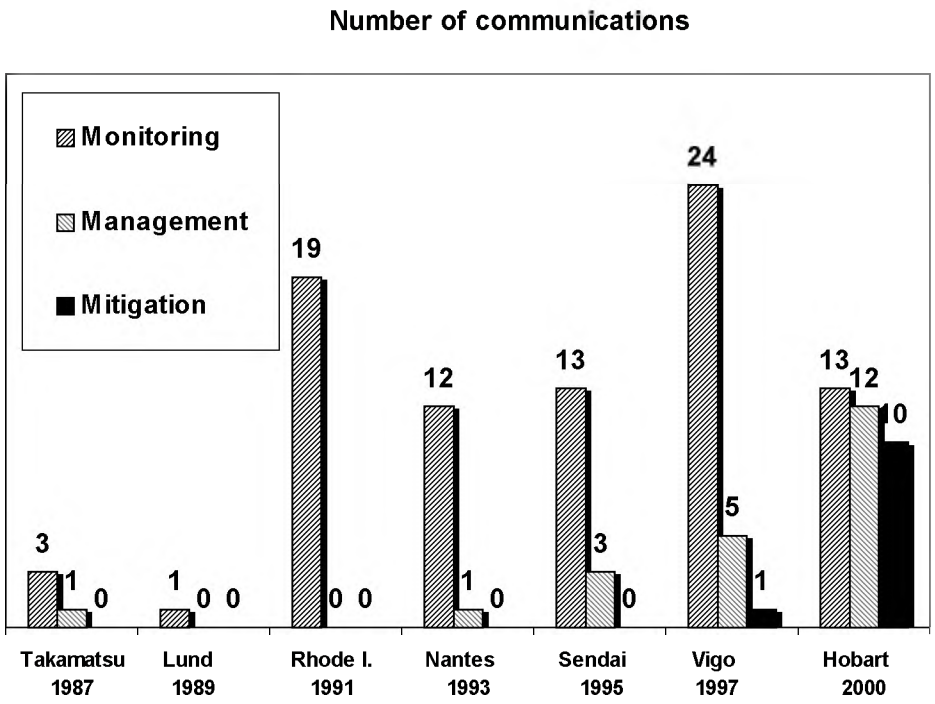

Figure 1

Implications for the future

The rapidly changing proportions in the categories of papers (Fig. 1) at the International HAB meetings may represent a function of both 1) current interest (by the selection committee) in the subject and 2) novel interest. If so, then interest in mitigation *sensu lato* relative to HABs overall, appears to doubling every year. No doubt, the exponent will decline when mitigation *sensu stricto* takes its place, complementing monitoring and management programmes, to minimise the harmful effects of algal blooms world-wide. We may suggest that world-wide, and more locally, algal harm minimisation programmes will require mitigation as part of their umbrella of protection. This is rather like health programmes, which also employ monitoring (epidemiological studies), management (including health and hygiene education programmes), and mitigation (actual medical treatment, including some which have harmful side effects) for when infections strike despite the preventative measures taken.

In a large-scale experimental phase, once the first direct mitigation techniques have been honed a little more, one could envisage stockpiles of substances, such as clays or cysteine compounds, strategically placed where problems could be foreseen. It would be important to have someone suitably trained to apply these products in the correct dilutions.

Concurrently, research, both practical and conceptual, needs to be encouraged into the effects of direct mitigation on the environment, particularly the plankton and the benthos. In some cases mitigation *sensu stricto*, might be expected to transfer ecological problems elsewhere, and perhaps introduce new problems. Recent suggestions to fertilise coastal zones, e.g., that of Markels [21], will no doubt increase the likelihood of seeking direct mitigation solutions, either openly or secretly, when the inevitable HABs arise. This would pose a risk of double-whammies [22]. Environmental damage caused by overzealous mitigation could come to be associated (perhaps rightly - it would depend on how things develop) with that caused by the aquaculture itself. One way to reduce the pressure to use mitigation will be to do good monitoring and management, and implement procedures according to their findings.

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mitigation and controls of HAB" by Hak-gyoon Kim.

He explained that fisheries loss could be mitigated, to some extent, by practical strategies such as "protect- HAB victim and "punish-HAB violator" system, even though there are difficult issues of predicting the extent and persistence.

With respect to punish-the-violator scheme, clay is the most efficient and least impact strategy to control harmful algae, and Korea reduced fisheries damages from US $ 95 million in 1995 to US $ 1.6 million in 1997 and US $ 0.3 million in 1999 by this means. "Preliminary mesocosm studies of clay dispersal in Florida" by M. R. Sengco (WHOI) provided information on the deployment and effectiveness of clay dispersal; he reported that USA growing interest in the effects of clay for HAB control is growing. "Progress of studies on harmful algal blooms and mitigation with clays in China" by Zhiming Yu (Chinese Academy of Sciences) emphasized that Van der Waals interactions during coagulation of kaolin on alga are important factors. Other Korean presentations described aspects of the effectiveness of clays for HAB control, methods of dispersal, and the impacts of clays on shellfish such as oyster, ark shell and abalone. Some positive effects of clays such as the extermination of Scuticociliata in cultured olive flounder by powdered clays, and increase of physiological functions of fish exposed to soil suspensions, were also reported.

Although it was generally accepted that clay removes HAB organisms, the issue highlighted in this symposium was the influence of clay dispersal on coastal ecosystems, and the potential risks of clays to the culture of marine organisms were discussed, especially the relationship between clay dispersion and alteration of the benthic habitats.

The symposium concluded by recognizing the important effect of clay for HAB control, and noted the importance of collaboration among researchers in different HAB regions. Participants emphasized the importance of information sharing, and hope that this symposium will contribute to the future development of innovative research on HAB control.

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China Harmful Algal Bloom WebPages

Recently, with support from the Innovation Program of the Chinese Academy of Sciences, C-HAB Web Pages have been set up at http://www.china-hab.ac.cn , to provide basic knowledge to the public, summarize the main HAB occurrence in China, describe main HAB species, introduce research activities in China, and make links to international websites and projects. Recent HAB occurrences in China will be reported promptly at the Web Pages. The Web Pages have Chinese and English versions and are still under development.

◆ **Mediterranean Sea**

Production of domoic acid in another species of *Pseudo-nitzschia*: *P. multistriata* in the Gulf of Naples (Mediterranean Sea)

Species of the diatom genus *Pseudo-nitzschia* can attain high concentrations in coastal waters. The production of domoic acid (DA), the causative agent of Amnesic Shellfish Poisoning (ASP), is reported for at least eight species of this genus [1, 3]. *Pseudo-nitzschia* identification at the species level is not always possible in light microscopy and often requires SEM (Scanning Electron Microscopy) and/or TEM (Transmission Electron Microscopy) investigations to detect the distinguishing



Figure 1. Pseudo-nitzschia multistriata, light micrograph; girdle view, showing the characteristic sigmoid shape of the cells.

morphological characters. Moreover, toxic and non-toxic strains seem to be present within the same 'morphospecies', suggesting a high genetic variability among strains, and/or differences in the factors controlling DA production. All this calls for accurate investigations aimed at correctly identifying *Pseudo-nitzschia* species and testing their potential toxicity.

A long-term phytoplankton monitoring program is conducted in the Gulf of Naples since 1984. The most abundant *Pseudo-nitzschia* species recorded in the Gulf are *P. delicatissima* and *P. pseudodelicatissima*. In recent years, *P. multistriata*, a species described from Japanese waters [4, 5], is recurrently recorded. This species, to our knowledge, has been never reported outside the type locality. Cells (38-50 mm long 2.8 – 3.8 mm wide) are characteristically sigmoid in girdle view (Fig. 1) and linear with pointed ends in valve view (Fig. 2). TEM examination of several cultures revealed the ultrastructural features of this species (Fig. 3). The number of interstriae in 10 mm is 37-46, and the number of fibulae is 23-28. Two rows of circular poroids are present in each interstria and 11-12 poroids in 1 mm. A central interspace along the raphe is lacking.

Cultures of *P. delicatissima*, *P. pseudodelicatissima* and *P. multistriata* were collected in late exponential phase to test domoic acid content. Analyses were carried out according to the procedure in Hummert et al. [2] with an HPLC-MS API 165 PE SCIEX; the detection limit of the method is 3 ng DA absolute. Domoic acid was detected in the four cultures of *P. multistriata* with concentrations ranging from almost undetectable values in culture SZN-B27 to 0.645 pg cell^{-1} in culture SZN-B28. No domoic acid was detected in *P. delicatissima* and *P. pseudodelicatissima* strains.

This is the first report of domoic-acid production for *P. multistriata* and the first report of a potential source of ASP in the Mediterranean Sea.



Figure 2. Pseudo-nitzschia multistriata, TEM crograph of the whole cell in valve view.

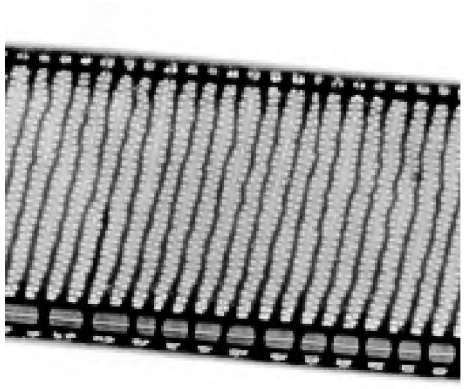

Figure 3. Pseudo-nitzschia multistriata, TEM micrograph of the middle part of the valve, showing fibulae, interstriae and poroid arrangement.

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- **Canada**

Shellfish Harvest Area Closure due to Domoic Acid - Mill River, Prince Edward Island

In late September, mussel samples (*Mytilus edulis*) collected for the Shellfish Program by the Canadian Food Inspection Agency (CFIA) at Mill River, western PEI (Fig. 1), were found to contain the neurotoxin domoic acid (DA) at a level of 33 µg DA per gram (wet weight) of mussel tissue; the acceptable limit is 20 µg DA/g. Harvesting of molluscan shellfish from the Mill River area was therefore closed by Fisheries and Oceans Canada on September 22. This is the first closure due to DA in western PEI, and the first anywhere in PEI since 1994.

The DA toxin was first found in November, 1987, in mussels from Cardigan Bay, eastern PEI, in what was the world's first episode of Amnesic Shellfish Poisoning (ASP) [1]. At that time, the toxin was found to be produced by the pennate diatom *Pseudo-nitzschia multiseries*. Blooms of *Pseudo-nitzschia* species occur each autumn in PEI estuaries and bays, but since 1994, they have been less intense [2]. Reasons for the change in bloom intensity are still under investigation, but it is thought that meteorological conditions are in part responsible. The autumn 1987 toxic *P. multiseries* bloom was preceded by a prolonged dry period in summer, followed by an unusually rainy autumn [3]. This could have provided nutrients, via river runoff, to the estuaries.

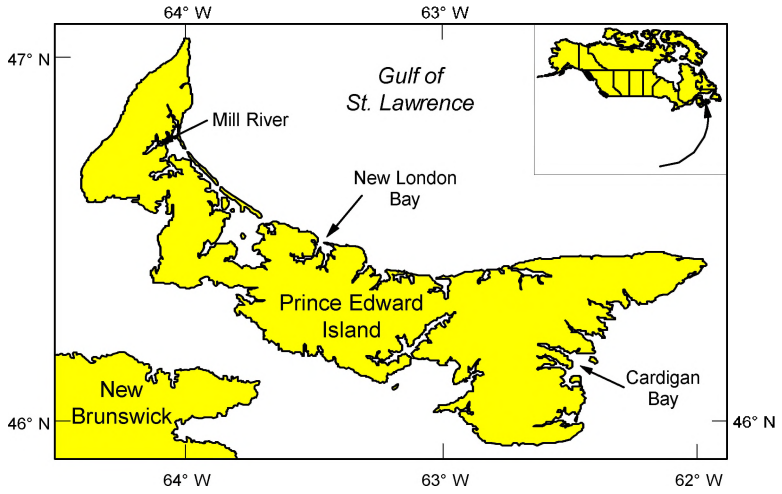

Figure 1. Location of the September 2000 domoic-acid-producing Pseudo-nitzschia multiseries bloom in Prince Edward Island, resulting in closure of shellfish harvesting in Mill River. Previous closures have been in New London Bay and Cardigan Bay.

Mill River is one of the few areas on PEI where water samples are not routinely collected for the enumeration of potentially toxic phytoplankton, because no previous incidences had been reported there. The first water sample was therefore only available

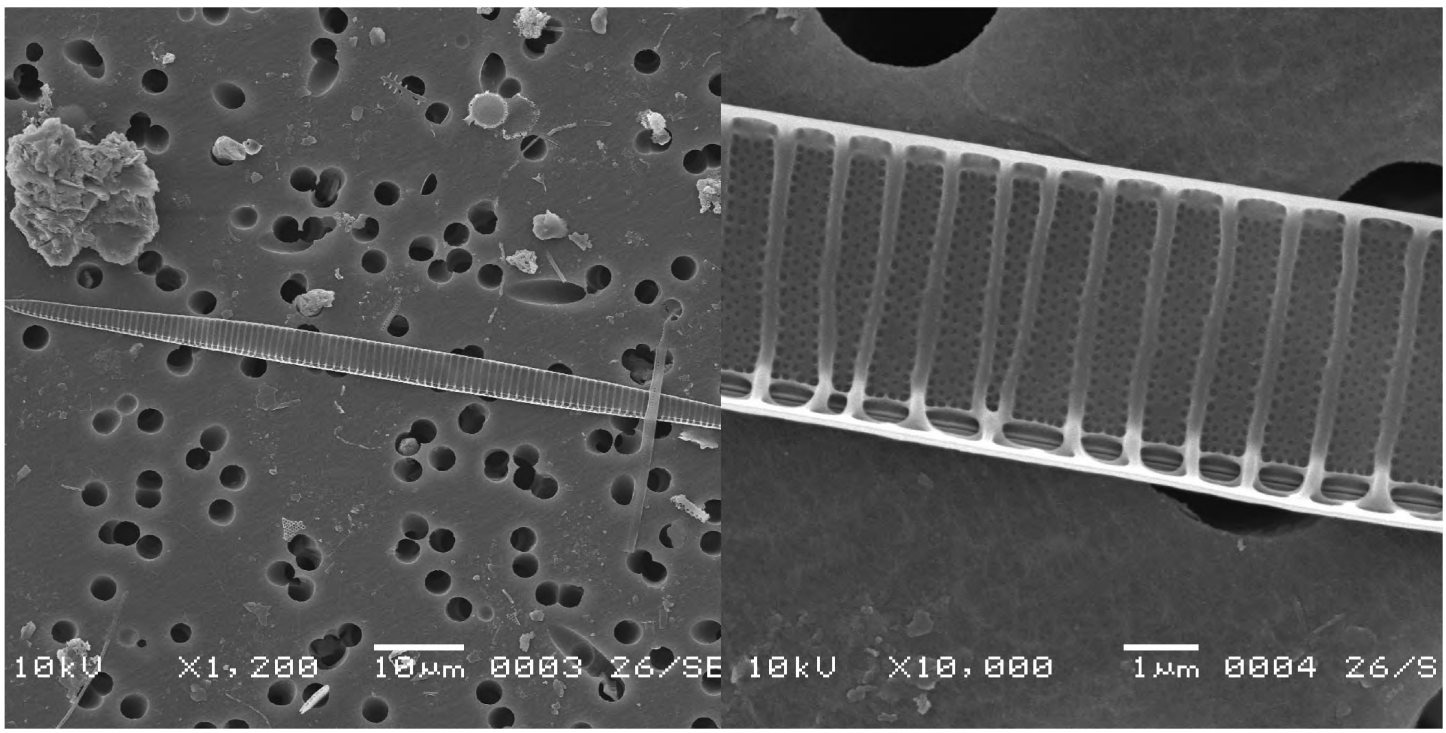

Figure 2. Scanning electron micrographs of Pseudo-nitzschia multiseries, taken from a sample collected in Mill River on September 23, 2000; the left panel shows a whole cell, and the right panel the detail of that cell, needed to confirm the species identity (images courtesy of Jim Ehrman, Digital Microscopy Facility, Mount Allison University, Sackville, New Brunswick).

one day after the closure, giving a cell count of about 220,000 *Pseudo-nitzschia* cells per litre. Scanning electron microscopy revealed only the toxin-producer *Pseudo-nitzschia multiseries* (Fig. 2); not one *P. pungens* cell was found in the sample. This is unusual, because blooms in late summer have usually been initiated with the non-toxic *Pseudo-nitzschia pungens* and then only later has the proportion of toxic *P. multiseries* increased [4].Since 1994, *P. pungens* has become dominant, with few or no observed toxic *P. multiseries* cells, and consequently no DA in shellfish - until now.

It is likely that we had witnessed only the tail end of a short, but intense bloom. Mussel samples analyzed by the CFIA only one week prior to the closure revealed no detectable DA. The *P. multiseries* cells in the Mill River water sample appeared in poor physiological condition. According to previous laboratory experiments, the majority of DA is produced when *P. multiseries* growth becomes limited by low concentrations of silicate or phosphate during stationary phase in batch culture [2]. Therefore, the cells in Mill River may have been highly toxic for a short period before the bloom crashed. Within 8 days of the peak in shellfish toxicity, only trace levels of DA (1 µg DA/g) remained in the mussels and the event was over. Shellfish harvesting resumed on October 6. The reasons for this short bloom, dominated by *P. multiseries*, are unknown. However, a rainy summer, combined with various nutrient sources upriver, may have provided opportune bloom conditions.

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◆ Gulf of California

First outbreak of *Cochlodinium polykrikoides* in the Gulf of California

From 15th to 28th of September 2000 several red patches were observed in the inner part of the coastal lagoon, La Ensenada de La Paz (24°10´ - 24°47´N, 110°20´ - 110°44´ W). Surface samples were collected and fixed with Lugol. Because Lugol is not a good fixative agent for this dinoflagellate, many cells became rounded, contracted, or disrupted. The identification of this species was difficult and required examinations of live cells. Water samples were also taken for the determination of photosynthetic pigments. The dinoflagellate *Cochlodinium polykrikoides* (Fig. 1) was responsible for water discoloration. Although *C. polykrikoides* is cosmopolitan in warm and tropical waters, this is the first record of it in Gulf of California waters. The patches of *C. polykrikoides* were found mainly in shallow areas (1-4 m depth), within a temperature range from 29 to 31°C. Nutrient concentrations during this bloom were: 0.02 - 0.90 µM NO_2+NO_3, 0.16 - 2.2 µM PO_4, and 1.0 - 35.36 µM SiO_4. Most cells of *C. polykrikoides* formed chains of four, rarely two. Cell density ranged from 360 x 10^3 to 7.05 x 10^6 *Cochlodinium*/L. Chlorophyll *a* concentration was 2.7 to 56.8 mg/m^3. *C. polykrikoides* is a species that kills fish in Korea [1], but few dead fish were associated with this event. This bloom occurred close to several fish and shrimp-culture ponds, and one of the ponds was infested with *Cochlodinium*. A total of 27 dead fish were found in the fish-pond, possibly caused by the dinoflagellate. There are no earlier records of *C. polykrikoides* in this area, but a *Cochlodinium* sp bloom has been recorded off Manzanillo, México [2]. Hydrographical and phytoplankton monitoring programs are necessary to prevent mortality marine animals, particularly cage species of economic interest. Work is in hand to obtain cultures of this species, and to evaluate its toxicity.

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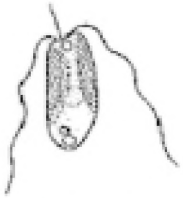
Figure 1. Light microscopy photograph of Cochlodinium polykrikoides

◆ **Morocco**

Blooms of *Prymnesium parvum* associated with fish mortalities in a hypereutrophic brackish lake in Morocco.



Prymnesium parvum N. Carter

In Morocco, harmful algae blooms studied so far have been caused by dinoflagellates which on the Atlantic and Mediterranean coasts [1], and by cyanobacteria in several lakes and reservoirs [2]. During a recent hydrobiological survey of Oued Mellah reservoir (Fig. 1; Table 1), autumnal Chrysophycean blooms of *Prymnesium parvum* occurred in November - December 1998 and September - October 1999. This was the first event of this kind recorded in Moroccan waters. The same site is also characterised by cyanobacterial blooms of *Microcystis ichthyoblabe* and *Aphanizomenon flos aquae* in spring and summer [3].

During the *P. parvum* proliferation, the water was characterised by moderate temperatures (15 – 23.5°C), elevated salinity (8.6 – 12.64 ‰), alkaline pH (7.67 – 9.04), a high content of organic matter (4.4 – 8.6 $mgO_2.L^{-1}$), elevated concentrations of total nitrogen (1,69 - 3,77 $mg.L^{-1}$), limited concentrations of nitrates (78.5 – 195.5 $\mu g.L^{-1}$) and undetectable amounts of orthophosphates. The chlorophyll a concentration (105.65 – 322.1 $\mu g.L^{-1}$) was mainly due to *P. parvum*, that dominated the phytoplankton (34 to 80% of total biomass). Cell counts were very high with maxima of $148x10^6$ cells.L^{-1} in 1998 and $169x10^6$ cells.L^{-1} in 1999. Such cell concentrations impart a yellow-gold colour to the water which is characteristic of haptophycean blooms.

Table 1. General characteristics of Oued Mellah lake – reservoir.

Lake	Oued Mellah reservoir
Date of construction	1928-1931
Geographical situation	33°30'N - 07°20'W - 25 km north of Casablanca
River	Oued Mellah
Total capacity	18 Mm^3
Useful capacity	8,8 Mm^3
Surface	250 ha
Maximum depth	9 m
Normal elevation	101,75 NGM
Bioclimate	Semi-arid Thermomediterranean with hot winter
Catchment geology	Primary schist surmounted by gréso-maghrébienne tile with triasic saliferous clays and altered basalts.
Use	Domestic water production - irrigation - electricity - Fishing and recreation
Ecological interest	National Biological and Ecological Interest Site (BEIS n°H8).

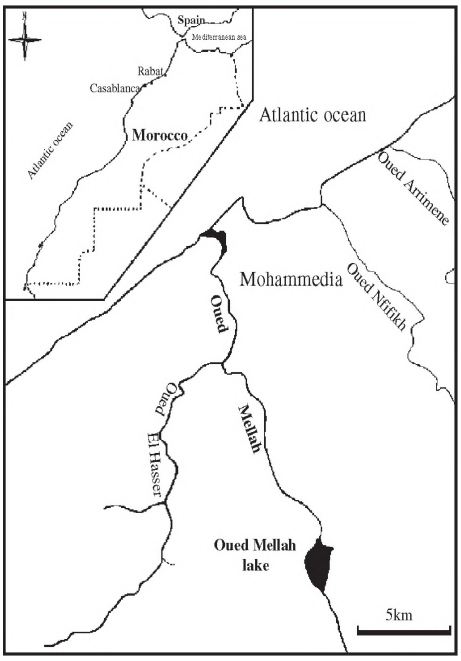


Figure 1. Geographic situation of Oued Mellah reservoir



Fish mortality caused by Prymnesium parvum bloom in Oued Mellaah reservoir (November 1998)

Blooms of this species cause extensive fish mortalities (carps, barbels, eels, sun-poles, gambusia…) (see photograph), and also kill other animals such as shrimps, bivalves and other invertebrates. The kinetics of *P. parvum* development showed that fish mortality began at the end of the growth phase and continued during the stationary phase of the 1998 and 1999 blooms. *P. parvum* is known to cause toxic blooms with catastrophic fish-kills in brackish waters in Europe, the Middle East, Ukraine, USA and Australia [4, 5]. Recently, Igarashi et al. [6] isolated and elucidated the structure of the causative ichthyotoxic and haemolytic compounds prymnesin-1 and prymnesin-2 from cultivated cells of *P. parvum*. Prymnesins show diverse biological activities including mouse toxicity, ichthyotoxicity, cytotoxicity and antibacterial activity [7,8].

In addition to the economic and ecological damages caused by these mortalities, the Oued Mellah lake lost its touristic value (Picnics, excursions, sport fishing, sailing...). These blooms constitute a high sanitary risk for the riparian population for two reasons, on one hand because of the deterioration of water quality, and on the other hand because of the uncontrolled commercialisation of large quantities of dying fish collected from the banks. Further autoecological and toxicological studies of isolated strains of *P. parvum* are in progress.

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Translated into English by I. Jenkinson.

◆ British Isles

A Key for the identification of blue-green algae of the British Isles

Eutrophication and blue-green algae (cyanobacteria)

Eutrophication is recognised by the Environment Agency of England and Wales as a national water quality issue and has become more widespread in recent decades, resulting in deterioration of the aquatic environment and serious problems for water-use. Cyanobacteria (blue-green algae) are a major recognised symptom of eutrophication, and generally occur during warm, dry summers, with plenty of sunshine and little wind mixing, although blooms can also occur during other periods. Cyanobacterial blooms and scums are natural features of many waterbodies and are likely to remain so, although successful eutrophication control strategies may limit the extent of their development.

Cyanobacterial toxicity

Cyanobacteria are capable of producing severely toxic chemicals potentially resulting in deleterious health effects, and even death in fish, livestock, pets and humans. Blooms may also have serious negative economic consequences for aquaculture (fish and shellfisheries).

Toxicity assessments of cyanobacterial scums/blooms continue to indicate a high incidence of toxicity (more than two-thirds of samples analysed are toxic). The Environment Agency, therefore, continues to adopt a precautionary approach, recognising the need to regard all cyanobacteria species and strains as capable of producing toxins, and hence to regard cyanobacterial populations as a threat to human and animal health and safety.

Environment Agency monitoring protocol

Monitoring of affected sites was extensive during the incidents of 1989 and 1990, when large cyanobacterial blooms were identified in UK inland waters, and intensive programmes were adopted for some sites. Since then, a reactive monitoring programme has been advocated, principally to cater for those water body owners and users who had not previously contacted the Agency. This approach reduced the earlier monitoring effort, allowing resources to be diverted into eutrophication control developments (aimed at reducing blue-green algal problems) – see www.environment-agency.gov.uk, 'Aquatic Eutrophication in England and Wales: a proposed management strategy' consultation document.

The Agency's position

The lack of suitable reference books for accurate naming of blue-green algae has been a major hindrance to algological work within the Environment Agency. Whilst there are both beginner's guides and specialised taxonomic works (usually in foreign languages), a practical blue-green algal flora has not been available.

A collaborative project was set-up with the University of Durham to produce a key for identification of blue-green algae from the British Isles to assist Agency staff engaged in monitoring work. The principal output is a computer-based interactive system built from LucID Professional, a specialised computer package for developing biological identification systems. The system was produced initially in CD-ROM format and has subsequently been made available nationally to Agency ecologists through the computer network on the Windows desktop.

The CD-ROM was officially launched by the Environment Agency and University of Durham on 30 May 2000.

In the future, it is hoped that following the success of this collaborative project there will be additional keys in CD-ROM format for other algal groups, adopting the LucID interactive system.

Background information

The key aids the identification of any blue-green algal sample collected in the British Isles. It includes 320 species in 65 genera of freshwater, terrestrial and marine blue-green algae, and contains many illustrations and colour images together with species descriptions. Freshwater and terrestrial species follow the Coded list of freshwater algae of the British Isles incorporating recent taxonomic developments. The key was based on LucID Professional software developed at the University of Queensland, Australia and operates as a multi-access key with the user answering questions for whatever information

they possess about the sample. LucID suggests possible names, and the user can then check the sample against the suggested species descriptions and images

Copies of R&D Technical Report E84, providing explanation of the LucID key and taxonomic accounts of genera, and R&D Technical Summary leaflet ES83 are available internally from the Regional Environment Agency Libraries/Management Support Officers and externally from WRc plc.

Contacts

CD-ROM availability and technical enquiries: Professor B.A. Whitton, Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, UK, Tel: 0191 3742427, Fax: 0191 3742417, E-mail: b.a.whitton@durham.ac.uk

Other enquiries: Dr Jan Krokowski, Environment Agency, National Centre for Ecotoxicology and Hazardous Substances, Evenlode House, Howbery Park, Wallingford, Oxon OX10 8BD, UK, Tel: 01491 828548, Fax: 01491 828427, E-mail: jan.krokowski@environment-agency.gov.uk

◆ North Sea

Chattonella sp. bloom in North Sea, spring 2000

Detection of the bloom

An extensive bloom caused by two raphidophytes, *Chattonella*-like cells, occurred from the German Bight almost to north Skagen between late April and the first half of May. On the 2 and 15 May,2000, two helicopter flights sampled along the North Sea coast of Schleswig-Holstein. On the first flight we found reddish discolouration in the coastal waters, 10 km off Sylt Island. The dominant species was *Chattonella sp* with more than 10 million cells per liter mixed with *Chattonella* cf. *verruculosa* and another unknown flagellate on the first sampling date. The corresponding chlorophyll concentration was 80.17 µg/l. On a routine cruise in April, Norwegian colleagues observed *Chattonella* cf. *verruculosa* with a concentration of 25-30 millions cells per litre, 10-18 kilometer off the coast of Esbjerg, Denmark on the 19-20 April 2000. On 4 May, Viborg County took samples about 20 km off Hanstholm and found 8.7-11 million *Chattonella* cells per liter. On 5 May, 1.8 million *Chattonella*-like cells per liter were found 10 km off Hirtshals by Danish scientists (Peter Henriksen, personal communication). On 15 May, *Chattonella sp.* Was still present, but the bloom had disappeared. This large bloom was also detected by MOS and SeaWiFS satellite images in cloud free conditions (Fig. 1). Peak concentrations may have been subsurface, especially in the sunny weather condition and not visible from the satellite.

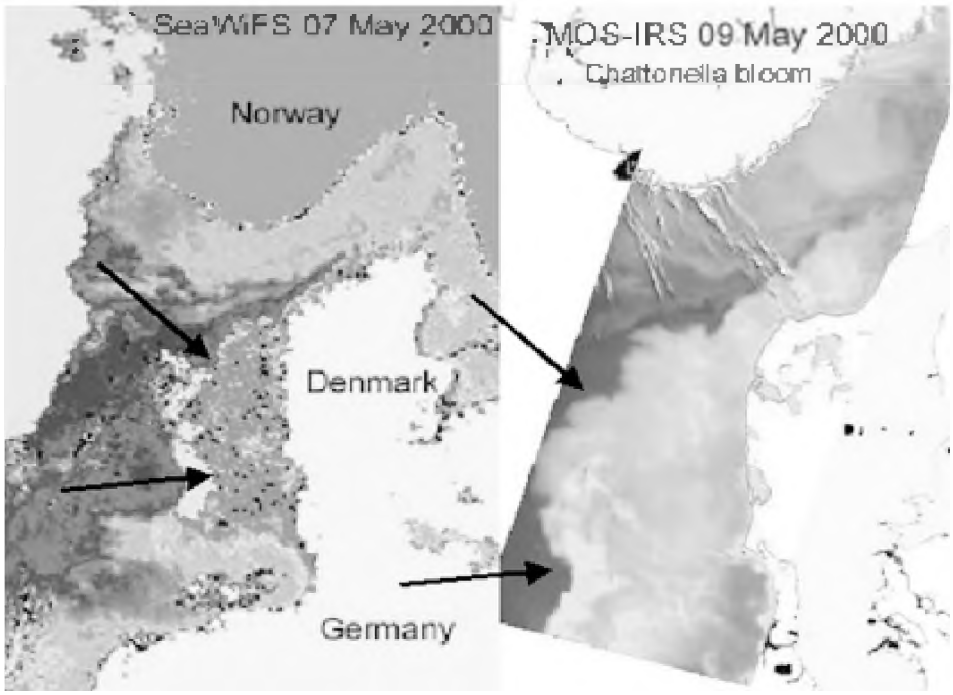

Figure 1. Chattonella sp. bloom was detected by two satellite sensors

Light microscope observations

1. Chattonella sp. (Fig. 2a)

Living cells are 12 - 23 µm long, of variable size, ovoid to irregularly ovoid, unlike *Chattonella verruculosa*; numerous, small chloroplasts; with two flagella , the shorter one as long as cell body, pulling the cell forward, pointed, slightly curved, in the swimming direction, the longer one twice cell length, trailing, close to the cell body, sometimes hidden.

The cells have mucocysts, and are phototactic. Living cells are very sensitive. They can die in a few minutes under light microscope. They stop swimming and lose flagella just before dying. Then the shape of the body changes immediately. Lugol-fixed cells usually lose their original cell-shape or break. However chloroplasts are visible and accumulated.

2. Unknown flagellate (Fig. 2b)

Living cells are 18-22 µm diameter, slightly flattened and roughly heart-shaped, flagella insertion marked by strong intrusion; numerous, irregularly plate-shaped chloroplasts; one flagella, about 3 times cell length; swimming behavior turning, sometimes stop, then continue; flagellum pushes cell forward. Swimming behavior is distinct from that of *Chattonella* species. Cells have big mucocysts, protruding from cell surface. Sometimes, during swimming or resting, the cells jumps straight forward, maybe caused by ejectosomes?

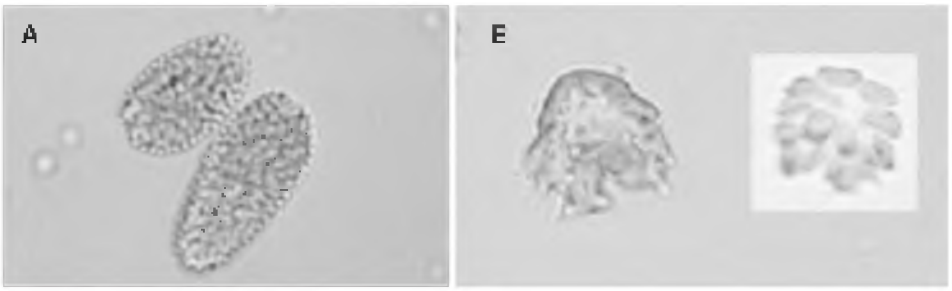

Fig.2. A. Chattonella sp. (living cells), 12-24 μm in length; B. Unknown flagellates, mucocysts are clearly visible (living cells), 18-22 μm in lenth

Fish kill and toxicity

Toxic *Chattonella* species are well known for fish killing in Japanese waters, where they have been observed since 1969. In European waters, *Chattonella antiqua* and *Chattonella marina* were first recorded in Netherlands coastal waters in 1991 [4]. A *Chattonella* cf. *verruculosa* bloom was first recorded In the North Sea in 1998 and killed more than 400 t of cultured salmon in southern Norway as well as some wild fish [5]. During the bloom this year, some dead herring and trout were found in Danish waters, which might be associated with this species (Peter Henriksen, pers. comm.). The killing mechanism of *Chattonella* to fish is not clear yet. It may related both to physical clogging of gills and to toxins. The toxicity of raphidophytes, though not yet fully investigated, seems to origin from neurotoxins as known from *C. antiqua* and *C. marina* [3].

Environmental aspects

The history of *Chattonella* in the North Sea is short, and it is not known whether these toxic raphidophytes are exotic or indigenous to European waters [1]. Because the cells are so small, fragile and difficult to preserve, they might be overlooked if cell density is low. On the other hand, *Chattonella* sp was never found in such high cell concentrations in this area before 1998. It seems that they have now established a niche and have the potential to recur and form extensive blooms in the North Sea. Does this phenomenon indicate some kind of environmental change?

Acknowledgement

Thanks are extended to Dr. Matthias Hetscher at DLR Remote Sensing Technology Institute in Berlin for kindly processing the satellite images.

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◆ Northwestern Atlantic

A parasite of *Dinophysis acuminata* in northwestern Atlantic

At the International Conference on Harmful Algal Blooms in Tasmania last February, Noren, Moestrup and Rehnstam-Holm presented their elegant work on the identity and life cycle of a highly infective flagellate, the parasite *Parvilucifera infectans*, of the newly introduced phylum Perkinsozoa. They astutely combined ultrastructural observations and molecular data. A photograph of an infected *Dinophysis norvegica* on their poster and in their talk immediately caught my eye and lead me to question whether this parasite sporangium could correspond to the large circular inclusions sometimes found in *D. acuminata* in coastal Rhode Island waters. Later on at the meeting, my suspicions came closer to certainty when Chretiennot-Dinet showed fabulous video recordings of parasitic zoospores of a very similar, perhaps identical (?), dinoflagellate-specific parasite from French coastal waters (part of this recording can be viewed from a CD-ROM available in a special issue of Estuarine, Coastal and Shelf Science, [1], or on line at http://www.academicpress.com/www/journal/ec/suppmat/contents.htm). Both groups of researchers wondered about the geographic distribution of these parasites outside western european waters. The present note reports on my observations in northeastern US waters of *D. acuminata* with inclusions suspected to correspond to or be affiliated with the above parasite(s).

In coastal embayments around Narragansett Bay, Rhode Island, significant populations of *D. acuminata* develop between May and October with peaks commonly occurring in June and/or July, especially at times of strong water column stratification [2]. Over the years, weekly sampling of this dinoflagellate from



Figure 1. Single large circular inclusion (arrow) inside Dinophysis acuminata similar to a developing sporangium of the parasite Parvilucifera infectans (Greenwich Cove, July 1 1995). Bright field microscopy.



Figure 2. Reticulate chloroplasts of a healthy D. acuminata. Epifluorescence microscopy.

Greenwich Cove (41°39.5'N, 71°27'W) has revealed a wide range of variations (number and size) in cellular inclusions observed both by light and epifluorescence microscopy. Except for some conspicuously large circular inclusions, most of the observed variations can usually be attributed either to volume changes in the pusular system or to the occasional presence of food vacuoles. It now appears very likely that the "large circular inclusions" seen in *D. acuminta* correspond to parasitic sporangia. My first observation of these large inclusions dates back to early July 1995, when *D. acuminata* reached 20,000 cells L^{-1}; a small proportion of cells (ca. 2%) harbored such round bodies (Fig. 1). Again toward the end of July 1999, large inclusions (one or two per cell) were recorded inside *D. acuminata*, but this time, some tiny swarmers were actively swimming inside as well as outside one of them, a particularly large inclusion within an otherwise almost empty *Dinophysis* theca. Unaware of the then unpublished findings of Noren and Erard-Le Denn and colleagues [1, 3], I questioned the nature of these round bodies, as others had done before [4].

When under blue excitation in epifluorescence microscopy, a healthy photosynthetic *D. acuminata* displays a flamboyant yellow-orange stellate to reticulate chloroplast (Fig. 2); however, the occasional "grape- or bead-like" appearance of certain chloroplasts has been puzzling. Variations in previous light history or nutrient status of the cells could be invoked but our inability to establish long-term culture of this dinoflagellate limits or complicates testing basic hypotheses. Noren *et al.* [3] report on the "granular appearance of the chloroplast" upon infection of *D. norvegica*. Thus parasitic infection may also account for some of the variation observed in the shape and size of the chloroplast. Interestingly, in July 1995, most *D. acuminata* displayed one or two chloroplasts looking like "clumps of grapes" (Fig. 3).

My observations need detailed ultrastructural and molecular analyses before the large inclusions in *D. acuminata* on this side of the Atlantic can be attributed with certainty to *P. infectans* or a close relative. However, taken all together and in light of the works mentioned above, these observations would suggest a parasitic nature for the inclusions.

The recognition that various pathogens may significantly contribute to regulation of protistan population dynamics has gained momentum in the past decade and has revived the idea that harmful algal blooms may be controlled via biological means [1, 3, 5]. Although the newly described potential candidate, the water-borne parasite *P. infectans*, is culturable and so far appears dinoflagellate-specific, it must be remembered that results of infection studies have been published on just a few diatoms and one raphidophyte, besides dinoflagellates. Hence many questions remain, mostly pertaining to conditions of infection, list of susceptible hosts (undesirable as well as desirable ones), effects of broad manipulations of food web dynamics and unintentional introduction into non-target areas. Erring on the side of caution would be prudent.



Figure 3. Beady chloroplasts of a D. acuminata cell during the putative parasitic episode (July 1 1995). Epifluorescence microscopy

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GEOHAB

The emerging new science programme sponsored by SCOR and IOC on the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) will soon publish its Science Plan (SP). The SP has been prepared by the IOC-SCOR Scientific Steering Committee (SSC) for GEOHAB. The SP was presented to, and adopted by, the SCOR General Meeting in October 2000 pending editorial changes. At a meeting of the SSC in La Paz, Mexico, 13-17 November 2000, the SP was revised based on the comments and advice of three independent reviewers appointed by SCOR

The SP is built around five programme elements: Biogeography and Biodiversity; Adaptive Strategies, Nutrients and Eutrophication; Ecosystem Processes; Observation and Modelling.

The SP will be available on World Wide Web for comments from the scientific community around 1 March 2001, and is expected to be published in April 2001.

The next step is development of an implementation plan for the Programme. The Implementation Plan (IP) will describe how the programme will approach the objectives of the SP, of which the primary one is to foster international research. This requires a coordinating framework to link existing national programmes and encourage and initiate more scientific interest in HABs.

The IP will be cross-cutting in approach, but will address each defined objective of the SP and will describe the actions required to achieve it. The IP will also consider the extent to which the objectives are already being undertaken, or standards are being set, by existing national programmes and how GEOHAB can help in international coordination. The development of the IP will require strong interaction with the scientific community and the SSC is charged with taking initiative for outreach activities in order to ensure such involvement.

The SSC, IOC, and SCOR are endeavoring to establish an International Project Office (IPO) to help implement

(Cont'd from p. 12)

GEOHAB. Funding for the IPO is being sought, and an official offer for partial support has been received. A major task of the SSC, SCOR, and IOC is to raise the remaining funding to establish the IPO.

You can follow the development of GEOHAB and in due time have access to the SP at http://ioc.unesco.org/hab/GEOHAB.htm, or contact the Chair of the GEOHAB SSC Dr.Patrick Gentien, CREMA, B.P. 5, 17137 L'Houmeau, FRANCE,

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The **ICES/IOC Working Group on Harmful Algal Bloom Dynamics** will meet in Dublin, Ireland, from 12B16 March 2001 to update the harmful algae event database (HAEDAT). It will also examin possible ways of analysing historical data and fossil records, and evaluate and assess the use of remote sensing and *in situ* optical sensing technology in HAB dynamics studies. There will be discussions of the potential sensitivity of HABs to climate changes; review of the implementation of GEOHAB relevant research in the ICES area; and preparation of a resolution for a workshop, possibly co-sponsored by regional programmes such as GEOHAB, GLOBEC and GOOS, on «Real Time Observation Systems applied to Harmful Algal Bloom Dynamics Studies and Global Ecosystem Functioning».

A Study Group on GEOHAB Implementation in the Baltic will meet in Dublin, Ireland from 12B13 March 2001 to create a plan for the implementation of GEOHAB in the Baltic Sea. SCOR and IOC will be invited to co-sponsor the Study Group. *Scientist from outside the North Atlantic (ICES area), can also join the WGHABD as it is IOC co-sponsored. Inquiries can be made to the Chair of the two groups, Dr. Kaisa Kononen, Maj and Tor Nessling Foundation, P. Hesperiankatu 3 A 4, FIN-00260 HELSINKI, FINLAND, Fax: +358-9-406 684, E-mail: Kaisa.Kononen@nessling.fi*

New Staff in the IOC-HAB programme



Ole Vestergaard joined the IOC Secretariat in Paris 1 April 2000. He is seconded to the IOC by Denmark as an Associate Expert. He will devote half of his time to the IOC HAB Programme. His responsibilities are development, co-ordination, and administrative back-up of the HAB Programme at IOC Headquarters in Paris. He works in close contact with the IOC HAB Science and Communication Centres in Copenhagen and Vigo. As a biologist he has been working with various aspects of aquatic ecology. Before joining the IOC he worked at the University of Copenhagen doing research on macroalgae diversity and distribution patterns in marine and brackish waters. He has also been working with Arctic marine phytoplankton and with monitoring and environmental assessments of aquatic ecosystems. Besides working for the HAB Programme, Ole also functions as Technical Secretary for the Global Coral Reef Monitoring Network (GCRMN), and a number of other IOC science programs.

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Mónica Lion is since 15 May 2000 head of the "IOC-IEO Science and Communication Centre on Harmful Algae" in Vigo, Spain. Mónica has been part of HAB community since 1996 when she started as a PhD student in the Centro de Investigaciones Marinas (Xunta de Galicia) in Vilanova de Arousa, under the guidance of Dr. J. Blanco. In her new job, she relies on the valuable help of all the HAB staff in the Instituto Español de Oceanografia and other experts from the Galician research laboratories working on different HAB aspects, to carry out training, assessment and communication activities according to the priorities of the IOC-IPHAB, with special attention to Latin American countries.

The main activities of the Vigo Centre are: HAB related research and managerial questions; development and maintenance of the IOC-ICES HAEDAT (Harmful Algae Events) data-base; compilation and co-edition of "Harmful Algae News"; organization of annual international training courses on "Toxic Phytoplankton and Phycotoxins"; assistance in development and implementation of relevant IOC HAB programme activities; free-of-charge dissemination of manuals, reports, conference proceedings etc. to Spanish and Latinamerican institutions; databases of Iberoamerican experts, a bibliography, etc.

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International Ballast Water Treatment Symposium & Workshop

The International Maritime Organization (IMO), with funding provided by the Global Environment Facility (GEF) through the United Nations Development Programme (UNDP), has initiated the Global Ballast Water Management Programme (GloBallast).

This programme is aimed at reducing the transfer of harmful marine species in ships' ballast water, by assisting developing countries to implement existing IMO voluntary *Guidelines for the control and management of ships' ballast water to minimize the transfer of harmful aquatic organisms and pathogens* (Assembly Resolution A.868(20)), and to prepare for the anticipated introduction of an international legal instrument on ballast water management currently being developed by IMO member countries.

The Need for and Purpose of the Symposium & Workshop

A core objective of the GloBallast programme is to assist developing countries to implement the existing IMO voluntary guidelines on ballast water management. The main management measure recommended under these guidelines is ballast exchange/ reballasting at sea. It is widely recognized that this approach has many limitations, including serious safety considerations that limit its applicability, and the fact that translocation of species can still occur even when a vessel has b een able to fully implement ballast exchange/reballasting at sea.

It is therefore extremely important that alternative, effective ballast water management and/or treatment methods are developed as soon as possible, to replace reballasting at sea. Significant research and development (R&D) efforts are underway by a number of scientific and engineering research establishments around the world, aimed at developing a more complete solution to the problem. All of these possibilities currently require significant further research effort. Major barriers still exist in scaling these various technologies to deal effectively with the huge quantities of ballast water carried by large ships (e.g. about 60,000 tonnes of ballast water on a 200,000 dwt bulk carrier). Treatment options must not interfere unduly with the safe and economical operation of the ship and must consider ship design limitations.

One of the problems currently faced by the global R&D and shipping community is that apart from the general criteria above, there are currently no internationally agreed and approved standards and criteria for the evaluation and approval of new ballast water treatment systems that are developed.

Most parties involved in the ballast water issue, including the Ballast Water Working Group of IMO's Marine Environment Protection Committee (MEPC), have identified the current lack of such standards and criteria as the single largest obstacle to finding a solution to the problem of marine organism transfers in ballast water.

In addition, many groups are working in isolation from each other. There are no formal mechanisms in place to ensure effective lines of communication between IMO, the R&D community, governments and ship designers, builders and owners on ballast water treatment issues. These are vital if the R&D effort is to succeed.

To help address this situation, the GloBallast Programme Coordination Unit (PCU) is organizing an international symposium 26-27 March 2001 (open) and workshop 28-30 March 2001(invitation only) on ballast water treatment

Further information

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◆ **Spain**

IOC-AECI- IEO IV Course on Toxic Microalgae and Marine Phycotoxins

Once again, the IOC-IEO Science and Communication Centre on Harmful Algae organized a Course on "Toxic Microalgae and Marine Phycotoxins" from 1-17 June 2000, at the Instituto Español de Oceanografía (IEO) in Vigo, Spain. It was sponsored by the IOC, the Agencia Española de Cooperacion International (AECI) and the IEO.

Thirteen scientists from different countries (Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Guatemala, Haiti, Mexico, Italy, Puerto Rico, Venezuela) participated in the course that is part of the training activities included in the Harmful Algal Bloom Programme of IOC. The purpose of the course was to impart theorical and practical knowledge about four aspects of toxic microalgae: Cultures, Taxonomy, Toxin determination and Benthic harmful microalgae.



All participants were invited to work with their own samples and presented a status report on harmful algal blooms in their countries. Complementing these activities, some visits to local research laboratories working with HAB were programmed.

Lectures were given by staff from IEO, Instituto de Investigaciones Marinas (IIM-CSIC), European Union Reference Laboratory on Marine Biotoxins, Centro de Control de Calidade do Medio Mariño and Centro de Investigacions Mariñas (Xunta de Galicia), ANFACO-CECOPESCA and Universities of Vigo and Santiago de Compostela.

The general impression of the course was very positive and we hope it will enable the participants to improve their capabilities and get valuables contacts for future collaborations.

This IOC training course is organized every year. Additional information can be required from "IOC-IEO Science and Communication Centre on Harmful Algae" (fax +34 986 492003 or e-mail: vigohab@vi.ieo.es)

◆ **Europe**

BIOHAB, A European Initiative

Biological control of Harmful Algal blooms in European coastal waters (BIOHAB) is a new EU research program with participants from 7 European institutes. It started March 2000 and will continue for 3 years. BIOHAB addresses one of the key issues of pollution, namely nutrients. Harmful Algal Blooms (HABs) occur in many European marine waters and may have increased in frequency with increased nutrient inputs from land.

The overall objective of BIOHAB is to determine the interplay between (anthropogenic) eutrophication and biological control of the losses and gains to HAB populations. The ultimate goal is to find ways to manage algal blooms in European coastal waters so that harmful species are avoided or at least that their negative effects are minimised. The project involves co-operation between several European countries, representing distinctly differing regions (the eutrophic North Sea, the low salinity Baltic, the oligotrophic Norwegian Sea, and the P-limited Mediterranean).

The scientific objectives are (1) To determine the susceptibility of HABs to biological control such as grazing (copepods, ciliates, hetero- and mixotrophic dinoflagellates) and infection (virus, bacteria, parasites) when growing under nutrient deficient and sufficient conditions. (2) Investigate the roles of HAB infochemicals in relation to grazing and infection. (3) To examine the general and unique patterns of growth and decline of HAB-species in various coastal regions. (4) To develop a generic or species-specific model for the development of HABs and their mitigation. (5) To obtain and grow HAB species-specific pathogens (viruses, bacteria, parasites) with potential to control HABs.

The Helsinki (HELCOM) and Oslo Paris (OPARCOM) Commissions have the primary task to protect the marine environments of the Baltic and North Seas. BIOHAB aims to improve knowledge of biological factors affecting key HAB-species, and may indicate potential ways to control HABs.

Contact person: Marcel J.W. Veldhuis (co-ordinator). Email: veldhuis@nioz.nl, website: www.nioz.nl/projects/biohab

New home for the Phycotoxins mailing list

For the past 30 months, PHYCOTOXINS has been residing on the Listquest.com servers but the company encountered unspecified difficulties and ceased most services on September 27. As we could not establish contact with the company by various means, about ten days passed before we decided the problems were terminal. A new service, using LISTSERV software, was launched using Canadian Food Inspection Agency (CFIA) servers on October 12. Phycotoxins continues to be managed by Don Richard (richarddj@em.agr.ca) of the CFIA in New Brunswick, Canada with backup support by Bill Silvert (wsilvert@ipimar.pt) at Instituto Nacional de Investigação das Pescas in Lisbon, Portugal.

There are currently about 550 subscribers throughout the world. Topics include the reporting of bloom events, fish kills, poisonings, conferences and workshops announcements, requests for information, job postings (but not requests for employment), and other issues related to phycotoxins. The relocation and formatting of the archives is a work-in-progress but should be completed in the near future. To join the phycotoxins list, send an e-mail to **listserv@www.agr.ca** with the line **«subscribe phycotoxins yourfirstname yourlastname»** in the body of your message. Should you require assistance, send the command **«info refcard»** to the same address or contact the owner at richarddj@em.agr.ca. Mailing lists rely on the members for information so please post relevant articles as you see fit. The list will be moderated for the next few months so as to keep errors and spam to a minimum.

Don J.A. Richard, Biotoxins Unit, Canadian Food Inspection Agency, 343 Université Ave., Moncton, NB, Canada E1C 9B6, tel.: 1-506-851-7831, e-mail: RichardDJ@em.agr.ca

Dr. William Silvert, Instituto Nacional de Investigação das Pescas, Departamento de Ambiente Aquatico, Avenida de Brasília, 1449-006, Lisboa, Portugal

HAB Capacity building

In August 2000, the Seventh International IOC-DANIDA Training Course on the Taxonomy and Biology of Harmful Algae was held at the IOC Science and Communication Centre on Harmful Algae at the Botanical Institute, University of Copenhagen. Over the years, the courses have in addition to provision of specific training established many fruitful working contacts and networks, some of which have resulted in cooperative research projects. The courses have benefited enormously from the close and successful cooperation between the Universities of Copenhagen, Tokyo, Nagasaki, Lund, Jena, Rhode Island, and the Spanish Institute of Oceanography, in composing the programme and providing the lecturers. The first course took place in 1993, and it has for some time been a wish from former participants who are still very active in HAB research, to have an opportunity to upgrade and up-date their knowledge even further. The IOC will accommodate this wish in 2001 by introducing a high level advanced HAB identification course with special focus on related techniques and difficult taxonomic groups. The details of the programme will be decided in cooperation with the selected trainees. The course will be open both former course participants and other applicants with the similar background. In 2001 the introductory level to HAB species identification will be addressed at regional courses. The IOC HAB training courses in 2001 will be announced early 2001 at http://ioc.unesco.org/hab/train.htm

Future events

JUNE 2002

4th INTERNATIONAL CONFERENCE ON MOLLUSCAN SHELLFISH SAFETY, Santiago de Compostela, Galicia, Spain, 4-8 June 2002. Hosted by "Centro de Investigacions Mariñas" (Marine Research Centre) and "Centro de Control da Calidade do Medio Mariño" (Galician Monitoring Centre). The sessions will be dealing with: microbiological contamination; chemical contamination and bioaccumulation; harmful and toxic algal events; shellfish contamination by marine biotoxins; depuration; management and mitigation; water quality at harvesting sites; public health; epidemiology and toxicology; monitoring, quality assurance programmes and regulatory controls; and allergies associated with shellfish consumption. Contact: ICMSS, c/o Dr Antonio Villalba.
Fax: +34 986 506788;
E-mail: villalba@cimacoron.org

OCTOBER 2001

The JOINT ASSEMBLY OF THE INTERNATIONAL ASSOCIATION FOR THE PHYSICAL SCIENCES OF THE OCEANS (IAPSO) AND THE INTERNATIONAL ASSOCIATION FOR BIOLOGICAL OCEANOGRAPHY (IABO), to be held in Mar del Plata, Argentina, on 21 - 28 October 2001, with Joint Symposia of the International Association of Meteorology and Atmospheric Sciences (IAMAS), the World Ocean Circulation Experiment (WOCE), the Climate Variability and Predictability Program (CLIVAR), the Joint Global Ocean Flux Study (JGOFS), the Global Ocean Ecosystems Dynamics Program (GLOBEC), and the International Association of Geodesy (IAG), has issued a call for papers. Abstracts must be submitted electronically, and must be in the format given on the Joint Assembly Web page at http://www.criba.edu.ar/2001_ocean (note that there is an underline between «2001» and «ocean» in the URL address).

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 22

• China

More Raphidophyte Blooms in South China Waters

There have been two Raphidophyceae blooms, as well as a number of other blooms recorded in Dapeng Bay in the early part of this year. A *Heterosigma akashiwo* bloom occurred in the inner bayments of Dapeng Bay. In April 2001, a mixed bloom of *Chattonella ovata* and *Chattonella globosa* was recorded in the same area.

The first Raphidophyceae bloom, *Heterosigma* sp., was recorded in South China waters in the late 1980s. No other Raphidophyceae bloom was recorded until 1993, when a *Chattonella marina* bloom occurred in Dapeng Bay. Since then, more and more Raphidophyceae species, e.g. *Chattonella antiqua*, *C. globosa*, and *C. ovata* have been found in these waters.

South China waters are one of the locations with the most frequent HAB occurrences in the world. In recent years, more and more new harmful species have been recorded in these waters. An historical bloom, which caused major fish kills in the South China waters affected by the bloom, was due to a newly identified species,



Figure 1. Chattonella ovata and Chattonella globosa

(Cont'd on p.2)

• Mediterranean Sea

Spreading of *Gymnodinium catenatum* Graham in the western Mediterranean Sea

Gymnodinium catenatum Graham is a naked, chain-forming dinoflagellate causing Paralytic Shellfish Poison (PSP) events in several parts of the world [1]. In Europe, *G. catenatum* was described for the first time in the Galician rias (NW Spain) in 1976 after a PSP event [2], hypothetically introduced by fishing ships from Argentina [3]. Another possibility for the origin is the historic presence of the species in inshore Moroccan Atlantic waters [3] where PSP events have been recorded since the 1970s, but *G. catenatum* was not identified there until 1982 [4,5]. It has also appeared along the coasts of Portugal and Gulf of Cadiz [6,7] and for first time in the Mediterranean Sea along the NW Alboran coast [8,9]. Recently *G. catenatum* has been reported in Eastern Mediterranean waters near the Suez Canal [10].

There are erroneous descriptions of the geographical expansion of *G. catenatum* due to confusion with two non-toxic species [11,12,13]. Along the European Atlantic coasts these records corresponded to *Gymnodinium nolleri* Ellegaard et Moestrup [14,15] and in the Mediterranean Sea correspond to *Gyrodinium impudicum* Fraga et Bravo [16,17,18].

From Lugol fixed samples, the primary evidence for the differentiation between *Gyrodinium impudicum* and *G. catenatum* is colony size. *G. catenatum* can form colonies of up to 64 individuals whereas the colony size of *G. impudicum* comprises generally 4 or rarely 8 individuals [19,13]. Before the description of *G. impudicum*, the presence of short colonies was attributed

(Cont'd on p.2)

The publication of Harmful Algae News is sponsored by the Botanical Institute, University of Copenhagen, and the Spanish Institute of Oceanography, Vigo.

(Continues from p. 1)

Karenia digitata [1]. A number of other species new to these waters have also been recorded [2].

Why are there more and more new harmful species or new records found in South China waters? One answer is definitely that there have been both more and intensive monitoring programs in the area in recent years, as well as new techniques and well-trained personel applied to phytoplankton monitoring programs. However, there has been a Red Tide Monitoring Program in Hong Kong since the 1970s, and every single bloom has been recorded in a red tide database which clearly reveals this increasing trend.

Chattonella blooms frequently occur in Japanese waters, but were not recorded in the subtropical waters of South China before 1993. Why have more and more *Chattonella* species and blooms been recorded recently in these waters? Are they exotic species carried in by ballast water, water currents, or other means? We intend to try to find answers to these questions.

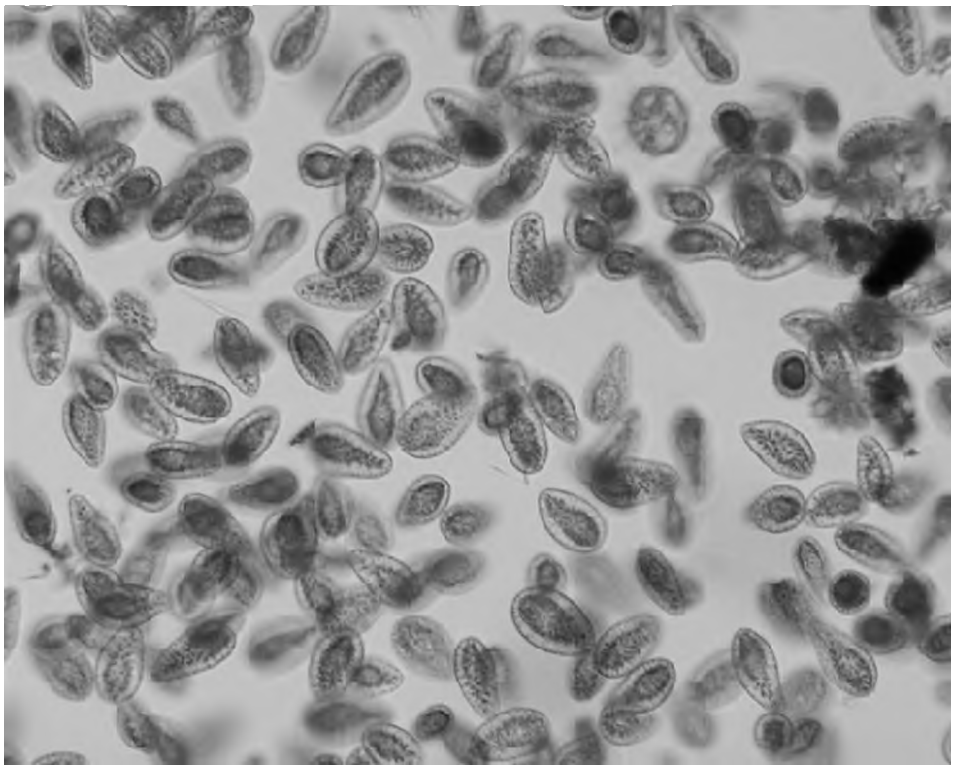

Figure 2. Bloom of Chattonella

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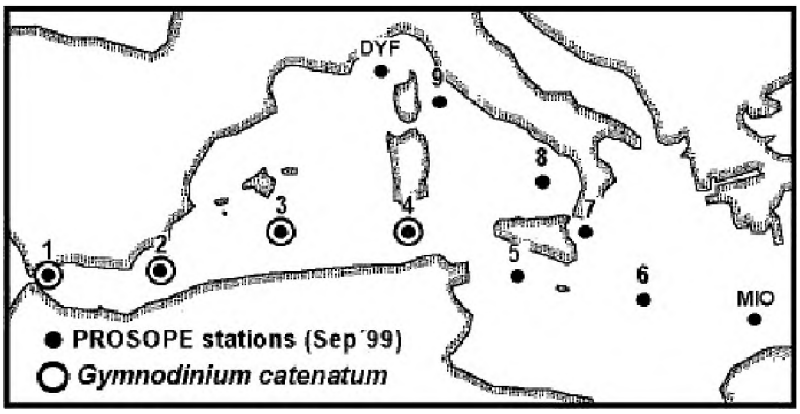
to morphological adaptation to new environments [16].

To our knowledge, clear identifications of *Gymnodinium catenatum* in the western Mediterranean were restricted to the Strait of Gibraltar and Alboran Sea [8,9] associated with PSP events [9]. Therefore, it can be postulated that the Almeria-Oran front acts as a barrier to the eastward expansion of this species in the Mediterranean.

During a survey aboard R/V "Thalassa" (September 8-30th, 1999) 11 stations were sampled in the Western Mediterranean and Ionian Sea (Fig. 1). Discrete water samples were collected and phytoplankton cells were counted by the Utermöhl method.

Gymnodinium catetatum was observed in the 4 stations from the southern basin of the Western Mediterranean Sea (Alboran Sea, Algerian basin and Sardinia channel; Table 1).

Agitation affects the samples in the Utermöhl chambers. As a consequence the average number of cells per colony in Table 1 is smaller than in the collected living cells due to fracture of the colonies. Despite this, colonies higher than 20 cells were usually



observed. The abundance of *G. catenatum* in the Algerian basin (Sta. 3) was close to the values that caused high PSP toxicity levels in the Alboran Sea [9]. The occurrence along the Algerian basin implies possible expansion towards the Tunisian and Italian coasts or the Balearic Sea according to the circulation patterns of the Modified Atlantic Water in the Western Mediterranean [20].

It seems that harmful algae events are increasing in the Mediterranean Sea [21] and if there are favourable habitats, this allochthonous species could be the 'protagonist of future red tides events.

Acknowledgements

We thank Dr. S. Fraga for confirming the identity of the species. Thanks to all participants and crew of the R/V "Thalassa" for their co-operative work during the cruise. This study was supported by JGOFS-France PROSOPE and operations of the PROOF-program.

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Table 1. Abundance (individuals per litre; # l^{-1}) and average colony size.

Station 1			Station 2			Station 3			Station 4		
depth	# l^{-1}	Ind/col.	depth	# l^{-1}	Ind/col.	depth	# l^{-1}	Ind/col.	depth	# l^{-1}	Ind/col.
5 m	0		5 m	0		-	-	-	7 m	140*	14*
20 m	1680	4	30 m	310	10	25 m	2050	8	27 m	0	
39 m	680	7	45 m	70	7	55 m	980	9	60 m	0	
59 m	0		70 m	0		80 m	0		90 m	0	

**Only one colony observed.*

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• Argentina

Cyanobacterial blooms in Paso de las Piedras reservoir (Buenos Aires, Argentina)

Paso de las Piedras reservoir, located at south Buenos Aires Province, Argentina, is used for drinking water supply to the cities of Bahía Blanca and Punta Alta, with a population of about 400,000 inhabitants. During April and May, a very noticeable odour was detected associated with a dense algal bloom. The odour, similar to that of an organochloride insecticide, was perceived both in lake-side areas and in the distribution system. Simultaneously, the presence of high contents of particulate material was reported in tap water.

These phenomena provoked the reaction of the users who urged the community authorities and the water supply company for immediate solutions to improve the service. As a result of the demands several studies were undertaken in raw water, in the treatment plants and in the distribution lines. In every case qualitative and quantitative analyses of phytoplankton, physicochemical analyses of water and acute toxicity mouse bioassays were carried out.

Microscopical analyses revealed that two species of Cyanobacteria: *Anabaena circinalis* and *Microcystis aeruginosa* were numerically dominant. The abundance of *Anabaena circinalis* ranged from 48320 to 84032 cells/ml in raw water and from 276 to 2472 cells/ml in tap water.

Acute toxicity bioassays were conducted using water samples taken from tap water, lyophilized and resuspended in physiological solution. Animals used were male mice distributed in two lots: a blank lot, intraperitoneally injected with physiological solution and a sample lot injected with the lyophilized suspension. The result was negative because after the recommended observation period none of the animals injected died and the typical symptoms caused by cyanobacterial hepatotoxins or neurotoxins were not observed. Likewise, no modification of eating behavior or sleep was detected during the experience and the evolution of body weight was similar in both lots.

The strong odour was due to the presence of Geosmin, a metabolite released by *Anabaena circinalis*.Complementary studies revealed the presence of other relevant compounds such as Trihalomethane (x: 70.5 µg/l), Copaene (tricyclo 4.40.02,7 dec-3-ene,1,7-dimethyl-8-(1-methylethyl)-steroisom) (CAS n°: 3856-25-5), by Gas-Chromatography-Mass Spectrometry (GC/MS), Bromocyclohexane (x: 57 µg/l. Very low concentrations of Xylene (x: 110 ppb), Ethyl benzene (x: 78 ppb) and Toluene (x: 157 ppb) were also detected. The results suggest that Copaene is a precursor of Geosmin and thus it would be very important for the early detection of the odorous compound.

The incident that took place at Paso de las Piedras reservoir in relation to potentially toxic cyanobacteria revealed to the private company and to community authorities involved in water supply service and control, respectively, the need to undertake preventive studies aimed to preserve the quality of water used for human consumption.

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• Argentina

Observations on *Prorocentrum lima* from North- Patagonian coastal waters (Argentina) associated with a human diarrhoeic disease episode

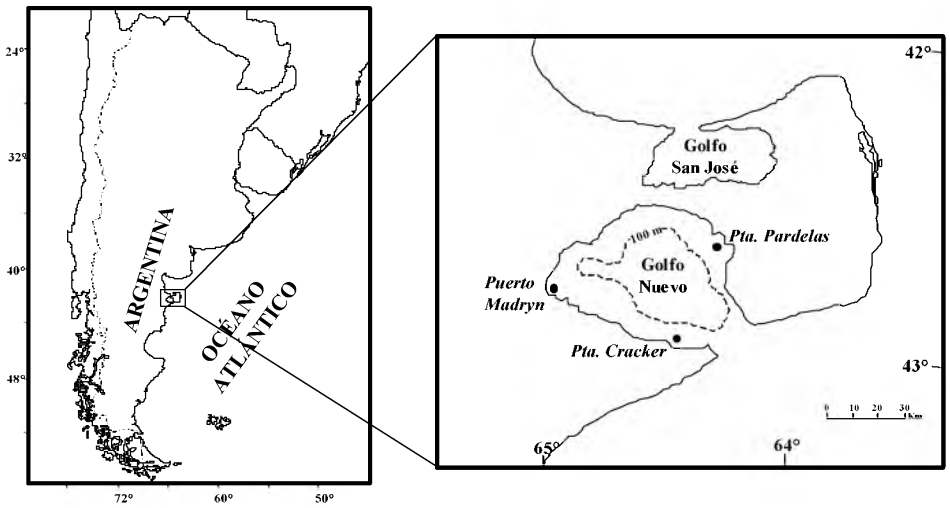

Figure 1. Map indicating the location of the North-Patagonian gulfs.

We are carried out a research project on the dynamics of harmful phytoplankton blooms in the North- Patagonian gulfs (Argentina). Recurrent algae blooms of the toxic dinoflagellate *Alexandrium tamarense* have been registered annually since 1980 in the Golfo Nuevo and Golfo San José (Fig. 1) causing human illness and economic losses. The capture and industrialization of shellfish in the region generates 1.0-2.5 million dollars per year provides employment for 150-250 people. The objectives of the project are to determine, through frequent sampling, the population dynamics of *Alexandrium tamarense* and other species known to cause harmful blooms; to study related environmental factors; to search for cysts of dinoflagellates in the bottom sediments; to contribute to the formation of graduate students in taxonomy and ecology of harmful phytoplankton, and to the awareness of human health risks of harmful algal blooms. A major objective of the project is to develop a conceptual model to relate key factors with the onset, magnitude, duration and localization of harmful algal blooms in the North-Patagonian gulfs.



Figure 2. Microphotograph of Procentrum lima cell in a water sample.

As part of the project, water and shellfish samples are taken every 2 weeks at 3 stations in Golfo Nuevo, Chubut, Argentina (Fig. 1). The stomach contents of at least 5 specimens of the mollusc *Aulacomya atra* ("cholga" or ribbed mussel) are examined under the microscope to identify and estimate the abundance of microalgae, especially noxious one, in the shellfish. *Aulacomya atra* usually feeds on planktonic and benthic microalgae. Last March, approximately 40 persons who ate blue mussels and cholgas at a celebration developed diarrhoeic shellfish poisoning; with diarrhea, nausea and vomiting (the patients were attended at the public hospital in Puerto Madryn). This episode coincided with the presence of the epibenthic *Prorocentrum lima* Ehrenberg (Dodge) (Fig. 2) in the stomach content of *Aulacomya atra* and *Mytilus edulis platensis* and in water samples from Golfo Nuevo and Golfo San José (Chubut Province, Argentina). This species is known from the area but not previously associated with human illness [1]. Samples of *Aulacomya atra* and *Mytilus edulis platensis* were run on a fluorometric phosphatase assay with positive results, indicating the presence of diarrhoeic toxins in both species (Steve Morton and Lucie Maranda, personal communication)

This brief note aims to alert people to the occurrence of diarrhoeic shellfish poison associated with *Prorocentrum lima* during early autumn in the North Patagonia region. PSP problems associated with *A. tamarense* are usually over by March, and the area is frequently open at that time of the year for shellfish harvesting.

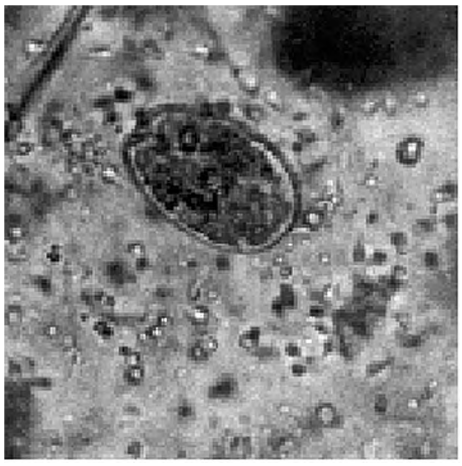

Figure 3. Aulacomya atra stomach content showing a cell of Procentrum lima.

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• Egypt

Toxic red tide species are on rise in Alexandria waters (Egypt)

A monitoring program was carried out in the Eastern Harbour of Alexandria (a relatively, shallow, semi-enclosed basin located in the central part of the city, sheltered from the open Mediterranean Sea by a breakwater of two inlets, area about 2.53 km^2, average depth 5 m, and a water volume of about 15.2x10^6 m^3, receiving drainage water of 35.2 x10^6 m^3, annually), during the three years 1998 – 2000.

Samples were collected to test the adequacy of short time-scale sampling to fully describe phytoplankton variability and ambient environmental conditions in a dynamic and eutrophic marine basin. The results indicate that red tide blooms (of 24 species, monospecific or in combination) gradually intensified in the neritic waters of Alexandria, and their intensity and geographical extent seem to be on rise; the phenomenon became of regular occurrence in the warm seasons.

At present, the toxic phytoplankton, *Alexandrium catenella*, *Alexandrium ostenfeldii*, *Chattonella antiqua*, and *Gymnodinium mikimotoi*, which are not indigenous in the Eastern Harbour, can be found, representing newly recorded species in Alexandria waters. *C. antiqua* and *G. mikimotoi* appeared suddenly in the harbour during the three year survey, sharing visible phytoplankton bloom periods with different causative species. Inquiries to fishermen and other people from the coastal areas around the harbour, and observation, have revealed that water discoloration was first observed west of Alexandria in Mex Bay (12 Km away from the harbour, a highly eutrophic bay, receiving a daily water input of about 6.6x10^6 m^3, mixed with agro-chemical, industrial and municipal waste waters), about two days earlier than their massive occurrence in the harbour. Thus, these blooms may have been populations that were transferred to the harbour from Mex Bay by local diffusion, since hydrographic processes must be responsible. Yet, the progressive eutrophication in Alexandria waters must share responsibility. Reduced salinity may be partly responsible for the development of *C. antiqua* and *G. mikimotoi*. The results are given in Table 1.

Table 1. Maximum occurrence of C. antiqua and G. mikimotoi in the Eastern Habour of Alexandria from 1998 to 2000.

Species	Peak Day	Cell. l^{-1} x10^0	Major Species
Chattonella antiqua	30 Sept. 98	0.54 (7.3%)	*Pyramimonas* sp. (5.35x10^6, 73.14%) *Cyclotella nana* (13.3%)
	27 July 99	0.85 (28%)	*Skeletonema costatum* (1.5x10^6, 49.5%) *C. nana* (12.8%)
	21 May 2000	1.14 (26.64%)	*S. costatum* (1.37x10^6, 32%)
	24 Sept.	0.65 (4.96%)	*S. costatum* (8.43x10^6, 64.24%)
	7 Oct.	0.31 (5.47%)	*S. costatum* (2.94x10^6, 52.41%)
Gymnodinium mikimotoi	14 Sept. 98	0.5 (12.7%)	*Leptocylindrus minimum* (1.5x10^6, 38.4%) *Cyclotella nana* (20.4%) *N. longissima* (12.7%) *S. costatum* (11.8%)
	15 Oct. 98	0.13 (9%)	*C. nana* (0.49x10^6, 34.8%)
	11 Aug. 99	0.93 (14.65%)	*N. longissima* (3.7x10^6, 68%)
	30 May	1.8 (29.6 %)	*T. nitzschioides* (3.87x10^6, 64.8%)
	9 Oct. 2000	0.42 (10.75%)	*L. minimum* (2.24x10^6, 57.5%)

During peak abundance (mixed mainly with diatoms) limited wild fish and faunal mortality was noted, as well as stunned or abnormally swimming fish, providing some evidence of toxicity.

The thermohaline stratification of the water column was a permanent character. *C. antiqua* occurred within a temperature range of 21-26 °C, most frequently between 24.5-26 °C, salinity range 34.5-38.5 psu, mostly 34.5-35 psu. These ranges were close to those for *G. mikimotoi* (22.5-27.2 °C, 34-36.5 psu). In all cases the surface water temperature was > 20°C. Nutrient concentrations were relatively low.

The density of *A. catenella* increased in 2000. The species appeared irregularly from late May to early October, with a peak (10.7x10^3 cell l^{-1}) on 23 May (21.5 °C, 35 psu, nitrate 0.8 mM, phosphate 2.3 mM). Also, this peak was recorded within a multi-species bloom of *S. costatum* (26%), *Thalassionema nitzschioides* (17%), *P. minimum* (14.3%), and *Chaetoceros curvesitus* (13%). *Alexandrium ostenfeldii* occurred less frequently than *A. catenella*, but was more abundance. The species in 1998 was seen from mid-September until mid-October, with 14.4x10^3 - 18.2x10^3 cell l^{-1} between 27-29 September (24.8-26 °C, 37.5-38 psu, weakly stratified water column and relatively high nutrients), accompanying a bloom of *Pyramimonas* spp. (2.73x10^6 cell l^{-1}, 83.8%) on the last day. The survey in 1999 revealed small numbers on 29 May (0.6x10^3 cell l^{-1}) and on 4 June (0.5x10^3 cell l^{-1}). The species on 11-16 September 2000 reached 14.3 x10^3-36.5 x10^3 cell l^{-1}, highest on 13 September (26.2 °C, 36.5 psu, Dt 1.2°C, Dpsu 2, nitrate and phosphate of 2.2 mM for each). This peak shared a water discoloration period of with *R. delicatula* (0.84x10^6 cell l^{-1}, 39.75 %), *Thalssiosira* sp. (27.6%) and *S. costatum* (12.8%).

These results suggest that an effective monitoring system should be established urgently, and that predictive techniques should be developed for toxic flagellates and other noxious species in the near-shore and open sea of Alexandria. To this end, it is necessary to examine the ecology of these organisms, particularly in their original sites and in the initial phase of population growth and the relation with the physical mechanisms of dispersion within these embayments. If the initial site of bloom formation of the red tide organisms can be determined, the monitoring of phytoplankton species at this site may allow prediction of red tide outbreaks.

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• Mexico

Unusual HABs in Manzanillo Bay, Colima, Mexico

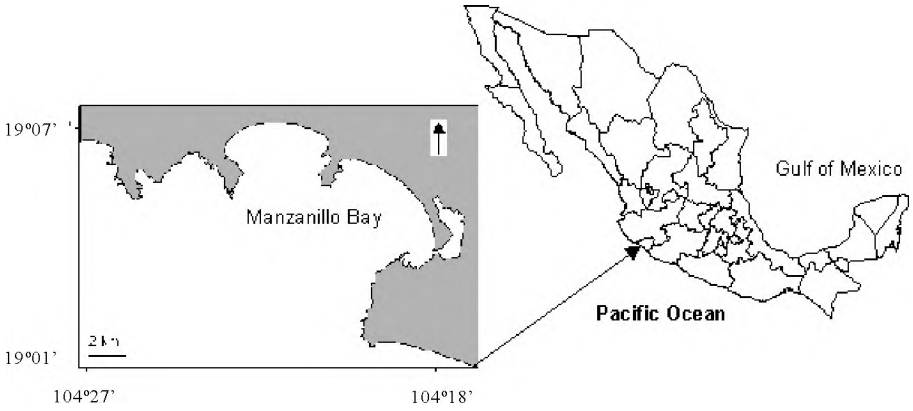

Figure 1. Geographic situation of the Manzanillo Bay, Colima, Mexico.

In Manzanillo Bay (19°01′-19°07′ N, 104°18′-104°26′ W, Fig. 1) red tide events are recorded annually at the end of winter and in spring, tides occur in patches and last one or two weeks, and reappear twice or three times in that season according to the oceanographic conditions. In 1999 we observed that some blooms increased in time and space, staying for more than two months and occupying the whole Manzanillo Bay [1]. From weekly monitorings (January to June, 2000), different events, in time and space, were recorded: five phytoplanktonic species and one ciliate were identified to be associated with changes in the color of the marine surface.

At the end of January two red tide patches were dominated by the dinoflagellate *Ceratium furca* and the ciliate *Mesodinium rubrum*, but during February, March and part of April the responsible was *Cochlodinium polykrikoides*, with exception of 11 and 12 March, when *Mesodinium rubrum* was the dominant; maximum cellular densities were 3258 cel/ml and 7003 cel/ml, respectively. At the end of April two patches were also observed, one due to the silicoflagellate *Dictyocha fibula* and the other due to the diatom *Thalassiothrix sp.*, whereas the last event was recorded in June, with the dinoflagellate *Scrippsiella trochoidea* as the dominant species, with densities of 6486 cel/ml.

Cochlodinium polykrikoides, reported from the first time in spring 1999 in the zone, and subsequently during winter and spring, 2000, develops reddish brown blooms, dense populations and extends widely, which resembles a monoculture. Chains of 8 cells (Fig. 2) and up to 16 cells, with an average of 4 cells, have been found, but no mortality of fishes has been reported [2].

The greatest intensity of these events, in terms of time and extension, occured when the surface temperature decreased from 25.5°C, at the end of February, and reached to 21.2 °C in the beginning of April; in this period, salinity increased from 34.56 to 34.68 psu, with variations of ±0.24.

In comparison with 1999, during 2000 the occurrence of red tides increased notably, and they are related with the lowest temperatures recorded in the area since 1995, condition probably due to the negative anomalies of temperature encountered in the North Pacific Ocean during the period of La Niña [3], as well as the development of internal waves in the area.

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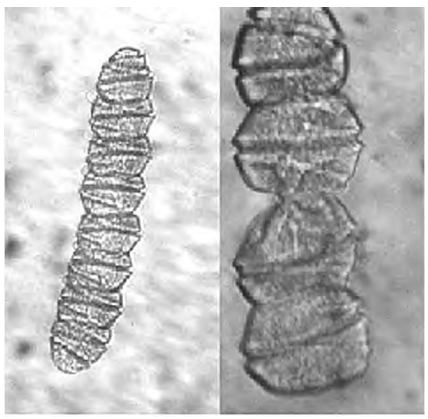
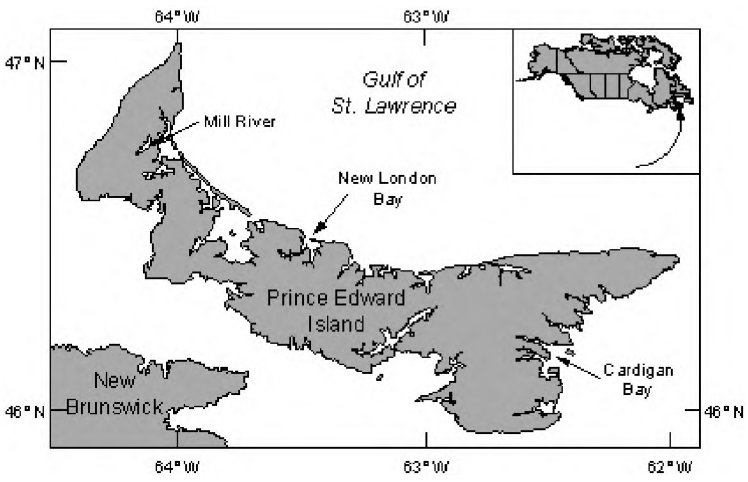


Figure 2. Cochlodinium polykrikoides. Light microscopy photograph, motile form

CORRIGENDUM

This figure replaces Fig. 1 of the article by Drs Stephen Bates and Don Richard, «Shellfish harvest area closure due to domoic acid - Mill River, Prince Edward Island» which appeared in HAN #21, pp 6-7.



• Ecuador

Red Tides - in Ecuador during La Niña 2000

Large - scale oceanic and atmospheric circulation patterns led to warmer than normal El Niño conditions in the tropical Pacific during 1997-98, followed by nearly normal and cold episode La Niña conditions during 1999-2000.

Consistent cold episode related oceanic features include: below normal sea surface temperatures (SSTs), with negative anomalies greater than - 1° C in the western and central Pacific, and below normal subsurface temperatures in the eastern equatorial Pacific.

Surprisingly few biological data have been published for La Niña years, although outstanding records of high primary production, higher zooplancton biomass and high fish catches were recorded during those years. Red tides have occurred in Ecuadorean waters almost every other year. *Mesodinium rubrum* is the most common causative organism of red tides, related to continual, but less intense upwelling. Blooms can persist for long periods of time, the autotrophic and motility of the organisms making the nutrients of the photo zone and below it available to them. On several occasions red tides of *Gyrodinium instriatum* have been reported in the Gulf of Guayaquil among other organisms (Jiménez, 1993). Large scale red tides were recorded during La Niña, 2000, associated with strong upwelling off the coast of Ecuador.

Along the coast of Ecuador and in the Gulf of Guayaquil, surface temperatures were between 26° and 28° C during February-March, 2000; but below the shallow thermocline at 20 meters depth were between 18° and 20° C. During this period, several large scale algal blooms were recorded along the coast from 4 to 20 March, 2000, and intensive blooms of *Mesodinium rubrum* occurred along the central coast of Ecuador.

Water samples showed a concentration of 3.500 cell/ml of *M. rubrum* with a minor concentration of *Gyrodinium instriatum* of 2.000 cell/ml. The diversity of dinoflagellates was high, mainly due to tropical species of *Ceratium* spp: *C. carriense, C. horridum, C. tripos var. semipulchelum, C. fusus, C. furca, C. trichoceros, C. macrocerus, C. porrectum, C. vultur, C. lunula* and *Protoperidinium pentagonum, P. oceanium, P. brochi, P. pellucidum, Prorocentrum gracilis, Pyrophacus steinii* and minor concentrations of diatoms such as *Coscinodiscus radiatus* and *Proboscia alata.*

On March 30, 2000, an extensive red tide from the northern part of the Gulf of Guayaquil to the southern part of Manabi province was again recorded. The bloom discolored the water and formed long scattered bands extending about 90 miles along the coast and 10 miles wide. The causative species was identified as *Gyrodinium instriatum* with concentrations higher than 10.000 cell/ml. The huge bloom of *G. instriatum* lasted about two weeks. At that time the concentration of *M. rubrum* decreased to 100 cell/ml and a high diversity of dinoflagellate, especially *Ceratium* spp., was again recorded. During the event we observed for the first time in oceanic or coastal waters of Ecuador, chains of *Gymnodinium catenatum*, a toxic dinoflagellate involved in paralytic shellfish poisoning (PSP), although in small concentration about 5- 10 cell/ml.

This organism is now isolated and maintained in culture. Preliminary findings by bioassay suggests that *G. catenatum* accounts for toxicity. These findings highlight the need for close monitoring of the coastal waters of Ecuador where important shrimp and fish culture facilities are located.

In mid-April blooms of *Ceratium trichocerus* and *Ceratium macrocerus* were recorded along with a high diversity of dinoflagellates. From May to July, 2000, SSTs were lower in the Gulf of Guayaquil and along the coast of Ecuador, from 18° to 19° C. In July a new red tide of *M. rubrum* was observed and low concentrations of *G. catenatum* was still present.

Until November, 2000, no further red tide were reported along the coast of Ecuador, although the cold conditions of La Niña persisted in the tropical Pacific and off the coast of Ecuador until the end of the year.

During these successive red tides, no mortalities of marine fauna was detected.

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IOC Manuals and Guides No. 41

Potentially Harmful Microalgae in the Western Indian Ocean
Microalgues Potentiellement Nuisibles d'Ouest de l'Ocean Indien

By Gert Hansen, Jean Turquet, Jean Pascal Quod, Loïc Ten-Hage, Charles Lugomela, Margereth Kyewalyanga, Mira Hurbungs, Peter Waiye, Bernard Ogongo, Shandrack Tunje & Henri Rakotoarinjanahary

This publication is a result of a cooperative research project, WIOHAB, which was implemented between 1998 and 2000 by the Kenya Marine and Fisheries Research Institute (Mombasa), Institute of Marine Science, (Zanzibar), Albion Fisheries Reseach Centre (Mauritius), National Centre for Oceanographic Research (Madagascar), Agence pour la Recherche et la Valorisation Marines(Reunion Island), and the IOC Science and Communication Centre on Harmful Algae at the University of Copenhagen (Denmark). Up to now, there have been no comprehensive publications on harmful algae in the Western Indian Ocean.

The project was implemented within the framework of the IOC Sub-commission for the Western Indian Ocean, IOCINCWIO, and was funded through the IOC by Sweden (SAREC) and Denmark (DANIDA).

The guide is bilingual, English-French. Available from October 2001.

Copies can be ordered at: p.boned@unesco.org; fax + 33 45685812; and: IOC of UNESCO, 1 rue Miollis, 75732 Paris cedex 15, Paris, France, attn. P. Boned.

Announcing a New Journal: Harmful Algae

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This new journal aims at providing a forum to promote knowledge of harmful microalgae, including cyanobacteria, as well as monitoring, management and control of these organisms. Both original research and review papers will be considered.

Papers dealing with the following aspects of harmful microalgae and cyanobacteria in marine and fresh waters will be considered:

- the distribution, life histories and taxonomy of harmful microalgae;
- the physiology and toxicology of harmful microalgae;
- harmful microalgal bloom ecology;
- trophic, socio-economic, public health and aquacultural impacts of harmful microalgal bloom events;
- occurrence, methods of detection and chemical structure of toxins in harmful microalgae, cyanobacteria, foodwebs and seafood;
- factors controlling toxin production, biosynthesis and chemical ecology.

Note: Papers dealing with pharmacology fall outside of the scope of the journal.

All papers will be subject to peer review. Authors will receive proofs. The editors, referees, and publisher will make every effort to expedite publication, the co-operation of authors in this task is welcomed.

Initially the journal is planned to appear quarterly, the first issue is expected to appear early 2002.

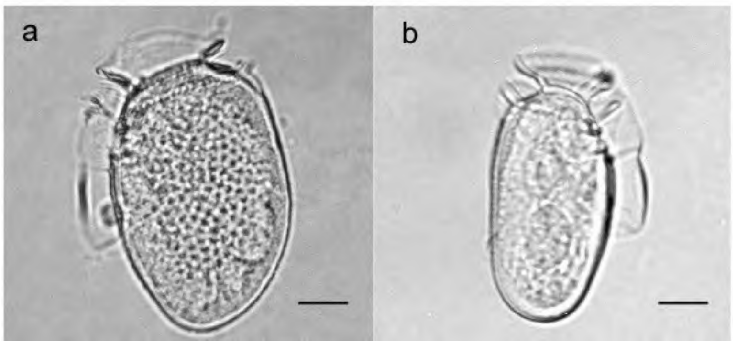
Authors of papers published in the first volume of Harmful Algae will receive a one-year subscription to the journal.

Visit the *Harmful Algae* website for full information on this new journal: www.elsevier.com/locate/hal

New image database of HAB species on the Web

A consortium of engineers and marine scientists have developed a software tool for automatically labelling HAB species (European contract MAS3-ct98-0188). Classification is based on a specimen´s contour as is the case of *Dinophysis* species. The team developed a software called "Dinoflagellate Categorisation by Artificial Neural Network" (DiCANN). DiCANN can be trained to recognise patterns of the problem dinoflagellates in a given locality provided it is trained with a minimum of 40 pictures of each species. It uses six analysis channels in this process, which are area, shape, local Fourier contrast, texture, texture density and internal junction structure. Performance has been shown to be proportional to morphological variance of the species. In previous work a version of the software was demonstrated to achieve 85% accuracy on 23 species of dinoflagellate (MEPS 139:281-287, 1996). Because DiCANN is trainable, it does not require the calibration normally required by image analysis software.

During the project over 1000 digital images were archived on the consortium´s distributed web database. The species are *Dinophysis acuta, D. acuminata, D. caudata, D. rotundata, D. tripos, D. fortii, D. sacculus* and the small forms of these species. These are now available to the scientific community at http://www.dist.unige.it/dicann.



Light microscopy observations of a) D. acuminata; b) D. sacculus from Galician Rías. Scale bars = 10 μm

The database is searchable, all images are in bmp format and can be downloaded.

The consortium also have available a database in excess of 5000 images of over 23 species of dinoflagellates from the Eastern North Atlantic Ocean (see http://newlyn.cis.plym.ac.uk/dicann. for details. These images are in jpeg format.

The consortium´s publications are also available at this web site, including: Dinoflagellate categorisation by artificial neural network (DiCANN) in: Hallegraeff, Blackburn, Bolch and Lewis Harmful Algal Blooms, IOC of UNESCO (in press), Proceedings of the 9th Int. Conf. on HABs Tasmania, February 2000.

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• Mexico

First North-American and Caribbean Training Course on Marine Toxins and Harmful Algal Blooms

After years of planning and expectancy raised during the last five years, the *IOC-CIBNOR First North-American and Caribbean Training Course on Marine Toxins and Harmful Algal Blooms* was held at the Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR), Mexico from 24 November to 10 December, 2000. The course was jointly sponsored by the Intergovernmental Oceanographic Commission (IOC) of UNESCO, CIBNOR, Mexico, as well as by other institutions and private companies.

The course was organized by Arturo P. Sierra-Beltran (CIBNOR, Mexico) and Roberto Cortés Altamirano (National Autonomous University of Mexico, Mexico), with the support of the IOC-IEO Science and Communication Centre on Harmful Algae, and hosted by the Postgraduate Studies Programme, CIBNOR (Mexico).

Yasuwo Fukuyo (University of Tokyo, Japan), Isabel Bravo (Spanish Institute of Oceanography, Vigo, Spain), José M. Franco (IIM-CSIC, Vigo, Spain Sergio Licea Duran (National Autonomous University of Mexico, Mexico), Casimiro Ramirez Camarena (National Fisheries Institute, Mexico), Berta Olivia Arredondo Vega, Ma. Lourdes Morquecho, Daniel Bernardo Lluch Cota, Salvador Emilio Lluch Cota, and Jose Bustillos Guzmán (CIBNOR, México) were invited to give lectures. The 8 participantscame from 6 countries: CHILE, COLOMBIA, COSTA RICA, GUATEMALA, TRINIDAD & TOBAGO and MEXICO.

The objective of the course was to disseminate basic knowledge on toxic phytoplankton, including the taxonomy and distribution of harmful algae, the determination of phycotoxins, and the development of monitoring programs. It was a theoretical and practical training course.

Lectures and seminars on the general aspects of the HAB problem initiated the activities (two working days), followed by specific subjects: climatology and oceanography, taxonomy and biogeography, toxin action and determination, remote sensors, public health, resource protection and monitoring programs among others (12 working days).

Participants were trained on practical taxonomy of phytoplankton including diatoms and cyanobacteria by the use of light microscopy. Dinoflagellate and cyst collection, isolation, concentration, cleaning, culturing and cloning, and cyst germination were subjects also covered with theoretical and practical lectures, as well. Accordingly with the variety of toxins present in the area, different methods for detection of toxins were carried out by the participants or demonstrated: mouse bioassays, ELISA tests and HPLC. Pigments analysis by HPLC and fatty acids determination by MS-GC were also performed by the participants.

The activities were directed to scientists of medium and high level that have working knowledge of the harmful marine algae and/or toxins, and are presently or in the near future will be involved on monitoring programs for research or protection purposes. The opportunity to CIBNOR of serving as a mean to pass the experience of a numerous group of experts towards the Caribbean and Central American countries, including as well some in South America, is more important than ever, if we look for a well integrated global economy and social development.

The gathering of experts to discuss special issues on the topic of HAB was stressed for establishing the similarities and differences between distinct geographical regions. The discussions held during the course will help to improve the skills of the participants (including the lecturers) in taxonomy, biogeography, toxin chemistry and toxicology, and will enable them to implement (or improve) reliable research and monitoring programs related to harmful algal events at the coastlines of their respective countries.

A series of books, manuals and materials were provided as teaching aids . Among them, highlights the effort of Dr. Y. Fukuyo who negotiated the fast publication by ANESC, Tokyo University, of the Technical Guide for Modern Dinoflagellate Cyst Study (Guia Tecnica para el Estudio de Quistes de Dinoflagelados Actuales) and also to Dr. Beatriz Reguera from the Oceanographic Center in Vigo (COI-IEO), who, in a very tight time schedule, translated the Spanish version on time to be distributed during the course. Each participant was provided with a take-home training course guide, summarizing the state of the art (according to the lecturers) of the harmful microalgae taxonomy and identification, toxin chemistry, ecophysiology and culturing. This training course guide is the core work for the elaboration of an improved Spanish version of the IOC Manual on Harmful Algae (currently under editing process), which we hope will see the light during 2001.

Each participant received a copy of interactive CDs to be used as an aid on the study of Harmful Marine Algae: 1) Atlas of Dinoflagellates v.1.5 & HAB Related Photograph Sets v.1.0, 2) Diatoms from the South China Sea 3) Colección de Información Ambiental para el Pacífico Mexicano and 4-7) Application of Satellite Remote Sensing on the Detection of Algal Blooms.

There were more than two weeks of very long working sessions but it worth it. We end up with a bunch of friends along the Americas and also new partners on the ANCA Project. At the moment, we will wait for feedback to evaluate the possibilities of continuity of training activities alike on the IOCARIBE region.

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• Tunisia

On the presence of potentially toxic algae in the lagoons of Tunisia

Since 1995, a national monitoring programme has been in operation in the areas of bivalve mollusc production in Tunisia (Gulf of Gabès, Boughrara Sea, Lake Tunis and Lake Bizerte), with the objective of microbiological control of live bivalve molluscs and the detection of potentially toxic microalgae in order to evaluate the toxicity.

The Tunisian lagoons (Fig. 1) have been subject to several research studies since the dystrophy crises in the autumn period. Guelorget (1992) reports the presence of the toxic species *Gymnodinium nagasakiense* in the Boughrara Sea with a concentration of $8x10^5$ cells/L during severe moralities in aquaculture operations in Southern Tunisia (AST). Romdhane et al (1998) explains the mortalities of fish in the lagoons of Boughrara and Ghar El Melh as due to toxicity of blooms of *Gymnodinium aureolum* and *Alexandrium minutum* respectively. The maximum concentrations reached $5.3x10^5$ (August 1991) and $2x10^5$ cells/L (June 1996), respectively. Furthermore, according to Arzul (1998), the haemolytic activity of *Gymnodinium cf nagasakiense* (sample from Gulf of Gabès) diminishes if anoxia or oxygen super-saturation occurs.

During the period from September 1996 to September 1999, we did not record mortalities of fish in Lake Bizerte or in the lake north of Tunis although increased concentrations of dinoflagellates were found. There were blooms of *Gymnodinium nagasakiense* in December 1996 ($1.27x10^6$ cell/L) and *Protoperidinium quinquecorne* in August 1999 ($14.5x10^6$ cells/L) which caused restricted areas of red water. In Lake Tunis (northern part) and in Tunis Channel, blooms recorded during the autumn were due to *Gyrodinium impudicum* with concentrations from $1.02x10^5$ to $1.73x10^6$ cells/L.

With respect to other potentially toxic microalgae, concentrations of *Dinophysis* varied from 0 to 1280 cells/L and rarely exceeded concentrations above 500 cells/L in Lake Tunis (northern part). The dominant species *D. sacculus* was accompanied by *D. caudata*, *D. acuminata* and *D. fortii*. These occurred primarily during October and November, and secondarily during May and June. In Tunis Channel, we found the same species and the lowest *Dinophysis* concentrations. Furthermore, we noted the presence of *Pseudonitzschia spp* in June 1998, at densities at $4.6x10^6$ cells/L.

In Bizerte Lagoon, concentrations of *Dinophysis spp* reached values in the order of $2.6x10^4$ cells/L in May 1999. The dominant species were *D. sacculus* and *D. caudata*, which appeared in December. We also detected the presence of *Ostreopsis siamensis* in Lake Bizerte and Tunis Channel at concentrations of 250 and 100 cells/L respectively.

In addition to the phytoplankton analyses, we carried out sampling of phanerogames (*Cymodocea nodosa*) and found *Prorocentrum lima* at a density at 3362 cells/100 grams of wet weight in a sample from Lake Tunis (northern part). Lawrence et al (1998) have shown that *P. lima* is an epiphyte on macroalgae like *Pilayella littoralis* and is often responsible for the production of DSP toxins and toxicity of mussels in Nova Scotia (Canada). In the Ghar El Melh lagoon, increased levels of *Prorocentrum lima* was recorded (densities above $1.5x10^4$ cells/L, December 1996). Turki and El Abed (1999) have also reported the sporadic presence of *P. lima* in Lake Bizerte and in Tunis Channel. The *Prorocentrum* species found in Tunisian coastal waters are: *P. micans*, *P. gracilis*, *P. triestinum P. minimum P. concavum*, *P. mexicanum*, *P. compressum* and *P. belizeanum*. The first three species, and in particular *P. triestinum* are very abundant in Lake Tunis and Lake Bizerte.

The coastal lagoons and lakes represent in general a zone of high productivity. It can be concluded that these fragile ecosystems are subject to anthropogenic disturbance. In fact, the presence of algal blooms and the dominance of dinoflagellates as compared to diatoms characterises a degradation of the biological quality of the water. Furthermore, the periodic dystrophy events in the different lagoons are quite surely linked to the phenomena of anoxia, and causes high turbidity of the water due to large amounts of organic matter.



Map of Tunisia, lagoons location.

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In Memoriam: Estela de Sousa e Silva (1921-2000)

Dr. Estela de Sousa e Silva was born on the 21 August 1921 in Lisbon where she died on the 6 September 2000.

E. Sousa Silva obtained her degree in Biology in 1943 at the Faculty of Sciences of the University of Lisbon. After a three years period of teaching she became interested in phytoplankton and developed several research studies as an investigator at "Junta de Investigação do Ultramar" and later at "Instituto de Biologia Marinha" where she stayed until 1971. She developed her scientific career at these Institutes, and also spent periods abroad with famous masters such as Trygve Braarud.

She made detailed studies on the marine microplankton, dinoflagellates, diatoms, and also Tintinoidea present in Portuguese coastal waters, and also in Angola, Moçambique, and Dakar, this last region through the interest of the "Institut Français d'Afrique Noire".

As a consequence of the occurrence of severe poisoning episodes with paralytic symptoms after consumption of shellfish from Obidos Lagoon (80km north Lisbon) in 1946 and 1955, she was asked to study phytoplankton communities in this lagoon. She identified several dense populations of dinoflagellates associated with biotoxicity. By the 50's, the microplankton in Obidos lagoon had become her main research interest. She implemented unialgal culturing for the dinoflagellates isolated from Portuguese marine waters, as a means of species identification and cytological and toxicological research.

In view of the health implications related to toxic dinoflagellate blooms, Dr. Estela de Sousa e Silva continued her career at the "Instituto Nacional de Saúde" and directed the Laboratory of Experimental Microbiology (LME), where she remained from 1971 until her retirement in 1991

E. Sousa Silva made several contributions to the science of harmful algal, promoting cytological and ultrastructural studies aimed at understanding the origin of dinoflagellate toxicity. She was also involved in the implementation of the shellfish toxicity monitoring programme along the Portuguese coasts. She found intracellular bacteria in some dinoflagellates, and initiateded several research lines related to toxicity based on the ultrastructural relationships between these micoorganisms and the role of bacteria in toxins production. Simultaneously she studied the growth, cytology and nuclear cycle of dinoflagellates and assessed the role of small cells observed in dense populations both in clonal cultures and in natural bloom population in their life history.

She published a considerable number of articles from 1948 to 1995 during her long and fruitful career. Her research on dinoflagellate biology and HAB is being continued and the algal culture she iniciated is maintained as the "LME E. Sousa Silva algal culture collection". It is being expanded with cyanobacterial clones isolated from Portuguese freshwater reservoirs.

A brief list of some of the more significant publications of Estela de Sousa e Silva follows:



Pinto, J.S. & Silva, E.S. 1956. The toxicity of *Cardium edule* L.and its possible relation to the dinoflagellate *Prorocentrum micans* Ehr. *Notas e Estudos do I.B.M.*, **12**: 1-20

Silva, E.S. 1962. Some observations on marine dinoflagellate cultures II. *Glenodinium foliaceum* Stein and *Gonyaulax diacantha* (Meunier) Schiller. *Botanica Marina* **2**: 75-100

Silva, E.S., 1963. Les "Red Waters" a la Lagune d'Obidos. Ses causes probables et ses rapports avec la toxicité des bivalves. In: Proceedings of the 4th International Seaweed Synposium (Pergamon Press), pp.265-275.

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Silva, E.S. & Faust, M.A., 1995. Small cells in the life history of dinoflagellates (Dynophyceae):a review. *Phycologia* **34**: 396-408.

Announcing the II International Conference on Harmful Algae Management and Mitigation

Qingdao, China 12-16 November, 2001

Harmful algae and their toxins can have serious impacts on health, prosperity and commerce. These impacts include contamination of seafood, damage to aquaculture and fisheries, direct human exposure (dermal, aerosol, ingestion), and aesthetic degradation. Furthermore, concern over the presence of algal toxins in seafood, or the proliferation of harmful algae, can lead to impediments to otherwise fruitful international trade.

The First International Conference on Harmful Algae Management and Mitigation was held in the Philippine Islands in May, 1999. Continuing the work begun there, this second conference will review the nature of harmful algae and their impacts, and then explore options for their management and mitigation.

The goal of the HAMM conferences is to develop practical strategies for minimizing the impacts of harmful algae and their toxins on human well-being and commerce, throughout and beyond the Asia-Pacific region.

For information please visit the HAMM conference web page at: http://vm.cfsan.fda.gov/~frf/sfhamm.html or contact:

Sherwood Hall, Ph.D., FDA, HFS-426, 200 C Street SW, Washington, DC 20204, phone: 1 202 205 4818, fax: 1 202 205 4881, Email: shall@cfsan.fda.gov and spindrift@erols.com

Future events

JUNE 2002

4 th INTERNATIONAL CONFERENCE ON MOLLUSCAN SHELLFISH SAFETY.

The web site of the 4th International Conference on Molluscan Shellfish Safety has been updated with the second announcement. Call for contributions and information on travel and accommodation, registration, and instructions for abstract preparation is included.

Web site: http://www.atlanticocongresos.com/moluscos/index.html

The topics of the conference will include:

- Microbiological contamination
- Chemical contamination and bioaccumulation
- Harmful and toxic algal events
- Shellfish contamination by marine biotoxins
- Depuration
- Management and mitigation
- Water quality at harvesting sites
- Public health, epidemiology and toxicology
- Monitoring, quality assurance programmes and regulatory controls
- Allergies associated with shellfish consumption

Help to divulge this information would be deeply appreciated.

Conference secretariat address:

ICMSS/Antonio Villalba, Centro de Investigacións Mariñas, Apdo. 13, 36620 Vilanova de Arousa, Pontevedra, Spain. Phone: 34 986500155, Fax: 34 986506788, Email: icmss@cimacoron.org

OCTOBER 2002

TENTH INTERNATIONAL CONFERENCE ON HARMFUL ALGAE, October 21-25 2002, St. Pete Beach, Florida, USA. Convener: Karen Steidinger. Further intomation at: http://www.state.fl.us/fwc/redtide/

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

A Programme of International Co-operative Research on HABs in Marine and Brackish Waters






GEOHAB Mission:

Foster international co-operative research on HABs in ecosystem types sharing common features, comparing the key species involved and the oceanographic processes that influence their population dynamics.

For further information and copies of the GEOHAB Science Plan visit the web at: http//ioc.unesco.org/hab,

or contact the IOC Science and Communication Centre on Harmful Algae, Copenhagen (see below)

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 23

• Mexico

Distribution of *Gymnodinium catenatum* Graham, in coastal waters of México

Gymnodinium catenatum Graham 1943 (Fig. 1) is a PSP toxin producer, originally described for the Gulf of California, México. Recent reports show a wider distribution of this species, with occurrences in Argentina, Australia, Italy, Japan, Portugal, and Spain. Along the Mexican Pacific coast, *G. catenatum* is distributed from Oaxaca northward to the central part of the Gulf of California (29°N) (Fig. 2). In the first report of this species in the central part of the Gulf of California, densities reached concentrations up to 1 million cells/L [1]. The first documented PSP outbreak due to *G. catenatum* occurred almost thirty years later (1979) in Bahía Mazatlán near its entrance to the Gulf of California. During that episode, three human deaths and an extensive fish kill occurred. PSP ranged from < 20 to 7640 µg/STX eq/100g in oysters, clams, and lobsters, and cell densities were as high of 6.6 x 10^6 cells/L [2]. Thereafter, important blooms have been reported for Bahía Mazatlán and Manzanillo, and Acapulco harbors [3, 4, 5, 6]. In the Acapulco blooms, PSP concentrations ranged from 120 to 250 µg/STX eq/100g in oysters, and *G. catenatum* abundance varied from 7.6 to 37.6 x 10^3 cells/L. However, no human fatalities have occurred. This fact may result from the relatively low toxin content of the local strains [7].

Since the 1980s, our group has carried out several phytoplankton studies in Bahía Concepción and Mazatlán; two important water bodies located near the middle and mouth of the Gulf of California,

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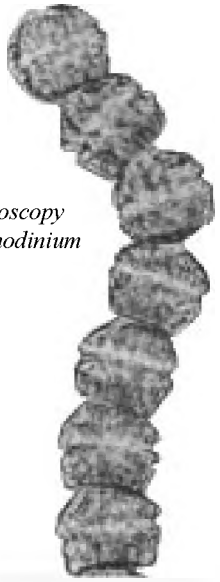

Figure 1. Light microscopy photograph of Gymnodinium catenatum

• China

Prorocentrum donghaiense - a high biomass bloom-forming species in the East China Sea

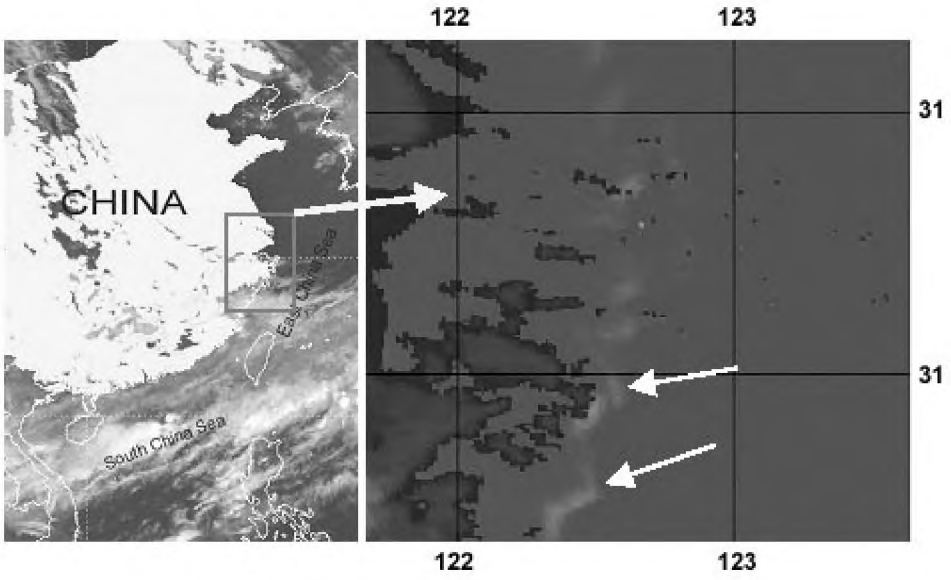

Fig. 1. Prorocentrum donghaiense bloom detected by FY satellite

Bloom occurrence

Since 1995, *Prorocentrum donghaiense* Lu 2001, a recurrent and high biomass bloom-forming species, has been observed in the plume front between coastal water of Zhejiang and Taiwan warm current [1]. It forms massive blooms in the convergence zone of the Yangtze (Changjiang) River Estuary and the coastal waters of Zhejiang province (Fig. 1). The bloom mainly appears in the surface layer of the water column but in some case the thickness of this kind of bloom can reach 10 meters. The coverage of discolouration (looks like diluted soy-sauce) can be several hundred, sometimes even a few thousand km^2. The bloom appears in April and May almost every year. The maximum recorded cell concentrations were up to 36 x 10^7 dm^{-3} and the corresponding value of chl a was

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The publication of Harmful Algae News is sponsored by the Botanical Institute, University of Copenhagen, and the Spanish Institute of Oceanography, Vigo.

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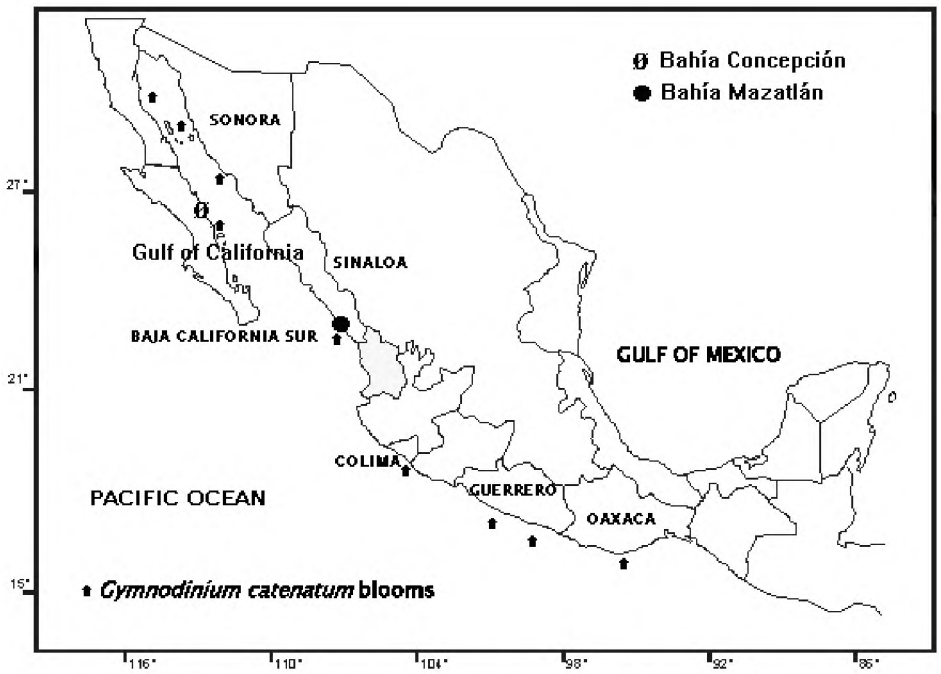

Figure 2. Distribution of G. catenatum blooms in Pacific coastal waters of México.

respectively. In the Gulf of California, several PSP-toxin producing species, such as *Alexandrium catenella, A. molinatum*, and, *A. minutum*, have been identified [3, 8, 9], but *G. catenatum* has been the most common bloom-forming species in the last four years. Therefore, special attention has been focussed on this species. Most *G. catenatum* blooms occur between February and May in the water temperature range 17 to 25 °C [3, 5, 8, 9]. Preliminary results of PSP toxin analyses from Bahía Concepción showed that neoSTX, dcSTX, dcGTX2, B1-2, and C1-2-3 were quantitatively the most important in phytoplankton and scallop flesh samples from areas with moderate and high abundances of *G. catenatum* [10]. Toxin profiles of a bloom of *G. catenatum* samples from Mazatlán were composed of C2, dcGTX2, and dcGTX3 toxins. As previously suggested, if saxitoxin analogue composition is a distinctive feature of different *G. catenatum* populations [7, 11], then our results suggest that the presence of neoSTX in plankton and scallop samples could be used as a distinctive characteristic of *G. catenatum* from Bahía Concepción. The characterization of toxin profiles of *G. catenatum* from different localities of the Mexican Pacific coast, as well as long term monitoring programs, are needed if prevention of harmful red tide effects are to be avoided in the future.

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IOC Taxonomic Reference List of Toxic Plankton Algae

Problems pertaining to toxic microalgae are being worked on and discussed by many categories of researchers: ecologists, taxonomists, toxicologists, by persons involved in monitoring the environments for harmful effect of toxic algae, by politicians, journalists, etc. It is a source of confusion that the scientific ('Latin') names of the algae sometimes change when the organisms become available for more detailed studies due to improved methodology or after establishment of the organisms in pure cultures, and such changes may give rise to misunderstandings.

At the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms, Vigo, Spain, 30 June-2 July 1997, it was decided to establish a Task Team on Algal Taxonomy with the aim of providing an agreed reference list of harmful algal species, including correct citation of the author(s), date of valid publication, and a list of synonyms.

The Committee comprised Ø. Moestrup (Copenhagen) as Chair, G.A. Codd (Dundee), M. Elbrächter (Sylt), M.A. Faust (Washington D.C.), S. Fraga (Vigo), Y. Fukuyo (Tokyo), G. Cronberg (Lund), Y. Halim (Alexandria), F.J.R. Taylor (Vancouver), and A. Zingone (Naples).

During the HAB 2000 Conference in Hobart, Tasmania, February 2000, the Committee met and discussed the contents and the format of the list. Following additional discussions over the e-mail, it was decided to prepare all entries in the list as follows:

(Cont'd on p. 5)

• Norway

A new bloom of *Chattonella* in Norwegian waters.

Since the first observations of *Chattonella* in 1991 outside the Netherlands [1], there have been several observations of the genus in northern Europe. The first large bloom was in 1998, covering an area from the west coast of Denmark, along the west coast of Sweden to the southern coast of Norway [2]. A second bloom occurred two year later in the southern North Sea [3]. In spring 2001, the raphidophytes *Chattonella marina* and *Heterosigma akashiwo* formed a massive bloom in the northeast part of Skagerrak. At its maximum, the bloom cover an area from the Kattegat, along the West Coast of Sweden and along the southern coastline of Norway up to Farsund. The first observations in Norwegian water were made in routine sampling in the Bay of Flødevigen at the beginning of March. The annual diatom spring bloom had started and was approaching maximum density of 2 x 10^6 cell l^{-1}, mainly dominated by *Chaetoceros* spp. During this period *Chattonella marina* was only observed in low numbers. As water masses with lower salinity from the east arrived in the bay, resulting in stronger stratification, the density of *Chattonella* started to increase from 16th March (Fig. 1). In the early phase of the bloom, large elongated cells was most common, reaching maximum concentration of 2 x 10^6 cell l^{-1} on 23rd March. This phase was partly overlapped by an increase in small cells of *Chattonella* and *Heterosigma akashiwo* that reached a maximum density of 9.5 x 10^6 cell l^{-1} at the end of March. On 26th March, the research vessel "G.M. Dannevig" set out to monitor and collect environmental data in the bloom area between Arendal and Langesund.

There was strong stratification in the whole area, with a pycnocline between 5-10 m depth. Above the pycnocline the salinity was 22-28 psu. The water temperature was 1 and 3°C down to 5 meter, and was rather uniform over the whole area. Above the pycnocline nitrate and phosphate concentrations were low at most stations. Silica, however, was relatively high throughout the water column, with a minimum around the pycnocline.

The cell counts showed that *Chattonella* and *Heterosigma* were mainly distributed above the pycnocline, and were absent at most stations below it. The maximum density of *Chattonella* was observed in the upper 2 meter. The picture that emerges from cell counts was verified by the chlorophyll *a* measurements, which showed high levels in the upper meters, 4 to 11 µg l^{-1}. The density of *Chattonella* was relatively high at most stations, where 10 out of 19 stations had > 5 x 10^6 cell l^{-1}, with a maximum concentration of 12.5 x 10^6 cell l^{-1}. Only one stations showed concentrations < 1x10^6 cell l^{-1}. *Heterosigma* was present at all stations, with a maximum density of 13.5 x 10^6 cell l^{-1}, but only four stations had concentrations ≥ 5 x 10^6 cell l^{-1}. In addition to the two dominating species, representatives of *Apedinella*, *Pseudopedinella*, and *Chrysochromulina* were present at all stations, along with several species of unidentified flagellates (<10 µm). Diatoms were observed above the pycnocline at a few stations, but were more common in deeper water masses. By the end of the cruise, an unidentified flagellate appeared and became rather common. This flagellate matches the description of the unidentified flagellate which appeared during the *Chattonella* bloom in 2000 [3].

Blooms of *Chattonella* have resulted in fish mortality in Japanese waters and other area in the past [4, 5, 6]. During the bloom in 1998, approximately 350 ton of salmon died in farms along the southern coast of Norway [2]. It has been estimated that this year's bloom killed approximately 1100 ton of Atlantic salmon. The precise mechanism behind the fish killing remains unclear, but studies indicate that interactions with the gills resulting in suffocation could be the cause [7]. It has been reported that *Chattonella* may produce a neurotoxin that could lead to fish mortality [8]. Investigations at the National Veterinary Institute and National Veterinary School, Norway, found no sign of toxins in the algae, fish or mussel meat during the bloom this year (C. Miles and T. Aune, personal communications).

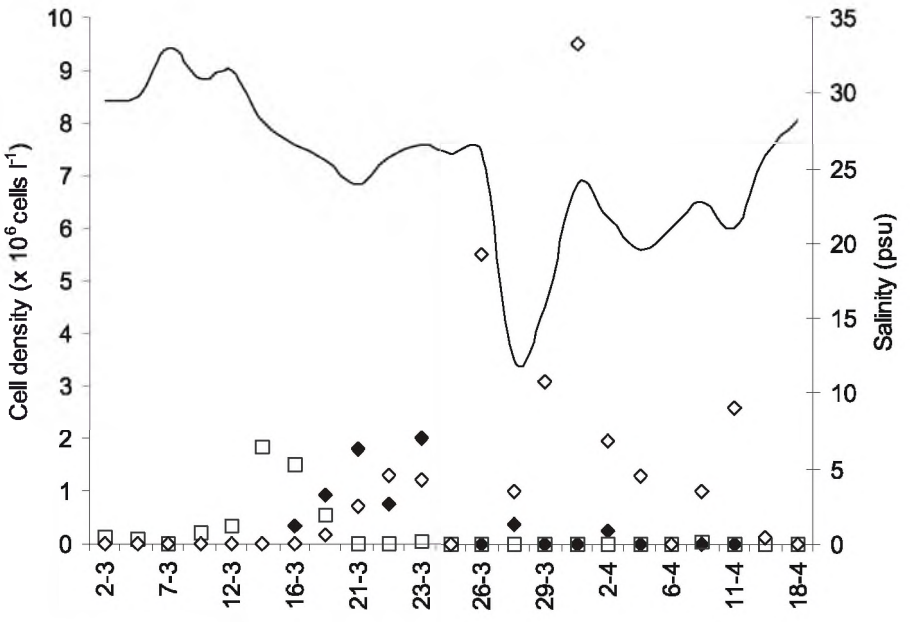

Figure 1. Routine sampling in the Bay of Flødevigen from the 2nd March to 19th April. Cell density (10^6 cells l^{-1}, left-hand axis) of diatoms (□), large Chattonella marina (♦), and small C. marina and Heterosigma akashiwo (◊). Line indicates the salinity (psu, right-hand axis) at 1 meter depth.

Acknowledgment

Thanks to the technical staff at Flødevigen Marine Research station, Lena Omli, Terje Jåvold, & Anita Reisvaag, and the crew at "G.M. Dannevig" for their contributions to this work. The Norwegian Research Council (Project No. 146052/120) and the Institute of Marine Research funded the investigation.

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more than 200 µg/ dm^3. During blooming, water temperature was 17-20°C and salinity range was about 20-28 PSU. The discolouration of the surface water caused by blooms of this species is so extensive that it has been observed by several different satellite sensors during cloud free weather in the last a few years.

Morphological Observation of the species

This species usually occurs as single cells but sometimes also as two or four cell chains (Fig. 2). Cells are asymmetric and elongated with slightly indented anterior end and rounded posterior end, 16-22 µm long, 9.5-14 µm wide and 7-9 µm thick. The periflagellar area on the right valve is v-shaped and has one flagellar pore and more than one smaller auxiliary pore surrounded by apical collars. The spines on the thecal plates are dense and knob-like. There are 3 spines per µm. Valve pores are round with a distinct wall. Trichocyst pores are distributed mainly around the margin of the valves (Fig. 3). The megacytic zones of old cells are broad with dense tiny knobs. This species is morphologically different from *Prorocentrum dentatum* and *Prorocentrum obtusidens* (see table 1) [2, 3, 4, 5, 6].

Environmental aspects

The bloom-forming tendency of *P. donghaiense* is very similar to that of *P. minimum,* which is one of the most important potentially toxic bloom-forming species in the Oslofjord, Kiel Fiord (Baltic Sea), and other coastal areas around the world, especially in estuaries. The blooms of both species, typically occur in the surface layer of the water column of stratified water masses, where there is a large supply of mineral nutrients above the pycnocline.

The species was recorded at the beginning of the 1990s but originally confused with other *Prorocentrum* species in the East China Sea. Massive blooms of *P. donghaiense* were not recorded until 1995. This may indicate some kind of environmental change has taken place here.

The dynamic processes of this typical high biomass bloom species need to be elucidated in the future. This is one of the major tasks of the new national research project (CEOHAB)– the Chinese Ecology and Oceanography on Harmful Algal Blooms programme. CEOHAB also much concerned with how to link and co-ordinate

Table 1. Diagnostic characteristics of relevant Prorocentrum species

Species	*P. donghaiense*	*P. dentatum* Stein	*P. obtusidens* Schiller
Type locality	Yangtze Estuary (ECS)	Atlantic Ocean	Mediterranean Sea
Cell shape			
	cell asymmetric and elongated, ovular posterior end	with pointed anterior extension at one side and tapered posterior end	anterior contours of the cell almost parallel, with a distinct blunt extension at one side
Cell size (Original description)	16-22 µm L 9.5-14 µm W 7-9 µm T	50-60 µm L	36 µm L 16-22 µm W
Index = L:W	1.5 - 1.7	2.12	1.6 - 2.3
Index (different authors)		1.9-2.5 (Dodge) 1.5 (Yoo & Lee) 2.52 and 2.9-3.2 (Horiguchi)	2.3 (Throndsen) 2.4 - 2.6 (Schiller)
Anterior spine visible in LM ?	not distinct, appears as apical collar	no	no
Valve pores **visible in LM ?**	yes, surrounded by wall, grouped no	yes yes	yes yes
Valve spines visible in LM ? **Shape (SEM)** **Spine density**	yes no short and knob like 3 spines/µm	yes yes pointed	?
Trichocyst pores	mainly near margin of the plates	mainly around margin	?
Structure of megacytic zone (old cell) visible in LM ?	5 rows of knobs/µm 8 knobs/µm on each row yes	yes	yes
Social behavior /harmful events	Single cell, sometimes two or four cell chain. bloom-forming in coastal water, discoloration of water (like diluted soy-sauce).	Single cell	sparse, solitary

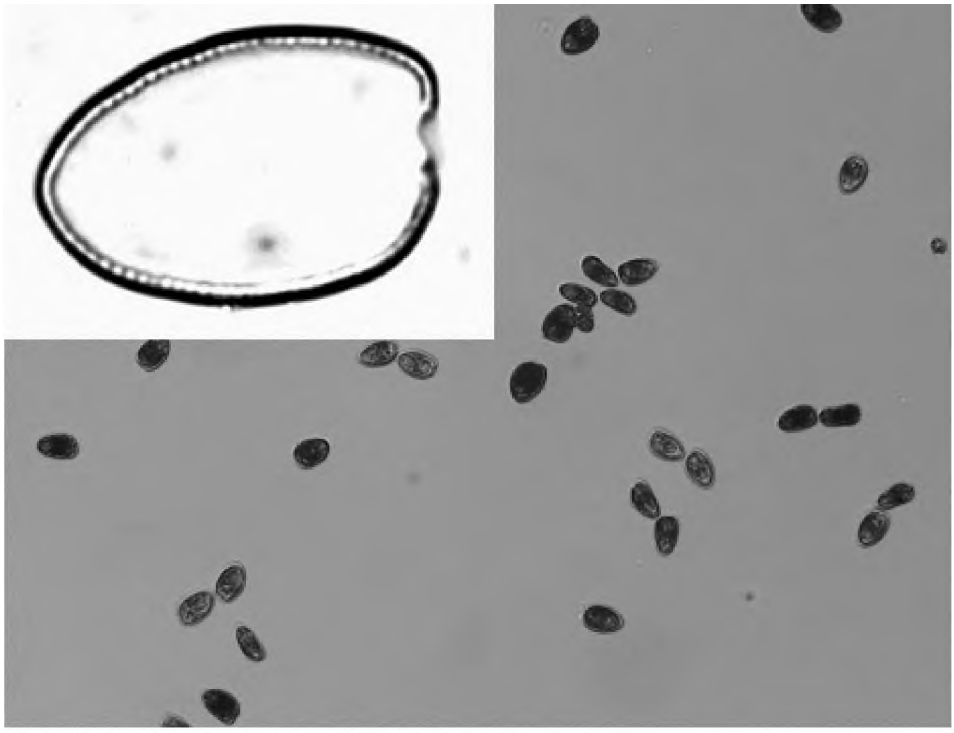

Fig.2. The Prorocentrum image under light microscope

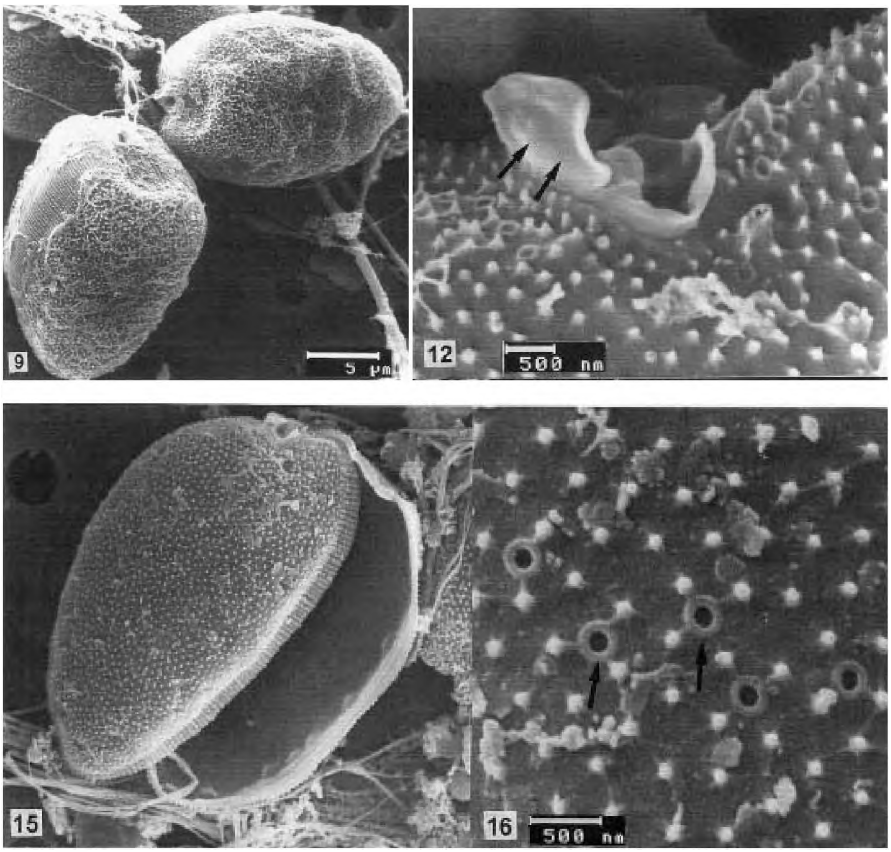

Fig.3. SEM image of Prorocentrum donghaiense

CEOHAB with GEOHAB in the near future and to obtain more support from the international HAB community.

Acknowledgements

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1. Correct name according to the International Botanical Code of Nomenclature, followed by author(s) and year it was given its present name
2. Basionym (the first name applied to the species, including year)
3. Reference to the article in which the species was first described
4. Reference to the article in which the species was given its present name
5. Type locality
6. Synonyms, including year
7. Main harmful effects
8. Up to (usually) 3 references with information on toxicity, toxins or toxic effects
9. Notes (if applicable)

The list of eukaryotic algae was completed in early 2002. The cyanobacteria (blue-green algae) will be added when the list of freshwater algae has been completed (mainly blue-green algae). In addition to a

(Cont'd on p. 7)

• Brazil

First report of *Microcystis* toxicity in Goiás (Central Brazil). Evidence of hepatic damage in mice.

Abstract

The presence of *Microcystis* genus in several water supplies of Goiânia city (State of Goiás, Central Brazil) was monitored. Extracts for mouse bioassay were prepared from both concentrated *Microcystis*, using a 20 mm mesh net and from colonies cultivated in ASM-1 liquid culture medium. Extracts from just one of the sampling stations studied were found to be toxic. Cultivated colonies showed very slight signs of toxicity in mice, but extract from concentrated ones induced 100% lethality in tested animals. Damage on liver tissue such as hepatocyte degeneration, coagulation areas and lymphocyte infiltration was also observed.

Introduction

Since a severe microcystin poisoning occurred in Caruaru (North Eastern Brazil) in 1996, when 69 persons died after an hemodialysis session, toxic *Microcystis* strains have been reported and studied in several Brazilian regions [1, 2, 3, 4]. In the State of Goiás, such studies about identification of toxic cyanobacteria had not been developed yet, even though most of surface water supplies for human consumption are lagoons, dams or similar reservoirs of still water, mainly in rural zones. Moreover, no monitoring neither educational programs exist in order to prevent such intoxication events.

The results presented here belong to a project that is being developed in the Catholic University of Goiás, aimed to the identification of toxic cyanobacteria in our State and to study environmental, seasonal and culture conditions of toxin production.

Methods

Sampling: Samples were collected monthly in four water supplies from Goiânia city intended for human consumption (Water Treatment Station and Agronomy Faculty Lagoon) or recreation (Buritis lake and Rosas lake, both located in urban parks). Sampling was conducted according to previously published recommendations [5, 6, 7].

Cyanobacteria were concentrated with a 20 mm mesh nylon net or isolated with Pasteur pipettes and cultivated in ASM-1 culture medium [8, 9] with a nitrogen/ phosphorus ratio of 4, under 12 hours light period and constant shaking.

Toxic extracts: Freshly obtained biomass was filtered through Whatman No.1 paper and then extracted with 0,5% ammonium acetate in methanol, concentrated in vacuum rotation evaporator to totally remove methanol and resuspended in 0,9% sodium chloride.

Mouse bioassay: Extracts equivalent to 1 g of cells per ml were injected intraperitoneally in male adult *Swiss* mice, three animals per sample. Volume injected represented 1% of body weight for each animal (1 ml/100 g). In order to observe chronic effects on liver, animals were injected daily during a whole week. Control mice were treated with 0,9% NaCl solution.

Toxic effects on liver: Livers from both control and treated animals were removed and weighed in analytical balance to calculate relative weight as the percent value to total body weight. The material was fixed in formaldehide/phosphate buffer. After paraffin inclusion, liver slices for microscopic observation were prepared using a microtome cutter Leica RM-2123-RT and stained with hematoxiline-eosine. Images were made using an optic microscope Olympus CX-40 coupled to a digital camera Olympus C-3000.

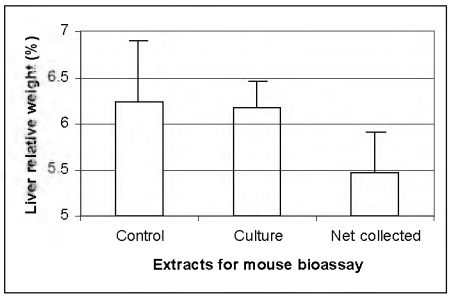

Figure 1: Effect of extracts from cultivated and net collected Microcystis on livers relative weight.

Results

Extracts from cultivated *Microcystis* strains isolated from all sampling stations showed no lethality in mice, although those from Buritis lake induced signs of neurological intoxication (dorsal hair standing on end, closed eyes and stretched out extremities) during the first 2 – 3 hours after the injection, and then quickly recovered.

Extracts prepared from *Microcystis* populations directly collected from water surface of Buritis lake were lethal in mice, as shown in tables 1 and 2.

As shown in figure 1, relative weight of treated animals liver was slightly lower as compared to those from control animals, though differences were statistically not significant. Moreover, no visible alterations were observed on liver surface, which was apparently undamaged.

Microscopic analysis of tissues stained with hematoxiline-eosine dye revealed hepatocyte degeneration, coagulation areas due to local haemorrhage and severe lymphocyte infiltration in the liver of mice treated with extracts prepared from net collected *Microcystis* (Fig. 2).

Discussion

Cultivated *Microcystis* from Buritis lake did not induced lethality in mice but just signs of slight intoxication, while colonies collected *in situ* were hepatotoxic in 100% of tested animals. This difference might be due to different nutrient concentration, expressed as nitrogen/

Table 1: Mouse bioassay response to Microcystis *extracts from different sampling stations.*

Sampling station	N/P ratio	Net concentrated colonies	Cultivated colonies
Water Treatment Station (running water)	19	No colonies found	---
Agronomy Faculty lagoon (still water)	6,5	Non toxic	Non toxic
Buritis lake (still water)	10,5	TOXIC (see table 2)	Slightly toxic
Rosas lake (still water)	14	Slightly toxic	Non toxic

Table 2: Lethality in mice of Microcystis *collected in Buritis lake.*

Time (h)	24	48	72	96	120
Mortality (%)	33,3	-	66,6	-	100

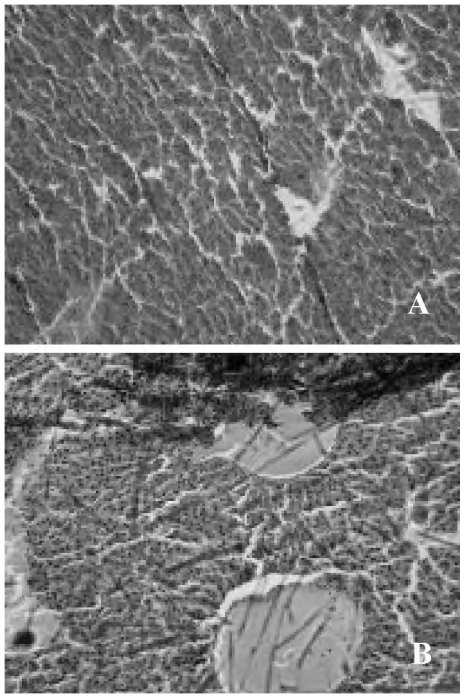

Figure 2: Effect of Microcystis *extract on mouse liver. A) Control. B) Treated with* Microcystis *extract from Buritis lake, showing lymphocyte infiltation (black arrow) and coagulation areas (white arrow).*

phosphorus ratio (N/P) in culture medium (N/P = 4) and in Buritis lake natural water (N/P = 10,5), and suggests the need of further studies on nutrient composition of culture medium, in order to increase toxin yield and to define optimum production conditions.

The fact that relative weight of treated mice liver was lower (though not significantly) then in control animals, was surprising, for most authors have described an increase of the liver weight in animals treated with hepatotoxins [9,10], even one day after the first injection. Hepatocyte degradation and haemorrhage have been probably responsible for our results. Hepatocyte injury and coagulation zones in liver tissue might be an evidence to the presence of microcystin in the tested extract. Lymphocyte infiltration reveals a severe infection, which is not necessarily due to microcystin, but it might be a consequence of using raw non sterile extract.

Buritis lake is not intended for human consumption but just for recreational purposes and toxic *Microcystis* colonies were present in concentrations not enough to form scums. Nevertheless, their presence is a fact that evidences the need of more complete environmental studies and monitoring programmes, in order to prevent future intoxication events. Such a surveillance would be extended to other regions of the State, where still surface waters are being used for human consumption.

Acknowledgements:

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printed edition, the list will be made generally available on the internet and updated at regular intervals. Suggestions for changes and additions to the list should be forwarded to the IOC Centre on Harmful Algae in Copenhagen Jacob Larsen: jacobl@bot.ku.dk with a copy (cc) to the Chairman of the Committee (moestrup@bot.ku.dk). The latter will evaluate the suggested changes, if needed after consultation with members of the Committee.

The Committee hopes to extend the list during the coming years, and to include additional information, including illustrations.

http://ioc.unesco.org/hab/data4taxlist.htm

New bibliographic database on HAB

The IOC/ASFA Bibliographic Database on HAB, HAB-BIB, contains more than 6000 references on potentially harmful algae, primarily from marine and brackish waters. It is based on an extract from the FAO-IOC-UNDOALOS-UNEP Aquatic Sciences and Fisheries Abstracts (ASFA) bibliographic database.

HAB-BIB covers the time period 1978 to 1999 (we are working on 99-02), as well as selected references from 1927-1971. The pre-1971 references and supplementary references 1971-99 have been entered electronically into the ASFA database by the IOC Science & Communication Centre on Harmful Algae at University of Copenhagen. Literature from the period 1971-1977 are at present not available on-line from ASFA, but only in the ASFA printed journals. In the near future all records will be electronically available through ASFA.

The search results may be exported either as a text file or by the use of RIS Auto Export Plug-In, directly into Reference Manager or Procite software (can be downloaded for free)

An attractive feature of HAB-BIB is that you may search by the ASFA geographical codes.

http://ioc.unesco.org/hab/HAB-BIB.htm

• Greece

Intense DSP outbreak associated with a *Dinophysis* bloom in Thermaikos Gulf (N.W. Aegean Sea, Greece)

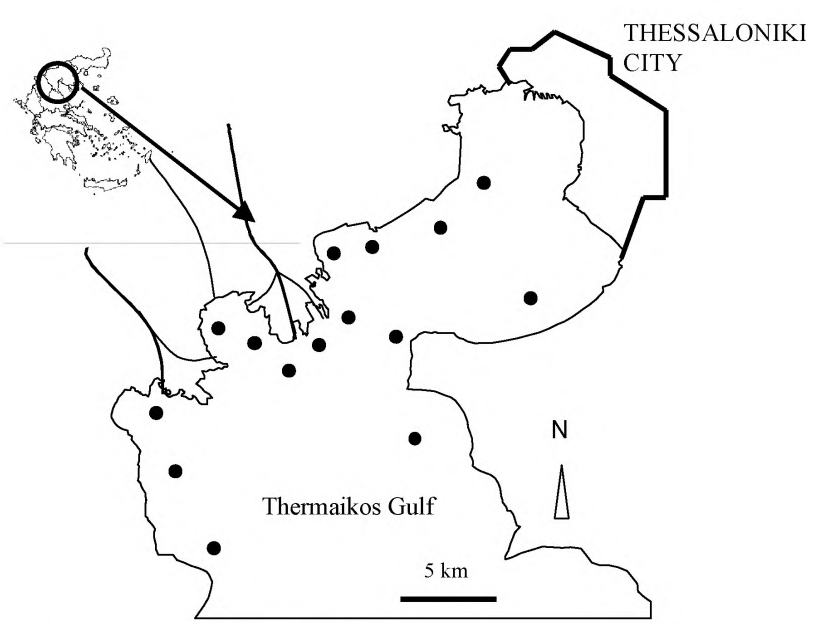

Figure 1. Map of Thermaikos Gulf, showing the sampling stations.

Several species of *Dinophysis* were observed in the past in low cell numbers in Thermaikos Gulf [1] (Fig. 1), which is the major shellfish cultivation area in Greece (95% of total production). During the harmful algal bloom monitoring programme in Thermaikos Gulf, a few *Dinophysis* (< 200 cells l^{-1}) were observed at the end of December 1999. At the beginning of samplings in 2000, high numbers, 3 x 10^4 cells l^{-1}, of *Dinophysis* (Fig. 2) were observed. The highest abundance reached, 5.4 x 10^4 cells l^{-1}, in the northeast part of the gulf in early February. The main part of the *Dinophysis* population was observed in the mid-depths (5 – 7 m) and occasionally near the bottom (13 – 16 m). The outbreak lasted until March 2000.

Among the *Dinophysis* cells, some individuals were morphologically similar to *D. acuminata* and others to *D. sacculus*. Since the identification between *D. sacculus* and *D. acuminata* is problematic [2], we cannot yet say whether these individuals belong to *D. acuminata* or *D. sacculus*.

Two size classes of *Dinophysis* were observed during the outbreak, large cells (44 –52 µm L; 32 – 38 µm W) and small cells (32 – 36 µm L, 22 – 28 µm W). In the initial stages of the outbreak, the *Dinophysis* population consisted of 50% small and 50 % large cells. During the outbreak, the decrease in the population was followed by the reduction in the percentage of small size cells.

Dinophysis was observed in water ranging in temperature from 9.5 to 11.5 °C, in salinity from 35.5 to 37.5 ‰, in PO_4-P from 6.5 to 390 µg l^{-1}, in SiO_2-Si from 12 to 140 µg l^{-1} and total inorganic nitrogen from 20 to 120 µg l^{-1}.

During this period the water column (maximum depth 16m) was homogeneous both in temperature and salinity. No relationships were found between numbers of *Dinophysis* and temperature, salinity and inorganic nutrients concentrations

The National Reference Laboratory for Biotoxins found DSP toxins in mussels with the mouse bioassay method, during the period of the *Dinophysis* outbreak. The closing of production zones (four months) caused damage to 40% of the total bivalve mollusc production of Thermaikos Gulf.

The same organism was also observed in the period March – May 2001 in Thermaikos Gulf. This outbreak was of shorter duration and lower abundance but also toxic.

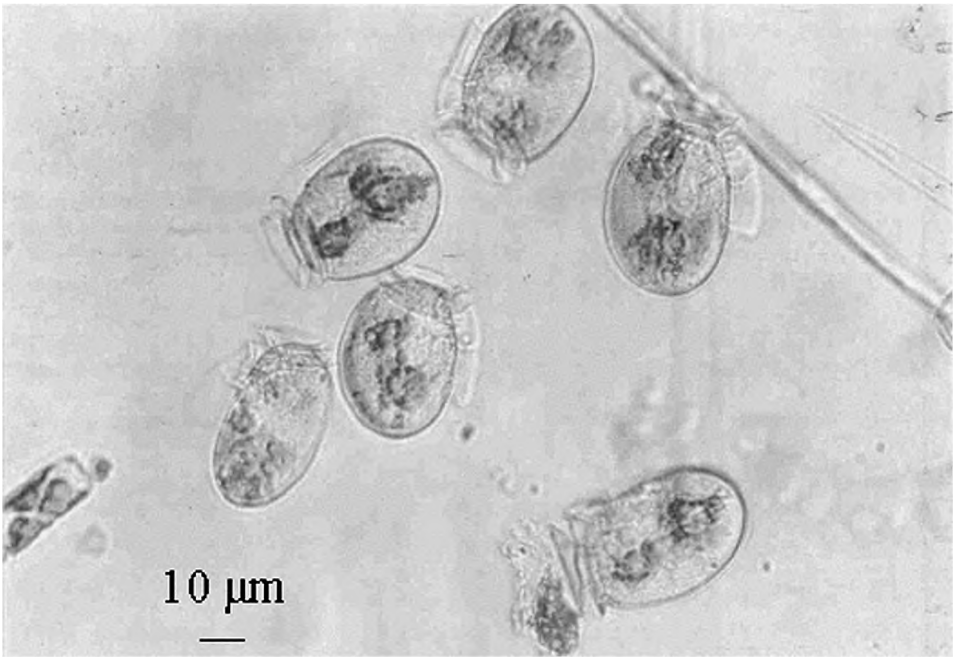

Fig. 2. Living Dinophysis cells in LM.

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• Chile

Application of remote sensing of ocean colour to forecast HAB events in Chile

A very intense event of HAB was detected at the beginning of the austral summer of 2002 in the south of Chile, due to the presence in high concentrations of the species *Alexandrium catenella* [1].

More than 30 people were intoxicated and one dead was registered in the X region of the country, in the last March.

There were strong economic impact in the area around the Archipelago of Chiloé, were the main population resource is the extraction of shellfish.

The region was declared as a catastrophic area by the president of the country in April.

This was the first event registered in this area, and the Northern limit of distribution of the species, which has expanded to north. Until this year the species was registered to affected the Southern regions (XI and XII) of this country.

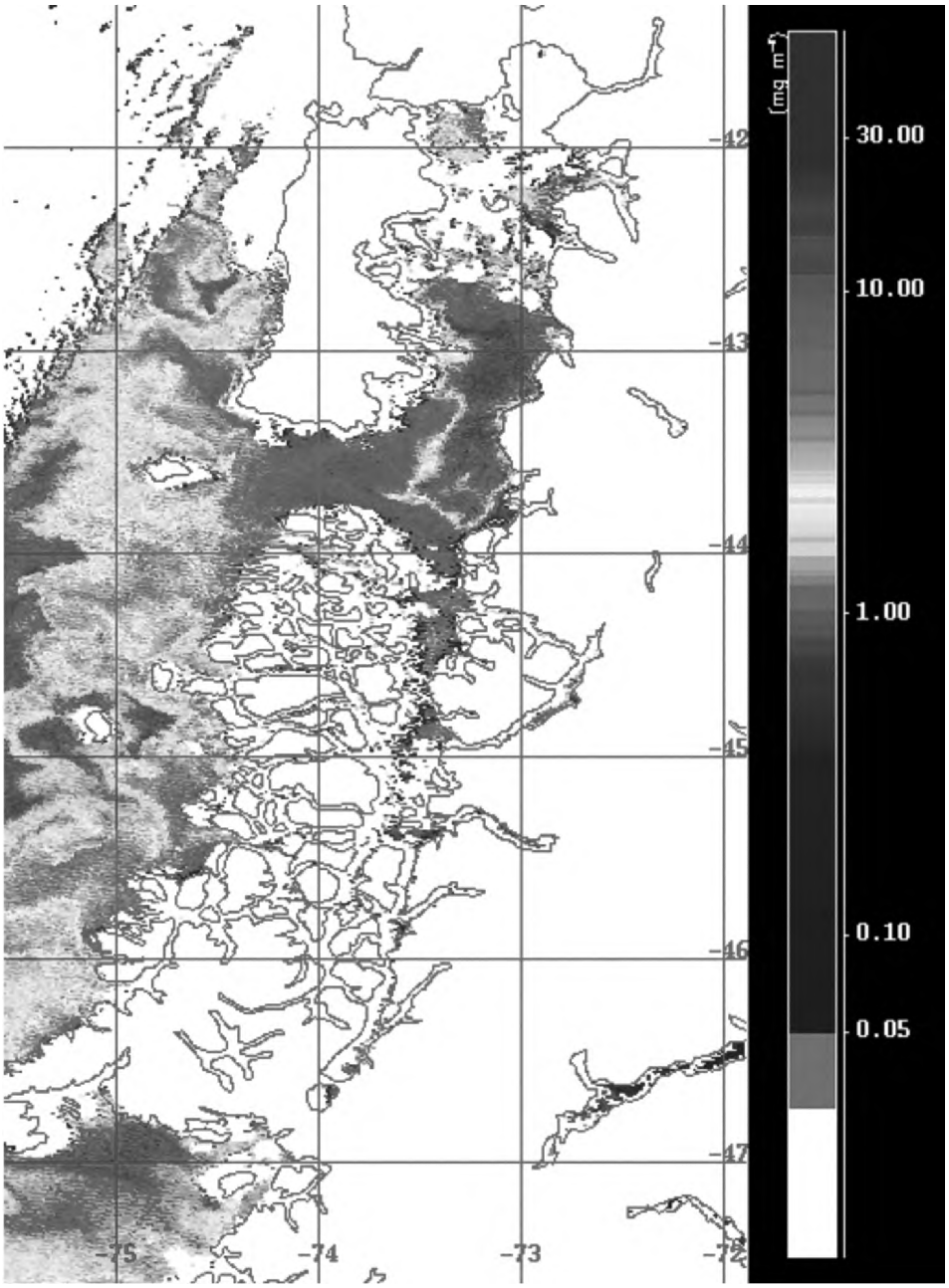
Remote sensing data has not been exploited for the moment for this application, in Chile. The method has been generally recognised as useful to identify high-risk blooms, and target in situ sampling [2].

During the beginning of this year new cooperations between Institutions and companies were established to promote remote sensing in Chile, as a contribution to monitor HAB.

This technology, supported by additional local knowledge and ancillary data was presented was presented by Dr. C. Rodriguez, for this application during the IV Spatial Conference of Latin America (Cartagena, Colombia, May 2002) to support this field of research. The proposal was accepted in the workgroup 3, in the session of prevention and mitigation of natural disasters.

The region affected by the high toxicity bloom is shown in the following image. This SeaWiFS data corresponds to 16th February 2002. The image was obtained and process at the Centre for Space Studies under agreement with Orbimage/NASA.

Image: Chlorophyll concentration ($mg \cdot m^{-3}$) around Chiloe Island. The high concentrations correspond to the algal bloom development during the last austral summer in this Chilean region. SeaWiFS Image from 16th February 2002.



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• Tyrrhenian Sea

Blooms of *Alexandrium taylori* in the Tyrrhenian Sea (Vulcano, Aeolian Islands)

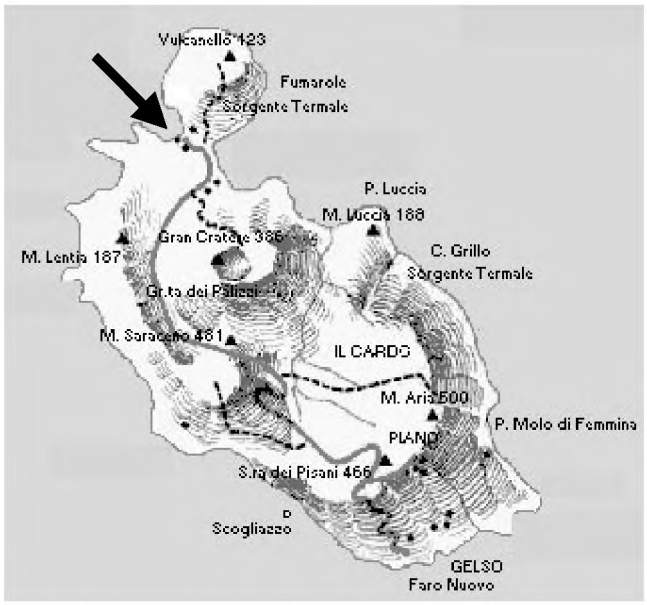

Fig. 1. Location of the West Bay of Vulcano (arrow), Tyrrhenian Sea, where the A. taylori *blooms take place in the summer.*

Since summer 1999 recurrent blooms of the dinoflagellate *Alexandrium taylori* Balech have been recorded off Vulcano Island [1]. *A. taylori* was first described in 1994 from the Atlantic, but in recent years (1997-2001) an increasing number of coastal areas in the Western Mediterranean Sea have been affected by blooms of this species [2]. During the summer periods 1999-2001, high-density blooms of *A. taylori* took place also off Vulcano Island (SW Mediterranean), where the beach of the West Bay (Fig. 1) is regularly affected by these events involving a greenish-yellow discolouration of the surface waters. The *A. taylori* densities and chlorophyll *a* concentrations ranged between 10^6-10^7 cells×L^{-1} and 0.2-120 µg×L^{-1}, respectively. In summer 2000, the population of *A. taylori* (Fig. 2) often represented the highest percentage of the total phytoplankton, constituting up to a 97% of the whole community. The remaining 3% of the phytoplankton community was composed of small diatoms. In summer 2001, phytoplankton blooms were entirely represented by *A. taylori* (100%), forming large patches mainly nearshore, in shallow waters of the West Bay (0.5-5 m depth). Water temperatures ranged from 28 to 30°C.

The location and extension of the yellowish patches were wind-driven, being confined to the beach under SE winds. Unlike other observations from the northern part of the Mediterranean basin [3], nutrient concentrations in the waters of the West Bay seemed to play a relevant role in the development of the *A. taylori* blooms, as well as in their maintenance in time. In the summer 2000, the seawater samples contained high concentrations of nutrients, above all ammonia and total phosphorus, with values up to 14.0 µM of NH_3-N and 3.2 µM of TOT-P. These concentrations were apparently connected with the hotel sewage during periods of increased anthropogenic pressure, as well as with the partial treatment of the wastes discharged into the sea, resulting in an enrichment of the waters. In contrast, the presence of riverine outputs or water contamination by bacteria were never observed in the West Bay during the study periods.

The nutrient load, together with the confinement of the West Bay of Vulcano - a semi-closed beach with calm waters and high summer temperatures – could have been responsible for the growth of *A. taylori* during most of the summer. As a final result, events of water discolouration due to persistent blooms of this dinoflagellate are more and more causing damage to the tourist industry on the island during the summer season.

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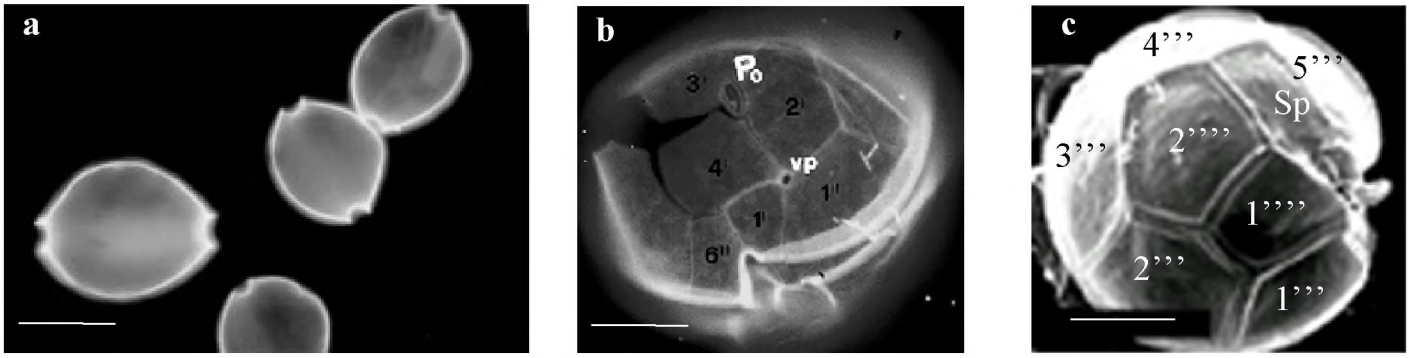

Fig. 2. A. taylori micrographs. a) General cell shape. LM, fluorescence. b) Epithecal details under epifluorescence, LM. c) Antapical view showing the hypothecal plates, SEM. Scale bars = 10 µm.

• International

LIFEHAB workshop held in Calvià, Majorca



The LIFEHAB workshop was conceived as a forum for discussion among specialists in taxonomy, life history strategies, cellular and molecular biology, geology and modelling of microalgal species. It was sponsored by the Marine Science and Technology Programme of the European Commission.

The workshop was held in Calvià, Majorca, Spain, October 24-27, 2001. Organised by the Institut de Ciències del Mar, CMIMA-CSIC (Barcelona) and the Calvià Town Council (Majorca, Balearic Islands).

The main goals were to: 1) review current knowledge of the life-cycles of phytoplanktonic organisms, focusing on HAB species; 2) identify the role of heteromorphic life cycles in population dynamics; 3) define future HAB research directions to fill existing gaps in knowledge; 4) discuss the most appropriate approaches and methods; and 5) promote the development of co-operative scientific initiatives.

The workshop included 25 presentations covering relevant aspects of life cycle studies in phytoplankton, ranging from updated reviews of the information available for the major algal groups, the role played by different life history stages in population dynamics, and the new tools available for addressing research questions. Discussion sessions focussed on the main gaps of knowledge and research needs.

The meeting allowed the updating of the available but frequently scattered knowledge on the topic, and created a scientific forum for discussion and clarification of different points. Discussions dealt with up-to-date methodologies and current knowledge to propose research recommendations (priority topics, methodology, modelling) on the life history of HABs.

The extensive discussion which took place during the Workshop highlighted a number of research needs which are common to all algal groups, as well as specific requirements related to life-cycle modalities peculiar to distinct algal divisions. The main gaps identified in our knowledge and priority research lines were:

Characterization of species. Knowledge of the morphological and functional complexity of life cycles, and of the modes of sexual reproduction, is needed in order to provide a more comprehensive framework for the definition of distinct taxa.

Elucidation of life cycles. Basic information on life histories should be gathered for a wide range of species to gain information on general life-cycle patterns within the different algal groups.

Life histories and toxicity. It is crucial to unravel the mechanisms and effects of toxin production in different life-cycle stages, which requires more sensitive methods for the quantification and detection of toxin profiles at the single-cell level.

Coupling of life histories and *in situ* population dynamics? The main challenge is to understand how distinct stages of life cycles interact with environmental and biological variables so as to produce harmful blooms, contribute to the persistence and expansion of harmful species, and to the make-up of their genetic diversity.

Kinds of methodological and technological developments needed. One of the major needs and challenges is the development and validation of molecular probes for rapid and reliable identification of HAB species and their different life history stages in the natural environment. Intraspecific genetic variability, as well as different physiological-related responses of the target organisms, should be investigated in order to design and validate molecular probes. Technological advances are required for improving *in situ* detection methods and instrumentation.

How can different disciplines contribute to the development of knowledge on HAB life cycles? The mechanisms underlying life cycles of harmful algae and their negative effects encompass a wide range of biological, physical and ecological scales, from genes to populations, from the interstitial environment in the sediment to large-scale movements of water masses, from cell-to-cell relationships to ecosystem interactions.

Cooperation among different research disciplines, coupled with the application of new technologies and the development of new models, represent special challenges for future research.

The proceedings of the workshop are available on the web: http:// www.icm.csic.es/bio/projects/lifehab/

For more information please contact: Esther Garcés, Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CMIMA, Passeig Marítim de la Barceloneta, 37-49,

COURSE ANNOUNCEMENT

Taxonomy of Harmful Microalgae

The Intergovernmental Oceanographic Commission (IOC) of UNESCO announces a regional training course in Taxonomy of Harmful Microalgae in collaboration with University of the Philippines, University of Tokyo, University of Tasmania, the IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, and the Virtual University of the International Ocean Institute.

The training course is a pilot-project within a project to establish an Internet portal on harmful algae for South East Asia, the HABSEA Portal. The course consists of 2 parts: the first part is a 3-months Internet distant learning course and second part is a 10-days practical microscope course in species identification.

The course is open for participants from South East Asia, and 16 persons will be accepted for this course. The IOC of UNESCO will cover all direct costs such as travel, accommodation, and allowances, but not Internet connections and fees for working hours in connection with the distant learning part of the course.

Schedule and venue: The distant learning part of the course will include a 3-day introductory workshop on 3-5 February 2003, held in South East Asia, followed by 3 months work at your home laboratory. The estimated workload is one day per week. The microscope course will take place at the University of the Philippines, Manila, ultimo May 2003.

Applications. Application forms are available from http://ioc.unesco.org/hab/train.htm. Applications should be send to Rhodora Azanza, IMS, the University of the Philippines by 15 November 2002. The selected participants will be contacted directly by the organizers, and have you not been contacted by 15 December, you should not expect to participate in the course.

All applications must be endorsed by an appropriate authority including an approval that the applicant is permitted to use working time equivalent to one day per week for 3 months to follow the distant learning part of the course and to participate in the microscope course. It should also be approved that appropriate computer facilities are at the disposal of the applicant. Internet browser and CD drive is required. Full details will appear in due time at http//:portal.unesco.org/seahab.

Contact: Prof. Rhodora V. AZANZA, Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, PHILIPPINES, Tel: (63) 2 98 96 76 to 79, local 7414, Fax: (63) 2 921 59 67, E-mail: rhod@upmsi.ph

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NEW HAB SOURCE BOOK FOR SOUTH AMERICA

The Spanish Institute of Oceanography (IEO) and the Intergovernmental Oceanographic Commission of UNESCO (IOC) are pleased to announce the publication of the book:

"Floraciones Algales Nocivas en el Cono Sur Americano" by E.A. Sar, M.E. Ferrario & B. Reguera. *Instituto Español de Oceanografia*, 2002, 312 pp. (ISBN: 84-95877-01-5; NIPO: 406-02-001-9). (Spanish only)

The preparation of this book was planned following the second meeting of the IOC Working Group on Harmful Algal Blooms in South America (FANSA, Mar del Plata 1995). The IOC-IEO Science and Communication Centre on Harmful Algae (IEO, Vigo) supported the editing and production of it.

The book is divided into four parts. The first part provides information and discussion on how to implement a monitoring programme of phytoplankton and phycotoxins, and on analytical and updated assay methods according to the EU Directives, for the detection, identification and quantification of phycotoxins. The second part deals with taxonomic aspects of different groups of harmful algae; the third part, Regional Reports, gives a thorough description on harmful events that have occurred in the different countries of the region , and their ongoing monitoring and research projects. The authors are mainly South American scientists, including the national focal points of the FANSA WG, and a few foreign scientists. The book will be distributed and mailed free to Iberoamerican institutions. Experts from other countries can get a free copy provided they accept to pay the mailing costs on arrival.

Orders should be placed with:

IOC-IEO Science and Communication Centre on Harmful Algae, Instituto Español de Oceanografia, Centro Oceanografico de Vigo, Apdo. 1552, 36200 Vigo, Pontevedra, SPAIN, Phone: 34 986 492111, Fax: 34 986 492003, e-mail: vigohab@vi.ieo.es. A PDF version of the whole book is available at http://ioc.unesco.org/hab

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MONITORING AND MANAGEMENT STRATEGIES FOR HARMFUL ALGAL BLOOMS IN COASTAL WATERS

APEC and IOC are pleased to announce the availability of the technical report: *Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters*, by D. M. Anderson, P. Andersen, V. M. Bricelj, J. J. Cullen, and J. E. Rensel. APEC Report # 201-MR-01.1, Asia Pacific Economic Programme, and Intergovernmental Oceanographic Commission of UNESCO, Technical Series No. 59, Paris, France.

The APEC HAB project supported the editing and printing of this report, and the IOC is assisting in distribution and mailing.

This 268-page report provides a broad review of the many programs, technologies, and policies used worldwide in the monitoring and management of harmful algal blooms in coastal waters.

To order a hardcopy or to download the PDF: http://ioc.unesco.org/hab

HARMFUL ALGAE NEWS

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No. 24

• Kenya

A cyano-anomaly? Cyanobacterial toxins as contributors to Lesser Flamingo mass deaths.

Microscopic analysis, over 70 years ago, of the stomach contents of the Lesser Flamingo, *Phoeniconaias minor*, confirmed what is apparent from the birds' ecology, feeding habits and bill structure: they feed on cyanobacteria [1]. The massive populations, at times over a million, of Lesser Flamingo at Kenya's Rift Valley saline, alkaline lakes, receive nutrients and energy from the local blooms of cyanobacteria, mainly *Arthrospira* spp. (formerly termed *Spirulina*). Although flamingos also feed on diatoms and invertebrates, e.g. brine shrimp, it is thought that the cyanobacteria can serve as the sole food of *P. minor.*



Over the past decade, episodic mass mortalities of Lesser Flamingos have occurred at the lakes [2]. The locations involved, including Lakes Nakuru, Bogoria

(Cont'd on p. 2)

• Mexico

First record of *Fibrocapsa* cf. *japonica* in Matanchen Bay, Nayarit, Mexican Pacific coast.

Abstract

In the present study, significative HAB'S bloom was confirmed in Matanchén Bay during Febrary 2003. The Raphidophice *Fibrocapsa* cf. *japonica*, Toriumi *et* Takano 1973 (=*Chatonella japonica*) has dominated (97%) red tide event occurring lately in coastal waters of Nayarit state, with high cell concentration of 4-6 millions of cells per liter of water. The duration of this bloom could not be determined exactly due to limiting sampling. There have been no reports of fish kills or human intoxication in the locality. This study represents the first survey on the distribution of *Fibrocapsa* cf. *japonica* in the Mexican Pacific coast. The finding adds Raphidophyceans to the list of harmful algal blooms and toxin producers in Mexico.

Key words: Fibrocapsa cf. *japonica*, Raphydophyte, Harmful algal bloom (=HAB) blooms, Nayarit State, Pacific Mexico.

Introduction

Routine phytoplankton monitoring in Matanchén Bay has revealed the presence of several discolorations on February 2003, however the duration of this bloom could not be determined exactly from beginning to lasting due to limiting samples. The discoloration was concentred in the middle of the bay near to Aticama village and Arrollo de La Palma turning seawater reddish-brown appearance. Matanchén Bay extends from 21° 25' 24" and 21° 30' 40" latitude N and 105° 12' 00" and 105° 15' 00" longitude W, a little below the Tropic of Cancer. The rain precipitations are in the order of 1000 to 1500 mm per year with climate warm and sub-humid with rains in summer. This Bay of Mexico is under the influence of fresh water particularly from San Cristóbal estuary in the north, Sauta River, Arroyo De La Palma and Santa Cruz River (Fig. 1).

(Cont'd on p. 3)

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(Cont'd from p. 1)

and Elmenteita, are important national wildlife and tourism resources. They are also subjected to increasing human pressures as receiving waters for anthropogenic diffuse and point pollution. Multiple factors are thought to be involved in the mass die-offs, including septicemia, avian tuberculosis, pesticides and heavy metals. Environmental analyses, feeding and body burden estimates have indicated that heavy metals would not have been sufficient alone to account for the deaths.

Analyses of flamingo carcass livers and cyanobacterial samples from Lakes Bogoria and Nakuru were carried out at both the Leibniz Institute for Freshwater Ecology and Inland Fisheries in Berlin, in the framework of the BIOLOG (Biodiversity and Global Change) programme of the German Federal Ministry of Education and Research and at the University of Dundee with support from the UK Natural Environment Research Council. Both groups of investigators found cyanobacterial toxins in dead flamingo livers: two hepatotoxins (microcystin-LR and –RR) and the neurotoxin, anatoxin-a, at estimated harmful concentrations [3]. The total extractable microcystin concentrations in the bird livers were 0.21 to 0.93 µg microcystin-LR equivalents, and that of anatoxin-a, 1.06 to 5.82 µg per g fresh weight. These toxin concentrations may have been sufficient alone to have caused the bird deaths, that of anatoxin-a being consistent with observations of staggering and convulsions in the flamingos before death and with opisthotonus, post-mortem.

Our investigations indicate that the microcystins and anatoxin-a should be included among the major agents contributing to the bird deaths. The only known sources of these toxins are cyanobacteria and it follows that the exposure of the flamingos to the toxins is largely via their diet. Further research with materials from Lake Bogoria Lesser Flamingos has identified the hepato- and neurotoxins in bird stomach and intestine contents and faecal pellets [4].

Shifts in cyanobacterial bloom composition, towards toxigenic genera, perhaps due to human intervention, could present the birds with an additional poisoning hazard. This may have occurred at Lake Nakuru, where the plankton population changed from one dominated by *Arthrospira* to one dominated by *Anabaenopsis* and *Anabaena* spp. [3]. The latter two genera include microcystin-producing species/strains and anatoxin-a can be produced by *Anabaena* spp. In addition, the birds may be exposed to cyanobacterial toxins other than via their primary food. At Lake Bogoria, the Lesser Flamingos drink in the vicinity of hot springs on the lake margin, where salinity is lower. The cyanobacterial mats around the hot springs include genera with toxigenic members (*Oscillatoria*, *Phormidium*; [4]) and it is possible that the flamingos may be exposed to the toxins via incidental ingestion of detached cyanobacterial mat biomass and/or drinking from the water nearby.

Mass die-offs of Lesser Flamingos do not appear to have been recorded during earlier decades of intensive study at these lakes [1, 5, 6], suggesting that the mass mortalities of the 1990s are relatively modern phenomena [2]. The extent to which increasing modern pressures from visitor numbers upon the breeding colonies, and pollution input from industry and agriculture interact with microbial threats to bird health, is a complex problem. The production of cyanobacterial toxins in the lakes and their presence in birds, dying after displaying typical signs of intoxication, indicate that their primary food can present benefits and costs. The characteristics of the toxigenic cyanobacteria in the lakes, and their regulation, require investigation to further understand the multiple influences upon the health and population dynamics of the Lesser Flamingo.

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• International

X HAB Model Group

At the tenth international conference on harmful algal blooms (HABs) at St. Pete Beach, Florida a special session on MODELS AND MYTHS was convened by J.J. Walsh on 23 October 2002. This workshop addressed the status of ecological modeling of HABs, from simple concepts of one dimensional processes that must be considered in any process model to three dimensional computer homomorphs of the "real world", where circulation models drive those of plankton competition. Ted Smayda presented an insightful introduction on "Models we should build". Present "Models we have built" on the Bay of Biscaye, Gulf of Maine, and the West Florida shelf were then discussed by Patrick Gentien, Dennis McGillicuddy, Bob Weisberg, and John Walsh. Topics varied from actual model results to future constraints in modeling HABs, i.e., initial conditions, data assimilation algorithms, sampling networks, and validation methods. These oral presentations will be part of the written proceedings of the conference.

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(Cont'd from p. 1)

Surface phytoplankton samples was taken from central bay region of Matanchén Bay on February 21 and 23, 2003. The samples were stored in a 250 ml plastic bottle, and then preserved with Lugol-acetate solution. Live whole specimens were analyzed using light microscope from which photographic records were made. Phytoplankton abundance was determinate by the standard Utermohl technique [1], and algal quantification, was made with 100x magnifications in aliquots of 10 ml using a 1 mm^2 scale Wipple disc with double cross pattern of 100 µm. Temperature was registered with a 0.1°C precision bucket thermometer and salinity was analyzed with a 1 psu precision refractometer.

Sampling results from identification and phytoplankton abundance during the bloom (Table 1) indicate 97% of total dominance of *F.* cf. *japonica* from over all community, although was associated with 27 species those represent only 3%. The identification of *F.* cf. *japonica* was based on morphological examination *in vivo* specimens which shape was spherical (possibly cyst stage) to ovoid. The vegetative cells showed their two characteristic flagella emerging from an anterior gullet and the mucosysts in posterior region consistent with the description of Loeblich III & Fine (1977) and Hara (1990) [2,3]. Another evident characteristic was the number and organization of chloroplasts (Fig. 2) and the spherical cell size of 15 µm, whereas in ovoid cells was 15-22 µm long and 15-17 µm wide. Living cells were fragile and difficult to preserve changing the body shape immediately, they can die in few minutes under light microscope. In addition this alga can be somewhat pleomorphic (it can have varied shapes).

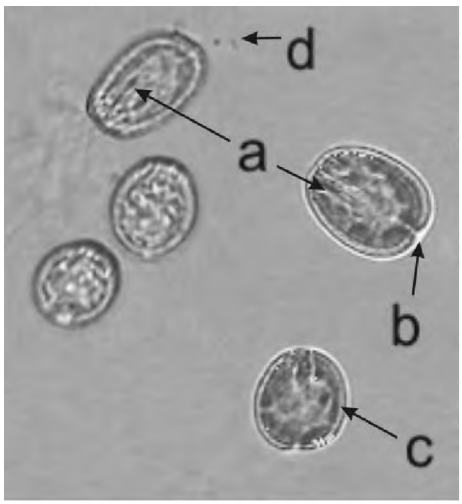

Fig. 2. Images of Fibrocapsa cf. japonica observed in living conditions. a)mucocysts b)gullet, c)chloroplasts arrangement d)flagella.

There have been no reports of fish kills or human intoxication in the locality although high proliferation of this specie in marine water may cause serious issues in fish industry and local aquaculture. No harmful algal bloom events have been recorded in coastal Nayarit State [4,5]. This occurrence is the first identification and published record of *Fibrocapsa* cf. *japonica* in Mexican Pacific littoral extending their distribution to warm temperature regions. Although this specie has been detected in Point Loma San Diego California Bay [2] correspond to USA (32° latitude N), so similar than those of Japan (30°-35° latitude N) where reach large numbers. Here we found it, in warm and more temperature water >26°C and >34 psu salinity. Hence it is reasonable to assume the presence as an invader specie traslocated by their resting cysts now adapting in this region, or also as new specie. However, to prove it is necessary to know the cellular ultraestructure in electronic microscopy and compare with known species.

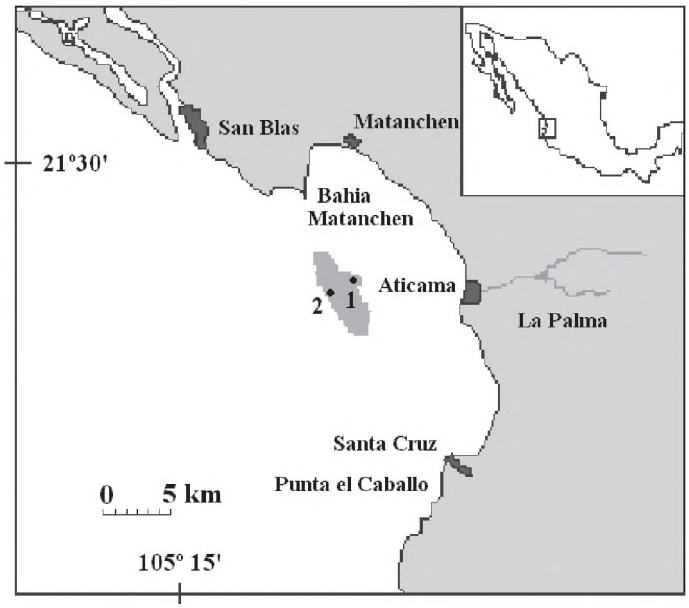

Fig.1. Map showing the study area of Matanchen Bay in Nayarit State México with location of sampling station and presence of discoloration.

Toxic Raphidophyceae have been documented from other geographical regions of the word: coastal waters of Japan, Australia, New Zealand, Brazil, northwestern Europe, California and Florida but, is in Seto Inland Japan where the populations showed devastating effects on mariculture [6]. Date no reported indicate fish mortality in Baja California Sur (Mexico) due Raphidophyceae in Todos Santos municipality of La Paz on July 22, 1999. So far, a few years ago flagellate resembling Chatonella sp. were recorded in water samples from Cabo San Lucas zone (Lat. 22° 30' N, Long. 110° 30' W) on march 1996 without blooming [7].

Toxicity PSP analysis by bioassay and chromatographic techniques was carried on by the Center for Biological Research (CIBNOR) with negative results, and conclude that the testing were not able to detect the presence of toxins. Recently in 2001 have been also reported fish kills in same geographical area of Nayarit State due Raphydophyceae assuming *Chattonella* cf. *antiqua* (Sierra-Beltrán, pers. comm.). Furthermore, a researcher from a red tide working group who usually visit this area, has been recognized the specie but in very low abundance (Gómez Aguirre, pers. comm.). The presence of these species in Nayarit State is particularly significant since they are known as NSP producers or brevetoxins-like compounds (named fibrocapsin) and to be the cause of fish kills [8].

It's clearly dominated the culture of this microalge and in unfavorable environmental condition induce cyst or non-motile spherical cells, very common feature in our samples. The precise mechanism behind the ichtyotoxic effects of the Raphidopyceae is still under debate and could be a combination of the different toxins. Furthermore, recent studies demonstrated that this microorganism flagellate is one of the reactive oxygen species (ROS), which generates superoxide O-(/)2 and hydrogen peroxide H_2O_2 under normal condition and may causes severe damage to fish [9]. Their results suggest that the generation of reactive oxygen species (ROS) is a common feature of this alga. There are no specific thresholds or critical values for the number of cell that pose a health risk. The abundance reported here occurred with cell numbers reaching 4 to 6 million cells L^{-1} and should be considered as higher cell concentrations, hence is necessary to determine toxicity of the organism under consideration. This survey on the alga *Fibrocapsa* cf. *japonica* indicate that currently environmental conditions are conductive to exceptional *Fibrocaspsa* cf. *japonica* bloom formation.

(Cont'd on p. 4)

(Cont'd from p. 3)

Table 1.- Microalgal abundances form Matanchén Bay bloom.

species	Feb-21-03 cels.ml^{-1}	%	Feb-23-03 cels.ml^{-1}	%
Fibrocapsa japonica	4,751	97.13	6230	97.66
Thalassionema nitzschioides	3.1	0.06	141	2.21
Coscinodiscus granii	0.4	0.01	0.3	0
Nitzschia closterium	73.1	1.49	0.7	0.01
Gyrosigma sp.	0.3	0.01	0.3	0
Pseudonitzschia sp.	0	0	1.4	0.02
Amphora sp.	0	0	0.1	0
Chaetoceros sp.	0	0	0.5	0.01
Navicula sp.	0.2	0	0.2	0
Thalassiosira sp.	0.2	0	0.8	0.01
Proboscia alata	0	0	0.1	0
Skeletonema costatum	0	0	2.1	0.03
Biddulphia mobiliensis	0	0	0.1	0
Leptocylindrus danicus	0.5	0.01	0	0
Detonula pumila	0.1	0	0	0
Protoperidinium nanum	51.6	1.05	0	0
Scrippsiella trocoidea	4.8	0.10	0.4	0.01
Peridinales	2.3	0.05	0.2	0
Ceratium falcatum	0	0	0.1	0
Prorocentrum gracilis	0	0	0.2	0
Gonyaulax spinifera	0.8	0.02	0	0
Dinophysis rotundatum	0	0	0.1	0
Gyrodinium sp	2	0.04	0	0
Silicoflagellate	0.3	0.01	0	0
Ciliate:*Mesodinium rubrum*	0.1	0	0.1	0
Euglenophyte	0	0	0.2	0
Cianophyceae	0.8	0.02	0.4	0.01
total	4,892	100	6379.3	100.00

Due to its high toxicity and periodic appearance, is essential for Nayarit local goberment increase scientific investigation of the autoecology of *Fibrocapsa* cf. *japonica* (or new specie) to minimize further impacts and damage on fisheries, aquaculture and healty ecosystems.

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• USA

ECOHAB PNW, A New West Coast, USA, Multidisciplinary Program

ECOHAB PNW is a new project whose goal is to study the physiology, toxicology, ecology and oceanography of toxic *Pseudo-nitzschia* species off the Pacific coast of Washington (WA) and British Columbia (BC). The project is funded jointly by the Division of Ocean Sciences NSF and by NOAA's Coastal Ocean Program. Recent studies suggest that the seasonal Juan de Fuca eddy, a nutrient rich retentive feature off the WA-BC coast, serves as a "bioreactor" for the growth of phytoplankton, including diatoms of the genus *Pseudo-nitzschia* (Fig.1). Specific study objectives are: 1) To determine the physical/biological/chemical factors that make the Juan de Fuca eddy region more viable for growth and sustenance of toxic *Pseudo-nitzschia* than the nearshore upwelling zone; 2) To determine the combination of environmental factors that regulate the production, accumulation, and/ or release of domoic acid (DA) from *Pseudo-nitzschia* cells in the field; and 3)

To determine possible transport pathways between DA initiation sites and shellfish beds on the nearby coast. A summary of results leading to the formation of this new program and a description of the research plans are given below. The ECOHAB PNW team welcomes collaboration with other interested scientists. For more detailed information about our program please visit our website at http://www.ecohabpnw.org.

Background

While DA poisoning was first recognized in an outbreak on Prince Edward Island, Canada [1], most of the known toxic events since that time have occurred on the U.S. west coast. DA was first implicated in the illness and death of brown pelicans and Brandt's cormorants in Monterey Bay, California in 1991 [2]. About one month following the toxic bloom in California, levels of DA above the regulatory limit of 20 mg/g shellfish tissue were found in the edible parts of razor clams (*Siliqua patula*) and Dungeness crabs (*Cancer magister*) on the Washington coast [3]. In 1998, impacts of DA to the health of marine life and to the fisheries economy were documented in several regions along the west coast. In particular, California sea lions (*Zalophus californianus*) in central California were severely affected by DA poisoning [4] and high levels of toxin in razor clams in Oregon and Washington resulted in beach closures for more than a year and a half [5].

Beach and harvest closures resulting from the toxigenic *Pseudo-nitzschia* blooms have a severe economic impact on both coastal economies and on tribal communities. In 1991, the closure of Washington State beaches to recreational and commercial shellfish harvesting resulted in a $15-20 million revenue loss to local fishing communities [6]. The commercial Dungeness crab industry on which Washington's Quileute tribe depends for employment lost 50% of their income in 1998 due to harvest closures. The entire razor clam harvest of the Quinault tribe, on which they depend for both subsistence and commercial revenue, was also lost in the fall of 1998 [7]. Razor clam beaches have again been closed this winter and spring (2003) and devastating economic impacts have resulted in an estimated $10 million loss to the recreational razor clam industry alone. With sufficient warning, tribal fishers could seek alternative buyers for eviscerated crab, and shellfish managers might have longer lead times to schedule closures. Moreover, mitigation strategies to reverse or moderate cell toxicity may soon be available [8].

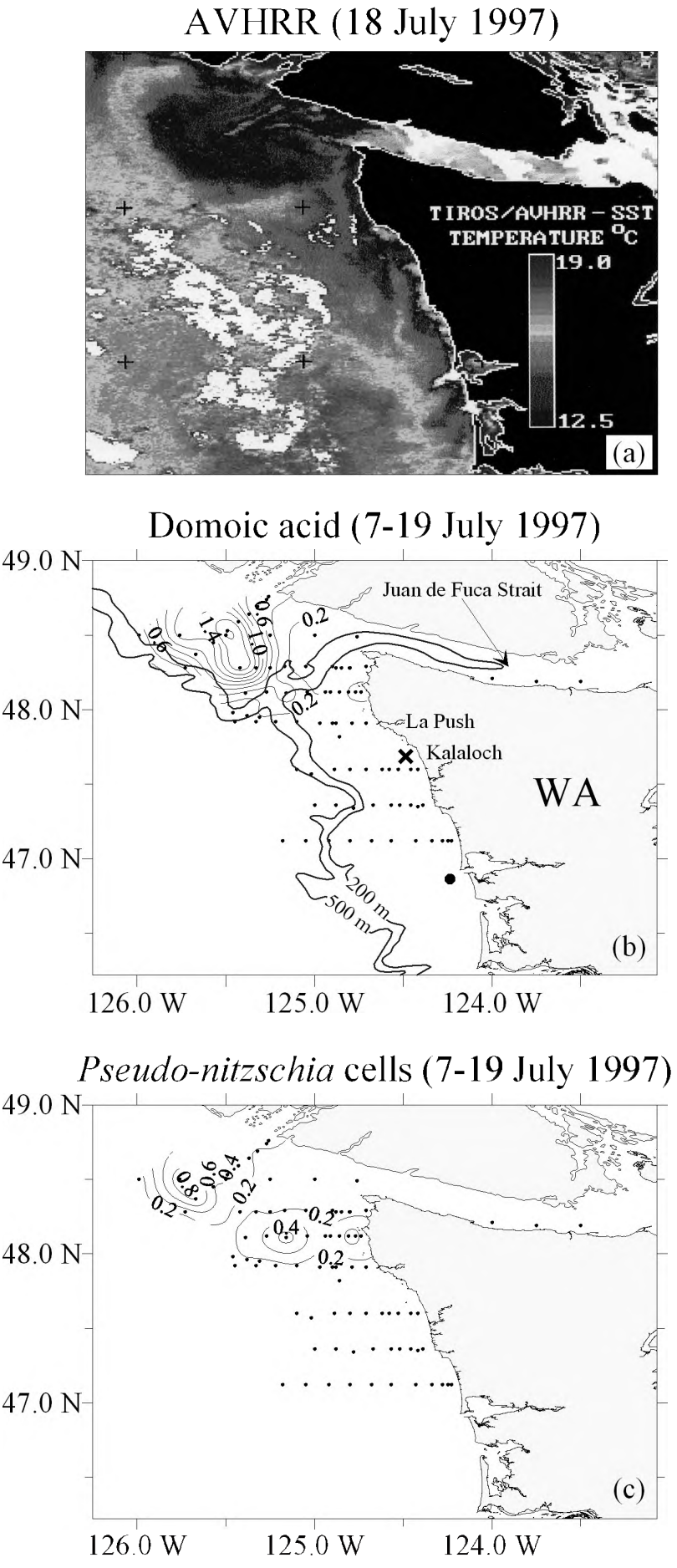

Fig. 1. Satellite-derived sea surface temperature, particulate DA (mg/L) and total Pseudo-nitzschia *cell numbers (10^6 cells/L) in surface seawater in July 1997 (Trainer et al., 2002). Dots represent all sampling stations where DA measurements and* Pseudo-nitzschia *cell counts were made. Spatial patterns show a coincidence of colder temperature, higher DA and greater numbers of* Pseudo-nitzschia *cells offshore of Juan de Fuca strait. The colder offshore water is indicative of the Juan de Fuca eddy. Colder water next to the Washington coast is indicative of local upwelling at the coast.*

Both the species of *Pseudo-nitzschia* (including *P. multiseries*, *P. australis*, and *P. pseudodelicatissima*) and the relative levels of toxicity [4, 9, 10, 11, 12] vary in time and space along the west coast of North America. Moreover, it is not uncommon for potentially toxic *Pseudo-nitzschia* cells to be present without detectable DA [8, 11]. The environmental regulation of DA production by *Pseudo-nitzschia* has not been determined in field populations due primarily to the ephemeral nature of these toxic events. Based on laboratory studies of unialgal cultures (primarily *P. multiseries*), two predominant triggers for the production of DA have been suggested: 1) the degree of cellular stress based on Si and P availability [13, 14, 15], and 2) the effects of micronutrient (Fe, Cu) conditions [8, 16].

A survey of DA along the entire U.S. west coast continental shelf in summer 1998 [11] suggests a strong relationship between DA concentration and mesoscale topographic features. Off northern California where large coastal promontories and hence rapid offshore transport occur, DA levels are low. However, at more retentive sites along the coast, such as offshore of the Strait of Juan de Fuca, over Heceta/Stonewall Bank in Oregon, offshore of Monterey Bay (inshore of the Farallone Islands) and near the Santa Barbara Channel, DA levels are higher. Recent studies suggest that the seasonal Juan de Fuca eddy is an initiation site for toxic blooms of *Pseudo-nitzschia* that impact shellfish on beaches along the Washington coast. Measurements made during cruises and beach sampling of seawater and shellfish are all consistent with the possibility that during some years DA from this eddy appears to move southward in prolonged upwelling events and then onshore during the first major storm of the fall season, where it results in high levels of DA in razor clams on coastal beaches [5].

Study Strategy

To test our hypotheses on the origin of toxic blooms, multidisciplinary field surveys and drifter deployments will be performed in the region of the Juan de Fuca eddy and the nearshore coastal upwelling region (Fig. 2). The temporal context for observed variability as well as seasonal changes will be provided by an array of moored sensors measuring PAR and *in vivo* fluorescence, currents, winds, temperature, conductivity as well as time-dependent water samplers (preserved plankton and DA) deployed in both eddy and coastal environments as well as in the mouth of the strait. Results from the field studies will be used to configure and test numerical physical and biophysical models to determine bio/chem/physical conditions conducive to bloom and/or toxin production as well as transport pathways of *Pseudo-nitzschia* or toxic *Pseudo-nitzschi*a to the coast.

The backbone of this project will be 6 three week cruises scheduled in July and September of 2003, 2004 and 2005. The length of the cruises was selected to ensure that a variety of growth regimes, including both upwelling and relaxation or downwelling, will be studied. *In situ* process studies will be made both in the eddy and coastal upwelling regimes as well as following aging water from each of these areas. Coastal *Pseudo-nitzschia* and DA data from the ORHAB (Olympic Region Harmful Algal Bloom) program and related state monitoring programs will be used to determine when and where toxic *Pseudo-nitzschia* arrive along the Washington coast resulting in toxification of razor clams. The field sampling plan, moored sensor arrays at key locations and drifter deployments will allow us to:

- Contrast characteristics of the nutrient-rich eddy with nutrient-rich nearshore upwelling areas. We will determine whether physical and biological factors that control DA production differ significantly in the two regimes.
- Contrast healthy and aged natural assemblages of *Pseudo-nitzschi*a to compare and contrast the environmental controls on DA production in cells at different stages of growth *in situ*.
- Determine the biophysical mechanisms of *Pseudo-nitzschia* advection to the coast, resulting in shellfish toxification. Possible mechanisms include the following scenarios: a) a healthy *Pseudo-nitzschia* population is advected directly from the offshore eddy to coastal shellfish during a storm event; b) an aged *Pseudo-nitzschia* population is advected from the eddy to the coast where it becomes a "seed" population that becomes toxic only when later supplied with nutrients from local coastal upwelling; or c) the nearshore, "seed" population toxifies the coastal shellfish directly after local upwelling followed by a storm.

The integrated field and modeling studies of ECOHAB PNW described above will make significant strides toward satisfying our long term goal—to develop a mechanistic basis for forecasting toxic *Pseudo-nitzschia* bloom development here and in other similar coastal regions in Eastern Boundary upwelling systems.

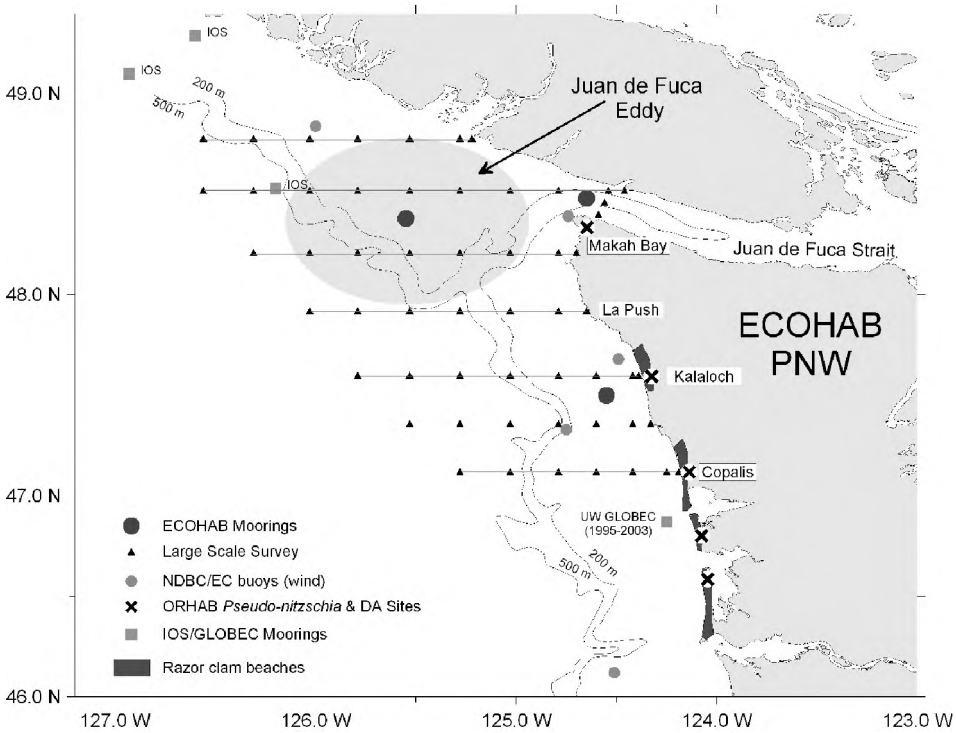

Fig. 2. Schematic large scale survey and locations of three interdisciplinary moored arrays. Locations of existing moorings, wind measurement buoys, razor clam beaches, and ORHAB sampling sites are also shown. The approximate location of the Juan de Fuca eddy is drawn as a lightly shaded area. Institute of Ocean Sciences (IOS) moorings are instrumented with current, salinity and temperature sensors at 3-4 depths between about 25 m and the bottom. ECOHAB PNW moorings will be instrumented with T, S, ADCP profilers and other current meters, time-dependent water samplers, PAR, wind and fluorescence.

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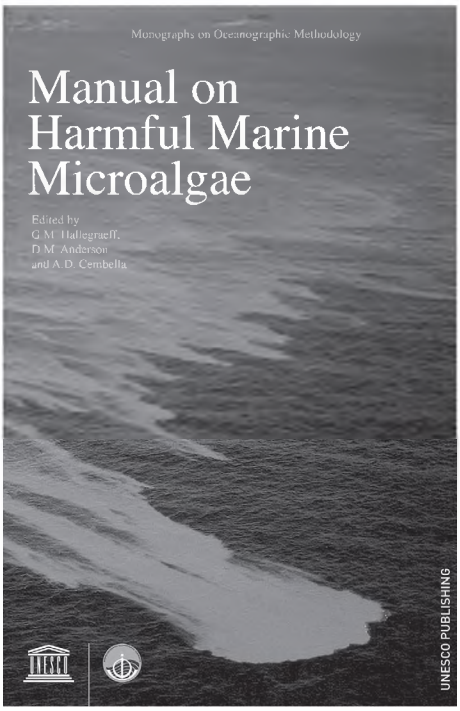

MANUAL ON HARMFUL MARINE MICROALGAE

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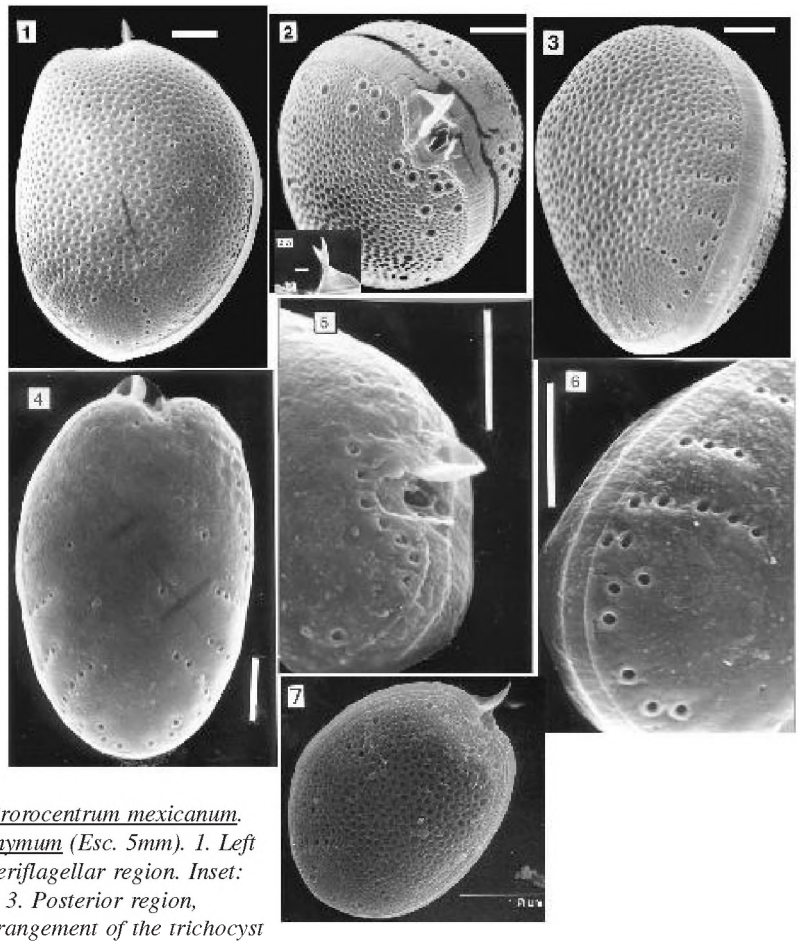
• Mexico

Prorocentrum mexicanum and *P. rhathymum* are different species

Osorio-Tafall (1942)[1] was the first to describe *P. mexicanum* under light microscope (=LM). He found the cells collecting plankton samples off Oaxaca, into the Gulf of Tehuantepec on the Mexican south Pacific. Loeblich III *et al.* (1979)[2] described *P. rhathymum* by scanning electron microscope (=SEM), from the clone 486 of samples obtained from surface waters in Cinnamon Bay, St.Johns, Virgin Islands. Later, since 1983, the species were marked as synonyms and further records and studies were labeled as *P. mexicanum* [3]. When observing samples of red tides dominated by *P. balticum* in Mazatlán Bay, México, during March and April, 1999-2000, we found several cells that perfectly fit the original description of Osorio-Tafall. When a detailed analysis of the morphology was made by SEM, we found several differences between *P. mexicanum(=Pm)* and *P. rhathymum(=Pr)* [4]. While *Pm* shape resembles a letter "D", *Pr* is neatly oval (Figs. 1 and 4 respectively). The periflagellar region and in particular, the spines, are different with two or three points in *Pm* (a unique characteristic of the species) (Figs. 2 and 5). Furthermore, trichocyst pores in the periflagellar rim are present in both valves in *Pm* while in *Pr* occur only in the valve where the periflagellar region is delimited, the right, according to Taylor [5]. The whole surface of the valves is covered by poroids increasing in size from the margin to the center of the cell of Pm. On the contrary, in Pr, the surface is smooth or rough, and the few trichocyst are aligned as radii along depressions or trenches (Figs. 3 and 6).

As mentioned above, at the moment, all records of *Pm* referred *Pr* like cells, the former is a planktonic organism and the natural habitat of the later is benthic as shown by its low mobility and the mucus layer that frequently produces. Nevertheless, *Pr* can be observed as ticoplanktonic, as the bloom reported in La Paz Bay, México [7], the origin of cells in Figs. 4, 5 and 6. Toxicity reports of *Pr-like* cells are common while in true *Pm*, the toxicity has not been proved yet. The known distribution of *Pr* is wide, being found in temperate and tropical regions. The knowledge of *Pm* distribution is scarce, in Mazatlán, is not very abundant and at the moment has not been observed causing discolorations by itself. The abundance of *Pm* seems to increment in direction to the Equator. *P. maximum* (=*Pmax*), a species which also may be easily confused with *Pm*, has not been re-described by SEM yet. Preliminary data obtained with samples collected in the Gulf of Nicoya, Costa Rica, indicates that the cells resembles *P. micans* and the spine in the periflagellar region is lanceolated (fig. 7) [8]. Cells mentioned as *Pmax* have been reported in estuaries in the Gulf of Guayaquil, Ecuador [9], with abundances close to 1000 cells/ml, dominating the area. A detailed (SEM) descriptions of the cells is not available, in particular the spine and periflagellar region, thus, it remains to be demonstrated to which of the three mentioned taxa they belong.



Figs. 1-3 Prorocentrum mexicanum. 4-6 P. rhathymum (Esc. 5mm). 1. Left Valve. 2. Periflagellar region. Inset: bifid spine. 3. Posterior region, annular arrangement of the trichocyst pores. 4. Right valva. 5. Periflagellar region, a single line of thrichocyst pores. 6. Posterior

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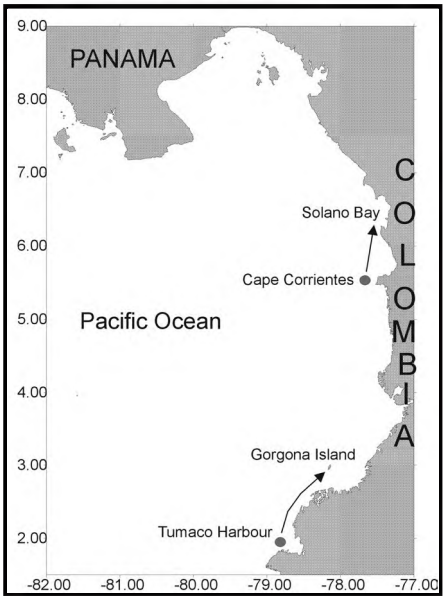
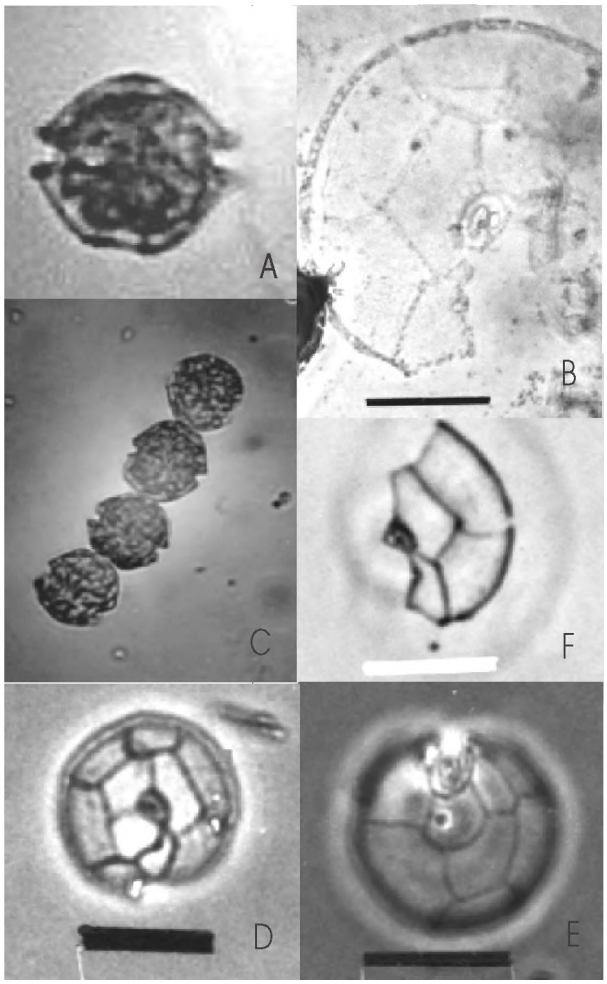
• Colombia

HABs due to *Alexandrium tamarense* on the Pacific coast of Colombia.

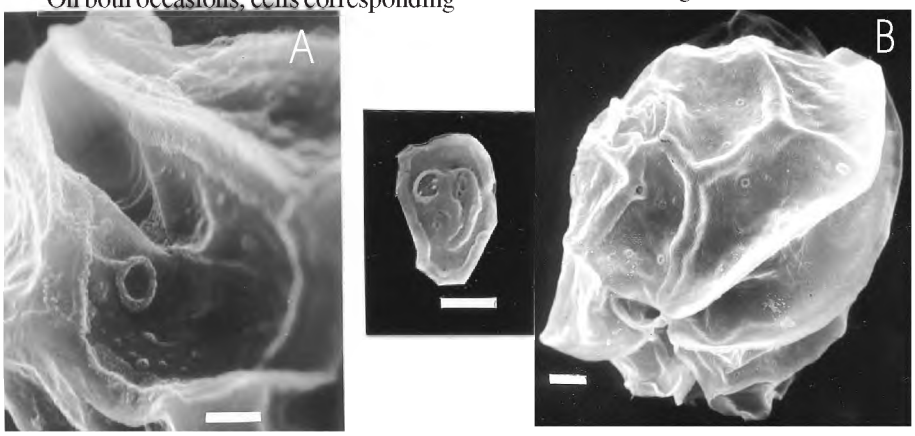


From 26 April to 15 May, 2001, a huge bloom was observed off Tumaco Bay, on the tropical Pacific coast of Colombia (Fig. 1 MAPA). This was the first time a harmful algae bloom (HAB) is reported in the region, reaching north to Gorgona Island. A year later (10 March, 2002), a new offshore occurrence of a HAB event developed from Corrientes Cape to Solano Bay, also on the Pacific of Colombia. Cell counts during these blooms reached $7.5x10^6$ cells.l^{-1} and $1.6x10^6$ cells.l^{-1}, respectively. During both events, low temperature and high salinity were recorded. While the average measurements in the area are 27-27.5°C and 30-31.5 psu, the values observed during the events were 24-24.6 °C and 33-34 psu, 3°C below the normal and more than 2.5 psu above the average values, indicative of local upwelling at the time of the events. During these events there were no reports of observable effects on the marine biota or human poisoning, since the blooms occurred offshore and far from exploited shellfish beds.

On both occasions, cells corresponding to the *Alexandrium* complex represented 99-100% of the biomass. Samples were preserved in Lugol-acetate solution. Phase contrast observations with the aid of Klein's silver stain, allowed detailed description of the apical pore complex, the first apical plate and the posterior sulcal plate of cells collected in 2002 (Figs. 2a-e FOTOS PHASE). Shape, dimensions (L= 25-40µ, X= 32µ, W= 25-37.5µ, X= 31.6µ, n = 50) and detailed structure of the plates were consistent with *Alexandrium tamarense* [1, 2]. It was difficult to differentiate the cells from *Alexandrium catenella*, but the presence of short chains of only 4 cells (single cells represented most of the biomass) was suggestive of *A. tamarense*. Some cells were gold/palladium-coated and observed in SEM. Following the description of Fukuyo [1], the shape and position of the ventral pore on plate 1' and a cell shape slightly longer than wider confirm the identification (Fig. 3 FOTO SEM).



Alexandrium tamarense is normally found in temperate waters (16°C in average) [3], but is also reported in warmer regions (=*Gonyaulax tamarensis f. excavata*) [4, 5]; it has not been reported before in the tropical Pacific. In 1976 a red tide was recorded in summer (July and September) North of Cape Corrientes but the causative organism was not reported [6]. The Centro de Control de Contaminacion del Pacifico of the Colombian Navy has been monitoring the area since 1994 without previous records of this species or HABs, and it may represent an introduction which is favoured by local upwelling.



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Elusive red tides in Kuwait coastal waters

The shallow (<33 m), well mixed, and illuminated but eutrophicated Kuwait Bay waters constitute a highly stressed ecosystem in the arid Arabian Gulf region. These waters contain about 15 potentially harmful phytoplankton taxa some of which attain red tide proportions (>20 µg chl a l^{-1} and $5x10^6$ cells l^{-1}). During the year 2000, two red-water episodes occurred which were ephemeral and massive (>4525 µg chl a l^{-1} and about 2 billion cells l^{-1}) with the patches extending from 50 m^2 to $15x10^4$ m^2. The dominant species differed each time e.g. *Prorocentrum* spp. *Pseudo-nitzschia seriata*, *N. longissima*, *Leptocylindrus* sp. and *Trichodesmium erythraeum*. The blooms were orange greenish-brown and appeared as thick foamy rafts, meshes and strands entraining gas bubbles. In experimental growth studies, eolian dust rich in essential micronutrients for phytoplankton growth had a positive effect on summer phytoplankton growth and yielded 526.8 mg chl a m^{-3}, similar to red tide proportions. Blooms of *Karenia* sp. during September-October 1999 were implicated in the mortality of 30 tons of wild mullets and 150 tons of caged sea bream at a cost of $7 million, but the cause could not be linked to phycotoxins as no toxin analyses were done. Suggestions are presented for a regional institutionalized long term approach to generate a high frequency sampling over extensive areas, to elucidate physiological ecology of suspect phycotoxin producing algae and to investigate the role of dust storms as a major ecological force in the formation of algal blooms.

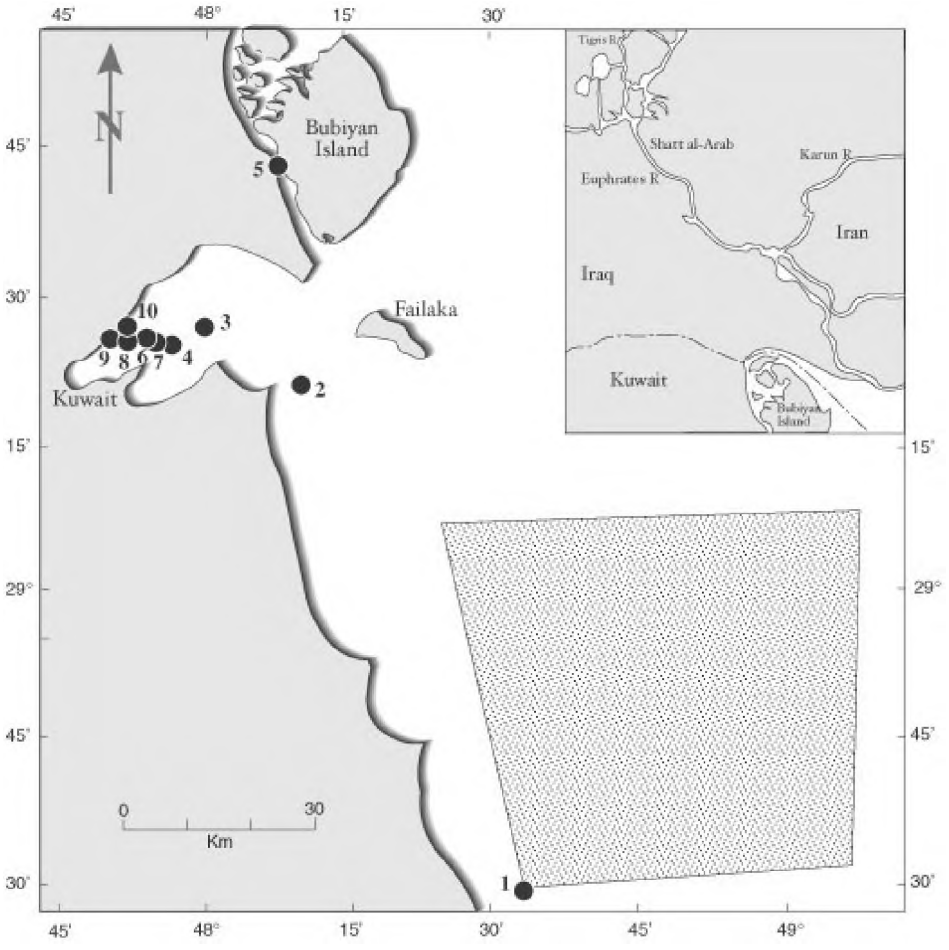

Fig. 1. Kuwait Bay and stations where red tides occurred during May 1997 to July 2000. Numbers represent the sequence of occurrence of blooms (see Table 1). Shaded area represents Trichodesmium erythraeum blooms on 19th May 2000.

The Arabian Gulf is a shallow (mean depth 30 m), sub-tropical, hypersaline, and enclosed basin. The Tigris, Euphrates and Karun rivers join to form the Shatt al-Arab River which empties into the northern region of the Gulf (Fig.1). The Shatt al-Arab outflow of about 1456 m^3 s^{-1} [1] is nutrient rich and is the main fresh water input to the Gulf [2]. As evaporation exceeds run off and because of its semi-enclosed nature, the Gulf acts as a negative estuary [3] and a trap for pollutants as well [1]. With an annual spill of about 200,000 barrels of oil and associated products the Gulf is extremely polluted [4].

To the south of Shatt al-Arab is Kuwait Bay, a highly stressed body of water. The sources of stress include nutrients from the Shatt al-Arab, discharges from 12 major industries including two petrochemical companies, three refineries, discharges of raw sewage, thermal and chlorinated effluents from six dual purpose desalination plants, wastes from slaughter houses, hospital wastes and pollutants associated with the extensive shipping industry [5]. For example in addition to the 0.003 x 10^6 km^3 d^{-1} sanitary wastewater, 0.023 x 10^6 km^3 d^{-1} wastewater from petrochemical refineries and fertilizer and polypropylene industries [6] and 0.282 x 10^6 km^3 d^{-1} raw or partially treated sewage are discharged into the Bay [7]. This sewage has the highest organic content amongst the Gulf countries [8]. Daily discharges from the power plants amount to 33.6 km^3 chlorinated cooling water and about 17 metric tons of residual oxidants [9]. Because of infilling, dredging of the shipping channels has to be continuous with 4.66 x 10^6 m^3 silt dredged annually. On the tacit assumption that the ongoing urbanization of the coastal areas in these arid Gulf countries will increase, stresses on the marine environment are also bound to intensify.

These stressed territorial waters of Kuwait and Kuwait Bay support a variety of biotopes including some of the world's most northerly coral reefs [10]. The Bay waters are well illuminated with 325-575 W m^{-2} and 83-275 W m^{-2} near the surface and the bottom respectively, sufficient for phytoplankton growth [11]. With a pronounced (3.5 m) tidal amplitude [12], and absence of thermal stratification, the water column is well mixed, and oxygenated except in waters deeper than 30 m where a strong cold bottom current exists in contrast to the upper layer (J.M. Al-Hassan unpublished). These waters are eutrophic and never stripped of the macronutrients i.e. phosphate, silicate and nitrate [11] but cannot be included under the 'very eutrophic category' [13]. Phytoplankton biomass, however, was usually low [10] and ranged between 0.01 to 12.8 mg chl a m^{-3} in individual samples and in the column from 3.8 mg chl a m^{-2} to mostly less than 90.0 mg chl a m^{-2}.

Red tides are almost unknown from the arid zone waters. The absence of

pronounced typical or atypical buildup of biomass in the nutrient rich, well illuminated and well mixed waters off Kuwait, is of prime interest. Red tides have escaped attention, save for a few anecdotes from this region. One of us (J.M. Al-Hassan) observed red tides in 1981 and 1982, but did not report them. Subba Rao *et al.* [14] reported qualitative and quantitative changes in algal species, size fractionated biomass and their primary production characteristics of a red tide from Kuwait coastal waters in 1997. In this paper we have integrated recent observations which illustrate how red tide episodes off Kuwait are frequent, ephemeral and massive.

Surface waters were collected by bucket from a private yacht, stored in polypropylene bottles and transported in ice-coolers to the laboratory for standard chemical and plankton analyses. Salinity was determined using the Orion Conductivity/Salinity Meter, Model 140. Nutrient analyses were based on procedures described in the ROPME manual [15] using Per Storp Analytical Autoanalyzer System and Beckman DU Series 600 Spectrophotometer. Unfortunately on 19 May 2000, phosphate levels, which would have been of significance in view of the Trichodesmium bloom could not be determined. Following examination of live water samples under a microscope, samples were preserved with 1% paraformaldehyde+ glutaraldehyde (50:50 volume). Phytoplankton cell densities were enumerated using an inverted plankton microscope. Chlorophyll a was estimated by the fluorometric method [16].

Following three days of calm weather in the waters between 28°30' and 29° 05' Lat. N and 48°20' to 49°05' Long. E, (Fig. 1, Station #1 and hatched area) massive blooms of the blue green alga *Trichodesmium erythraeum* occurred on 19th May 2000]. These blooms had >900 µg chl a l^{-1} and consisted of 100 million cells l^{-1}; nitrate and silicate levels were 3 and 39 µg at l^{-1} respectively. From our field observations, captured in figure 2 (panels A to D), it is possible to reconstruct the formation of the local red tide patches. The patches consisted of strands and lattice-like structures (panel A) at the surface, which coalesced into extensive floating mats (panel B). The algal aggregates varied from a thin sheen to approximately two cm thick; the thick layers included foamy oily complexes with heterogeneous strands, lattice, or rope-like structures in hues of green, blue, pink, red, orange, brown, and gray (panel C). The approximate size of the patches varied from 50 m^2 to 15 x 10^4 m^2. Intact patches at the sea surface had a smell similar to decaying vegetables, mechanical agitation resulted in the release of a pungent odor, which affected the human nervous system causing difficulty in breathing, nausea, coughing, choking, irritation to the eyes, a lethargic feeling and loss of co-ordination. Upon storage the odor worsened over 24 h making removal of samples for analysis difficult. Biotoxicity tests could not be carried out because of a lack of facilities. By the following day (20th May) a strong northerly wind and the ebb tide caused the blooms to advect south and disappear.

On 7 July, 2000 (Fig.1, Station #2) massive blooms with *Pseudo-nitzschia seriata* cell densities (749.5-1008.9 millions per litre) were present in the scum thus at least an order of magnitude higher than any literature values from seawater. Other cell densities (millions per litre) were for picoplankton (720.1-962.8), *Prorocentrum* spp. (2.9-4.3), *Nitzschia longissima* (10.1-11.5), *Leptocylindrus* sp. (5.76-10.09), *Gymnodinium* spp. (2.9). Chlorophyll levels were very high (2906.75 – 4525.47 µg chl a l^{-1}). The salinity of the water ranged from 42.2- 42.4 PSU. Despite the heavy blooms nutrients were not exhausted and ranged (µg at l^{-1}) between 0.02 and 1.20 PO_4, 0.06-0.24 NO_2, 0.01-0.52 NO_3, and 11.65-37.07 SiO_3. The algal cells formed orange greenish- brown thick foamy rafts, meshes and strands that could be scooped from the surface (Fig. 2, Photos A-D). These patches were ~20x30 m in extent and a 12 cm perched bird – Sylvia nana (Mutrag in Arabic) could not free itself (Fig. 2, Panel D). Upon storage this foam turned greenish-brown. Although there were several entrained live phytoplankton cells, these rafts contained many dead and decayed phytoplankton cells (unusually large die-offs of the bloom species). Gas bubbles, of undetermined chemical composition, were entrained in the scum for more than 48 h. Under the influence of southeast winds, the associated currents and the high amplitude of tides these patches meandered into Kuwait Bay (Stations #3, 4, 6 to 10). By the third day the blooms had advected out of the bay

Table 1: Summary of occurrences of algal blooms and their biomass (Chl. a µg or x 10^6 cells l^{-1}) in Kuwait Bay

Occurrence	Station	Taxonomic group	Taxa	Biomass	Reference
Nov. to Jan., March and May 1987 to 1988	Kuwait Bay	Haptophyte	*Phaeocystis* sp.	136 *a*	Al-Hassan *et al.* 1990 #28
October 1996		Ciliates	*Myrionecta rubra*	156 *a*	Al-Yamani *et al.* 1997 #29
May 1997	1	Diatom Dinoflagellates	*Nitzschia* spp. and *Gymnodinium* sp.	501 *a*	Subba Rao *et al.* 1999a #19
May 2000	9	Cyanobacteria	*Trichodesmium erythraeum*	900 *a*	Present report
July 2000	10	Diatoms	*Pseudo-nitzschia seriata*	749.5 to 1008.9 cells 2906.75 to 4525.47*a*	Present report
	10	Diatoms	*N. longissima*	10.1-11.5 cells	
	10	Diatoms	*Leptocylindrus* sp.	5.8-10.1 cells	
	10	Cyanobacteria Picoplankton		720.1 to 962.8 cells	
	10	Dinoflagellates	*Prorocentrum* spp.	2.9 to 4.3 cells	
	10	Dinoflagellates	*Gymnodinium* spp.	2.9 cells	

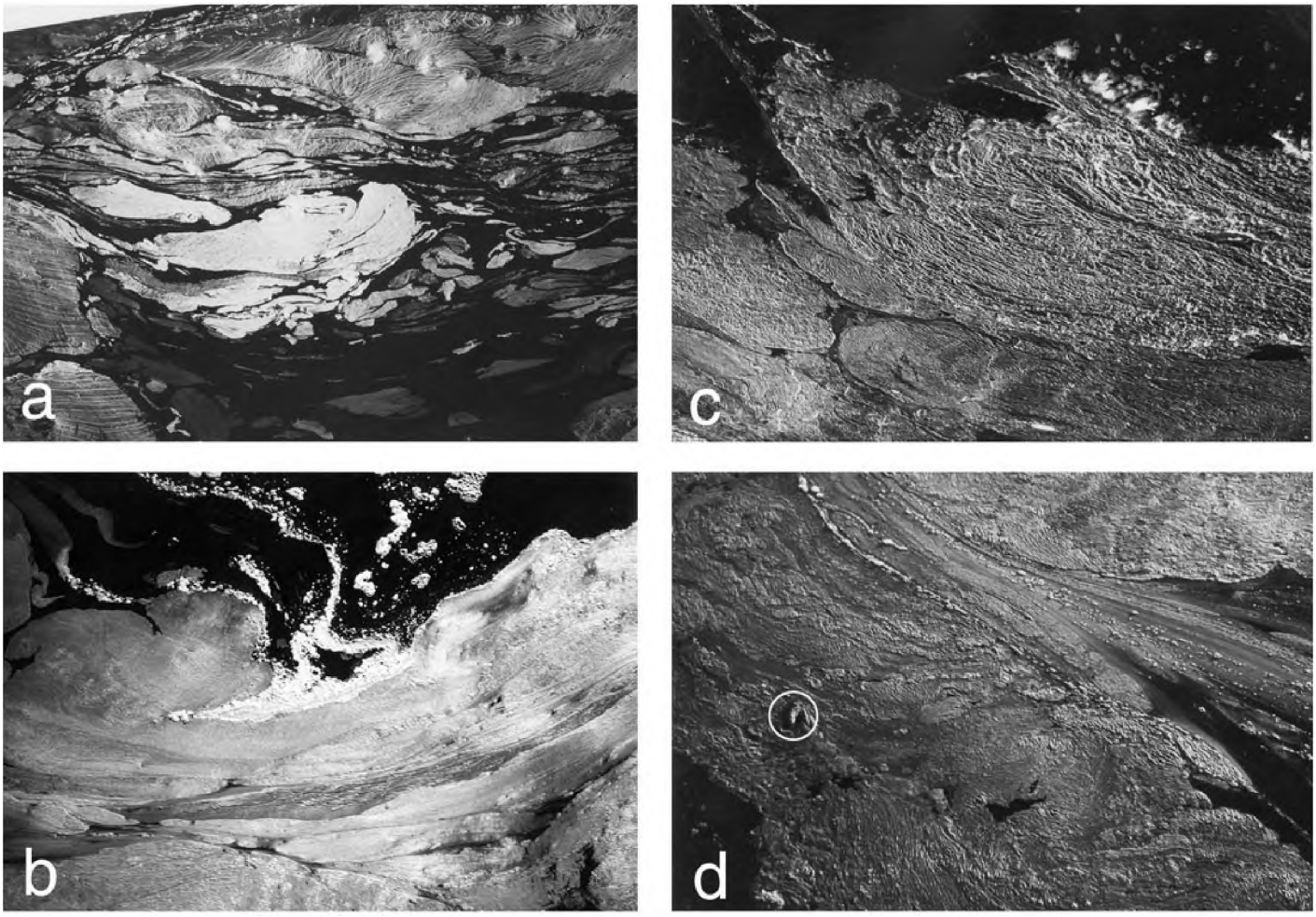

Fig. 2. Panels A,B,C and D showing progressions and patterns of discoloration due to red tide in Kuwait Bay. Panel D shows an entangled bird Sylvia nana.

eastwards (Station 5) towards Failaka Island and finally south into the open Gulf.

In this arid zone sea with no pronounced seasonal growth of phytoplankton, the time of occurrence of algal blooms, their constituents and magnitude varied widely in contrast to red tide episodes in the temperate embayments. Thus blooms of *Phaeocystis* species occurred during November-January, March and May [17] with 136 µg chla l^{-1}, with *Myrionecta rubra* (156 µg chl a l^{-1}) during August [18], *Pseudo-nitzschia* spp. and *Gymnodinium* spp. blooms (501 µg chl a l^{-1}, Station #1) during May 1997 [14], and with the benign diatoms *Chaetoceros curvisetus*, *Nitzschia longissima*, and the ciliate *Myrionecta rubra*, and the gymnodinian *Karenia* sp. [19] (265 µg chl a l^{-1}) during September-October 1999 (Stations #2, 3, 4, 6, 7 and 8).

On average Kuwait experiences 27 dust storms annually with an average annual dust fall of 33.38 g m^2 in Kuwait Bay [20]. Phytoplankton samples collected during summer and enriched with dust produced more rapid doublings of biomass than the control and attained chlorophyll values as high as 526.8 mg chl a m^{-3}, similar to red tide proportions [21]. The response of phytoplankton to the addition of eolian dust was rapid and dose dependent in the range of 10^5 to 10^7 mg m^{-3} enrichments; the differences in the doubling rates and biomass were statistically significant. Of the 29 species initially present, a small Naviculoid diatom (<6 x 3 µm) attained red tide proportions (23.1 x 10^6 cell l^{-1}) [21]. The dust contained trace metals in the following ranking: Fe > Zn > Cu > Mn > Co > Ni, V > Pb > Cr, some of which constitute essential micronutrients for phytoplankton growth. Because of their impact, the role of dust storms as a major ecological force in the formation of algal blooms must also be included in any environmental study. The "iron hypothesis" proposes that iron is the limiting nutrient in "high nutrient low chlorophyll" (HNLC) regions, and extensions of it suggest the iron can be supplied by dust storms. There is now quite a large literature about this, and Walsh and colleagues have suggested that wind blown iron makes nitrogen available to *Karenia* via N-fixing *Trichodesmium* blooms.

In Kuwait waters, the 1999 bloom was implicated in the death of 30 tonnes of surface feeding wild mullets and 150 tonnes of caged sea bream, resulting in a loss of $7 million and consumer confidence. Confirmatory data on the identity of the suspect toxigenic organisms, nature of the toxin, bioassy, total quantity of toxin delivered are not available but are crucial before implicating the ephemeral algal patches as causative agents of fish kills. Gills of sea bream from the cage cultures yielded levels (µg g^{-1}) of As (20-24), Fe (274-987), Ni (7-13.5), Cu (18-31) and Zn (45-252) much higher than the corresponding 4, 36, 1.6, 1.9, and 15 in the gills of wild sea bream. The Se:Ni, Fe:Ni, Zn:Pb ratios in the gills were higher than those in wild fish. It is plausible these metallic elements may have played a compounding role in causing mass mortality of the sea bream [19].

Because of the heavy sea traffic either for recreation, trade, fishing and defence, and because the population is mostly coastal in this arid zone kingdom, the users should be alert to any red tide sightings and report these to a central agency. A regional multinational, multidisciplinary institutionalized marine science program, backed by remote sensing to track red tides would be highly desirable.

Acknowledgements

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• Italy

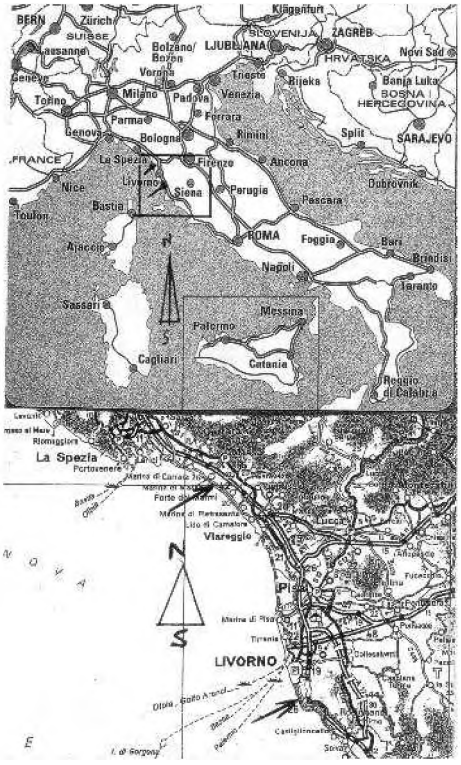
Harmful epiphytic dinoflagellate on Tyrrhenian Sea reefs

Epiphytic and benthic dinoflagellates present on the reefs of the Tuscany shore have been studied. at two main monitoring areas: one of them on the artificial reef located in submerged sand opposite Marina di Massa beach, to prevent erosion; the other, on natural reefs in the Livorno oligotrophic lagoon. Macroalgae samples (*Corallina mediterranea*, *Cystoseira spicata*, and others) were collected in bags with surrounding seawater. The macroalgae were shaken in sampling water to remove dinoflagellates and weighed. The seawater was filtered through 125 mm mesh net, and the volume measured. Cell counts were made with an inverted microscope at 100 x, while algae identification was carried on at 600 x UV after colouring thecal plates with calcofluor. The first stage of research showed a bloom of Ostreopsis ovata, see table 1 from Simoni et al (2002).

The results indicate that the *Ostreopsis ovata* quickly colonizes the algae of the natural and artificial reefs in late spring and in summer, when the temperature of water reaches 22 °C. *Ostreopsis ovata* is

Table 1

Sampling zone	Date	pH	water temperature	*Ostreopsis ovata* (density +,++,+++)
Marina di Massa	30/08/00	8.7	26.5 °C	+++
Marina di Massa	27/09/00	8.4	22.5 °C	++
Marina di Massa	01/12/00	8.3	14.5 °C	+
Livorno	01/12/00	8.8	17.5 °C	+
Livorno	14/02/01	8.8	12 °C	0
Livorno	29/04/01	8.8	15 °C	0
Marina di Massa	04/05/01	8.3	16.9 °C	0
Marina di Massa	01/06/01	8.3	20 °C	0
Livorno	22/06/01	8.4	22 °C	+++
Livorno	03/08/01	8.5	23°C	+++
Marina di Massa	09/08/01	8.4	24°C	+++



MONITORING AREAS

most abundant in summer, decreases in autumn and disappears in winter. We assume this species is able to produce resistant forms, such as spores or vegetative hibernating forms, which survive at lower temperatures.

Further studies, started in summer 2001, verified the presence of other harmful epiphytic dinoflagellates. Reef macroalgae were colonized by the potentially toxic *Coolia monotis* (29 x 44 µm), *Prorocentrum lima* (38 x 28 µm), *Prorocentrum emarginatum* (35x27 µm), as well as *Ostreopsis ovata*. The results of the quantitative monitoring are shown in Table 2.

The results seem to confirm the tropical characteristics of *Ostreopsis ovata*: it is not found in winter nor in early spring, when the water temperature barely reaches 11°C (lowest temperature limit in the Mediterranean). On the other hand, *Coolia monotis*, *Prorocentrum lima*, and *Prorocentrum emarginatum* are present all year. The results show that natural reefs are colonized by more species than artificial cliffs. Other flagellates found on these natural reefs are *Scrippsiella trocoidea*, *Goniaulax* sp., *Amphidinium* sp., and the cyanobacterium *Oscillatoria* sp. Diatoms prevail during the whole year.

On artificial reefs, where suspended sand and mud often make shallow waters turbid, the number of epiphytic and benthic species are noticably reduced. On the other hand, selective pressure caused by high water temperature (up to 27°-28°C) produce algal blooms of the thermophilic *Ostreopsis ovata*, on artificial reefs of Marina di Massa in August.

Ostreopsis ovata, *Coolia monotis*, *Prorocentrum lima*, *Prorocentrum emarginatum* have been found also on reefs in Elba, (Simoni 2002), Sicily, and Sardinia (Institute Talassografico CNR Messina), but only Ostreopsis ovata has been spotted on Viareggio (LU) artificial reefs, and on La Spezia natural reefs (ARPAL La Spezia) and near Bari (Bari Laboratory Marine Biology). These species are apparently absent in the northern Adriatic Sea.

Ostreopsis ovata develops intense bloom in August in the breakwater of Marina di Massa, where waters are calm and reach high temperatures owing to insufficient water circulation. In summer 2002, concentrations of *Ostreopsis ovata* in Marina di Massa reached 10550 cells L^{-1}, and of *Coolia monotis* 1025 cells L^{-1}.During blooms, *Ostreopsis ovata* were found associated with floating detached macroalgae in the warmest periods of the year. In some areas, this phenomenon has been associated with respiratory irritation in bathers. Epidemiological investigations are now in progress to establish a possible relationship between the two phenomena. Furthermore, shellfish and *Arbacia* sp. mortality has been observed during *Ostreopsis ovata* blooms on Marina di Massa reefs.

An additional study highlights the possible presence of ciguatoxins and DSP in the alimentary chain of the reef ecosystem; immunological methods (Cigua-check and DSP-Check) indicate the presence of DSP-like and ciguatoxin-like toxins (table 3):

These results indicate that ciguatera-like substances accumulate in the trophic chain. However, the lack of epidemiological evidence of neurological and gastro-intestinal symptoms in fish consumers does not presently allow evaluation of the potential toxicological risk to public health. Sampling and analysis will continue to verify the possible increase of the test-positivity in summer and autumn.

Table 3

Organism	Date	Tissue	Origin	Cigua-Check ppb	DSP-Check ppb
Scorpena porcus *Scorpena porcus* *Scorpena porcus*	15/01/02	flesh	Livorno fish market	0.9 0.9 0	<2
S. porcus *S. porcus* *S. porcus*	15/01/02	liver	Livorno fish market	1.2 0.9 0.8	1.3 <2
Murena helena	15/01/02	flesh	"	0.8	<2
M. helena	15/01/02	liver	"	1.2	3
Conger sp.	05/01/02	flesh	"	1.0	<2
Charonia sp *Charonia* sp	02/03/02	flesh	Cliff in Livorno	1.3 0.9	5
Patella sp.	08/03/02	flesh	Cliff in Livorno	0.8	
Octopus vulgaris 2 Kg *Octopus vulgaris* 2 Kg	05/03/02	tentacle	Cliff in Livorno	1.0	0.9
O. vulgaris 0.3 Kg	05/03/02	tentacle	Cliff in Livorno	0.9	
Mullus surmuletus	10/03/02	flesh	Cliff in Livorno	0.8	0.9
Crenilabrus sp. *Crenilabrus* sp.	10/03/02	flesh	Cliff in Livorno	0.8 0	0.6
Serranellus sp.	10/03/02	flesh	Cliff in Livorno	0.8	0.7
Mitilus sp.	08/03/02	flesh	Cliff M. di Massa	0.9	
S. porcus	24/04/02	flesh	Livorno fishmans	0.8	0.2
Labrus turdus	24/04/02	flesh	Livorno fishmans	0.8	0.5
M. surmuletus *M. surmuletus*	24/04/02	flesh	Livorno fishmans	0.8 0.8	0.5 0
Boops salpa	24/04/02	flesh	Livorno fishmans	0.8	1.8

Table 2

Date	Stations	pH	T. °C	in 1 gr of algae				in 1 gr of algae			
				O .ovata		*C. monotis*		*P. lima*		*P. emarginatum*	
				mean	range	*mean*	range	*mean*	range	*mean*	range
02/10/01	Livorno	8.23	21.5	67.8	16-94	18.1	9.4-35	15.4	5-40	0	0
04/10/01	M.di Massa	8.0	21.3	9.3	2-23	1	0-1.6	0	0	0.5	0-1.82
19/10/01	M.di Massa	7.9	20.8	2.2	0-3.5	0	0	0	0	0	0
29/10/01	Livorno.	8.0	21.5	15.7	0-32	14	0-38	10.8	0-25.7	5	0-15.5
20/11/01	M.di Massa	8.4	12.5	0	0	0	0	0	0	0	0
26/11/01	Livorno..	8.2	13.6	12.7	7.2-20	6.6	1.7-16	5.6	0-15.3	0	0
06/12/01	Livorno..	7.90	10.9	39.9	19-112	19.7	9-34	17.3	9-32	3.2	1-7
06/12/01	M.di Massa	8.0	10.5	0	0	0	0	0	0	0	0
03/01/02	Livorno	8.01	11.2	1	0-2	2.8	1-4	8.2	1-23	1.2	0-5
02/02/02	Livorno	8.01	11.8	1	0-6	16	0-57	3	0-12	1	0-3
05/03/02	Livorno	7.97	13.5	0	0	2.9	1.8-4.1	12.13	1.48-3.11	0.9	0-2.7
08/03/02	M.di Massa	8.28	14	0	0	0	0	0	0	0	0
19/04/02	Livorno	8.12	17.5	0	0	33	0-39	6	3-7	4	1-7
17/05/02	Livorno	8.02	19	0	0	25	2-53	7	1-20	7	3-20
20/05/02	M.di Massa	8.2	19	0	0	0	0	0	0	0	0
03/06/02	Livorno	8.28	21	0	0	10	1-24	16	5-21	10	8-15
03/06/02	M.di Massa	8.16	21	0	0	0	0	0	0	0	0

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GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Announcement of an Open Science Meeting on the Core Research Project: HABs in Upwelling Systems

LISBON, PORTUGAL

Instituto Nacional de Investigação Agrária e das Pescas - INIAP-IPIMAR

17-20 November 2003

Conveners

Teresa Moita, Portugal
Grant Pitcher, South Africa

Co-ordinating Committee

Francisco G. Figueiras, Spain
Raphe Kudela, USA
Trevor Probyn, South Africa
Vera Trainer, USA

GEOHAB

The GEOHAB Programme, endorsed by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO, is an international programme aimed at fostering and promoting co-operative research directed toward improving the prediction of harmful algal bloom events.

Core Research Project: HABs in Upwelling Systems

The GEOHAB Core Research Project on Harmful Algal Blooms (HABs) in Upwelling Systems must be comparative, interdisciplinary, and international. It will directly address the goal of GEOHAB of improved prediction of HABs by determining the ecological and oceanographic mechanisms underlying their population dynamics, integrating biological, chemical, and physical studies supported by enhanced observation and modelling techniques.

Upwelling systems can be classified according to their physical, chemical and biological characteristics. Development of a Core Research Programme on HABs in Upwelling Systems is built on the premise that understanding the ecology and oceanography of HABs in upwelling systems will benefit from a comparative approach. The comparative method is the method of choice when controlled experimentation is not practical. To the extent that experimental control in the study of marine ecosystems is problematic, comparison presents an alternative for drawing scientific inference. Comparisons will allow the grouping of harmful species from similar habitat types. The extent, to which HAB species respond in a similar way, in systems which share similar characteristics, will assist in establishing the oceanographic processes that influence HAB population dynamics and community interactions. Equally important will be identification of similar systems that do not have the same functional HAB species or groupings. Understanding the response of harmful algae to perturbations within upwelling systems will assist in prediction, and identification of divergences from predicted responses will also be informative. Sharing of expertise and resources, the formulation of common research objectives and methods, and the implementation of similar research activities and field investigations in each of the designated upwelling systems will permit comparison.

Invitation

This announcement serves as an invitation to the broad scientific community to participate in the formulation and design of a GEOHAB Core Research Project on HABs in Upwelling Systems. Scientists working in physical, chemical and/or biological disciplines related to harmful algal research, and on the development of relevant instrumentation and models are encouraged to participate.

Meeting Format and Objectives

Monday & Tuesday:
Presentations relating to our current knowledge and understanding of HABs in upwelling systems. Presentation topics will relate to:

- Identification of the HAB species in given upwelling systems
- Identification of the physical, chemical and biological processes that define or characterise upwelling systems and quantification of the response of HAB species to these processes
- Development of models of HABs in upwelling systems to support fundamental research and predictive capabilities.

Wednesday & Thursday:

- Review of current national and regional projects/programmes in order to identify elements of research that could contribute to the Core Research Project.
- Formulation and design of a plan to guide core research in upwelling systems.
- Identification of framework activities to support the research plan.
- Identification of interested participants and designated regions for comparative research.

Friday:

- A GEOHAB Core Research Project Planning Committee will meet in closed session to finalise a report of the Open Science Meeting.

For further information:

About the GEOHAB Open Science Meeting: http://ioc.unesco.org/geohabcore/

About local arrangements please contact: *Teresa Moita, Email: tmoita@ipimar.pt or Sofia Palma, Email: sofia@ipimar.pt, Instituto Nacional de Investigação Agrária e das Pescas, Av. Brasília 1449-006 Lisboa, Portugal, INIAP-IPIMAR (website: http://ipimar-iniap.ipimar.pt)*

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Announcement of an Open Science Meeting on the Core Research Project: HABs in Fjords And Coastal Embayments

VALPARAÍSO, CHILE

COMITE OCEANOGRAFICO NACIONAL

26-30 April 2004

Conveners

Leonardo Guzman, Chile
Allan Cembella, Germany

GEOHAB

The GEOHAB Programme, endorsed by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO, is an international programme aimed at fostering and promoting co-operative research directed toward improving the prediction of harmful algal bloom events.

Core Research Project: HABs in Fjords and Coastal Embayments

The GEOHAB Core Research Project on Harmful Algal Blooms (HABs) in Fjords and Coastal Embayments must be comparative, interdisciplinary, and international. It will directly address the goal of GEOHAB of improved prediction of HABs by determining the ecological and oceanographic mechanisms underlying their population dynamics, integrating biological, chemical, and physical studies supported by enhanced observation and modelling techniques.

The overall objective is to understand and quantify the critical processes underlying HAB population and community dynamics in fjords and coastal embayments at temperate latitudes.

Fjords and coastal embayments are combined in this GEOHAB Core Research Project because they share features such as the importance of geographical constraints on water exchange and bloom retention and the dominance of meso-scale structures. Classic fjords, usually characterised by a high ratio of length to width, a deep wedge-shaped basin, freshwater input, and a sill located toward the mouth, create retention and/or initiation zones that favour the proliferation of a particular suite of HAB species. Many groups of key species (e.g., *Alexandrium* spp., *Pseudo-nitzschia* spp., and various raphidophytes) are virtually identical in fjords in the Northern and Southern hemispheres at similar latitudes. Such ecosystems are often only marginally affected by human activities because of low population densities, thus they are usually not subject to eutrophication. Coastal embayments are a broader category of an ecosystem type; generally, such systems comprise relatively shallow nearshore marine environments, partially surrounded by land, and often affected by terrigenous run-off, but on a smaller spatial scale than open coastal or upwelling systems. As with fjords, the hydrodynamic processes may be complex, with an accentuated role of tidal flux, storm surges, wind-driven mixing, and salinity and thermal stratification. The physical processes associated with HABs in these systems are most often related to «density adjustment» problems, that is, buoyancy and frontal dynamics, geostrophic adjustment, establishment of a pycnocline after a storm and perhaps topographic frontal motion. The effects of benthic-pelagic coupling are likely to be crucial in understanding HAB dynamics in fjords and coastal embayments. Coastal embayments with limited exchange to the open coast may serve as «seed beds» for benthic cysts or relict populations of HAB species. Such systems are particularly vulnerable to anthropogenic changes in the biological and chemical regime, and the introduction of exotic species via deballasting and transfer of aquaculture stock. Many fjords and coastal embayments are well characterised in terms of long-term plankton records and toxicity events. Optical data sets on ocean colour and relevant plankton patches are becoming increasing available from these systems. Furthermore, basic circulation models (both 2-D and 3-D) are already available from several locations around the world.

Overall Objective: To understand and quantify the critical processes underlying HAB population and community dynamics in fjords and coastal embayments in temperate latitudes.

Invitation

This announcement serves as an invitation to the broad scientific community to participate in the formulation and design of a GEOHAB Core Research Project on HABs in Fjords and Coastal Embayments. Scientists working in physical, chemical and/or biological disciplines related to harmful algal research, and on the development of relevant instrumentation and models are encouraged to participate.

For further information:

http://ioc.unesco.org/geohabcore/ or http://www.jhu.edu/scor/OSM2Program.htm

About local arrangements please contact: Leonardo Guzmán (lguzman@ifop.cl)

HARMFUL ALGAE NEWS

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Phytoplankton blooms in the North Atlantic: results from the Continuous Plankton Recorder survey 2001/2002

The Continuous Plankton Recorder (CPR) survey was instigated by Alister Hardy in 1931 and has since evolved over a period of seven decades into a unique marine monitoring programme. The survey contains biological information on over 450 taxa of phytoplankton and zooplankton and provides the scientific community with its only measure of the state of oceanic plankton over oceanic space and decadal time scales. The self-contained automatic plankton recorders are towed at monthly intervals by ships-of-opportunity along a number of transects that continuously collect plankton from a standard depth of 7 m. Using this simple but highly cost effective technique, CPRs have been towed by volunteer ships for over 4 million nautical miles, resulting in the collection of nearly 200,000 plankton samples from all over the North Atlantic. On certain standard routes there now exists a virtually unbroken monthly coverage going back to 1948. In this brief article we highlight some of the CPR surveys phytoplankton results recorded in the North Atlantic for the year 2001/2002. In the case of the CPR survey, the term 'unusual' or 'large' in reference to phytoplankton blooms refers to a population increases greater than four standard deviations above the species recorded baseline mean (baseline mean: 1980-2000). Notable blooms that are geographically large refer to patch sizes greater than 100 km in diameter. The majority of data analysis has been performed to highlight the year 2001 in context to the long-term dataset. 2002 sample data is presented here as preliminary and has not yet undergone quality checks.

The spring bloom in the North Atlantic 2001

In early February 2001 in the North Sea a couple of early diatom blooms occurred including *Odontella regia* and *Thalassionema nitzschioides*. A very

(Cont'd on p. 2)

• Japan

The «Red Tide Watcher» Project

Eutrophication of the coastal waters in Asia has been accelerated by rapid economic growth, leading to increased frequency and intensity of red tide occurrences in the region. This demands an efficient monitoring of red tides to minimize their negative impacts on fisheries resources, marine ecosystems and human health. Considering the application of satellite remote sensing techniques to monitor red tides that spread widely in a short time, the «Red Tide Watcher» project sponsored by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) was initiated in October 2002. This project aims at

(Cont'd on p. 9)



Participants of the first workshop on red tide monitoring in the Asian coastal waters.

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(Cont'd from p. 1)

early bloom of *Thalassionema nitzschioides* developed in the second week of February on Georges Bank (north-west Atlantic) which was also present the following month. *Rhizosolenia imbrica shrubsolei*, *Thalassiosira* spp. and *Thalassionema nitzschioides* dominated early March and April blooms in the North Sea. Peak spring biomass reached a maximum in the third week of March in the North Sea, one week earlier than the baseline mean (1948-2000). Early April saw the development of a large bloom of *Thalassiosira* spp. off the Celtic Sea shelf edge and in the Bay of Biscay. The dominating species in the shelf areas of the north-west Atlantic (Georges Bank, Scotian Shelf and the Grand Banks) around this time were *Rhizosolenia hebetata semispina* and *Chaetoceros* spp. The northern shelf edge of the Grand Banks continued to experience very large blooms of *Chaetoceros* spp. well into June.

Overall phytoplankton biomass 1948-2001

Figure 1 shows the long-term monthly values of phytoplankton colour (an index of total phytoplankton biomass) in the central North Sea from 1948 to 2001. It is obvious from the figure that there has been a large increase in phytoplankton biomass since the late 1980s. From the late 1940s to the late 1980s, the majority of biomass was restricted to the spring and autumn bloom periods, i.e. diatoms dominated the phytoplankton assemblage. Since the late 1980s, biomass has increased throughout the seasonal cycle. While spring and autumnal blooms values have remained relatively the same compared to the baseline mean (1948-2000), phytoplankton colour has significantly increased during the winter and in particular the summer months. This suggests that the phytoplankton community has shifted from a traditional dominance of diatoms to flagellates and dinoflagellates. 2001 continued with this trend with particularly high values recorded in July. In other parts of the North Atlantic, high increases in phytoplankton biomass are also seen off the Newfoundland Shelf (with an increase in winter blooms), the Scotian Shelf and the Labrador Sea. In the northern North Atlantic and in the sub-polar gyre, phytoplankton biomass has been declining over the last decade. Taking the North Atlantic as a whole, the large-scale spatial patterns over time show similarities to satellite derived estimates of chlorophyll over the same time-period.

Exceptional and Harmful Algal Blooms (HABs)

As well as providing an index of phytoplankton biomass (phytoplankton colour), the CPR survey identifies approximately 170 phytoplankton taxa. Figure 2 shows the occurrence of exceptional phytoplankton blooms in the North Atlantic and the North Sea in 2001 (the majority of diatom species have been removed, see spring bloom text). Apart from diatom species, by far the most common bloom forming taxa recorded by the CPR survey in 2001 was Coccolithaceae in particular the species *Coccolithus pelagicus*. In early June the CPR survey recorded a massive bloom of Coccolithaceae between Scotland and Iceland approximately 600 km in diameter. Large blooms of Coccolithaceae were also recorded in the northern North Sea and at the entrance of the English Channel in July. An exceptionally large bloom of *Ceratium longipes* occurred off the Scotian Shelf in early July (~ 400 km). Apart from geographically extensive blooms, of particular note are the occurrences of Harmful Algal Blooms (HABs) in the North Sea. Large blooms of *Dinophysis* spp. occurred off north-east Scotland in July and August and off the northern Danish coast also in July. Although not considered toxic, large blooms of *Ceratium furca*, such as those recorded in the northern North Sea in July and August, can lead to hypoxia which have been known to have a detrimental effect on benthic fauna. The red-tide forming species *Noctiluca scintillans* occurred in July off the Dutch coast and in August

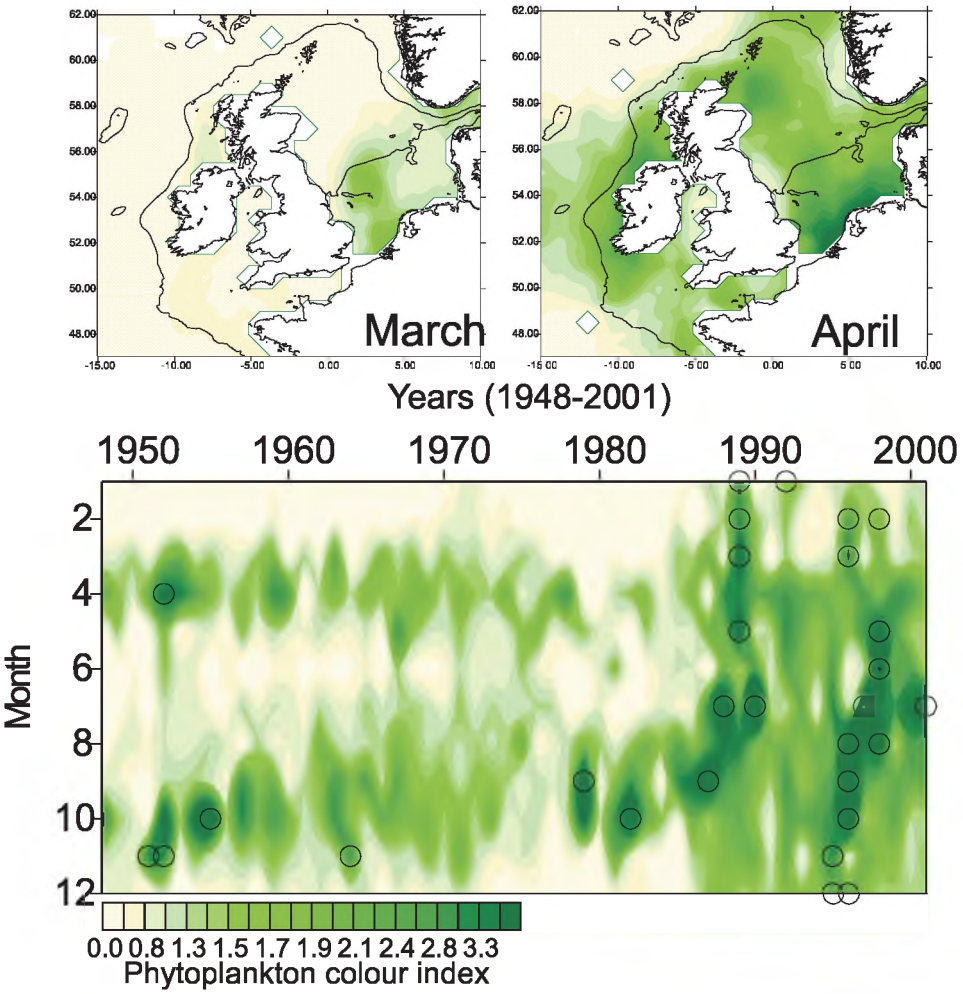

Fig. 1 (top) Typical early development of the spring bloom in the north-east Atlantic, beginning on Dogger Bank, the Southern Bight and at the entrance to the Skagerrak in March and spreading to other shelf areas in April; (bottom) long-term monthly values of phytoplankton colour in the central North Sea from 1948 to 2001. Circles denote colour values >2 SD above the long-term monthly mean.

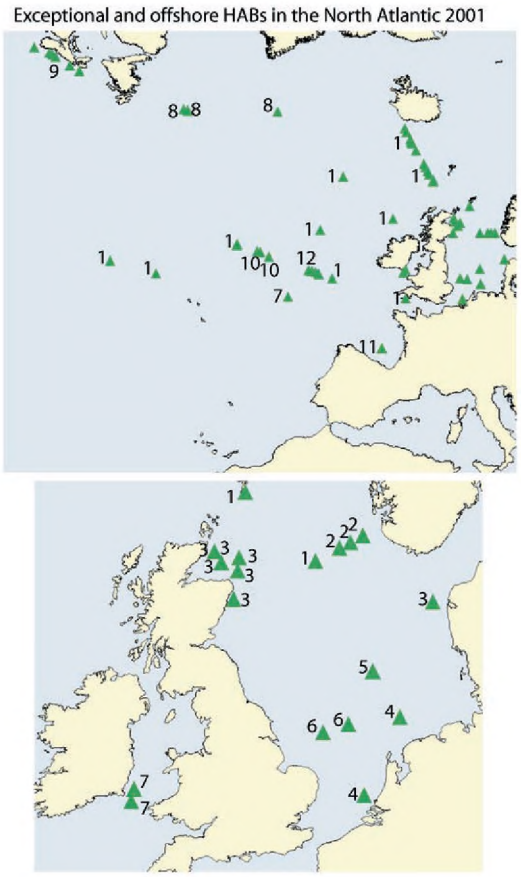

Fig. 2. Exceptional and offshore HABs recorded by the CPR survey in the North Atlantic (conical projection) and North Sea in 2001. Exceptional blooms include Coccolithaceae and all dinoflagellates and species associated with Harmful Algal Blooms (all diatom species except Cylindrotheca closterium, Leptocylindrus danicus *and* Thalassiothrix longissima *have been excluded). 'Exceptional blooms' are species that have occurred 4 standard deviations above the species recorded sample mean (baseline mean 1980-2000).*
1: Coccolithaceae, 2: Ceratium furca, *3:* Dinophysis *spp., 4:* Noctiluca scintillans, *5:* Ceratium macroceros, *6:* Ceratium fusus, *7:* Cylindrotheca closterium, *8:* Thalassiothrix longissima, *9:* Ceratium longipes, *10:* Exuviaella *spp., 11:* Leptocylindrus danicus, *12:* Gonyaulax *spp.*

in the Southern Bight. Of other note was the occurrence of *Cylindrotheca closterium* recorded in June at the southern entrance to the Irish Sea. This diatom species has been associated with the production of foam and mucilage. Preliminary evidence for 2002 suggests that there have been extensive blooms in the Labrador Sea of *Ceratium acticum*, *Detonula* spp. and *Navicula planamembranacea*. Large blooms of *Thalassiothrix longissima* have also been recorded in the central North Sea in early spring 2002. The presence of this species in such large numbers is associated with large influxes of Atlantic water and hence nutrients into the North Sea. Future work using the CPR survey results will include an investigation into whether phytoplankton blooms are increasing over the last few decades in the North Atlantic and in particular around suspected coastal eutrophicated regions and whether phytoplankton blooms are predominately driven by climatic or anthropogenic changes.

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CALL FOR RESEARCH

GEOHAB invites research consortia and individual researchers to submit outlines of their proposals or already funded projects for GEOHAB endorsement.

GEOHAB will only be successful when a critical mass of research is networked and thereby can benefit from interaction and common framework activities.

GEOHAB in particular invites research on HAB in upwelling systems, and fjords and coastal embayments at the present time.

See all details on participation in GEOHAB at http://ioc.unesco.org/hab/GEOHAB.htm

? Taxonomy problems ?

The IOC Taxonomic Reference List of Toxic Microalgae is regularly updated and is now presented in a new attractive format.

The list is intended as an authoritative reference that reflects current use of names and synonyms.

The Editors are Ø. Moestrup, (Chair), G.A. Codd, M. Elbrächter, M.A. Faust, S. Fraga, Y. Fukuyo, G. Cronberg, Y. Halim, F.J.R. Taylor and A. Zingone.

http://ioc.unesco.org/hab/data.htm

CODEX revision

The CODEX ALIMENTARIUS Committee for Fish and Fisheries Products has recommended that an expert review be made with the view to revise the CODEX Code of Practice for Marine Biotoxins. An WHO-IOC-FAO Expert Consultation has been set up to address this and a first workshop was held at the Food Authority of Ireland, 22-24 March. The final expert consultation will be held in Oslo, Norway, in September. This work has merged with the IPHAB Task Team on Biotoxins and is chaired by the IPHAB Vice Chair, Dr. Phil Busby, New Zealand. More information at http://www.who.int/foodsafety/en/ under 'Upcoming Events'.

• Italy

Microcystin concentrations in water and ichthyofauna of Massaciuccoli Wetlands (Tuscany)

Summary/Abstract

The present report deals with the analysis of the water and the ichthyofauna of the Massaciuccoli wetland (Tuscany, Italy), where blooms of *Microcystis aeruginosa* have been observed since about ten years ago. A monitoring study based on monthly collections of water samples was carried out from June to November 2003 and the results show microcystin levels greater than 160 μg L^{-1} in superficial waters. Microcystin reaches the highest levels in the liver of *Mugil cephalus* (10,900 μg L^{-1}) and in the "head" of the allochthonous crustacean *Procambarus clarkii* (1,092 μg L^{-1}). When compared with the control group, an increase of the concentration of the toxin was detected in the liver of *Carassius auratus* experimentally reared for one month in lake water collected during blooms of *Microcystis aeruginosa.*

Introduction

Massaciuccoli Lake, which originated from the sea, lies within a large wetland, partly reclaimed, situated near the coast between Torre del Lago and Viareggio (Lucca); the lake covers an area of approximately 700 ha, has an average depth of 1.6 m, a maximum depth of 2.5 m and an average volume of about 11,000,000 m^3.

The lake is connected to the sea by its only effluent, the Burlamacca channel, situated on the northwest side of the wetland. The waters of the Massaciuccoli ecosystem are brackish, with the highest concentrations of chlorides - about 4,000 mg L^{-1} - near the hydraulic doors, located at the end of the Burlamacca channel; the lowest concentrations of Cl^- (600 to 1,000 mg L^{-1}) can be found within the lake basin. The lake has no affluents: mainly water is pumped into the lake by the water-scooping machines of reclamation works, located for the most part on the southern side of the lake. Human pressure on this ecosystem consists mainly of emissions from sewage purification plants of the neighboring villages, and of water draining from intensive agriculture, and has caused a rapid increase in eutrophication in the last 50 years. In 2003 the average concentration of chlorophyll *a* was 48.19 μg L^{-1}, with the highest level of 184.3 μg L^{-1}; lake clarity, measured by Secchi disk transparency, was 0.43 m, with a minimum of 0.15 m; the euphotic zone depth (Zeu 5%) was 0.85 m, and the average concentration of Cyanophyceae was 825,000 cells mL^{-1}, which corresponds to 94% of the total number of cells [1].

Since 1972, endemic blooms of the microalga *Prymnesium parvum* have occurred in Massaciuccoli Lake, causing large periodic fishes mortalities [2,3]. Increasingly large blooms of *Microcystis aeruginosa* have been observed in the last ten years, and often lead to the formation of dense scums. The blooms of *Prymnesium parvum* occur in wintertime and in early spring, while the blooms of *Microcystis aeruginosa* take place in summer and autumn. The regular alternation of the blooms of the two species shows a reciprocal allelopathic effect induced by each other toxins.

In this framework, we performed the present research to: 1) monitor the concentrations of *M. aeruginosa* and microcystin in the waters of the Massaciuccoli ecosystem; 2) study the intake and the accumulation of toxins in body tissues of the ichtyofauna; 3) identify the fish species and tissues where the toxin reaches highest concentrations. This study should be regarded as well as an attempt to assess the potential threat to public health posed by the ingestion of fishes or crustaceans contaminated by algal toxins: according to the World Health Organization [4], the TDI (tolerable daily intake) of microcystin LR (the most toxic for humans), in relation to hepatic pathologies, is 0.04 μg kg^{-1} of body weight. Therefore a 70 kg adult should not exceed a daily dose of 2.8 μg of microcystin.

Material and methods

Water samples (3 liters) were collected monthly from June to November 2003 with a set of three one-meter-long PVC pipe cores, dipped perpendicular to the surface, which could be closed at one end.

M. aeruginosa counts were performed in 1 or 2 mL of water with an inverted microscope at 100X and 600X.

Microcystin concentrations in water were determined with the ELISA immunological procedure; in fishes and in *Procambarus* the tests were conducted on water extracts (1:10) of the flesh, of the liver [5], or of the whole body (small Caspian sand smelts - *Atherina mocon* - and crustaceans).

Results

The results of tests conducted on fish (liver, flesh, eggs) and crustacean samples are shown in Table 1, which shows the average, lowest, and highest concentrations of microcystin (μg kg^{-1}) in 44 samples of flesh, liver and bowels, two samples of eggs, three of whole Caspian-sand smelts, four of *Procambarus clarkii* (head and tail), and one whole specimen of *P. clarkii*; Table 1 also reports the amounts the can be safely ingested by a 70 kg adult.

Flesh and liver from *Mugil cephalus* from the Northern Ligurian Sea were analyzed as a control (Table 2), and shown to have extremely low levels of microcystins, probably due to specific links between the g-globulins on the surface of the wells of the test kit and the liver extracts from the fish; an alternative reason may be that *Mugil cephalus* sometimes feeds in estuaries where occasional blooms of Cyanophyceae occur.

As a further control, two specimens of *Carassius auratus,* bought in an aquarium shop, were kept respectively: one in the water of a part of Massaciuccoli wetland called San Rocchino, at a time when there was a consistent scum of *M. aeruginosa,* and the other in tap water. After one month, the concentration of microcystin in the liver of the specimen

Table 1

Species and tissues	Nr. of specimens examined	Microcystin average concentration	Microcystin highest concentration and sampling area	Microcystin lowest concentration	Kg of fish corresponding to 1 TDI for a 70 kg human*
Micropterus salmoides (Lac.) (flesh)	5	1.86	2.7 (Cava Sisa)	1	1.03
Micropterus salmoides (liver)	5	3.42	5.4 (Cava Sisa)	1.2	0.52
Cyprinus carpio (L.) (flesh)	8	1.7	1.57 (Fosso Morto)	1	1.7
Cyprinus carpio (liver)	8	1.62	3.1 (Fosso Morto)	1	0.90
Cyprinus carpio (eggs)	1	1.4	1.4 (Fosso Morto)	1.4	2
Mugil cephalus (L.) (flesh)	10	2.10	5.09 (P. Grande)	1	0.47
Mugil cephalus (liver)	10	5,296	10,900 (P. Grande)	634	0.26 g
Mugil cephalus (eggs)	1		8.5 (P. Grande)		330 g
Iactulurus sp. (flesh)	4	1.23	1.6 (Piaggetta)	1	1.75
Iactulurus sp. (liver)	4	2.67	4 (Piaggetta)	2.1	0.7
Scardinius sp. (flesh)	3	1.40	1.77 (Incrociata)	1.1	1.6
Scardinius sp. (liver)	3	3.70	5.3 (Incrociata)	1.95	0.53
Carassius sp. (flesh)	10	1.24	1.3 (P. Grande)	1	2.6
Carassius sp. (liver)	10	3.23	8.1 (P. Grande)	1	0.35
Atherina sp. (whole)	three 30g specimen	4.2	2.35 (Lake)	1	1.12
Procambarus clarkii (tail)	4	1.43	1.64 (Canale "Le 15")	1	1.70
Procambarus clarkii (head)	4	21886	1092 (Canale "Le 15")	16	2.56 g
Procambarus clarkii (whole)	1	120	120 (Canale "Le 15")	120	23 g
Lepomis gibbosus (L.) (flesh)	2	1.16	1.54 (Canale "Le 15")	1	1.8
Lepomis gibbosus (L.) (liver)	2	12.7	16 (Canale "Le 15")	10	0.17
Anguilla anguilla (L.) (flesh)	1	1.47	1.47 (San Rocchino)	1.47	1.9
Anguilla ang. (L.) (bowels)	1	1.6	1.6 (San Rocchino)	1.6	1.75

* *The TDI is expressed in kg unless otherwise specified*

reared in San Rocchino water was 16 times higher then the concentration in the specimen reared in tap water.

Discussion

The results of this investigation of water samples and ichthyofauna of Massaciuccoli Lake show first of all that blooms of *Microcystis aeruginosa* occur mainly in summer and autumn, either in dry or rainy periods. Besides, during the collection of samples we noticed that scums of *Microcystis aeruginosa* occur on the water surface most frequently on the north-west side of the lake, namely in the west stretch of the Burlamacca channel and in site indicated as San Rocchino. The highest concentrations of *Microcystis* were detected in June and in November (Table 3), while the highest concentrations of microcystin were detected in August, September, and October (Table 4). During the presence of the scums, the concentration of *Microcystis* in superficial waters was very high (200,000 cell mL^{-1}), as well as the concentration of microcystin (> 160 $\mu g\ L^{-1}$). There are no indications that the blooms of *Microcystis aeruginosa* can cause death of fishes. Dead mallards and fishes were occasionally found during the presence of scums, but the causes of their death could not be established.

The highest microcystin concentrations were found in the liver of *Mugil cephalus* and in the head of *Procambarus clarkii*. The high toxin concentration in the flesh of the 10 *Mugil cephaluss* of our sample (5.08 $\mu g\ L^{-1}$) show that a 70 kg adult should ingest 0.47 kg of fish in order to reach the TDI. Therefore, if the *Mugil cephalus* are accurately disemboweled the toxicological risk is relatively low. On the other hand, if the liver is ingested, the TDI is reached after eating 0.5 g of tissue containing the concentration of microcystin detected on average in our study (5,296 $\mu g\ kg^{-1}$), or eating only 0.26 g of tissue with the highest levels of microcystin (10,900 $\mu g\ kg^{-1}$). These results show that ingestion of only one fish liver affected by such high levels of microcystin may pose a serious hazard to humans. The ovaries from *Mugil cephalus* in which the highest concentrations of microcystin are present in their livers do not show particularly high levels of the toxin. In fact, the TDI is reached after the ingestion of 300 g of eggs at the concentrations detected in the present study.

Tests conducted on the head of *Procambarus*, where the bowels of the animal are located, confirm the potential hazard posed by this crustacean. The average concentration of

Table 2

Species and tissues	Nr. of specimens examined	Microcystin average concentration	Microcystin highest concentration and sampling area	Microcystin lowest concentration	Kg of fish corresponding to 1 TDI for a 70 kg human*
Sea *Mugil* (flesh)	3	1.35	1.7	1	1.64
Sea *Mugil* (liver)	3	2.20	2.4	2	1.27
Carassius without *Microcystis* (flesh)	1	1			2.8
Carassius without *Microcystis* (liver)	1	9.8			0.28
Carassius with *Microcystis* (flesh)	1	1			2.8
Carassius with *Microcystis* (liver)	1	159			17 g

* *The TDI is expressed in kg unless otherwise specified*

Table 3

Sampling area	Nov 02	Dec 02	Jan 03	Feb 03	Mar 03	Apr 03	May 03	Jun 03	Jul 03	Aug 03	Sep 03	Oct 03	Nov 03	High	Low	St. Dev.
Middle of the lake	250	50	0	0	0	10	400	6,000	4,400	2,000	1,500	3,200	1,200	6,000	1,500	2,037
Malfante	60		30	0	0	0	0	15,000	3,000	2,600		4,000	2,600	15,000	2,600	4,664
Burlamacca	30		0	0	0	0	0	12,500	3,200	6,600	58,634	3,700	600	58,634	3,200	17,349
S. Rocchino	30	0	0	0	0	0	0	12,000	3,400	1,000	1,520	3,400	1,500	12,000	1,000	3,470
Incrociata	120		0	0	0	0	0	14,500	600	2,500		5,400	1,200	14,500	600	4,626
Canale "Le 15"	95	0	2	30	0	10	50	12,000	1,000	1,200	2,010	0	800	12,000	0	3,412
Fosso Morto	0		0	80	0	0	0	13,000	0	2,600		14,500	800	14,500	0	5,725
Centralino	120		0	0	0	0	0	10,000	0	1,400		4,200	15,000	10,000	0	3,247
Barra					0		0		5,200							
Punta Grande						10										
S. Rocchino surface												200,000				
Highest values	250	50	30	80	0	10	400	15,000	5,200	6,600	58,634	14,500	15,000			
Rain (mm)	*123.2*	*161.5*	*43.5*	*15.0*	*22.7*	*80.2*	*0.6*	*48.4*	*1.8*	*35.9*	*61.6*	*93.4*				

Table 4

Sampling area	Nov 02	Dec 02	Jan 03	Feb 03	Mar 03	Apr 03	May 03	Jun 03	Jul 03	Aug 03	Sep 03	Oct 03	Nov 03	High	Low	St. Dev.
Middle of the lake	0.60	0.19	0.10	< 0.1	< 0.1	0.20	>1.6	1.42	1.02	< 1	3.31	2.90	<1	3.31	1.02	1.25
Malfante	0.70		< 0.1	< 0.1	< 0.1			4.81	1.67	1.44		5.60	1.1	5.60	0.70	2.20
Burlamacca	< 0.1		< 0.1	< 0.1	< 0.1	< 0.1		3.61	1.70	3.58	> 16	6.90	<1	6.90	1.70	2.16
San Rocchino	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		3.94	< 0.1	< 1	12.45	>16	6.9	>16	3.94	6.02
Incrociata	< 0.1							4.11	< 0.1	1.22		2.00	<1	4.11	1.22	1.50
Canale "Le 15"	< 0.1	< 0.1					0.34	2.43	< 0.1	< 1	< 1		<1	2.43	0.34	1.48
Fosso Morto	< 0.1							3.71	< 01	> 16		5.00	<1	>216	3.71	0.91
Centralino	4.20				0.20			2.78	< 0.1	> 16			<1	>16	0.20	2.03
Incr. Superf.									2.25					2.25	2.25	
Barra							0.7									
S. Rocchino surface												>160				
Highest values	4.2	0.19	0.10		0.20	0.20	>1.6	4.81	1.70	>16	>16	>16	6.9			

microcystin was 218.9 µg Kg^{-1}, with a peak of 1,092 µg Kg^{-1}, and the TDI could be reached after the ingestion of only 2.56 g. The microcystin concentration of a homogenated extract of three full *P. clarkii* was 120 µg Kg^{-1}, and the TDI corresponded to the ingestion of 25 g of specimen.

In the other species, the TDI was reached after a daily intake of flesh > 1 kg and the risk of toxic effects for the consumer is very low.

Based on these results, fishing of *Mugil cephalus* and of *Procambarus clarkii* as well as sport fishing have been forbidden in Lake Massaciuccoli and in the wetland; recreational activities have also been prohibited in the periods and in the areas where the presence of scums was observed.

Acknowledgments

We would like to thank Dr. Ercolini and a number of professional fishermen for the help provided us in collecting samples.

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PICES Working Group HAB, Honolulu

At the PICES Annual Meeting in Honolulu, Hawaii from 15-23 October, the a joint PICES-IOC workshop on 'Developing a North Pacific HAB data resource-II' will be held with Hak-Gyoon Kim (Co-Chair), and Vera Trainer (Co-Chair)) and Henrik Enevoldsen (IOC) as conveners. At last year's joint PICES/IOC Workshop on "Harmful algal blooms – Harmonization of data", representatives from PICES member countries accepted an offer from IOC/ICES to utilize their successful harmful algal event meta-database (HAE-DAT) format on a trial basis. The goal of the 2004 workshop is to provide an interim "report card" on the use of this database. The central tasks are to ascertain how well the database process worked and to identify any difficulties in data delivery from member nations.

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• Mexico

Marine die-offs from *Chattonella marina* and *Ch.* cf. *ovata* in Kun Kaak Bay, Sonora in the Gulf of California

In Kun Kaak Bay, Sonora, Mexico, fishermen observed extensive fish mortality during early April 2003. They also observed large numbers of dead pen shells, clams, octopus, and sea cucumbers in the bottom layers and sea floor. These observations were concurrent with reports of green-yellow discoloration at the surface. During May, underwater surveys were carried out and samples were taken to assess phytoplankton and nutrients where surface discolorations were densest (Fig. 1). Abiotic data were obtained for temperature using a bucket thermometer (precision ±0.1°C), salinity with a hand-held refractometer (precision ±1 psu), and nutrients using a commercial kit (HACH) [1]. Surface samples taken with a bottle were fixed (1 ml Lugol's acetate in 100 ml sample) for plankton observations. Samples of 10 ml were allowed to settle for 24 hours and observed with a phase contrast inverted microscope adapted with a Whipple disc (1 mm^2 divided in 100-µm squares). Photomicrographs were obtained using an inverted phase contrast microscope at 200X.

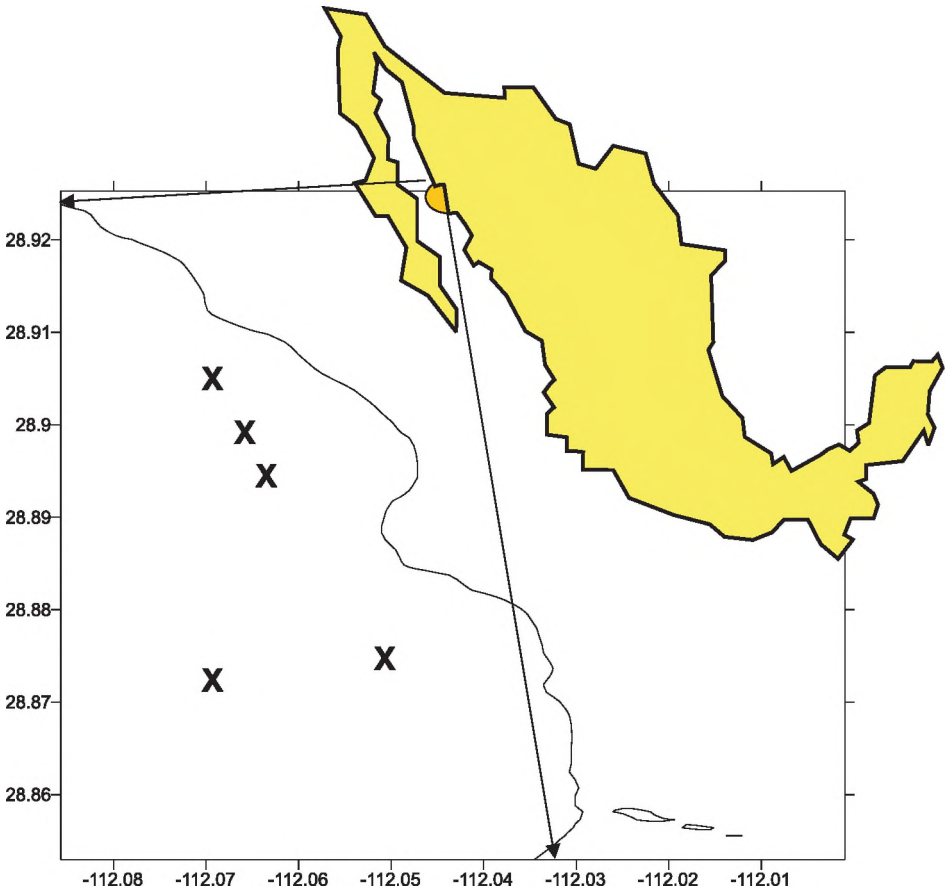

Fig. 1. Map of the Kun Kaak Bay area, showing location of the sampling stations and extent of the red tide.

Results

Underwater observations detected a thick viscous layer on the bottom, sometimes forming balls. Fish and mollusks were in an advanced stage of putrefaction, precluding its use for detecting toxins. Estimated area covered by this event was 95 km^2 (Fig.1). This area is recognized as a natural reservoir of *Aterina tuberculosa* (pen shell) and *Callinectes sp.* (blue crab), where stocks were very abundant. Both fisheries were severely damaged. Currently, very few individuals of these two species are found, severely affecting the livelihood of more than 60 diver-fishermen. Global Greengrants Fund is currently assessing the damage suffered on the different fishing banks. Stranded fish collected at the local beaches were used to study affected species (Table 1). It was not possible to assess the volume of stranded fish because local authorities removed the dead fish daily. Fishermen observed that fish were carried southward by local currents to distant beaches. In the region most severely affected, laboratories raising post-larvae shrimp were in operation. They reported bad odours coming from the water and up to 40% loss in post-larvae. Mortality level was controlled by increasing ozonization of the intake water.

Quantitative plankton analyses, based on shape and size [3], indicate a strong dominance by *Chattonella marina* (Fig.2). Those with size >70 µm may represent *Chattonella* cf. *ovata* (9%) (Fig. 2a). Abundance reached 480 to 568 cells ml^{-1} at coastal sampling stations, 324 cells ml^{-1} one km off shore, and 142 cells ml^{-1} on the sea bottom (Table 2). Even though many ce-

Table 1. List of species stranded in high numbers during the HAB in April, 2003

FISH	
Common name	Scientific name
Burrito	*Haemulopsis nitidus*
Tieso del Pacífico???	*Ophichthus triserialis*
--------	*Congriperla estriada*
--------	*Ophiodon galaeoides*
Jawfish	*Gnathypops snyderi*
Lenguado tapadera (flatfish)	*Citarichthys gilberti*
BENTHOS	
Callos de hacha (penshells)	*Atrina maura*
	Atrina tuberculosa
	Pinna rugosa
Almeja reina (clam)	*Dosinia ponderosa*
	Megapitaria aurantiaca
Almeja chocolata (clam)	*and Megapitaria squalida*
Almeja china (clam)	*Chione gnidia*
Caracol rosa espinoso (conch)	*Hexaplex erythrotomus*
Almeja amarilla, botijona, o hiluda (clams)	*Laevycardium elatum*
Pulpos de diferentes especies (octopi of several species)	

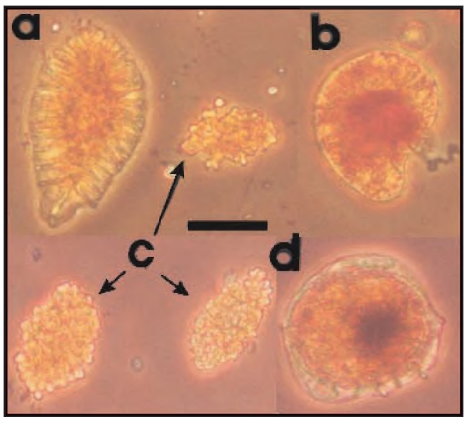

Fig. 2. (a) Vegetative cell of Chattonella marina*; (b) Vegetative cell of* Chattonella *cf.* ovata*; (c) Small cells of* Ch. Marina*; (d) Cyst or sphaeric non-motile cell. Scale bars = 30 µ. Cells fixed with acid Lugol.*

uncommon event and that this species is well adapted to local conditions. This species was recently reported in Mexican waters [4], but it was not recorded as forming blooms and contributing to fish and shellfish mortality. The causes for this HAB may be related to physical conditions that prevailed at this time. Available data indicate that SST was optimal 25.9-26.9°C (25°C [5]), but salinity (36-38 psu) was well above the reported optimum (20 psu [5]). A high concentration of phosphate was depleted over the following few days (Table 3). The sources of phosphate are natural seasonal upwelling and surplus outflow from aquaculture enterprises in the area (larvae-producing laboratories and extensive shrimp farms). The phosphate allows blooms to last longer, hence severely affecting marine biota and the marine environment, in general. Nevertheless, available data does not help us determine the contribution of each source, but common sense suggests a few ways to control anthropogenic inputs to the environment. This includes construction of retention dams and natural filters to reduce organic matter and excess nutrient by-products of aquaculture.

Table 2. Phytoplankton abundance (cells ml^{-1}) in Kun Kaak Bay, Sonora, Mexico on 17 May 2003

Raphidophyceae	E-1	%	E-2	%	E-2 f	%	E-3	%	E-4	%	E-5	%
Chattonella marina	568	25.53	324	51.8	142	24.7	518	57.9	555	53.8	480	35.5
Dinophyceae	691	31	232	37.1	36	6.31	187	20.9	311	30.2	128	9.48
Bacillariophyceae	952	42.7	58	9.28	388	67.4	173	19.3	129	12.5	728	53.8
Euglenophyceae	4	0.17	0	0	0	0	0	0	6	0.55	0	0
Dictyochophyceae	13	0.6	11	1.76	10	1.67	17	1.9	19	1.86	17	1.27
Cyanophyceae	0	0	0	0	0	0	0	0	12	1.12	0	0
Total	2229	100	625	100	576	100	895	100	1031	100	1353	100

Table 3. Nutrients (mg l^{-1}) in samples from Kun Kaak Bay,

	17 May 2003		22 May 2003	
Sampling Place	NO_3	PO_4	NO_3	PO_4
E1	0.2	1.09	1.5	0.29
E2	0.1	1.09		
E2F	0.0	0.72		
E3	0.1	0.54		
E4	?	?		
E5	0.1	>3.0	1.4	0.3
Canal Entrance			1.8	0.26

lls were deformed, the cells were easily distinguished and measured: (n = 43); length 30-80 µm (½ 49.7) and width 15-47 µm (½ 29.7). Mean length was slightly less than the reports of Band Schmidt *et al.* [4] (a difference of 12 to 27 µm). According to Imai *et al.* [5] *Ch. marina* (Fig. 2b) may be present in a range of sizes, as reported here. *Ch. marina* cells >50 µm represented 35% (Figs. 2 a and b) and cells <50 µm amounted to 65% (Fig. 2c). Some cysts were observed as well as circular non-motile cells [4] (Fig. 2d). We observed all cell cycle stages reported by Imai *et al.* [5] including pre-encystment, haploid and diploid small cells, and vegetative G1 and G2 phase cells. These data support the idea that this was not an

Consequences

Kun Kaak Bay has been recognized as a zone of high productivity for some time [6]. It is under the influence of upwelling currents and is well known for high primary productivity of diatoms, of which, *Stephanopyxis palmeriana* is distinctive [7]. Recently, dominance has shifted to dinoflagelates and rhaphydophytes, a well known indicator of euthrophication. Consequently, there is a serious risk to aquaculture and fishing in the region from noxious and toxic blooms, as shown here. This event should be considered a warning: blooms may increase and become periodic, in turn disrupting primary economic activities and impacting human and marine animal health. This event also calls for development and support of permanent monitoring activities and research that may provide information for better management of HABs, including mitigation.

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(Cont'd from p. 1)

the establishment of mechanisms for dissemination and exchange of red tide related information among scientists and technicians in organizations responsible for the monitoring in Asia and the neighboring countries.

To seek a suitable approach to be use of remote sensing for red tide monitoring, the first Workshop on «Red Tide Monitoring in the Asian Coastal Waters» was held at the University of Tokyo in Tokyo, Japan, on 10-12 March, 2003 as a part of the «Red Tide Watcher» project. This workshop provided a forum for both red tide biologists and the remote sensing community to exchange information for enhancing mutual understanding of the problem, and to share common ideas on key issues. The organizers sincerely intended this workshop would be the first step towards establishing a red tide surveillance system in Asia.

The workshop was attended by more than 75 participants from 13 countries representing Japan, Korea, China, Hong Kong, Vietnam, Thailand, Malaysia, Philippines, Singapore, Indonesia, Spain, New Zealand and the USA. Thirty papers were presented covering eight sessions/groups, viz, 1) Introduction, 2) Korean Waters, 3) China Sea, 4) Japanese Waters, 5) Thailand and Philippines Waters, 6) Vietnam, Malaysia and Indonesia Waters, 7) Other Areas, 8) General discussion.

The workshop was fruitful in establishing mutual understanding between remote sensing oceanographers and red tide biologists. A summary of the outcome of this workshop was as follows: 1) Ocean color remote sensing techniques have proven to be a useful tool to detect meso-scale discolorations caused by high-biomass microalgal proliferations (various terms were suggested, such as algal bloom, phytoplankton proliferations, meso-scale discoloration), 2) It was realized that ocean color as well as other remote sensing data (chlorophyll, sea surface temperature, wind etc.) is useful to understand the processes for development and decay of red tides, 3) An understanding of oceanographic processes will allow us to analyze how to use remote sensing data for red tide research and monitoring, 4) Accumulation of knowledge on red tide occurrence was confirmed in various regions, 5) It was strongly felt that cooperation between biologists, satellite experts, oceanographers, and meteorologists is essential. Mutual communication and understanding among the disciplines is a pre-requisite, 6) Species level information is essential for red tide monitoring, 7) An optical approach is promising in identifying responsible functional groups of organisms, 8) In order to derive red tide information from remote sensing data, optical research on species-specific characteristics should be enhanced, 9) Specification of *in situ* database should be redefined to fit interpretation of satellite derived data, 10) Development of algorithms for optically complex water masses should be promoted. 11) The combined use of *in situ* mooring instruments is recommended, 12) Future real-time monitoring system of red tide should be discussed on the basis of scientific and technological researches using *in situ* and remote sensing methods.

After the workshop, several activities of the project followed. These included: 1) A small symposium for the standardization of species identification was held in Nagasaki, Japan, on 21-27 September, 2003, to list important red tide species/genera that cause taxonomic problems, to identify the quality of the problem in each species/genus, and to establish strategies to solve the identified problem. 2) The first Korea-Japan Workshop on Ocean Color (KJWOC) was held in Nagasaki, Japan, on 10-12 January, 2003. The 1st KJWOC was attended by more than 30 participants. Twenty-two papers were presented covering seven sessions, viz, 1. Opening, 2. Satellite Systems, 3. Atmospheric Correction and In-Water Algorithms, 4. Red Tide, 5. North Pacific Ecosystems, 6. North Pacific and Other Ecosystems, 7. General discussion and Closing. The 1st KJWOC was fruitful and a good first step for collaboration between Korea and Japan. 3) A multi-disciplinary survey on a whole-bay scale red tide was conducted in Manila Bay from March 19 to 23, 2004. The survey aimed to understand bio-optical properties and physiological ecology of green *Noctiluca* (*Noctiluca scintillans* with the endosymbiont *Pedinomonas noctilucae*). Subjects examined during the cruise include spatial distribution of phytoplankton, growth, grazing, photosynthesis and respiration of *N. scintillans*, and bio-optical properties of the *Noctiluca* bloom.

Cooperation among satellite oceanographer and red tide biologists, coupled with efficient use of *in situ* and remote sensing techniques, would strengthen the future real-time monitoring system of red tide.

For further information, please visit our website: http://fol.fs.a.u-tokyo.ac.jp/rtw/

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HANA Network: Harmful Algae of North Africa

At the «Regional Training Course on Harmful Algae» organised by IOC of UNESCO in cooperation with the AECI, NAUTA, IEO, COPEMED and DANIDA, from 1 to 12 December 2003 at the INSTM, Salammbô/La Goulette, Tunisia. Following the initiative of Prof. Y. Halim, the participants established a network for Harmful Algae of North Africa «HANA».

The HANA network was conceived with two general objectives in mind: to promote organized research and monitoring in the region and to establish a link between the HANA region and the international programmes on HABs.

The HANA region (Mauritania, Morocco, Algeria, Tunisia, Libya and Egypt) has considerable marine resources consisting among others of fish and shellfish mariculture and tourism. Economic losses due to harmful blooms are caused by the proliferation of a variety of species belonging to Bacillariophyceae, Cyanophyceae, Dinophyceae, Cryptophyceae and Prasinophyceae. In this region more than elsewhere knowledge about the processes driving the incidence of such blooms is far from satisfactory. Reports on HABs are scattered and often result from unassisted individual efforts. HANA will encourage the exchange of information with the aim of creating a continuously updated data and metadata-base. Three types of information will be collected, archived and made available:

1) The incidence of HABs, the species involved and the concomitant environmental conditions

2) The personnel involved, their areas of specialization and their levels of expertise



Participants and teachers of the IOC Regional Training Course on Harmful Algae.

3) The relevant regional publications.

With the assistance of IOC, HANA will work towards further enhancing research capabilities in the region. Advanced training workshops will be organized and a digital identification guide to harmful species of the region will be developed. Working groups will bring together scientists and managers for exchange of information and experience and for updating their knowledge. Some selected scientists will be given the opportunity to interact with the international scientific community by attending relevant meetings and conferences, as happened with the Working Group on Harmful Algal Bloom Dynamics in Corsica, from 5 to 8 April 2004.

Some steps have already been taken to collect the above information:

- The regional publications
- An inventory of the researchers of the region.

Progress towards the implementation of HANA objectives can only be achieved through intra-regional cooperation in one or more Pilot Projects supported by IOC.

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South America HAB Internet Portal

IOC-UNESCO is for 2004-2005 funding the development of an internet portal on HAB for South America. The project will be implemented by the South American IOC Working Group on HAB (COI FANSA). The portal will include a web based learning component. The project will be initiated at a workshop in Itajai, Brazil, 4-6 June 2004. Contact: Chair COI FANSA, Dr. Leonardo Guzmán, lguzman@ifop.cl

IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB)

The IPHAB is scheduling its next session for March 2005. The IPHAB is established to set priorities of international HAB issues and activities. You can find the IPHAB Terms of Reference, List of Members, and IPHAB reports at http://ioc.unesco.org/hab/act2.htm

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GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

OPEN SCIENCE MEETING: HABs IN UPWELLING SYSTEMS

The Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) programme was initiated in 1999 by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO to develop a research program on the ecological and oceanographic mechanisms underlying the population dynamics of harmful algal blooms (HABs). The end goal of this research is to allow the development of observation systems and models that will enable prediction of HABs, thereby reducing their impacts on the health of humans and marine organisms, as well as their economic and social impacts on societies.

The GEOHAB *Implementation Plan* (GEOHAB, 2003) specifies the formation of Core Research Projects (CRPs) related to four ecosystem types—upwelling systems, fjords and coastal embayments, eutrophic systems, and stratified systems. These CRPs are to be initiated through small, focused open science meetings. The first of these open science meetings was hosted at the Instituto Nacional de Investigação Agrária e das Pescas (INIAP-IPIMAR), in Lisbon, Portugal from 17-20 November 2003, and focused on HABs in upwelling systems. The meeting served to identify interested participants and research regions and to bring together the international community to design core research. Meeting participants discussed a wide variety of research topics related to HABs in upwelling systems, which the meeting planning committee [co-chaired by Grant Pitcher – South Africa and Teresa Moita – Portugal, and including Francisco Figueiras – Spain, Raphael Kudela – USA, Trevor Probyn – South Africa, and Vera Trainer – USA] distilled into 8 high priority research activities:

1 An ecologically based classification of the different harmful species based on their adaptation to the multiple sub-habitats characteristic of upwelling ecosystems. Included in this classification of HAB species in upwelling systems will be the functional role of morphological, physiological, behavioural and life-history characteristics, at the cellular level.

2 Identification of the seed strategies employed by HAB species within upwelling systems. Establishment of the sites of HAB initiation and characterisation of environmental influences on the life history stages of HAB species in upwelling systems is considered a priority in developing a predictive capability.

3 Determination of the influence of small-scale physical processes on the growth and dispersion of HAB species. Turbulent mixing determines much high-frequency environmental fluctuation and in so doing can control nutrient, irradiance, and phytoplankton patchiness, and is also known to affect plankton growth rates. Varying responses in terms of the succession of species within and among upwelling systems will allow inferences of the properties of the upper water column regulating species succession and the development of HABs.

4 An investigation of the nutritional physiology of target species as related to the natural variation in nutrient signals. Although time series, field measurements of nutrient concentrations can provide valuable insight to nutrient dynamics, provided that transboundary fluxes are quantified, direct measurements of regeneration and assimilation rates need also to be performed using isotope tracer methodology. These measurements will serve to provide meaningful input to biogeochemical models that can be employed in a predictive manner when coupled with the primary hydrodynamic forcing typical of upwelling ecosystems.

5 An assessment of genetic predisposition versus environmental conditions in the toxin production of target species in different upwelling systems. Variability in toxin production is likely caused by a combination of genotype and environmental conditions and elucidation of these respective roles in toxigenicity is critical in developing a predictive capability. Differences in the absolute toxicity of a given species in separate upwelling regions may be exploited to allow characterization of genes important in toxin synthesis.

6 Determination of the importance of coastal morphology and bathymetry on the dynamics of HABs in upwelling systems. These influences are responsible for creating alternating patterns of active and passive upwelling circulations along the coast which may serve in creating sites favouring bloom initiation, retention, dispersion, etc. Characterisation of these sites will assist in understanding their role in the dynamics of HABs.

7 Field based observations incor-



The GEOHAB Open Science Meeting on HABs in Upwelling Systems was hosted by the National Institute for Agricultural and Fisheries Research (INIAP-IPIMAR), Lisbon Portugal.

porating measurements of cross-shelf and along-shore advection and their role in the initiation, transport, accumulation and dispersion of HABs. These observations should be made with reference to both vegetative and resting stages of HAB species.

8 Identification of climate indicators as predictors of HAB events in upwelling systems. Evidence exists to suggest that variations in upwelling intensities and locations, and also ecosystems have occurred in concert with warming of the earth's climate. Research is required to relate the effects of climate change, and associated variation in the predominant physical and chemical forcing mechanisms, on HAB species and communities that typify coastal upwelling environments.

Our understanding of and ability to predict HABs in upwelling systems over the next 5-10 years will reflect the extent to which the GEOHAB CRP can answer these questions. The CRP – HABs in Upwelling Systems is built on the premise that understanding the ecology and oceanography of HABs in upwelling systems will benefit from a comparative approach, which is the method of choice when controlled experimentation is not practical. To the extent that experimental control in the study of marine ecosystems is problematic, comparison presents a potentially powerful alternative for drawing scientific inference. Comparisons with respect to HABs will incorporate the grouping of species from upwelling systems. Assessment of the extent to which these HAB species respond in a similar way within these systems will allow the oceanographic processes that influence HAB population dynamics and community interactions to be established. Equally important will be identification of upwelling systems that have dissimilar HAB species or groupings. In addition, understanding the response of harmful algae to perturbations within upwelling systems will assist in prediction, and identification of divergences from predicted responses will also be informative.

The GEOHAB Scientific Steering Committee (SSC) will help provide international coordination for the CRP – HABs in Upwelling Systems, through the establishment of a GEOHAB CRP Subcommittee. This Subcommittee will commit to the promotion of comparative research and the involvement of individuals from the Californian, Iberian and Benguela upwelling regions, and from other major upwelling systems. The subcommittee will be responsible for working with scientists involved in the CRP to ensure that they coordinate their research, using the same measurement protocols, sharing data, and contributing to observation and model development. One or two members of the CRP Subcommittee will be members of the international GEOHAB SSC, to ensure a strong linkage between the Subcommittee and the SSC.

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More detail at http://ioc.unesco.org/hab/GEOHAB.htm and http://www.jhu.edu/scor/GEOHAB.htm and http://ioc.unesco.org/geohabcore/

Working Group on HAB Dynamics

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics met at Corsica, 5-8 March. The tasks for 2004 included the review of existing phytoplankton population dynamics models with particular emphasis on prediction of HAB events, and the review of biological loss processes of selected HAB species. The WG also discussed environmental dynamics and impacts of individual phycotoxins and their metabolites enabled by new analytical technologies. The WG reviewed plans for a planned (2005) Workshop on New and Classic Techniques for the Determination of Numerical Abundance of HAB-species. Every year the WG collate and assess national harmful algal event reports and update the decadal mapping of harmful algal events for the IOC-ICES harmful algal event database, HAE-DAT. Progress was reported in the computerized production of decadal maps from country reports, including the revision of reports already in the database covering the last 10 years. A special task this year was to start preparations to summarize the distribution and number of harmful algal blooms in the North Sea for the period 2000-2004, and any trends over recent decades in the occurrence of these blooms for input to the ICES Regional Ecosystem Study Group for the North Sea in 2006. More detail at http://ioc.unesco.org/hab/act6.htm





The meeting was hosted by University of Liege (Belgium) at their marine field station STARESO in Corsica (France).



The 11th International Conference on Harmful Algae will be held under the auspices of the International Society for the Study of Harmful Algae (ISSHA) and the Phycological Society of Southern Africa (PSSA). The Conference will provide a broad forum for phycologists, microbiologists, toxicologists, physiologists, molecular biologists, aquatic ecologists and managers to address and exchange research findings and perspectives concerning all aspects of toxic and harmful algae. The venue for the Conference will be the Cape Town International Convention Centre on the Foreshore of the Cape Town Business District, South Africa.

Important Dates:
Final Date for Receipt of Abstracts
31 May 2004
Early Registration Deadline
15 August 2004
Late Registration Deadline
15 October 2004
Website: *www.botany.uwc.ac.za/pssa*

Conference Secretariat:
Elizabeth Danckwerts
Telephone: +27 21 683 5522
Mobile: +27 82 468 7504
Fax: +27 21 674 3269
E-mail: *aecon.e@mweb.co.za*

HAB-DIR: International Directory of Experts in Harmful Algae and Their Effects on Fisheries and Public Health

This international directory lists research scientists, fisheries managers, public health officials, aquaculture professionals, physicians, and animal health specialists who are experienced in dealing with toxic and harmful algal event and their impact on fisheries and public health. The directory is intended to improve and accelerate international information exchange and rapid access to experts who can respond quickly to these outbreaks and thus help reduce the consequences to fisheries and public health.

The first edition of this directory was published in 1990 by Woods Hole Oceanographic Institution Sea Grant program. The second edition was a cooperative effort by the U.S. Department of Commerce, U.S. NOAA National Marine Fisheries Service, U.S. Northeast Fisheries Science Center, and the Harmful Algal Bloom Programme of the IOC. The Directory is today an internet based product prepared by the IOC Science and Communication Centre on Harmful Algae, Copenhagen.
The Directory can be updated and amended on-line. Are you already in the Directory? Maybe your data needs to be updated?
http://ioc.unesco.org/hab/data.htm

PUBLICATIONS

The following publications can be ordered from http://ioc.unesco.org/hab/pub.htm:

Manual on Harmful Marine Microalgae, Hallegraeff, G. M. et al (Eds) UNESCO Monographs on Oceanographic Methodology, UNESCO 2003.

Villaba, A., Reguera, B., Romalde J., Bieras, L.R. (Eds) 2003. Molluscan Shellfish Safety, Proceedings of the 4th International Conference on Molluscan Shellfish Safety. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Vigo and Paris. 620 pp.

Microalgues potentiellement nuisibles de l'océan Indien Occidental - Guide basé sur une étude préliminaire / Potentially Harmful Microalgae of the Western Indian Ocean - a guide based on a preliminary survey, IOC Technical Series No. 41.

"Floraciones Algales Nocivas en el Cono Sur Americano" by E.A. Sar, M.E. Ferrario & B. Reguera. Instituto Español de Oceanografía, 2002, 312 pp. (ISBN: 84-95877-01-5; NIPO: 406-02-001-9). (Spanish only)

Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters, by D. M. Anderson, P. Andersen, V. M. Bricelj, J. J. Cullen, and J. E. Rensel. APEC Report # 201-MR-01.1, Asia Pacific Economic Programme, and Intergovernmental Oceanographic Commission of UNESCO, Technical Series No. 59, Paris, France, 2001.

Proceedings of the Ninth International Conference on Harmful Algal Blooms, Hobart, Australia, 7-11 February 2000, Hallegraeff, G.M., et al. (Eds). IOCof UNESCO, 2001.

ISSHA

International Society for the Study of Harmful Algae

http://www.issha.org/

ISSHA President's Corner

April 1-3, 2004 was an historic time for the International Society for the Study of Harmful Algae (ISSHA). It was the first meeting of the Council which consists of the President, two Vice-Presidents, Secretary, Treasurer (the Executive) and 9 of the 14 recently elected Council members from all over the world. Council members represent Harmful Algal Bloom (HAB) disciplines from Denmark, Norway, Sweden, England, Germany, Spain, Italy, Canada, United States, Japan, South Korea, and Australia. The meeting was held in Hornbaek, Denmark, a short distance from Copenhagen, in a rural, beautiful and quiet setting.

The goals of this first Council meeting were to review the objectives of ISSHA, highlight the benefits of ISSHA membership, and determine how ISSHA could affiliate with existing initiatives in the field of harmful algae without competing with those initiatives. Committees on Elections, Membership, Travel Awards, Achievement Awards, Finance, Conference Programs, and Publications were established and goals set. An additional Ad Hoc Committee on Special Projects was established to handle requests for participation in different activities such as HAB maps, expert panels, and support for training programs. Each committee has assignments with deliverables to help achieve Society objectives.

Some examples of assignments: funds will be solicited to support student travel to HAB conferences, including the 11th HAB2004 in South Africa; the ISSHA website will be modified to present more information to members; ISSHA will sponsor HAB conferences, in collaboration with local organizing committees, starting with the 12th conference in Copenhagen; ISSHA members will edit HAB conference proceedings and IOC will publish the proceedings. ISSHA will also consider producing special publications as warranted. Another merger of initiatives is in this Harmful Algae News issue. A section on ISSHA activities will appear in each HAN issue. Just as importantly, ISSHA Council members and other members have pledged to increase the contributions to HAN on a regular basis.

Please go to the ISSHA website at www.issha.org for the posting of the minutes from the recent Council meeting, including a listing of the benefits of ISSHA membership.

First came the Statutes that helped provide the framework for our Society and then this Council meeting gave us the momentum to move on. I would like to personally thank the Executive and Council for their dedication and belief in this young, growing Society of several hundred members. If you are not a member, please join. If you have not renewed, please renew. The membership form is contained in this issue. At 20 USD a year for a membership fee, it is one of the best values around.

See you in Cape Town.

Karen Steidinger, President of ISSHA



The ISSHA Council quorum 2004

ISSHA AWARDS: CALL FOR NOMINATIONS

Current members of ISSHA are invited to submit nominations for the following awards:

The Yasumoto Lifetime Achievement Award

This award is offered in recognition of a long, and outstanding record of contribution to harmful algal research. Previous recipients: Dr. F.J.R. (Max) Taylor (2000), Dr. G.R. Hasle (2002), and Dr. T. Smayda (2002). Other senior scientists similarly honored by the harmful algal research community before ISSHA included Dr. L. Provasoli and Dr. E. Balech.

The Young Scientist Achievement Award

This award is offered in recognition of outstanding achievement in any aspect of harmful algal research by a scientist early in their career (i.e. within ten years of receiving their PhD). The award is intended for an exceptional or "break-through" contribution to an area of harmful algae research, rather than a cumulative record of achievement. Previous recipient: Dr. C. Scholin (2002).

Any ISSHA member may submit nominations for either achievement award, which should provide a complete, yet succinct description of the nominee's contribution (not more than one page). Nominations should be sent by e-mail to Barrie Dale (barrie.dale@geo.uio.no), Chair of the Committee on Achievement Awards, to be received no later than 1 September 2004. It should be noted that ISSHA does not necessarily give achievement awards at every International HAB Conference. Nominations received by 1 September 2004, will be considered by the ISSHA Council, and eventual awards will be presented at the 11th HAB Conference in Cape Town, South Africa, November 2004.

The Maureen Keller Student Presentation Awards

These awards will be presented for the best oral and poster presentations at the 11th HAB Conference. A student is considered eligible for these awards for a period of up to one year following a successful defense of their thesis. A tribute to Maureen Keller may be seen at the following link: http://www.bigelow.org/mk.html.

Travel Awards

A committee has been formed to coordinate efforts to raise and distribute funds for travel to international HAB conferences. The committee is chaired by Don Anderson, with assistance from members of the ISSHA Council (Allan Cembella, Henrik Enevoldsen, Karen Steidinger, Yasuwo Fukuyo, and Beatriz Reguera), as well as other ISSHA members, to be named later. Assistance from additional members of the HAB community is welcome – please contact the chair (danderson@whoi.edu) with suggestions or if you wish to volunteer your services. Of particular interest are suggestions of sources of funds that could be contacted by the Committee.

Travel awards are anticipated for students, postdocs, and researchers, the latter generally being from developing countries. Among the criteria for selection will be the following:

- Membership in ISSHA
- Availability of "matching" funds
- Economic status of country of origin
- Prior travel awards to the same candidate
- Commitment to an oral or a poster presentation

Nominations sought for ISSHA Executive

The International Society for the Study of Harmful Algae is seeking nominations for the ISSHA Executive (President, two Vice-Presidents, Secretary and Treasurer). The current Executive (Steidinger, Cembella, Oshima, Bates, and Enevoldsen, respectively) are not eligible for the same offices they currently hold although they are eligible for other offices. Those nominated must be current ISSHA members. Following the nominations, the Elections Committee will prepare a ballot for mailout to ISSHA members on 01 August 2004. The ballots will be counted during the 11th International Conference on Harmful Algae (15-19 November 2004), when the new Executive will take effect.

Please submit your nominations by 01 JULY 2004 to Dr. Stephen S. Bates at batess@dfo-mpo.gc.ca or Fisheries and Oceans Canada, Gulf Fisheries Centre, 343 Université Ave., Moncton NB E1C 9B6, Canada.

President: _____

Vice-President: _____

Vice-President: _____

Secretary: _____

Treasurer: _____

Application for Membership: International Society for the Study of Harmful Algae

Name: _____

Address: _____

_____

_____

E-mail: _____

Tel: _____

Type of Membership ($US or equivalent Euros):

Regular ($20) ☐ Student ($10) ☐ Corporate ($40) ☐

Credit Card:

Visa: ☐ MasterCard: ☐ Eurocard: ☐ Maestro: ☐ Visa-Electron: ☐

Card number: _____

Expiry date: _____ / _____ (MUST be indicated)

Security code: _____ (last three digits on back of credit card)

Amount USD: _____

Signature: _____ Date: _____

This form is approved by PBS. VAT (or SE #): SE 21 41 61 42

If you prefer to pay by bank transfer please transfer via your bank or by home banking (e-banking) to the account of ISSHA: Nordea Bank, IBAN DK7920008473580633, Frederiksborggade 14-16, 1360 Copenhagen K, Denmark.

Checks are not accepted.

Send or fax this form to:
Henrik Enevoldsen, ISSHA Treasurer
University of Copenhagen
Øster Farimagsgade 2D
DK-1353 Copenhagen K

DENMARK

Fax: (45) 33134447

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

http://ioc.unesco.org/hab/news.htm No. 26

• Greece

The coincidence of a *Prymnesium parvum* bloom and the mass kill of birds and fish in Lake Koronia

An extremely dense bloom of the haptophyte *Prymnesium parvum* N. Carter (Fig. 1) occurred in August-September 2004 in the shallow Lake Koronia giving the water a yellow-golden colour. The bloom peaked from 9 to 11th September, 2004. This is the first record of a harmful *P. parvum* bloom in both inland and marine waters in Greece. Harmful algal blooms studied to date in Greek inland waters have been caused by cyanobacteria [for a review see 1].

Lake Koronia located in northern Greece (40º 40' 58" N; 23º 09' 33" E), at an altitude of 75 m above sea level has undergone a massive decrease in lake volume over the past 20 years, with dramatic decreases in surface area and maximum depth. In the1970's the surface area was 46.2 km^2 and the maxi-

(Cont'd on p. 2)

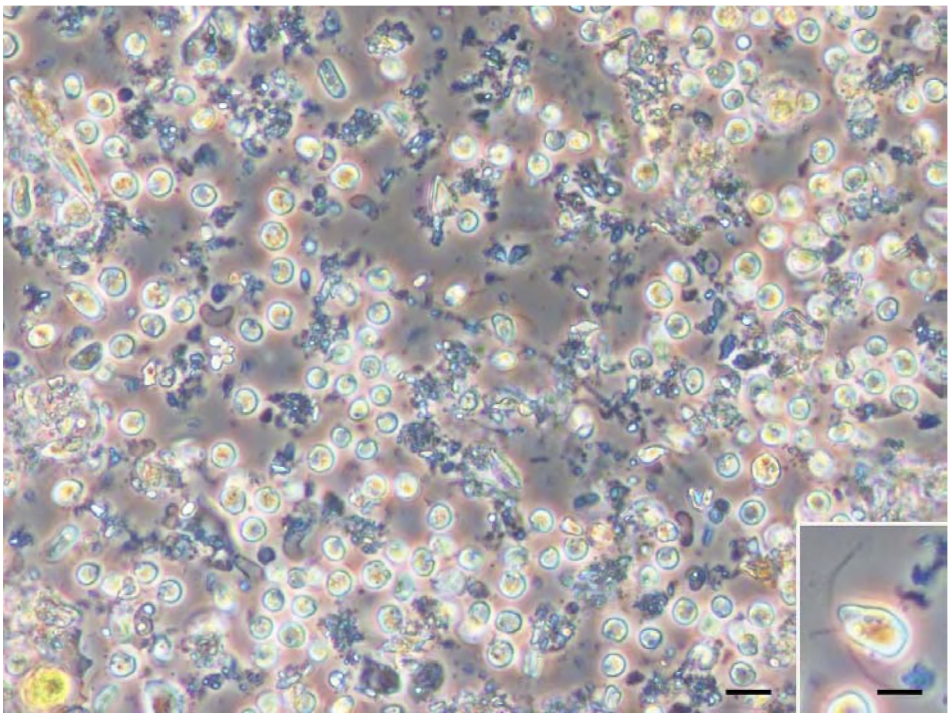

Fig. 1. Light micrograph (phase contrast) of a water sample, containing predominantly Prymnesium parvum, *collected from Lake Koronia on 11th September, 2004. Inset: Motile* P. parvum *cell with visible flagellae and haptonema. Bars indicate 10 µm; inset 5 µm.*

• Guatemala

Violet bloom produced by a cyanobacterium in a Guatemalan lagoon

During September 2003, violet discolorations due to cyanobacteria were observed in the water of Ipala Lagoon, 168 km from Guatemala City (Fig. 1), a crater of volcanic origin. These waters are drinkable and have been used by the nearby village for many years, without trouble. It is not known when these discolorations began, but the organism is now so abundant, that a sample in a flask has a strong violet colour, like paint (Fig. 2). Counts with a hematocytometer gave estimates of 94,400 cells/mL. This cyanobacterium is strongly pigmented comprised approximately 99% of total cell count; there were a few Pennales and many phytoflagellates difficult to identify in the fixed sample.

(Cont'd on p. 2)



Fig. 2.

The publication of Harmful Algae News is sponsored by the Botanical Institute University of Copenhagen, and the Spanish Institute of Oceanography, Vigo.

(Cont'd from p. 1)

mum depth was 8 m. In 1995 the surface area was 30 km^2 and the maximum depth 1 m and finally in the summer of 2002 the lake dried up completely. Early in 2003 water started accumulating again in the lake and to date the maximum depth is about 0.9 m.

The physical and chemical conditions of the lake water at the peak of the *P. parvum* bloom were as follows: the water temperature was 20.9 ºC, the pH 8.2, the transparency 0.18 m Secchi depth, the salinity 5.3‰, the conductivity 9.2 mS cm^{-1}, the surface water dissolved oxygen concentration 9.9 mg L^{-1} and the above bottom water concentration was 7.9 mg L^{-1}. The phosphate phosphorus concentration was 118.9 µg L^{-1}, the dissolved inorganic nitrogen concentration was 543.6 µg L^{-1} and the N:P atomic ratio was 10.1.

Prymnesium parvum population densities ranged from 120 to 1450 x 10^6 cells L^{-1}, at the peak of the bloom, at different sampling stations in L. Koronia. The phytoplankters *Pediastrum boryanum* (Turpin) Meneghini and *Cryptomonas* sp. were also present in abundance, with population densities of 1.8 x 10^6 and 2.1 x 10^6 individuals L^{-1}, respectively.

A massive bird kill was observed to coincide with the bloom peak of *P. parvum*, while one week later a mass fish kill was also observed. The number of dead birds reported at a meeting organised by the Prefecture of Thessaloniki was estimated to be in the tens of thousands. Thirty species of water bird were found dead, including over 200 young individuals of *Pelecanus crispus*, a world-endangered species, and individuals of *Platalea leucorodia* and *Egretta alba*, as reported by the Hunting Federation of Macedonia and Thrace. Reports from the local authorities estimate that the number of dead fish is in the order of hundreds. From the literature it is known that fish kills can be caused by *P. parvum* and *Prymnesium* sp. [2, 3, 4, 5]. For example, fish died in the epilimnion where the density of *Prymnesium* sp. was 10-40 x 10^6 cells L^{-1} [4]. However, to the best of our knowledge, there are no reports to date of bird kills associated with blooms of *P. parvum*. It is known that *P. parvum* produces prymnesins, potent haemolytic, ichthyotoxic and cytotoxic glycosides and other allelopathic substances [6, 7, 8]. Allelopathic compounds released by *P. parvum* induce changes in the plankton community structure [9].

Investigations are underway to elucidate the cause(s) of this ecological catastrophe in L. Koronia. The lake is covered by the Directives 79/409/EEC [10] and 92/43/EEC [11], the RAMSAR Convention (http://www.ramsar.org) and is a part of a National Wetland Park [12]. The ecological importance of Lake Koronia cannot be underestimated and it is an invaluable haven for large populations of resident and migratory birds.

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Email: cook@pp-mail.bio.auth.gr

(Cont'd from p. 1)



The dominant cyanobacterium is *Merismopedia aeruginea,* in colonies of 2, 4, 8 and 16 small cells arranged in quadrangular lamelliform colonies. The shape of the cells ranges from oval to hemispherical, the diameter is 2.5-4 µm and length 3-4 µm, with a very fine granular content of violet color, sometimes concentrated (Fig. 3). The colour distinguishes it from *Merismopedia glauca* (Ehr.) Naegeli, a species very common in fresh waters but of blue-green colour, though some authors consider it the same species [1]. Other authors [2, 3] differentiate it and give values of 3-5 µm diameter, and we did not find colonies this size. There are no signs of toxicity and there are no deaths related to its consumption. This is the first time the species has been known to cause discoloration, although it was already characterized for its violet color. After eight months the water still has this violet pigmentation, even though the organism is no longer present.

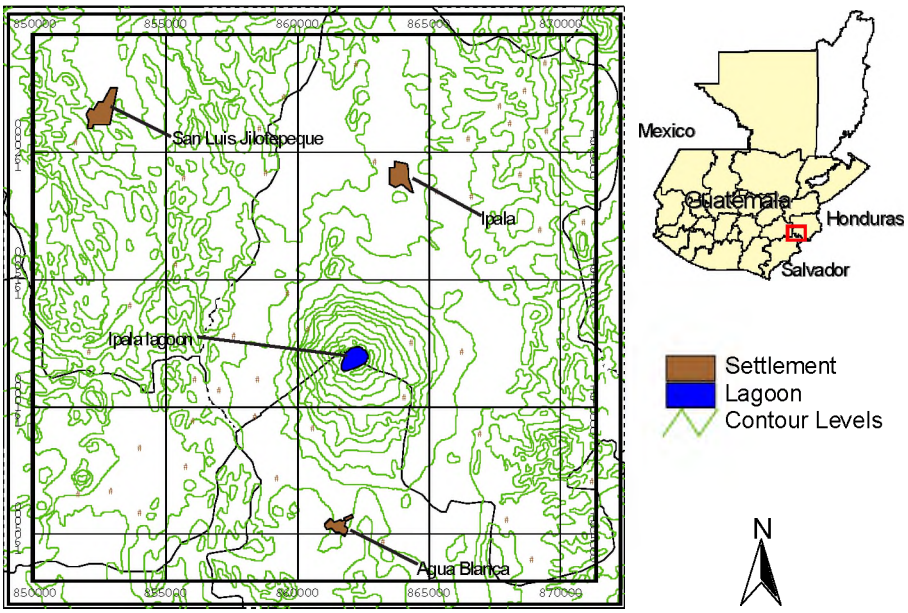

Fig. 1. Geografical location of Ipala Lagoon.

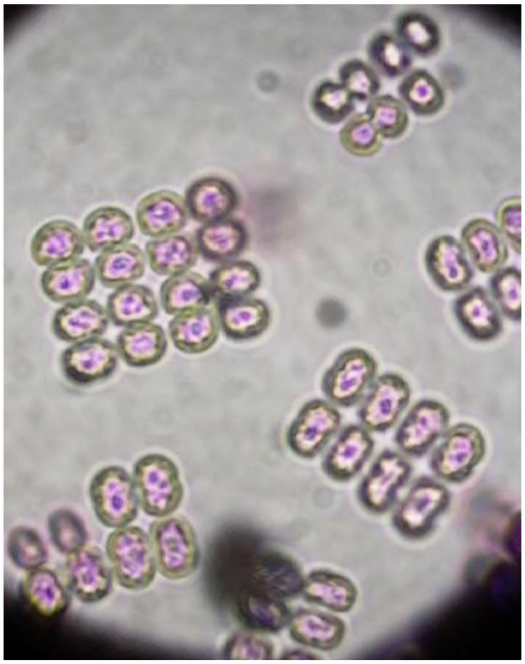

Fig. 3. Merismopedia aeruginea *(Digital zoom).*

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Fish kills or mass mortality events of fish. Monitoring of global fish kills and the life and work of Margarethe Brongersma-Sanders

Dr Sue Turner has been awarded a grant from the Schure-Beijerinck-Popping Fund of The Royal Netherlands Academy of Sciences for research into 'Fish Kills as Climatic Indicators' and to delve into the life and pioneer work of Dr Margarethe Brongersma-Sanders, especially her contributions to the understanding of fish kills and their relationship to harmful algal blooms. She was the first to understand the link between dead fish, upwelling currents and red tide events and the creation of oil. Later in life on the strength of her work she became a consultant to Shell. She classified fish kills into several categories with algal causes being uppermost.

A database of fish kill events is being compiled, especially in the poorly known southern hemisphere, to track causes in the fossil and recent record. Recent data will be compared with fossil to define a key of characteristics from different causes. The aim is to gain climatic, palaeoclimatic, palaeogeographic and palaeoecological information from fossil fish kill events and discern patterns such as those related to El Niño-La Niña. If anyone working on blooms, either marine or freshwater, has records of fish kills, please contact me.

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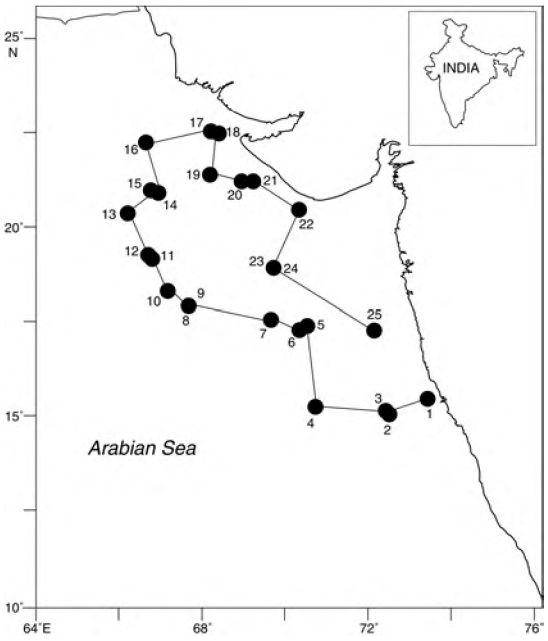
• India

Indian satellite IRS-P4 (OCEANSAT). Monitoring algal blooms in the Arabian Sea

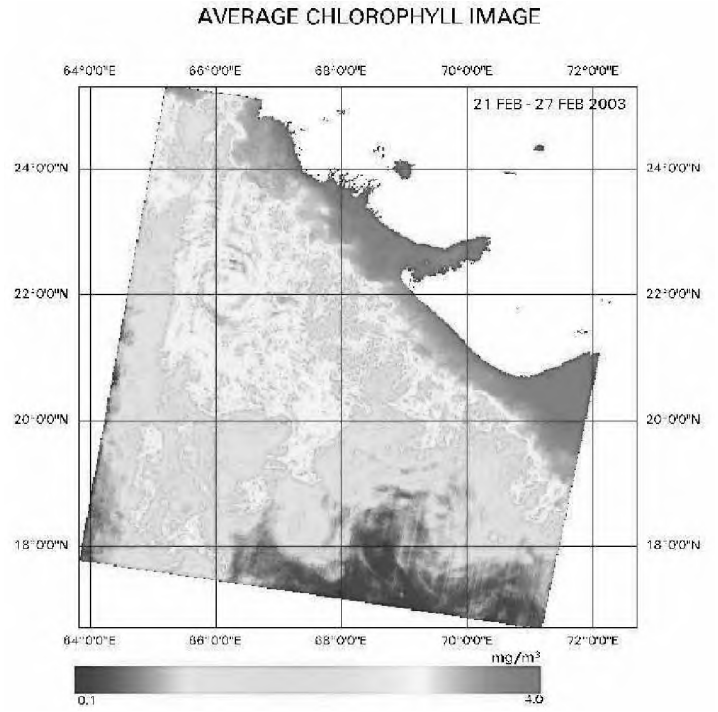


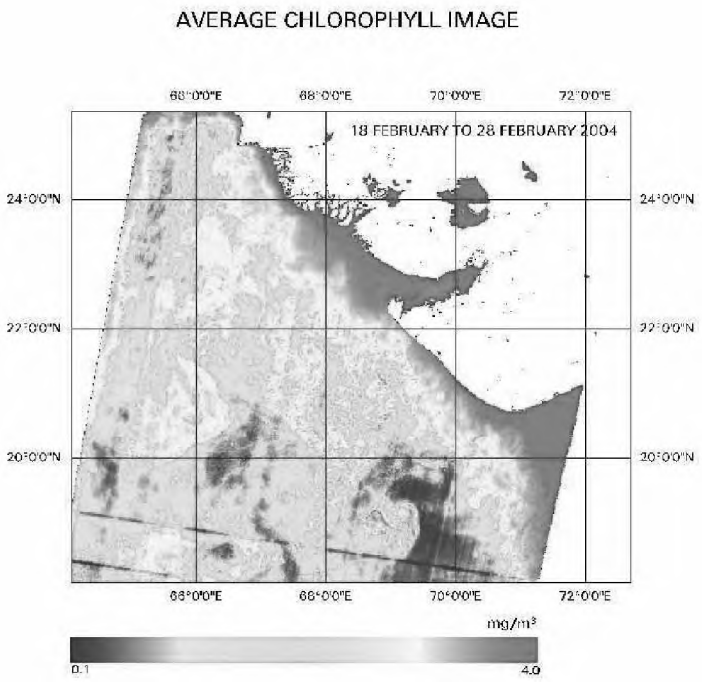
The launching of IRS-P4 (OCEANSAT), India's first operational ocean satellite on 26 May 1999, gave a much-needed fillip to the country's ocean research efforts. The satellite carries two sensors, MSMR (Multichannel Scanning Microwave Radiometer) for data collection on SST, wind speed, atmospheric water vapour etc., and OCM (Ocean Colour Monitor) for data on atmospheric aerosols, suspended sediments, chlorophyll concentration and detection and monitoring of algal blooms in the sea. Though there have been reports on sporadic occurrences of algal blooms in the Arabian Sea, the attention of Indian oceanographers was attracted only when harmful algal blooms in other oceans, and their adverse impacts on fisheries, came into focus.

With increasing awareness, and the availability of better observational and sea going facilities, several Indian research groups have reported on various aspects of blooms, e.g., of *Trichodesmium erythreum, Noctiluca scintillans* [1-5]. But all these observations were from the coastal waters and had limited spatial and temporal coverage. Assessments of effects on fish landings or fish mortality as sequels to the blooms were only circumstantial. This underlined the need for extensive spatial and temporal studies to cover the whole expanse of Indian waters, enumeration of the bloom forming species and understanding of the conditions that trigger blooms.

The collaborative efforts of the Space Applications Centre (SAC of ISRO) Ahmedabad, and the National Institute of Oceanography (NIO of CSIR), Goa, provided the impetus for this venture, and to fully utilize the OCM capability of OCEANSAT and oceanographic research vessels FORV *Sagar Sampada* and ORV *Sagar Kanya* of Department of Ocean Development (through CSIR/SAC/NIO) for sea truthing and validation. With this in view, all cruises had a major aim to perfect our techniques for use of ocean colour, gather data on physical, chemical and biological conditions of Indian waters, and assess primary productivity and build up a data base on bloom aspects.

The cruises of FORV *Sagar Sampada* (Cr. 212) in the year 2003 and a repeat cruise in 2004 (Cr. 222) during the same period and location (Feb 26 to Mar 15) between Goa and Porbander sector (NE Arabian Sea, 10-22°N and 66-75°E) were organized during the OCEANSAT satellite pass, for sea truthing and validation. An extensive bloom of *Noctiluca scintillans* that imparted a green colour was detected. The bloom was found gradually extending westward as far as Oman. This was very obvious from the time series, generated from the OCM data, and from earlier observations made during the years





2000 to 2002. We observed that the bloom generally begins by the end of January and persists until mid-March.

The blooms observed in surf to 70 m water depth showed varied density in *Noctiluca* cell counts. During cruise FORV *Sagar Sampada* (Cr. 212), the surface water samples showed 64 - 4128 *Noctiluca* cells/L and deeper waters (10-15 m) supported 1080-2542 cells/L. The bloom population was of 5-10 times larger than the total phytoplankton population in the area sampled. Considering the very large area that the blooms occupied, one can imagine their contribution to the carbon cycling in the ocean-atmosphere coupled system.

This is the first report of large-scale *Noctiluca* blooms from open waters of the NE Arabian Sea, and we plan to make further observations with longer cruises in the waters of the Arabian Sea, as well as the Bay of Bengal. Since the present observations were in repeated stations, data analysis will facilitate better understanding of the inter-annual variability in the productivity pattern and improve our knowledge of the blooming species.

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• Italy

Further investigation on blooms of *Ostreopsis ovata, Coolia monotis, Prorocentrum lima* on the macroalgae of artificial and natural reefs in the Northern Tyrrhenian Sea

Ostreopsis ovata, Coolia monotis, Prorocentrum lima, Prorocentrum sp., Amphidinium sp. have been detected on the Tuscany coast, Tyrrhenian Sea, on macroalgae on the artificial reefs of Marina di Massa and Versilia, and on the natural reefs of Livorno. The same dinoflagellates have been found on the islands of the Tuscany archipelago: Elba, Giannutri, Giglio [1, 2]. During these blooms, red tides caused by the detachment of such epiphytic algae from the macroalgae can occur [3]. The blooms have been considered responsible for inflammation of the upper respiratory tract and conjunctivitis in swimmers [3, 4].

The present study, from July 2002 to August 2003, was in the same areas monitored in our previous studies [1]: the artificial reefs of Marina di Massa (Massa), and the natural reefs of the locality of Calafuria south to Livorno (indicated as Livorno in text and tables). Ciguatoxins were detected with the ELISA immunological procedure, after a simple chromatographic extraction in methanol. The toxicological test with *Artemia salina* was done with filtrates of the microalgae homogenized in seawater, and on suspended cells captured with a phytoplankton net (mesh size: 20 mm). The material was frozen, treated with ultrasound for 20 s, and finally filtered. The test was repeated 2 times, each on 10 specimens of *A. salina* for 24 h. in a 10 mL sample. The results are expressed in % of dead specimens of *A. salina.* Cell counts were made in an Utermöhl chamber at 100x and species identification was done at 1000x UV in calcofluor. The cell density is expressed either as the ratio of the number of cells to 1 g of macroalgae (wet weight), or as the ratio of the number of cells to 1 mL of the seawater used for detaching the dinoflagellates, or as the ratio of the number of cells to 10 L of seawater filtered with a phytoplankton net (mesh size: 20 µm).

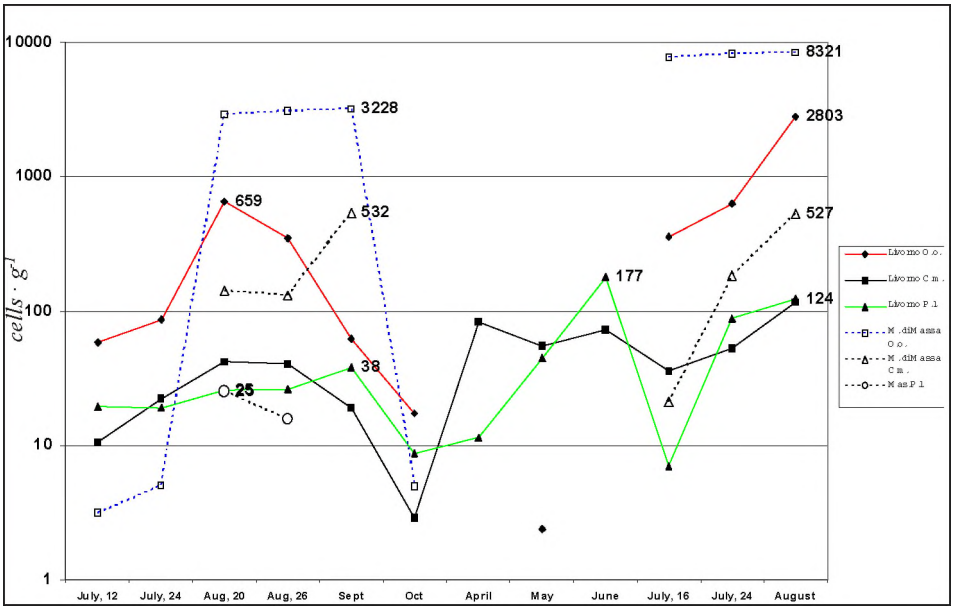

Fig. 1. Density of O. ovata, C. monotis, and P. lima. (July '02 - August '03).

The density of *O. ovata* in macroalgae increases with water temperature (Figs. 1, 2). During blooms, the density of *O. ovata* is greater on the artificial reef of Marina di Massa than on the natural reefs of Livorno. In summer, the increase of algal density from zero to the maximum value is extremely fast,

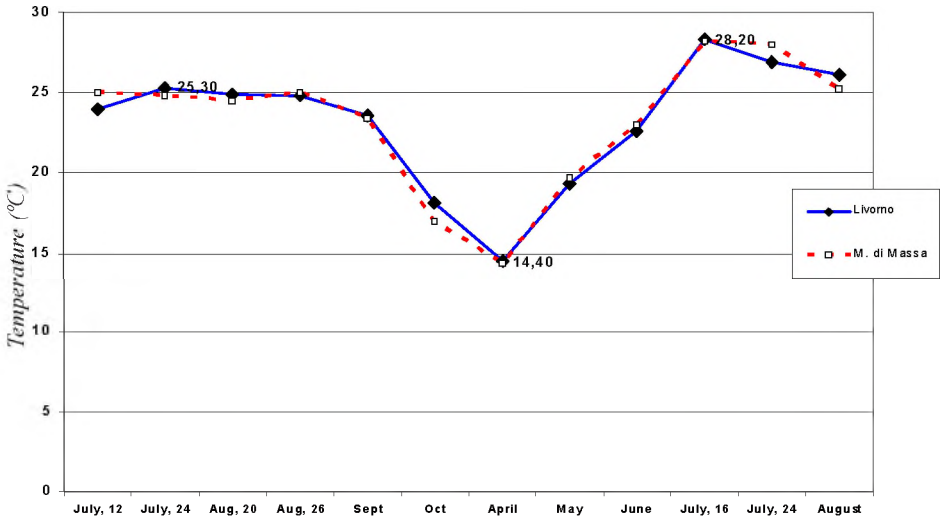

Fig. 2. Surface temperature of water (July 2002 - August 2003).

while the reduction of algal density and the apparent disappearance of *O. ovata* in autumn-winter is gradual. This pattern reflects the capability of this species to produce resistant forms, hardly detectable in the natural environment, which are activated when temperature rises above 20-25°C. Growth of *C. monotis* also depends on temperature: in Marina di Massa, *C. monotis* was found only in August-September 2002 and in July-August 2003, when the peak value was recorded, while in Livorno, *C. monotis* occurred all year and reached a peak in August. *P. lima* was found during the whole year on the reefs of Livorno, but only in small numbers in Marina di Massa, in August 2002. These results show that the selectivity of the ecosystem of the artificial reefs of Marina di Massa is greater then the selectivity of natural reefs of Livorno, in accordance with our previous studies [3]. During blooms on the macroalgae, high quantities of *O. ovata* and *C. monotis* are present in the water around the reefs. The highest density of *O. ovata* was observed in August-September 2002 and in July 2003 at Marina di Massa, and in September 2002 and July 2003 at Livorno. The highest density of *C. monotis* was detected in September 2002 and in August 2003, while *P. lima* was present in small numbers only at Livorno, in August 2002 and April 2003 (Table 1).

The toxicological tests with *A. salina* reveal a significant correlation ($p<1\%$) between the number of *O. ovata* in extracts of macroalgae from Marina di Massa and the percentage of dead crustaceans in 24 h, which varies from 65% to 100% while the number of *O. ovata* was in the range of 542 to 906 cells mL^{-1}. The correlation between the mortality and the algal concentration was not significant with *C. monotis, P. lima* and *Amphidinium sp.*

The liposoluble toxins extracted in methanol were tested by using specific antibodies and detected through colorimetric analysis. The concentrations, expressed in ppb, are shown in Table 2. No significant increase of Cigua-Check positivity in shellfish and fish was observed during the blooms. Thus shellfish and fish do not concentrate the cigua-like compounds. Tests at the Centre for Marine Research of Cesenatico (the Italian national authority centre for the study of algal toxins), treating mice with the same concentrations of toxins as those detected in our tests, have not shown any toxic effect [3].

Between winter and early spring *O. ovata* is not detectable, although motionless forms of *O. ovata* can exceptionally be found enclosed in a thick membrane. In vitro, a temperature decrease or a reduction in nutrients leads to round forms enclosed in a jelly envelope, identical to those observed in *O. siamensis* [5].

C. monotis, P. lima, and *Amphidinium sp.* are present all the year on the Livorno reefs, but never reach high concentrations there. On the Marina di Massa reefs, the blooms of *C. monotis* occur at the same time as those of *O. ovata*, are larger than at Livorno, but more ephemeral. *P. lima* and *Amphidinium sp.* never reach high concentrations. Since the toxicity of *O. ovata* has not been clearly proved yet, it is possible that *C. monotis* plays an important role in the potential episodes of ecotoxicity. The problem of the toxicity for humans following the ingestion of either edible shellfish or predatory fish could be effectively addressed with comprehensive studies on the ability of potentially toxic Mediterranean species to produce toxins (palitoxin and hemolysins *Ostreopsis sp.*, yessotoxin *C. monotis*) detectable by following chemical or biological tests, and promoting an adequate environmental and sanitary surveillance.

Table 1. Potentially toxic dinoflagellates detected in samples taken with a plankton net (cells/10 L^{-1}).

	Date	*Amphidium sp.*	*Dinophysis caudata*	*Dinophysis sacculus*	*Dinophysis rotundata*	*Alexandrium sp.*
Marina di Massa	20/08/02				197	
Livorno	26/05/03		8	118		
Livorno	11/06/03		79	126		39
Massa	24/07/03	39				
Massa	06/08/03	8				
Livorno	06/08/03	8				

Table 2. Cigua-Check (ppb).

Artificial reefs (Marina di Massa)					
	April 03	June 03	16 July 03	24 July 03	August 03
Mytilus sp.		<0.8	0.9	1	1
Patella sp.				0.9	
Natural reefs (Livorno)					
Mytilus sp.	0.9	<0.8	0.9	0.9	1.2
Patella sp.	0.95			0.9	
Murena aelena			0.8		
Octopus vulgaris			0.85		
Boops salpa			0.9		

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• Costa Rica

Extensive blooms due to *Cochlodinium polykrikoides*: New to Costa Rica

At the beginning of April of 2004 an extensive bloom produced by *Cochlodinium polykrikoides* was detected, embracing the whole Pacific coast of Costa Rica. The bloom was characterized by a red-oxide colour and was located in front of the beaches of Puntarenas and Caldera in the Gulf of Nicoya, Costa Rica (10°N, 85°W). This phenomenon had never observed before in this country; the species dominated other phytoplankton usually responsible for blooms. The phenomenon started during the previous dry season (from December 2003 to March 2004) showing an important increase in April.

During this event a penetrating and fetid smell was perceived that affected the affluence of tourism to some beaches; there was also a great quantity of foam. Near some coastal towns dead fish, mainly Carangidae, were observed. In Culebra Bay, mortality of coral reefs was observed due to dense cell concentrations that reached depths up to 6 meters (Carlos Jimenez, in press). Near the tourist aquarium Parque Marino del Pacífico, there has been damage such as deformation in larvae of *Lutjanus* (Lutjanidae), probably because the aquarium takes its water close to the blooms. Fishing activity was reduced, perhaps due to the presence of the large amount of mucus and decreased oxygen concentration in the water.

Since the year 2000, we have observed extensive algal blooms that last more than one year on the Pacific coast of Costa Rica, dominated by different morphotypes of *Pyrodinium bahamense* [1]. Prior to this, long lasting blooms were not observed. In May 2002, an extensive bloom was observed with similar characteristic to that reported in this note; on that occasion, 17 cases of human intoxication, with respiratory symptoms and burning feeling in the eyes were reported. This bloom was also dominated by *Cochlodinium polykrikoides* and the cyanobacterium *Trichodesmium erythraeum* [2].

Later on, during the rainy months of September and October 2003, several blooms of this dinoflagellate were reported, reaching maximum concentrations of 17.5 x 10^4 cells/L by mid-October 2003.

The time distribution of blooms produced by *C. polykrikoides* had increased from May 2002 until the present, mainly located on the central Pacific coast. In a same manner, this species has recently been reported in the Gulf of California [3, 4], and in Mexico. The Costa Rica blooms observed are generally monospecific, displacing other species usually present during this season. We wish to stress the need for research on HAB along the coasts of Costa Rica, where climatic changes or the increase in the anthropogenic eutrophication of coastal waters may be changing HAB patterns.



Fig. 1. Light microphotograph of Cochlodinium polykrikoides.

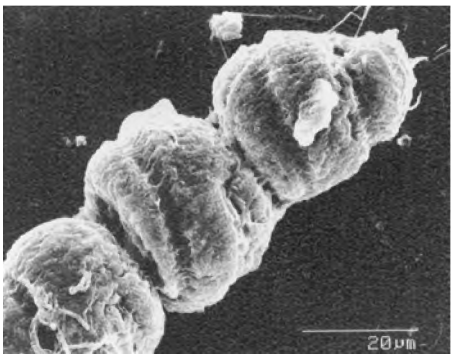

Fig. 2. SEM microphotograph showing the reticular cell and trichocyst.

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• Angola

Mortality caused by dinoflagellates bloom in Luanda

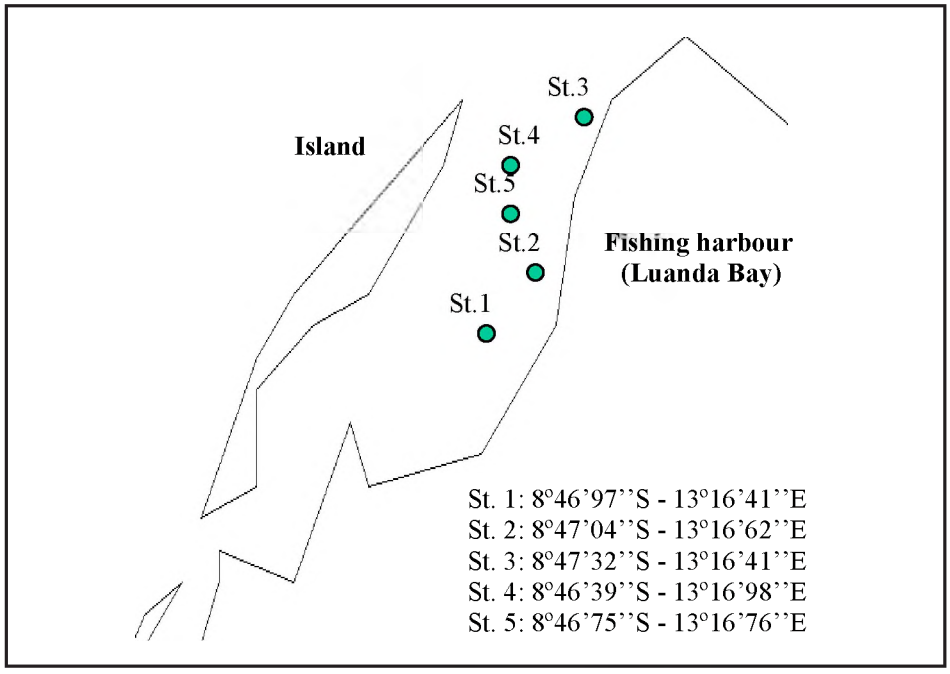

Fig. 1. Map of location of the samplings in the Fishing harbour, Luanda.

On 24-25 September 2002, a red tide and mortality of fish and crabs occurred in Luanda Bay, Angola, associated with dinoflagellates. This was the first phytoplankton bloom here with mortality in which the causative species were identified. Brownish water with an extent of approximately 2 km^2 lasted for 2 days. *Alexandrium* spp. (maximum 5.1x10^6 cells/L), and *Gyrodinium spirale* (maximum 6.5x10^5 cells/L) were the most abundant species found in the fish harbour area (8° 47'S, 13° 16'E). An unidentified species of *Chattonella* also contributed to the bloom with lower cell concentrations (9x10^4). This phenomenon caused mortality of *Dentex*, *Epinephelus* and *Merluccius* species and of crabs.

Surface samples were collected at 5 stations in the fish harbour on 24 and 25 September 2002 (Fig. 1).

Samples were either fixed in 2% formol or kept alive for some hours to help identification of species. For the qualitative and quantitative determination of the samples, the Utermöhl technique was used. Samples were studied using an inverted microscope with contrast, phase and epifluorescence Axiovert 200. Cell counts are shown in Table 1. Mortalities were first noticed on the morning of the 24th, intensified as the day progressed and decreased on the 25th. This event caused panic amongst subsistence fishermen in the area.

Alexandrium spp. were found at all stations, with a dominance of 100% at station 5 on the 24th and station 1 on the 25th. The dominance of this species was approximately 90% at stations 2 and 3, on the 25th. Species of *Alexandrium* and *Gyrodinium* produce PSP toxins and cause fish mortality [1]. *Prorocentrum* which occurs amongst the microalgae identified has previously been associated with fish mortalities in Angola by Silva [2]. The raphidophyte *Chattonella* was found in concentrations of 5.8x10^4 and 9x10^4 cells/L at stations 1 and 3 respectively. Although the mechanisms by which raphidophytes cause death are poorly known, physical damages to fish gills due to mucus secretion; hemolytic activity may also be involved [3, 4], as well as the production of free radicals [5].

It seems possible that increased nutrient levels in the area affected contributed to the occurrence of the bloom.

Most mass mortalities in the subequatorial southeast Atlantic have been recorded on the Namibian coast, especially in the Walvis Bay area in 1940s, with *Gymnodinium* implicated. A fish mortality in 1997 on the Swakopmund coast was associated with *Gymnodinium galatheanum*. Similar phenomena were also recorded in South Africa (St. Helena Bay in 1997), causing a mortality of sardines and attributed to the presence of PSP (Paralytic Shellfish Poisoning), produced by *Alexandrium catenella*. This was the first confirmed case of an organism pro-

Table 1. Abundance (n° cells/L) of the most representative species.

Species	St. 1	St. 2	St. 3	St. 4	St. 5	St. 1	St. 2	St. 3	St. 4	St. 5
			24/9/02					25/9/02		
Alexandrium spp.	4.6x10^5	7x10^5	5x10^5	2.5x10^3	51x10^5	51x10^5	10x10^5	30x10^5	5.8x10^4	2.5x10^3
Chatonella spp.	5.8x10^4		9x10^4							
Gonyaulax spinifera	2x10^4	2.5x10^3	2.5x10^3	4x10^4						
Gymnodinium spp.		4.6x10^4		6x10^4			2.5x10^4			
Gyrodinium spirale	7x10^5	4.7x10^5	8x10^4						30x10^3	6x10^4
Navicula spp.		5x10^3	3x10^4			2.8x10^4	6.9x10^4	20x10^4		
Nitzia closterium	2.5x10^3	2.5x10^3	5x10^3	2.5x10^3					43x10^3	
Prorocentrum micans	12x10^3	5x10^3	2x10^4	3.8x10^4			5x10^3	10x10^3	10x10^3	2.5x10^3
Scripsiella spp.	4.6x10^4	7.6x10^3	5x10^4	12x10^3			2.5x10^3	2.5x10^3	5x10^3	10x10^3

ducing PSP and fish mortality in the Benguela current [1].

An early record of fish mortality caused by a red tide of *Prorocentrum balticum* took place in 1951 on the Angola coast [2]. Other events related to discoloured water and marine mortalities occurred in Luanda Bay and the coastal area of Cabinda and Lobito Bay, in 1997 and 2002 respectively, (data of the Marine Research Institute of Angola).

We thank Dra. Lia Neto and Dr. Osvaldo Costa for sample collection and the National Direction of inspection for a small vessel.

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HABTech2003 Workshop, New Zealand

The second HABTech workshop was again held in Nelson, New Zealand, hosted and organised by Cawthron Institute. The aim of HABTech03 was to provide participants with a highly informative and enjoyable event, providing practical information on new developments in analytical methods for harmful algal species and marine biotoxins in shellfish and other seafood products. The plenary speakers were experts in their fields, many of them key international researchers. Two panels of experts were drawn together to give their opinions and answer the questions: «What is the future of the mouse in monitoring and regulations?», «What are the risks and benefits of shifting to new technologies for monitoring?» and «How can we speed up regulatory acceptance of new methods?». A summary of these presentations and Hot Topics can be found in the published Proceedings.

A unique feature of HABTech was the demonstration sessions where participants had 'hands-on' experience with the latest techniques for HAB and toxin detection. We endeavoured to provide an in-depth coverage of the field, including genetic techniques for algal identification and a wide range of assays for toxin analysis - functional, immuno and instrumental. At least 50 posters were presented, providing a wealth of detailed information to complement the plenary and demo session materials. Extended abstracts of the Posters and Demonstrations are included in the Proceedings.

Without APEC, NRC Canada, and the New Zealand Ministry of Research Science & Technology and their substantial sponsorship of the workshop we would not have been able to bring together the speakers and other key people from APEC countries to Nelson. The APEC connection was greatly facilitated by Dr Michael Quilliam from the Institute of Marine Biosciences, NRC, Halifax. His project *Development and Validation of Phycotoxin Analytical Methods, Standards and Reference Materials for Seafood Product Certification and Safety* in the APEC Red Tide program provided impetus for several of the research projects that were demonstrated and discussed. The project contributes to an effort by APEC to coordinate monitoring and management of HABs within the 21 APEC economies in order to facilitate a free flow of goods and services, and in particular, shellfish and fish products potentially contaminated with algal toxins. Key tasks of the project included methods development and validation, certified reference materials (CRMs), and toxicity data on pure toxins and information on all of these was presented at the workshop. Mike's enthusiasm, drive and exceptional knowledge of the area were appreciated during the planning and organising of the workshop.

Our thanks also go to those registrants who supported the workshop by supplying demos and to sponsoring companies for their loans of key equipment and consumables. In particular we acknowledge the Micromass Quattro Micro LC-MS system loaned by Waters Australia Pty.

The Proceedings can be purchased from Cawthron through the librarian or obtained free on request on CDROM. PowerPoint presentations and photos are included. Go to the Cawthron website for request information: www.cawthron.org.nz.

P. Holland, L. Rhodes & L. Brown (Proceedings editors), Cawthron Institute, PB 2 Nelson, New Zealand.

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GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

Open Science Meeting: HABs and Eutrophication

7-10 March 2005

Baltimore, Maryland USA

Eutrophication is recognized as one of the factors contributing to the increasing proliferation of harmful algal blooms in coastal areas worldwide. This meeting, the third in a series of Open Science Meetings convened by GEOHAB, is designed to bring experts together from around the world to review the state of knowledge with respect to our understanding of the role of eutrophication in the proliferation of worldwide HABs, and take the initial steps in designing the next phase of research on comparative, eutrophic ecosystems and species that will be necessary to address this critical, global issue.

GEOHAB is a programme of international co-operative research on HABs in marine and brackish waters.

The GEOHAB Scientific Goal is to improve prediction of HABs by determining the ecological and oceanographic mechanisms underlying their population dynamics, integrating biological, chemical, and physical studies supported by enhanced observation and modelling systems.

The GEOHAB Mission is to foster international co-operative research on HABs in ecosystem types sharing common features, comparing the key species involved and the oceanographic processes that influence their population dynamics.

Conference Topics:

- Global trends in eutrophication and HABs
- Physiology of HABs with respect to nutrients
- Comparative studies on HABs in eutrophic areas
- HAB programmes in global eutrophic areas



- Modelling of nutrients and HABs
- New methodologies for nutrient and HAB monitoring

Important Dates

First Announcement: 25 September 2004
Abstract Deadline: 10 December 2004
Registration Deadline: 10 January 2005

The community is invited to submit abstracts for contributed oral and poster sessions.

Information regarding Meeting Proceedings will be forthcoming.

Registration: US $200

One-Day Registration: US $50
Student Registration: US $120
Late Registration: US $250

Registration is limited to 200 persons, so please register early!

For more information, contact the Meeting Co-ordinator: Ed Urban, Executive Director, SCOR, Department of Earth and Planetary Sciences, The Johns Hopkins University, Baltimore, MD 21218 U.S.A., Email: Ed.Urban@jhu.edu

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http://www.jhu.edu/scor/GEOHAB-OSM3.htm

ISSHA

International Society for the Study of Harmful Algae

http://www.issha.org/

ISSHA President's Corner

Dear ISSHA members and HA community,

My tenure as President of the International Society for the Study of Harmful Algae will be over at the end of November 2004, when the new President of our Society will be announced at the 11th International Conference on Harmful Algae in South Africa. I want to take this opportunity to tell you of the progress we have made as a fledgling Society and to thank you for your interest in, and support of, ISSHA. As with everything else, PEOPLE make things happen. Without the dedication of members, particularly the elected Council members and the Executive, ISSHA would not have developed to the stage it has today. Most societies, in the beginning, travel a bumpy road. ISSHA was no exception but it weathered the initiation period. Through the efforts of the Executive over the last several years, particularly Stephen Bates (Secretary), Allan Cembella (Vice-President), Henrik Enevoldsen (Treasurer), and Max Taylor (Past-President), this Society was kept on course. Among our first hurdles was to draft ISSHA Statutes, review them, and present them to the membership for ratification. This happened! To me, this was a major accomplishment and was principally due to the efforts of Jane Lewis and Henrik Enevoldsen. Thank you both. Another accomplishment was the ISSHA website and here I would like to thank Allan Cembella, Nancy Lewis, Judy Kleindinst, and Stephen Bates. Following those advancements came the election of 14 Council Members from around the world and the first Council meeting in April 2004, in Denmark. The minutes of that meeting are posted on the ISSHA website at www.issha.org. At that meeting standing committees and their chairs were established. Each committee was given a direction with assigned tasks. One of the committees is the Committee Travel Awards, chaired by Don Anderson. Council members were successful in obtaining money for more than 15 students and scientists to attend the 11th International Conference on Harmful Algae. Again, my thanks to a dedicated Council. That brings us full circle to the next election, that of the new Executive.

I have truly enjoyed being a part of ISSHA and working with colleagues and friends to advance our Society.

Karen A. Steidinger, ISSHA President.

October, 2004.

Members vote for the new ISSHA Executive

Members of the current Executive (Karen Steidinger, President; Allan Cembella, Vice-President; Yasukatsu Oshima, Vice-President; Stephen Bates, Secretary; Henrik Enevoldsen, Treasurer; and Max Taylor, Past President) are nearing the end of their extended terms, after having served since February 2000. To put the election of the new ISSHA Executive into gear, a nomination announcement was posted on the ISSHA website and sent to all members via the ISSHA list server. The ballots were then mailed on 22 September, with a request that they be returned to our Treasurer by November 5th. Although we had sufficient nominations for all positions, many nominees declined due to work loads. Therefore, with positions where there was only one candidate willing to serve if elected, a second «write-in» candidate could be inserted.

The ballots will be brought to the Cape Town XI International Conference on Harmful Algae, where they will be opened and counted. The results will be announced at the ISSHA General Assembly, which will be held during the conference (Thursday, 18 November, late afternoon). The new Executive will serve a three-year term, starting with the General Assembly in Cape Town. ISSHA is fortunate to have Council members that are dedicated and active, and their continued service will help the new Executive.

Committee on Elections (Karen Steidinger and Stephen Bates).

Application for Membership: International Society for the Study of Harmful Algae

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HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

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Cape Agulhas

Cape Agulhas marks the geographical boundary between Indian and Atlantic Oceans, but the dynamic boundary where the Agulhas and Benguela Currents meet fluctuates, and usually lies nearer Cape Point where the Agulhas casts eddies into the Atlantic. Here Asian (*A. tamiyavanichi*) and American (*A. catenella*) clades of *Alexandrium* meet (**Carlos Ruiz Sebastián**). One of them may have been responsible for the deaths of half a million fur seals on Possession Island in the early 19th century [1]. This conjunction of members of different clades centred on distant waters leads to the question of whether there are genuinely cosmopolitan species. *Karenia brevis* was briefly regarded as cosmopolitan, but improved taxonomic knowledge has shown that it is not, and now we have several newly described ones to contend with (**Miguel de Salas**). Grethe Hasle recently reviewed the cosmopolitanism of *Pseudo-nitzschia* [2], and it is clear from her discussion that the concept is as much operational as biological. Morphologically 'difficult' species are more cosmopolitan than others [3], but sexual compatibility and genetic studies of *P. pungens* appear to support a cosmopolitan interpretation (**Griet Casteleyn**). If true cosmopolitanism (in Ekman's [4] sense) should eventually be rejected ('we are still far from a reliable assessment of taxonomic units that are phylogenetically significant [5]), yet genetic homogeneity of some widespread species convincingly demonstrated, then anthropogenic spreading by vectors like ballast water would be even more strongly indicated [6].

Studies of intra- and interspecific diversity make essential contributions to this debate, and several were presented at this conference, amongst them of *Alexandrium catenella* in the NE Pacific, in which a latitudinal gradient in toxicity is provisionally identified (**Rozalind Antropus**), similar perhaps to gradients described in the NW Atlantic and NW Pacific, of *Karlodinium micrum* (**Tsvetan Bachvaroff**), *Karenia mikimotoi* (**K.A. Soltysiak**; **Lisa Campbell**), *Cochlodinium polykrikoides* (**C.M. Mikulski**), and *Skeletonema costatum* (**Anna Godhe**). A new look at *Prorocentrum* leads **Shauna Murray** to wonder whether *Exuviaella* should be revived; the distinctions between *Gymnodinium galatheanum*, *K. micrum* (now found to be a synonym of *K. veneficum* by **Bergholtz**, see comment below), and *Karenia mikimotoi* are still not easily resolved and *cf aureolum* is back again (**Violeta Velikova**). There are suggestions that *Alexandrium tamiyavanichii* and *cohorticula* are conspecific (**Po-Teen Lim**), and also *A. ostenfeldii* and *peruvianum* (**Linda Percy**). The fallout after the revision/explosion of the genus *Gymnodinium* is continuing with several new *Takayama* and *Karlodinium* species (**Miguel de Salas, T. Bergholtz**). Max Taylor at an earlier conference in this series asked «What's in a name?» – a great deal, obviously.

(Cont'd on p. 2)



*Ehrenberg's sketches (from sheet 957) of «*Peridinium splendor-maris*», courtesy the Ehrenberg Collection, Museum für Naturkunde, Berlin. The entire collection of drawings is online an available free for non-commercial uses at: http://www.museum.hu-berlin.de:55080/Ehrenberg/.*

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The story here is that once upon a time there was a genus, *Gymnodinium* and a closely related genus *Gyrodinium*, including several harmful species, classified on the basis of external morphological characters. Then Daugbjerg *et al.* [7], based on ultrastructural characters and molecular information, took out of *Gymnodinium* several new genera (*Akashiwo*, *Karlodinium*, *Karenia*), moved *Gyrodinium impudicum* to *Gymnodinium impudicum* (provisionally), plus other things. Once new criteria were established to look at these 'naked' dinoflagellates (actually their cellulose plates are just thinner), a lot of new species started to be described, and the new genus *Takayama* was established for species similar to *Gyrodinium corsicum* [8].

No doubt such 'revolutions' are very beneficial for taxonomy, and for ecology as well (see later comment on *Karlodinium* in Spain).

Sympatric genetic differentiation within morphospecies such as *Pseudo-nitzschia delicatissima*, *P. pseudodelicatissima* **(A. Amato)** and *P. galaxiae* **(S.M. McDonald)** highlights our ignorance of mechanisms of speciation in phytoplankton. How is it that such similar cryptic species exist and cooccur? Did they stop mating and then diverge, or did they diverge in different areas and then meet again? McDonald finds that the three populations of *P. galaxiae* forming three consecutive blooms in the Gulf of Naples are genetically distinct, which suggests sympatric speciation could occur by fractionation of a temporal niche, perhaps by virtue of different life forms (the three populations are dominated by different size classes).

Decades ago, the canonical view held that *Karenia* blooms off western Florida developed offshore (in the Loop Current), and subsequently moved towards the coast; an offshore current inoculates coastal waters where upwelling injects nutrients, and blooms appear. Several regional variations on this theme were presented in Cape Town. Extensive blooms of *Prorocentrum donghaiense* in the East China Sea appear in the upwelling front after inoculation by the Taiwan Current **(Mingyuan Zhou)**; analogous blooms of the newly described *Karenia concordia* appear in upwelled water in Hauraki Gulf, New Zealand, following inoculation by the East Aukland Current **(Hoe Chang)**, of *Cochlodinium polykrikoides* at the confluence of the Kuroshio with South Korean coastal waters **(Changkyu Lee)**, and of *Phaeocystis* cf *globosa* off Vietnam **(DanLing Tang)**.

A different mechanism operates off British Columbia and Washington in the northeast Pacific, where the nutrient-rich semi-permanent topographically trapped Juan de Fuca eddy acts as a 'bioreactor' for *Pseudo-nitzschia*; populations growing there can be entrained by the upwelling plume and thence blown shorewards by gales **(Susan Geier**; **Nicholaus Adams)**; the eddy itself can collapse during downwelling conditions **(Michael Foreman)**. Models are being used, to explore the hydrographic variability of this region **(Amy MacFadyen)** and relations between nutrient budgets and toxicity **(Angelica Peña)**. River plumes, for example those of the Yangtze, Mekong, and Columbia Rivers in the preceding case studies, can exert important constraints on the different mechanisms mentioned, perhaps by providing nutrients, but more importantly by blocking upwelling.

Eddy-trapped populations were invoked too in the Gulf of California, where stratified Taylor columns provide (in the authors' words) «bed and breakfast for red tide producers» **(Arturo Sierra-Beltran)**, and in Biscay where **Patrick Gentien** suggests retention in small (10 nautical miles across) gyres or «holding zones» is essential for mating of *Dinophysis*. Do dinoflagellates lek? Yet another mechanism which brings blooms nearshore was described for the southwest approaches to Ireland and Britain; here the seasonal thermocline traps 'cold pools' of water marked by bottom fronts [9], and the resulting geostrophic jets which lie above the fronts can transport plankton into the coastal zone **(Robin Raine)**.

Near the coast is not quite on the coast where many commercially important shellfish live, and where many fish farms are located. Several contributions looked at this last hurdle to intoxification of resources by offshore and nearshore blooms. *Dinophysis* and *Karenia* can escape the cold pool jets just mentioned and be advected by local currents into fjords and bays such as Killary Harbour **(Caroline Cusack)** and Bantry Bay **(Georgina McDermott)** in western Ireland; interannual variations in upwelling and downwelling may also determine whether resources in Aveiro, Portugal **(Teresa Moita)**, or the Rias Bajas of northwest Spain **(Beatriz Reguera)** are



threatened by *Dinophysis acuminata* or *D. acuta* in particular years, or by both. Shelf populations of *Karenia mikimotoi* can enter the same rías with downwelling winds (**Francisco Figueiras**), and *Pseudo-nitzschia* can move in and out of Saldanha Bay as upwelling rates vary (**Claudio Marangoni**). Loss of topographic control may allow Gentien's holding zones to impact the shore.

What all these hydrodynamic mechanisms have in common is that plankton is moved from one place to another, just as etymology says it should be. A question infrequently asked is, how do the surviving progeny get back to where they started, for example to the cyst beds which seed annual blooms? Countercurrents at depths reached by vertical movement are one solution to the spatial problem, proposed for upwelling systems and estuaries respectively [10]. **Lincoln MacKenzie**, noting that *Gymnodinium catenatum* moves 'upstream', suggests a role for nearshore buoyant plumes as countercurrents. Control of vertical position can be used to achieve shoreward movement and might be the basis of the navigational challenge in this case too [11]. But what signals the cells need to decide *when* to navigate remain unknown.

A growing number of time series of harmful algae, and in some cases of their toxins, is becoming available. These give us the possibility of searching for the causes of decadal trends. The abstracts published in *Harmful Algae* **3** (June 2004) contain introductions to several long-term data sets now available, e.g., *Alexandrium fundyense* in Bay of Fundy (J. Martin, p238), *Prorocentrum* spp. in Narragansett Bay (T. Smayda, p250; for *Heterosigma akashiwo* there see also [12]), and the southward spread of PSP in Puget Sound (J.C. Weckell, p264). From this conference we can add *Pseudo-nitzschia* in Mexican waters 1979-2004 (**Sergio Licea**) and in the Gulf of Naples, 1984-2004 (**Adriana Zingone**), and *Alexandrium peruvianum* on the Peruvian coast, 1981-2004 (**Sonia Sánchez**). Multiannual trends in phytoplankton abundance and composition are variously attributed to eutrophication, introductions, climate change, and overfishing; all these possibilities were brought up at this conference.

The dynamics of *Aureococcus anophagefferens* (brown tide) blooms were once explained by the 'chem-tech' hypothesis [13], as a response to increased nutrient availability which raises carrying capacity coupled with pesticides which reduce grazing. More recent work indicates that overfishing of hard clams allows *Aureococcus* to pass a threshold concentration beyond which the clams are unable to control them, and assigns nutrients a secondary rôle (**Chris Gobler**) [14]. Another study, in parts of the Chesapeake system, comes to a comparable conclusion; there overfishing of eastern oysters combined with increased algal production following eutrophication are implicated [15]. There is no obvious link between HABs and nutrients from aquaculture in Scottish waters (**Matthew Gubbins**). The report of a study group recently convened by the ICES Advisory Committee on Ecosystems (ICES CM 2004/ACE: 04) records that «We find no convincing evidence that HAB events are nutrient enrichment driven events, either generally or for individual HAB species.»

Nevertheless, it is still plausible that HABs are driven at least partly by eutrophication, although the mechanisms must be more complex than simple more-in/more-out or bottom-up models indicate. **Patricia Glibert** provides a global view of the enormous increase in the use of urea as a fertilizer, and suggests that insufficient attention has been directed towards such organic inputs when they reach coastal waters. The European MOLTEN project has been exploring a possible way to reconstruct past nutrient environments based on transfer functions (**Marianne Ellegaard**) which may provide an additional tool for tackling these questions.

Based on two sediment cores representing a time span of about 2000 years, **Ana Amorim** maintains that *Gymnodinium catenatum* was introduced into Portuguese waters in the early 20th century; this species was not found in a 9000 year record in the nearby Ría de Vigo [16], which strengthens the liklihood that it is recently introduced to this region. The importance of controlling such introductions in so far as ballast discharges are responsible was stressed by **Martina Doblin**, and **Gustaaf Hallegraeff** summarized international progress on this front. In the long run 'ballast free ships' offer one possible solution to these problem [17].

Time series integrate variability over different time scales; in general high frequency events echo short spatial scales, and low frequency events larger ones (the spatial coherence of change is exemplified in Stommel diagrams [18]). An example of the former



was provided for Alfacs Bay in the Ebro Delta area of the W Mediterranean where the normally winter blooming *Karlodinium micrum* (now, following **Bergholtz**, *K. veneficum*) formed a summer bloom during an unusual combination of runoff and stratification (**M. Fernández-Tejedor**). Are the winter blooming and summer blooming *Karlodinium* two different species? The second hypothesis is attractive since **T. Bergholtz** describes *K. armiger* from Alfacs, and *K. veneficum* is there too.This reminds us that there is no good ecology without good taxonomy. The complication faced when species are split is enormous, but the obverse is that some ecological paradoxes (how can a winter species survive in summer conditions) are resolved by acknowledging the extent of diversity. Whatever our favourite species concept is, there is nothing simpler than to call different things by different names.

Several contributors invoked climatic indices to account for interannual trends in phytoplankton abundance on intermediate time scales. Trends in *Alexandrium catenella* since the 1970s in Chilean waters were linked to El Niño Southern Oscillation or ENSO (**Leonardo Guzmán**), and in *Dinophysis* spp in the Galician rías to the North Atlantic Oscillation or NAO (**Beatriz Reguera**). Changes associated with ENSO were also invoked to account for the appearance of *Gymnodinium catenatum* and other species in Pacific Mexican waters (**Alejandro Morales-Blake**) and *Alexandrium peruvianum* in Peru (**Sonia Sánchez**). Climatological indices like ENSO and NAO are part of the stock-in-trade of fisheries oceanography, and we shall probably hear a lot more of such indices as our algal time series lengthen.

A recent paper [19] based on North Sea time series indicates that dinoflagellates are in general phenologically more plastic than diatoms, thus while diatoms tend to express their flowering periods at fixed times in the solar year, responding for example to day-length or to internal clocks (like *Pseudo-nitzschia* in the Gulf of Naples), dinoflagellates flower at times determined by such events as the timing of stratification. Fisheries science has long recognized the existence of trends in stock abundance on decadal time scales related to climatic trends, and the occurrence of 'regime shifts'. Similar patterns were recognized by several contributors to this conference. Regime shifts based on bifurcation theory are suggested to lie behind changing HAB frequencies in the Bohai Sea, China (**J. Feng**), and there may be decadal trends in the range of *Gymnodinium catenatum* in New Zealand waters (**Lincoln MacKenzie**). Changing species dominance in the Black Sea (**Violeta Velikova**) and on the Sonora coast of Mexico (**R.C. Altamirano**), and the 'opening of a raphidophyte niche' in Delaware estuaries (**Kathryn Coyne**) may be further examples pointing to regime shifts. The absence of HAB problems - *Gymnodinium catenatum* has hardly appeared in Galician waters for a decade, and *Pyrodinium bahamense* has not been prominent in the Philippines for several years – may also be aspects of analogous phenomena.

Hydrodynamic processes set the stage for algal blooms, but as Australian entomologist A.J. Nicholson taught us [20], to understand population dynamics we need to «find which of the factors are influenced, and how readily they are influenced, by changes in the density of animals», or, in our case, algae. He meant in part that climatic events, being density independent, cannot *regulate* population numbers in any dynamical sense. At the end of *On the Origin of Species* (1859), Darwin wrote: «It is interesting to contemplate a tangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent upon each other in so complex a manner, have all been produced by laws acting around us. These laws ... being growth with reproduction; inheritance ... and ... natural selection ...»; these are the laws which *do* regulate numbers, and are the same laws Nicholson had in mind insofar as they act on ecological time scales.

The planktonic and benthic communities in which harmful algae live are also tangled in many ways. Zooplankton in some circumstances avoid phytoplankton, eg. *Chrysochromulina polylepis* [21], *Karenia mikimotoi*; it has been shown that two freshwater species of *Peridinium* can control their recruitment to the water column in response to grazer abundance [22], and thus gain a







head start. These are just a few examples. At this conference we heard of 'predatory' bacteria which lyse *Microcystis* cells **(Jabulani Gumbo)** as well as algicidal **(Patricia Blair)** and symbiotic ones **(Christoher Bolch**; **David Green)**, endosymbiosis of *Pedinomonas* in *Noctiluca* **(Ken Furuya)**, allelopathy and parasitism (see below), and of a variety of predator-prey relations; *Favella* eats *Alexandrium* **(Takashi Kamiyama)**, as does *Acartia* **(M. Laabir)**, *Amphidinium* eats raphidophytes **(Sascha Kloepper)**, *Prymnesium* eats bacteria and algae when starved of nitrogen **(Wanderson Carvalho)**, *Metridia* eats *Pseudo-nitzschia*, apparently without harm **(Evelyn Lessard)**, *Eurytemora* eats *Dinophysis* **(O. Setälä)**. These things at least *can* happen, whether they *do* is another matter [23]. Organ transplants too are a component of eukaryote technology **(Tsvetan Bachvaroff)**. There was also speculation about a *positive* predator-prey relation; grazing on the tasty stuff followed by nutrient regeneration allows other species to bloom **(Tore Johannessen)**.

Social interactions have been classified as mutualistic, altruistic, selfish, or spiteful [24]. These are the «non-predatory» forces [25] which integrate population and community ecology. Quorum sensing is a kind of mutualism, and was raised as a possibility in phytoplankton again **(Louis Peperzak)**. *Gyrodinium instriatum* zygotes produce cysts in the presence of other cells, but vegetative cells if cultured alone; thus «... zygotes are able to distinguish the existence, and further, the approximate density of other cells of the same population» [26], so that population growth rate is controlled socially. Maybe some similar mechanism accounts for the fact that planozygotes of several other species in some circumstances divide rather than encyst **(Rosa Figueroa)**. Spite is equivalent to allelopathy, it reduces the fitness of others, if only temporarily. Allelopathic effects were observed in food crops millenia ago, and some modern agronomists think they may in the future be able to use allelochemicals to replace synthetic chemicals for the management of weeds and pests.



Allelopathy is usually mediated by ectocrines, *secondary* metabolites (i.e., which influence ecological interactions, as opposed to *primary* metabolites involved in essential processes within the cell) released from the cells, and a standard if not entirely conclusive procedure (there may be direct competition for resources too, not easily manipulated independently) is to test the impact of filtrates from one species on the growth rate of another. The effectiveness of such strategies in natural conditions must depend on mixing rates, which will determine if functional concentrations can be achieved and maintained [27]. A simple logistic model of allelopathic interactions between two species suggests that the equilibrium is unstable, so that local extinction of one of the pair should result [28]; Ushida [29] uses a logistic model to look at the relations between co-cultured *Heterocapsa circularisquama* and *Gymnodinium mikimotoi*, and provides evidence that the different outcomes depends on contact rates as well as on ectocrines.

The potential importance of allelopathic mechanisms is perhaps indicated by invasive species when they form monospecific blooms, indicating that some strong competitive mechanism is at work; recipient communities may be naïve to newcomers' ectocrines. In the HAB context, several studies have emphasized that the compounds involved in allelopathy are distinct from those we usually refer to as toxins [30], and the same point was made several times at this meeting **(Julia Kubanek** for *Karenia brevis*, **Sanna Suikannen** for two cyanophytes (and see [31]), **Tilman Alpermann** and **Urban Tillman** for *Alexandrium*). The powerful toxins produced by *Pseudo-nitzschia multiseries* do not have any allelopathical effect **(Nina Lundholm)**.

Amoebophrya is a parasitic dinoflagellate whose vegetative phase infests various free-living forms including *Alexandrium*, *Scrippsiella* and *Dinophysis*, and which may offer «the somewhat remote possibility of biological control» of harmful species [32]. The infestation





rates are usually only a few percent, but in a Chesapeake Bay study, it was found that the dynamics of the host-parasite relationship are quite complex, and that epidemics are restricted to a thin layer near the thermocline [33], and that the parasites may be host specific [34]; so *A. ceratii* may contain several cryptic species, a point frequently made with unicellular organisms [35]. Coats reported mortality rates of infected *Gymnodinium sanguineum* of 5-8%/day, but epidemics were also observed in a thin layer near the pycnocline, with infection rates of 20 to 80% and mortality as high as 54%; mortality rates <5% from this cause were estimated in *Dinophysis norvegica* in the Baltic [36]. New details of this parasite's life and new hosts were reported - **Jong-Gyu Park** lists nine hosts from Korean waters, and **Mario Sengco** four which can be infected with *A. ceratii* derived from *Alexandrium*. *Amoebophrya* can infect *Karlodinium micrum* (= *veneficum*), and protects itself from the host's karlotoxin by imitating the dominant sterol (**Allen Place**) with which the host protects itself (**Jonathan Deeds**). Just how complex the responses of individual phytoplankton species in the tangled bank might be was explored using network analysis by **Ferenc Jordan**, who showed that the bottom of the marine food web, where HABs occur, and the top, where overfishing happens, can be surprisingly close to each other functionally; in agreement with Jackson *et al.* [37], he argues that overfishing may have substantial effects on phytoplankton species abundance.

Karlodinium micrum was first described by Braarud from Walvis Bay [38], and subsequently off southern Norway and in the North Sea, in the Swan and Canning estuaries in western Australia, in the Chesapeake system, and elsewhere. It is thus estuarine and 'cosmopolitan', and is linked to fish kills. It can be confused with *Pfiesteria*, *Gymnodinium estuariale*, and other taxa. Ballantine's *Gymnodinium veneficum*, isolated from Plymouth waters by Parke, is identical to *Karlodinium micrum* (**T. Bergholtz**), and since it was described one year before, the name to use from now onwards for both is *Karlodinium veneficum*. As we learned in Hobart (Tengs), *Karlodinium* shares a unique feature with *aureolum* and *breve*, namely plastids which contain chl1 + chl2 + fucoxanthins; these plastids distinguish this group from other dinoflagellates, and link them to haptophytes by tertiary endosymbiosis.

The *Ludi Saeculares* (secular games) of ancient Rome were celebrated at long but irregular intervals, and the word secular is sometimes used for events which occur every century or so. Maybe the most difficult question we can ask about domoic acid (DA) poisoning is, given what we now know about the widespread occurrence of toxic *Pseudo-nitzschia* species, why has there only been one recorded outbreak of poisoning in humans (caused by mussels from PEI, Canada, in 1987 [39]). DA in seaweed extracts (*Digenea*) has been used as anthelminthic in oral doses of 0.5 mg DA/kg body wt, but doses in the 1987 event ranged from 31 to 128 mg DA/100g muscle, much higher. Deaths of sea birds in Monterey Bay, California in 1991 led to the identification of domoic acid on the US West Coast, and since then several other mortalities of marine vertebrates have been attributed to DA. It is not always easy to identify sources of DA when it is detected, and, while nearly monospecific *Pseudo-nitzschia* blooms do occur (**Brian Bill**), mixtures seem to be the norm (e.g., **Keri Baugh**, NE Pacific; **Eileen Bresnan** and **Johanna Fehling**, Scottish waters; **C.I. Churro**, Aveiro, Portugal; **Roberta Congestri**, Tyrrhenian Sea), and toxic and non-toxic species can be dominant at different seasons (**Keith Davidson**). Cryptic diversity, already referred to, is widespread in *Pseudo-nitzschia*, and could partially explain variability in toxin content.

Christian Gottfried Ehrenberg, one of the 'fathers' of microscopy and micropalaeontology, witnessed a luminescent bloom in the waters of Sorrento (Gulf of Naples) in 1860; he called the organism responsible *Peridinium splendor-maris*, but in 1873 he made a new genus for the species, which became *Blepharocysta splendor-maris*; Stein in 1883 mixed everything up by illustrating *Blepharocysta splendor-maris* with images of a different organism, the one with no cingulum well known to taxonomists; **Malte Elbrächter** has now rediscovered Ehrenberg's material and demonstrated that it is an *Alexandrium* species, *A. balechii*, perhaps the same species as that which has been known to form massive, luminescent blooms in the neighbouring Gulf of Salerno in the



1980s [40]. How can a species given such a lustrous name escape detection for 120 years? [41].

On the lighter side, we have a new way to collect cysts – melt sea ice **(Janne-Markus Rintala)**, a new definition of the sea bed: «The bottom is the solid surface where distance between parts is smaller than a man's foot.» **(Dolors Blasco)**, and an example of how competitive HABs are - **Else Hegseth** told us of HAB species *under the midnight sun*, in the Lofotenfjord (in 68-70 degrees N latitude), but was upstaged by **Kin-Chung Ho** with his HAB species in Svalbard (in about 78 degrees N).

HAB2004 attracted about 400 participants from nearly 60 countries. The programme guide contains 428 abstracts, of which 320 were presented as posters. Apart from the seven plenary talks, other oral presentations were inevitably divided between parallel sessions. So, we could only listen to half the orals, and at a nominal five minutes for each poster, one would need 27 hours or approximately three working days to read them all. The preceding notes dwell on about 20% of these contributions, and large areas of the conference are not mentioned at all; no attempt has been made to include the many interesting presentations dealing with physiology, toxins, monitoring, public health, etc, and the editors would welcome contributions on these aspects from other participants. For a review of some of the contributions on cyanophytes, see the accompanying article by **Ian Falconer**.

Tim Wyatt & Adriana Zingone





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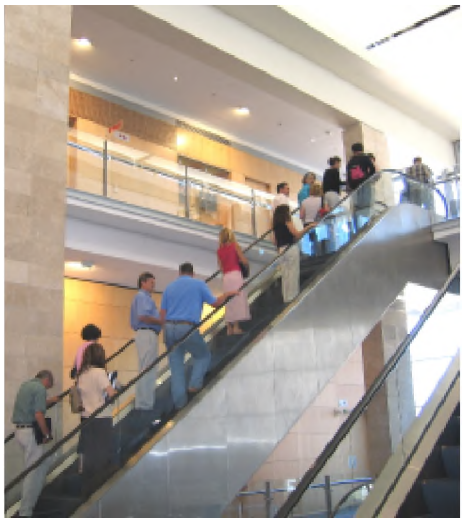
41. This is a relatively easy *Alexandrium* to identify, yet it has only been recorded from the South Tyrrhenian Sea, Greece and Florida, where it was re-described by Karen Steidinger. The nomenclatural implication of Malte's finding is that the name of the genus *Alexandrium* should rather be *Blepharocysta*, but happily the ICBN (International Code of Botanical Nomenclature) allows *nomina conservanda* (names to be kept despite the priority rule) in such special cases. But what we have called *Blepharocysta* for ages has to be renamed, and *Alexandrium balechii* should probably become *Alexandrium splendor-maris*.



• **Indonesia**

Mass Fish Mortality in Jakarta Bay: HAB organisms as the culprit?

National HAB Task Force Needed

SATURDAY, May 8th, 2004 seemed to be a bad day for local tourists when they went to Ancol Bay (or Jakarta Bay) for recreation and/or family gatherings. Instead of having fun with their family and breathing fresh sea air after a hard working week, they found thousands of dead fishes lying on the beach [2]. It was not only bad for the tourists, but also for the fishermen who live near the beach.

A week after the incident, people were starting to point at the culprit. The fishermen, because they were afraid to catch and sell the dead fish, would have no income for days, and accused the National Oil Company (Pertamina) of being responsible for the disaster, while some environment NGOs concluded that the main groups responsible were the industries that were discharging their wastes into the bay. A local government officer, however, told the press that the incident happened because local fishermen's boats spilled oil in the bay's water. Later, some research groups from reputable institutions such as the Oceanographic Research Institute – National Science Institute (P2O – LIPI), Oceanography Study Program – University of Indonesia (PSK – UI), and Oceanography Study Program – Bogor Agricultural Institute (PKSPL – IPB), found that the *causa prima* of the fish mortality was non-toxic HAB organisms such as *Noctiluca scintillans,* and *Trichodesmium erythraeum* [3, 7].

The main questions aired in the Indonesian atmosphere for almost a week were: «What is (or are) the cause(s) of the incident?» and «Who will take the responsibility?» No single answer found widespread support. Each group «stubbornly» maintained it had the best results and findings.

These disputes simply aggravated the situation. People became confused by the conflicting conclusions of the different groups. Local fishermen were not going to catch fish, while people were afraid to buy and eat fish as well as other seafood captured from the surrounding waters. Meanwhile, the government remained silent, as usual. Furthermore, it took more than a week to clean fish debris from the bay, and the odours as well.

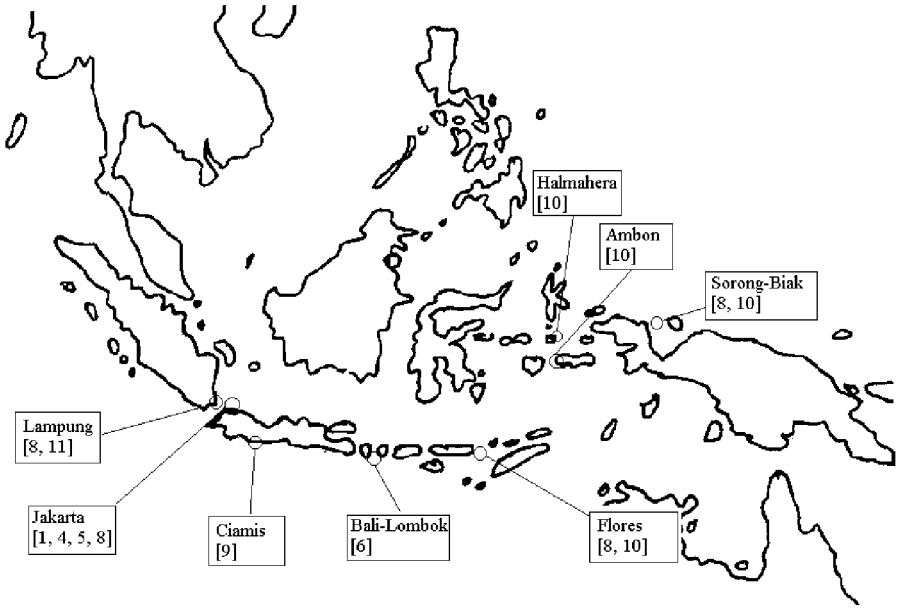


Figure. Map of Indonesian waters. Circles show the position of HAB organisms reported by the researchers. Numbers indicate the source cited.

Why did such a situation arise? Probably the most important reason is that, up to the present time, Indonesia has no official or National HAB Task Force as other Southeast Asian Nations (ASEAN) do, for instance, in the Philippines and Vietnam. Therefore, there were no official statements to keep the public informed of the official findings on such an incident. A HAB Task Force is assumed to comprise interdisciplinary expertise, such as marine biologists, fishery scientists, marine oceanographers, fishermen, NGOs, medical practitioners, politicians, government officers, and other group committed to HAB issues. Such a National HAB Task Force should receive a full mandate from the government to do monitoring and other activities related to HAB nationwide.

Why does Indonesia need such a Task Force? Some researchers in the field have reported that Indonesian waters are not free from HAB organisms (see Figure). Some HAB microalgae such as *Dinophysis* spp., *Alexandrium* spp., and *Pseudo-nitzchia* spp. are found off the coast of Jakarta [5], and others such as *Gymnodinium gracile* and *Dinophysis ovum* occur in the Bali-Lombok Strait [6], *Pseudo-nitzschia* spp., *Coscinodiscus* spp., and *Chaetoceros* spp. occur in Ciamis Bay [9] and the cyst of *Pyrodinium bahamense* var. *compressum* in Hanura Bay, Lampung [11]. Twenty years ago, *Pyrodinium bahamense* var. *compressum* was reported to cause the death of some fishermen who ate fresh shellfish captured at Lewotobi Bay, Eastern Nusa Tenggara and Kao Bay, Ambon [10]. The same organism is often found in the waters around Halmahera Island [8]. See also the table below.

What are the obstacles to establish such a Task Force in this country? First of all must be the government's political will, which has no strong policy for its national marine aspects. Second, Indonesia that has a very long coastline, about 81,000 km, a difficulty in term of management and monitoring activities. Third, there is still a dearth of researchers in this field in the country, and they live mainly in Java, the main and most populated island in the country. Fourth,

Table. HAB species reported from Indonesian waters

HAB Species	Location	Source
Pyrodinium bahamense var. *compressum*	Lewotobi, east Flores	[8, 10]
	Kao Bay, Halmahera	[10]
	Ambon Bay	[10]
	Kotania Bay	[8]
	Sorong and Biak Bays	[8, 10]
	Hurun Bay, Lampung	[8]
P. bahamense var. *compressum* cyst	Hanura Bay, Lampung	[11]
Noctiluca scintillans	Off Jakarta Bay	[1, 4, 5]
Trichodesmium erythraeum	Off Jakarta Bay	[4]
	Eastern coast, Lampung	[8]
Pseudonitzchia spp	Ciamis Bay	[9]
P. pungens	Jakarta Bay	[4]
Prorocentrum spp	Jakarta Bay	[4]
Peridinium sp	Jakarta Bay	[1, 4]
Skeletonema costatum	Jakarta Bay	[1, 4]
Tintinnopsis sp	Jakarta Bay	[4]
Dinophysis spp	Off Jakarta Bay	[5]
	Bali-Lombok strait	[6]
D. caudata	Jakarta Bay	[4, 8]
Alexandrium spp	Off Jakarta Bay	[5]
Gambierdiscus toxicus	Lewotobi, east Flores	[8]
Gymnodinium spp	Jakarta Bay	[4]
	Bali-Lombok strait	[6]
Ceratium furca	Jakarta Bay	[4]
Coscinodiscus spp	Ciamis Bay	[9]
Chaetoceros spp	Jakarta Bay	[1, 4, 8]
	Ciamis Bay	[9]

the regional monetary crisis of 1998 still affects the government's budget for research and development (R & D) for its huge marine resources. Fifth, up to the present time, there was no official and formal group of researchers in this field at the national level.

One good thing with regard to HAB issues in the country is that there is an informal mailing list group namely Indonesia_HAB@yahoogroups.com that has more than 20 members nationwide and actively discussing and exchanging information about issues related to HAB, both in the country and abroad. But the group still needs assistance to develop, and open for support from and joint-programmes with other groups.

The latest mass fish mortality in Jakarta Bay is an appropriate moment for the Government to take action. Through the Department of Marine and Fisheries, government can help people to prevent arguments such as those that followed the abovementioned incident. A National HAB Task Force is evidently the better choice in this regard than an independent group or two of researchers from research and/or academic institutions doing research and monitoring on HAB related cases in the country. Because their results, findings, and conclusions, more often than not, might still confuse and frustrate people.

Other than that, Jakarta Bay, which is assumed to be the front door of this country, badly needs continuous monitoring, not only for HAB organisms but also for other aspects as one-whole ecosystem. Jakarta Bay since 1981 has become the most frequent place in the country not only for HABs, but also for mass fish mortalities . Thus, government should prepare the supporting infrastructures including regulations, experts, and the most important thing is their will (especially their political will to do so).

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• USA

Cochlodinium polykrikoides in the Peconic Estuary

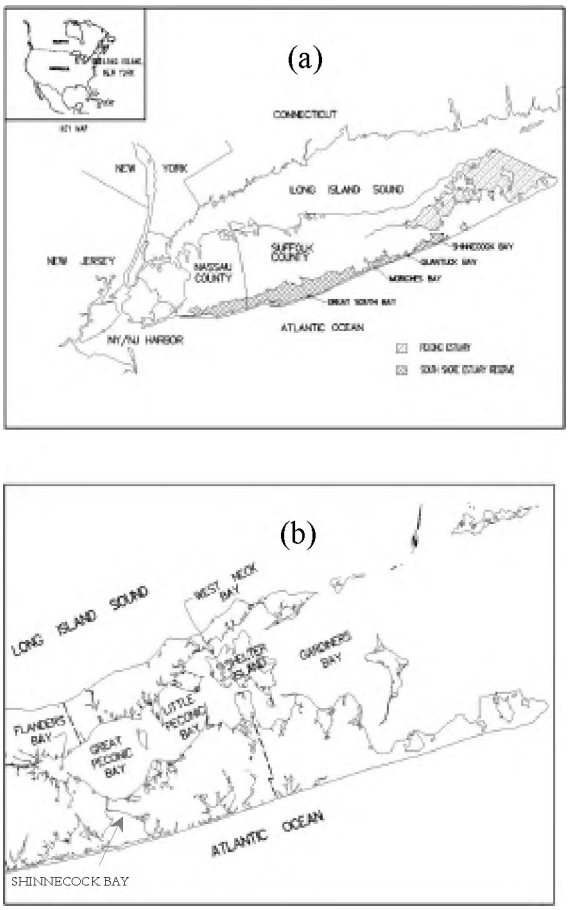

Fig. 1. (a) Long Island, NY. (b) Peconic Estuary.

A bloom of the dinoflagellate, *Cochlodinium polykrikoides* (syn. *C. heterolobatum*) has been identified in the Peconic Estuary, Long Island, NY, USA (Fig. 1), an area undoubtedly more familiar as the original focus of the «brown tide», *Aureococcus anophagefferens* [1]. The bloom occurred in late September-early October, 2004, when patches of red water were noted in Flanders Bay, extending into Great Peconic Bay and, ultimately, into neighboring Shinnecock Bay, through a one-way (south) flow canal.

The organism was found in chains of two to eight cells, with an occasional single cell noted. Cell size ranged from 25 – 40 µm, with the terminal cells somewhat longer than wide. The hyposome of the chain's terminal cell was bi-lobed. Cells within the chain were either spherical or slightly depressed dorso-ventrally. A large nucleus, and numerous chloroplasts were present, and the cingulum (girdle) made almost two turns around the cell. A stigma was contained within the episome (Fig. 2). As a live sample was not available, identification was made from lugol-preserved samples.

This had been thought to be the initial *Cochlodinium* bloom in the area, however, review of previous files and photomicrographs revealed the occurrence of a similar bloom in West Neck Bay, a subembayment of the Peconic Estuary, in early September, 2002. Cell numbers during the 2004 bloom were estimated, by counting all cells within a Palmer-Maloney counting chamber, to be over 2 x 10^6 per liter. No population estimate was made of the 2002 bloom, but it was noted that the waters, contained within a very poorly flushed area, were quite red.

Although *C. polykrikoides* has been implicated in kills of impounded fish in Japan [2], China and Korea [3] and, more recently the west coast of Canada [4], the Gulf of California [5], and the Philippines [6], no incidents of dead fish were reported during or after either bloom in the Peconic Estuary where motile species were free to leave the area. Hargraves and Maranda [7] reported a bloom in Pt. Judith Pond, Rhode Island, USA approaching 10^7 cells per liter, but also did not observe any fish mortality. *C. polykrikoides* (identified as *C. heterolobatum*) has also been reported to form intense blooms in the mouth of the York River, a tributary of Chesapeake Bay [8] and, beginning with a bloom in 1992, spreading into the lower bay [9].

Because *C. polykrikoides* is myxotrophic [10], engulfing phytoplankton species with equivalent spherical diameters of < 12 µm, its place in the food web, and its effect on estuarine ecology, regardless of its fish-killing potential, is difficult to determine. While larvae of the mussel *Mytilus galloprovincialis* have been shown to be capable of preying upon *C. polykrikoides* [10], its nutritional value relative to other species that may have been displaced has not been determined. Other studies have indicated that metamorphosis of oyster (*Crassostrea gigas*) trochophore larvae is retarded [11], and calcium uptake by larvae of the American oyster, *Crassostrea virginica*, is depressed, and mortality elevated by exposure to *C. polykrikoides* [12].

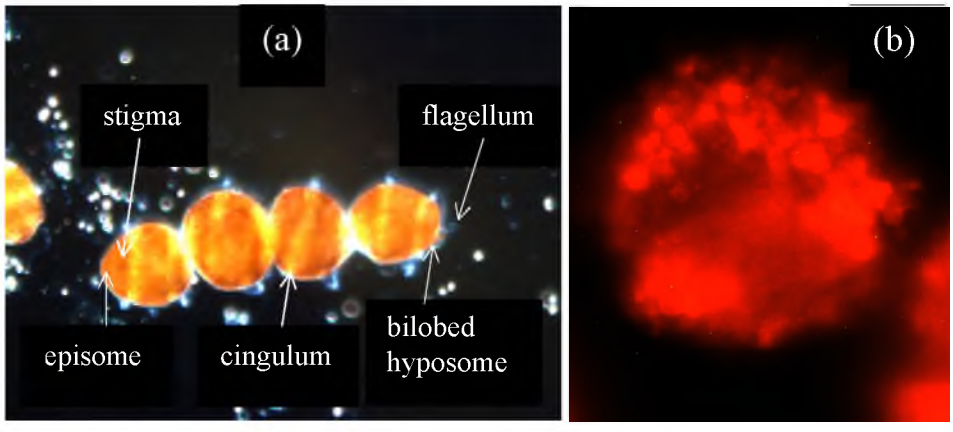

Fig. 2. (a) 4-cell chain illustrating the various structures. (b) Chloroplasts within a single cell visible under fluorescent microscopy.

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XI th International Conference on Harmful Algal Blooms, Cape Town Nov 2004

Cyanobacterial news

Those of us researching into toxic cyanobacteria were offered two excellent conference choices this year. The first was the International Conference on Toxic Cyanobacteria in Bergen, Norway in June, and the second the HAB conference in Cape Town in November. Few could afford both! Fortunately this coincidence does not occur in 2006, when the HAB conference is in Copenhagen, as the Toxic Cyanobacteria conference is in Brazil in 2007.

Among the cyano papers at the HAB conference was a fascinating account by **Karen Arthur** of tumours in green turtles feeding on seagrass covered by the filamentous *Lyngbya*. This organism is well known for causing severe skin burns on bathers rubbing against the filaments, but had not previously been recognised as an ecological problem. The turtles were exposed to *Lyngbya* toxins through feeding on the seagrass, and also through physical contact with the filaments. The tumours appear at the flipper junctions and on the face. The increased dominance of *Lyngbya* is attributed to nutrients from fertiliser and sewage increasing in the shallow coastal bays of southern Queensland. Karen received the award for best student presentation for this paper.

Two other papers also focussed on the carcinogenic effects of algal toxins, **Valerie Fessard** evaluated the genotoxic effects of okadiac acid (the toxin of diarrheic shellfish poisoning) on cultured mammalian cells, showing that it caused chromosome loss and therefore potential cancer. To identify whether this is a real risk will require epidemiological studies of people who were sick in past outbreaks of this shellfish poisoning.

In my paper on the health impacts and carcinogenicity of the cyanobacterial toxin cylindrospermopsin, both the acute poisoning and potential carcinogenicity were discussed. Preliminary data on cancer rates in a population known to have been acutely poisoned and whose water supply was contaminated by this toxin for several years, indicated an increase in gastrointestinal cancers.

The majority of research on cyanobacteria was located in the poster sessions, and included several studies of *Microcystis* and *Cylindrospermopsis* and their toxins. The sophistication of new techniques applied to these studies was impressive, with increased use of genetic markers for toxin gene detection coupled with chemical or immunological toxin measurement. One interesting ecological aspect was the generally toxic North American, South African, Indian and European strains of *Microcystis*, compared to tropical African lakes with huge *Microcystis* populations with negligible toxicity.

The increasing data on *Cylindrospermopsis* and cylindrospermopsin in Europe is fascinating. In an extensive survey of German lakes by **Claudia Wiedner**, **Jutta Fastner** and colleagues, no *Cylindrospermopsis* strains were found that produced cylindrospermopsin. However many lakes tested showed cylindrospermpsin in the water. It is apparent that some other species is producing the toxin in European waters, whereas in North America, Asia and Australia the most frequent source is *Cylindrospermopsis*. This implies that the presence of cylindrospermopsin in water supplies may be a much wider risk than was anticipated.

On Wednesday morning the cyanobacterial researchers gathered for a round-table discussion on research priorities, and the particular problems faced by southern Africa. The Global Water Research Coalition earlier in 2004 did a similar exercise, meeting in Australia. The key areas of the toxicity of cylindrospermopsin, management of reservoirs and on-line or direct monitoring techniques for toxicity were discussed. The need for very basic detection methods and drinking water treatment technology in less developed countries was highlighted.

Overall a good conference, well organised and in a delightful setting.

Ian R. Falconer, University of Adelaide, Australia.



NOTE:

NEW HAB SPECIES IDENTIFICATION GUIDE TO SOUTH EAST ASIA

A guide to potentially toxic microalgae of Vietnamese waters has been published as Opera Botanica No. 140. Although the guide builds on Vietnamese material it will be of interest to anyone working with harmful algae in the South East Asian region.

Larsen, J. & Nguyen, N.L. (eds), 2004. Potentially toxic microalgae of Vietnamese waters. Opera Botanica 140: 5-216. Copenhagen.

ISBN 87-88702-85-5.

Future events

MARCH 2005

GEOHAB: OPEN SCIENCE MEETING ON HABS AND EUTROPHICATION. March 7-10, 2005. Baltimore, Maryland, USA.

The conference topics include:

- Global trends in eutrophication and HABs
- Physiology of HABs with respect to nutrients
- Comparative studies on HABs in eutrophic areas
- HAB programs in global eutrophic areas
- Modeling of nutrients and HABs
- New methodologies for nutrient and HAB monitoring

Further information at: http://www.jhu.edu/scor/GEOHAB-OSM3.htm

APRIL 2005

MARINE AND FRESHWATER TOXINS ANALYSIS: FIRST JOINT SYMPOSIUM AND AOAC TASK FORCE MEETING. April 11-14, 2005. Baiona, Spain .

Convener: Department of Analytical and Food Chemistry. Universidade de Vigo, Spain.

The symposium will address new developments and validation efforts in the analysis of marine and freshwater toxins, and is held as a joint meeting with the AOAC Task Force on Marine and Freshwater Toxins.

The conference program is planned to maximize interaction between the Symposium and the Task Force: Presentations will address special needs of the community from toxin monitoring to new concerns in the intentional contamination of food and water.

Further information at: http://www.aoac.org/marine_toxins/task_force.htm

HARMFUL ALGAE NEWS

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The Intergovernmental Oceanographic Commission of UNESCO August 2005

HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

http://ioc.unesco.org/hab/news.htm No. 28

• *Tunisia*

Detection of toxic *Alexandrium catenella* (Whedon & Kofoid) Balech in clam production zone of North Lake and Channel, Tunisia

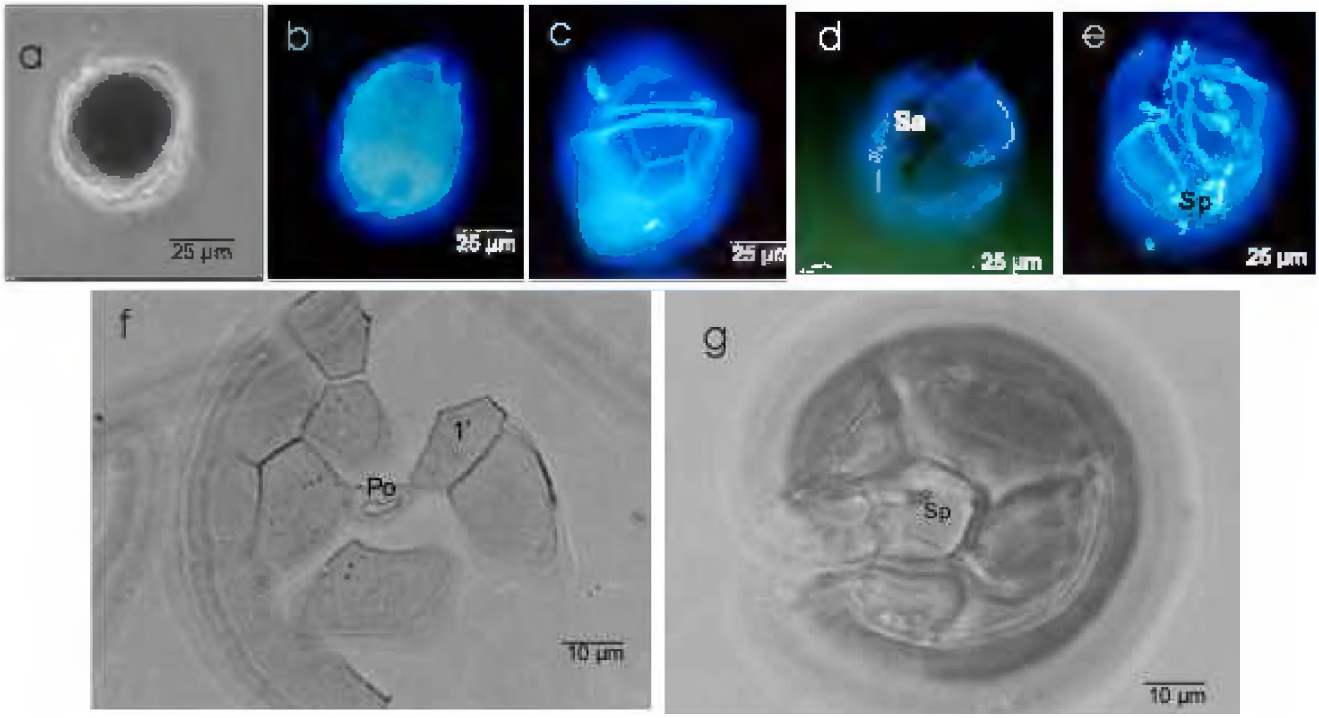

Fig. 2. Alexandrium catenella*: (a) microphotograph with phase contrast; (b) microphotograph with epifluorescence, ventral view; (c) dorsal view; (d) Po, 1' and Sa; (e) sulcal plates; (f) apical plates of epitheca; (e) Hypotheca.*

A dozen species of *Alexandrium* have been identified in the Bay of Tunis, of which two are toxic, *A. tamarense* and *A. minutum* [1]. During our monitoring of the production zones of bivalve molluscs in northern Tunisia, *A. catenella* was detected for the first time in the Tunis Channel in August 1997. This species seems to be well adapted to the Mediterranean, and may have been introduced with ballast water [2]. In addition, these are the kinds of ecosystem, estuarine and lagoonal, which are most vulnerable to invasions [2].

(Cont'd on p.2)

SEED: A new EU-US collaboration on HABs

The word SEED is not an acronym or abbreviation; it directly refers to seed as produced by plants. However, the indication 'SEED' refers to a new project entitled *Life cycle transformations among HAB species, and the environmental and physiological factors that regulate them*, for which we do not want an acronym.

The project is funded by the Global Change and Ecosystems Programme of the European Commission and the U.S. National Science Foundation. SEED started in March 2005 and will continue for three years. SEED is endorsed by the Global Ecology and Oceanography of Harmful Algal Blooms programme of the Intergovernmental Oceanographic Commission of UNESCO (GEOHAB) as a Targeted Research Project.

Two coordinators, a steering committee, and 12 partners make up the

(Cont'd on p.3)

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo and the Institute of Biology, University of Copenhagen.

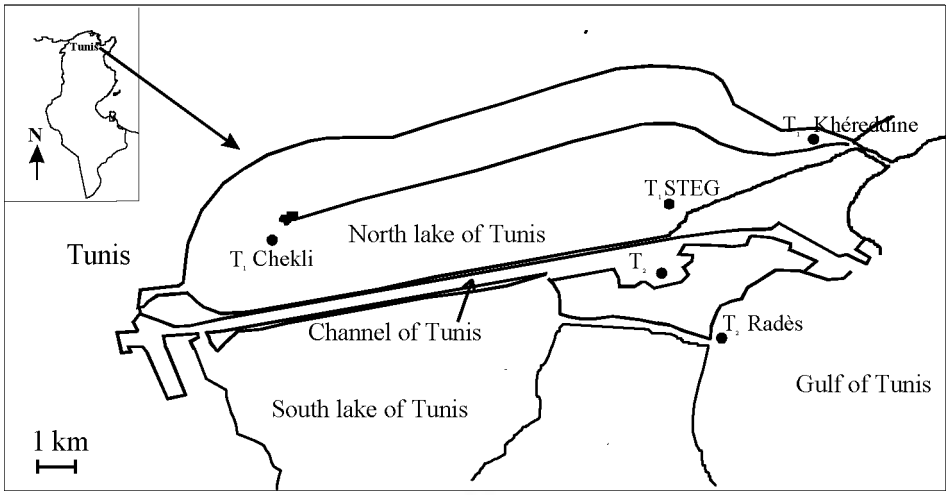

Fig. 1. Sampling stations in shellfish production area of North lake and Channel of Tunis.

In the Mediterranean, the first appearance of *A. catenella* dates from 1983 along the Catalan coast [3], and its distribution gradually extended along the northwest Mediterranean coast with recurrent blooms during the period 1997 to 1999 in the port of Barcelona, and with associated production of paralytic toxins [4]. Toxic episodes due to the presence of gonyautoxins and saxitoxins in L'étang de Thau are attributed to *A. tamarense* with *A. catenella* also suspected [5]. The latter species has also been noted in Sardinian waters (Tyrrhenian Sea) [6].

The present report follows the annual pattern of this toxic species in the North Lake and Channel of Tunisia (Fig. 1) where the main resources of the clam *Tapes decussatus* are concentrated. These two sites have been monitored for toxic planktonic microalgae since 1996 [7].

The North Lake of Tunis was earlier marked by the presence of recurrent 'green tides' of *Ulva lactuca*, and important ecological changes have occurred in the last dozen years following restoration works which were made in the period 1984 to 1988. In particular, there has been a net increase in macrophyte diversity, and marine phanerogams have appeared [8]. Among the toxic species mentioned in the clam production zones in the North Lake and Channel, only species producing diarrhetic toxins, *Dinophysis* spp and *Prorocentrum lima*, have previously been detected [9, 10]. The navigation channel is strongly perturbed by anthropogenic pollution due to the existence of three ports, with a direct impact on the benthic macrofauna which may extend as far as the adjacent Gulf of Tunis [11]. A first appearance of red water due to a bloom of *Noctiluca scintillans* was recorded in March

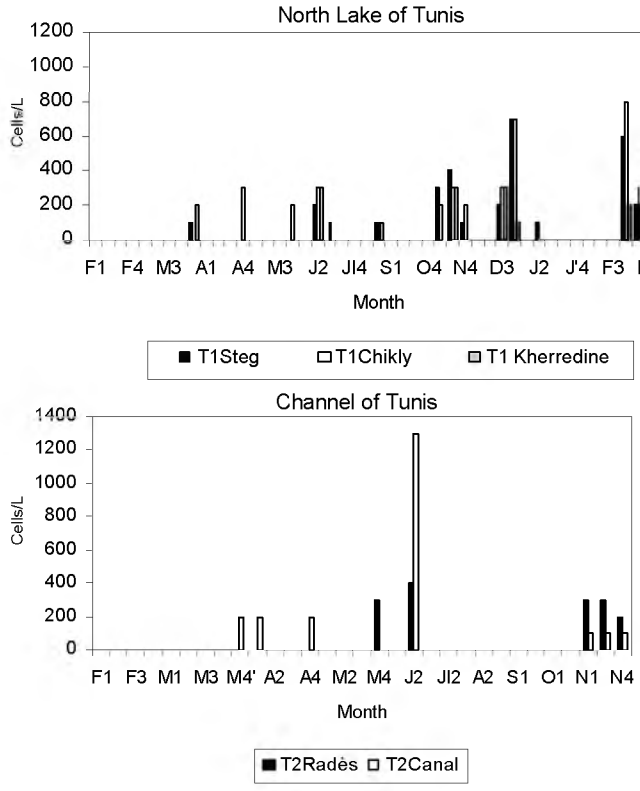

Fig. 3. Concentrations of A. catenella *(cells/L) in the clam production zone in the North Lake and Channel of Tunis (February 2004 - March 2005).*

(Cont'd from p. 1)

2003 along the southwest coast of the Gulf.

The characteristic morphology of *A. catenella* is shown in Fig. 2. These cells are solitary, and rarely seen in chains or pairs. Total length varies from 25 to 32.5 µm, and the transverse diameter from 19 to 32 µm. The epitheca is very fragile relative to the hypotheca, which sometimes lacks the pore at the level of plate Sp. This species is found at certain times of the year (March 2005) accompanied by *Scripsiella trochoidea* and *Gonyaulax spinifera*. Differentiation between these cells is made by means of epifluorescent microscopy after staining the cells with calcofluor.

Annual variations in the abundance of *A. catenella* above the critical level of 500 cells/L have rarely been found except in June in the Channel of Tunis, and in two periods, December and February to March, in the North Lake (Fig. 3). Estimated maximum concentrations are respectively 1300 cells/L in the Channel (T2) in March 2005 and in June 2004, and 1000 cells/L in the North Lake (T1 Chikly).

It is still too early to state that this species is increasing in abundance in the clam production zones in the North Lake and Channel of Tunis. Our future studies should allow us to follow the evolution of *A. catenella* which has been detected since its first appearance.

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consortium of SEED and include scientists from 7 European countries and the USA.

SEED aims to understand to what extent environmental and physiological factors may influence the non-vegetative stages of the life cycles of harmful algal bloom (HAB) species, thereby contributing to the increase in harmful algal blooms (HAB) in European marine, fresh, and brackish waters. SEED will focus on the life histories of some of the most relevant HAB species in Europe.

The areas to be studied comprise the regions bordering coastal sites in the Western Mediterranean Sea, Atlantic Ocean, North Sea, Baltic Sea, and Swedish lakes. All of these regions have heavy anthropogenic influences: fisheries, urban development, aquaculture and tourism. All are subject to the frequent occurrence of HABs, with a variety of detrimental impacts including human intoxications, closure of shellfish farms, water discoloration causing a negative impact on tourism, etc.

The approach of SEED is comparative, ranging from species to ecosystem levels. Despite considerable phylogenetic distance, many morphological and physiological traits of HAB species are convergent and common.

The specific objectives are:

1. To characterize and quantify the different stages of the life cycle in a bloom development of selected HAB species in comparative environments.

2. To characterize the physiological mechanisms and tolerances underlying dormancy stages/cysts, spores and akinetes, their formation and germination for key HAB species.

3. To explore the mating interactions of non-toxic and toxic HAB species to better understand the nature of species invasions or dispersions and to evaluate the use of non-toxic strains as a possible mitigation strategy in HABs.

4. To quantify the flux of cells of key HAB species in different areas from and to the sediments.

5. To investigate the role of sedimentary dynamics in the preservation, accumulation and dispersion of dormant stages.

6. To explore methods to identify specific life cycle stages of key HAB species through molecular or immunological techniques.

7. To use the newly acquired knowledge in simple numerical models to verify the relevance of life history features and to develop or refine predictive and conceptual models of bloom dynamics for selected HAB species, taking into account life-history.

8. To determine the role of dormancy stages of HAB species in different environments in relationship to the expansion/increase of HABs. To formulate principles that explain similarities between ecosystems.

To facilitate the development of conceptual and numerical models of HAB dynamics, it is imperative to recognize common patterns of response among species. In recognition of this convergence and to carry out cost-effective research, SEED will take advantage of existing model species while keeping the main focus on an array of target HAB species, which range from marine to brackish to fresh water organisms, and cover a broad range of phylogenetic types (dinoflagellates, diatoms, raphidophytes, cyanobacteria).

New results will be used to elucidate and evaluate the role of life cycle processes, especially dormancy stages, in bloom dynamics. Furthermore, one aim of SEED is to integrate the acquired knowledge into the development of new model formulations. This improved modelling will enhance prediction and support the development of new management and mitigation strategies for these species.

For further information on the project, visit our website: www.icm.csic.es/bio/seed/



From bottom to top and from left to right: Toni Jordi, Gotzon Basterretxea, Esther Garcès, Karin Rengefors, Donald Anderson, Mercedes Masó, Robin Raine, Jordi Camp, Silvia Anglès, Jordi Solé, Kees van Lenning, Rosa Isabel Figueroa, Antonella Penna, Marina Montresor, Magda Vila, Kalle Olli, Nicolas Touzet, Deana Erdner, Santiago Fraga, Sanna Suikkanen, Isabel Bravo, Anke Kremp, Jane Lewis, Hartmut Barth, Marta Estrada, Mariagrazia Giacobbe, Elena Bertozzini, Cecilia Satta, Antonella Luglié, Maria Laamanen, Carmen Hormigo, Dolors Blasco.

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• *Mexico*

Ostreopsis siamensis (Dinophyceae) a new tychoplanktonic record from Isabel Island National Park, Pacific Mexico



Fig. 1. Study area and sampling locations.

An early summer sampling programme was carried out from 18th May to 26th June 2004, around Isabel Island National Park (Fig. 1). Isabel Island is a major Pacific nesting site for seabirds, principally frigate birds, blue footed and yellow footed boobies, pelicans, swallows, sea gulls and brown footed boobies. Its origin is volcanic, dating back approximately 3.5 million years; it has been used as a temporary camp for fishermen in the area. The climate is tropical, with a long wet summer season (May to October) and a short dry winter season (November to April). The rainy season coincides with hurricanes and tropical storms in the zone. Diverse and abundant coral reef communities cover 4.93% of the island [1]. The study was done to describe the general phytoplankton community, and to document the distribution of toxic dinoflagellates. Until now, harmful algae have never been investigated in this island. We report for the first time the occurrence of the potentially toxic dinoflagellate *Ostreopsis siamensis* Schmidt 1902, and it's geographical distribution in the Mexican Pacific area.

Phytoplankton samples were collected during early summer at five stations in Isabel Island National Park. The samples containing *Ostreopsis siamensis* were obtained from surface net tows using a 62 µm pore size plankton net, towed by boat at a low speed for 4 minutes, very close to coral reef in shallow waters. The specimens were fixed immediately with 4% glutaraldehyde solution. Surface water temperature (25°C), salinity (35.3‰) and dissolved oxygen (6.15 mg.L^{-1}) were measured in-situ with an YSI model 85. Phytoplankton cells were identified using the Utermöhl technique and a phase-contrast microscope. Morphological details were observed through scanning electron microcopy (JEOL/OE jsm-6360).

The morphological characteristics of the organisms were in accord with the descriptions of Fukuyo [2] and Faust [3], which coincide in all plates of the genus, the epithecal (Fig. 2a), the hypothecal (Fig. 2b), ventral zone (Fig. 2c) and both kind of pores (Fig. 2d). The shapes and dimensions of the Po plate are characteristic of each species and different from the other species as shown in Fig. 3. The dorso-ventral distance ranged from 88 to 129 (½ 101)µm and transdiameter from 65 to 90 (½ 78.4)µm. Our report confirms the presence of *O. siamensis* as part of the natural biota in this latitude. It is possible that Isabel Island waters generate favorable environmental conditions for the proliferation of benthic dinoflagellates, which are transported later by currents to the plankton zone (= tychoplankton). The species has often been reported as benthic, epiphytic, but is most frequently associated with sand and as an epiphyte on seaweeds [4]. Further analysis will outline the

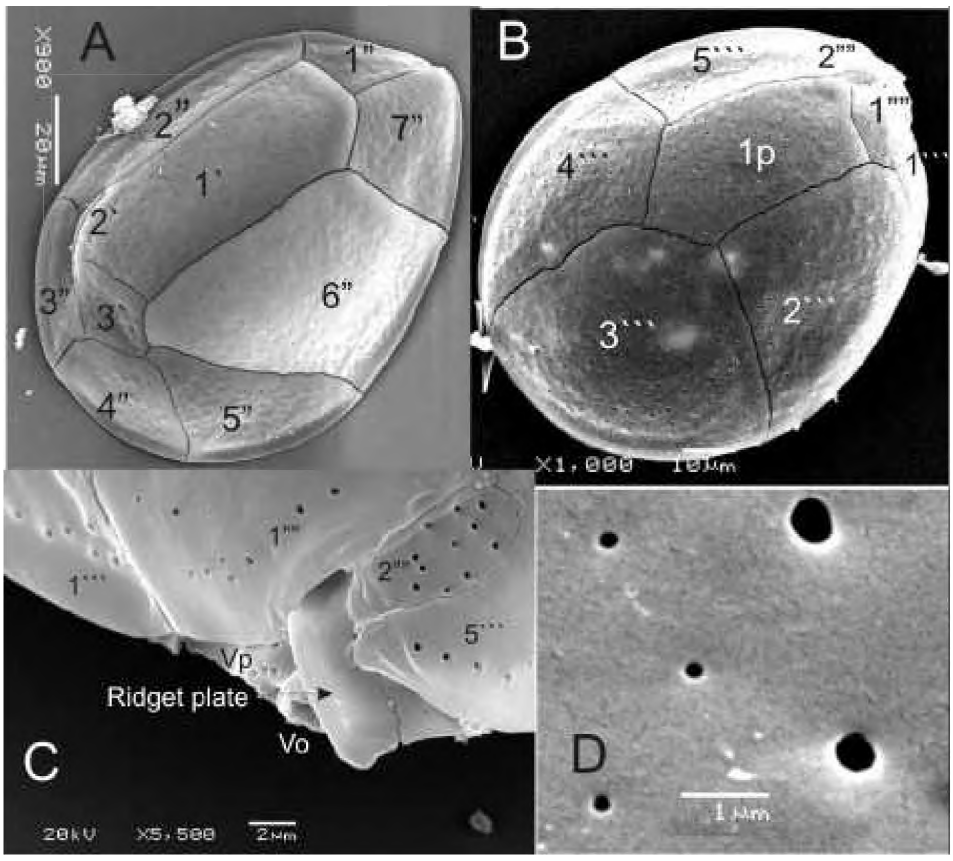

Fig. 2. Thecal plates of Ostreopsis siamensis: a) Epitheca, b) Hypotheca, c) Ventral region, d) Small and large pores.

seasonal distribution, outbreak times, summer-winter survival and species succession in this fishery location, as well as its abundance. According to Faust [3] the ecology of *O. siamensis* is diverse; the type species was identified from plankton samples collected by Schmidt from the Gulf of Thailand. Some years later, the morphology of *O. siamensis* was reexamined by Fukuyo [2] based on specimens from Ryukyu Island in Japan. *O. siamensis* is also known from South Florida, Florida Keys, Puerto Rico, U.S. Virgin Islands, Hawaii, Guam and New Zealand [5, 6].

This species is known to be toxin producer. Nikajima *et al.* [7] were the first, followed by Yasumoto *et al.*, [8] and Holmes *et al.* [9] to indicate the presence of toxins produced by *O. siamensis* populations, which produces ostreocin D, an analog of palytoxin, one of the most potent marine toxins known. Palyotoxin can accumulate in fish and crabs and is considered responsible for clupeotoxism, an illness linked to the eating of sediment-feeding fishes in Japan [6]. It is also thought to be the cause of the multiplicity of symptoms in the pathology of ciguatera [10]. The toxin affects first coral grazing fish, then piscivorous fish, notably groupers, and finally humans. Other organisms affected are snapper, mackerel, jack, goat fish, barracuda, parrot fish, and some gastropods [5]. Until now, no clinical case of ciguatera has been reported in the island by health authorities, but the high to moderate abundances of *Ostreopsis* cells observed raise the possibility that ostreocin or another kind of palytoxin, are responsible for different seafood poisoning (ciguatera, clupeotoxism, etc.). Strong coastal surface currents have been proposed as major players in the transport of cells to the island waters.

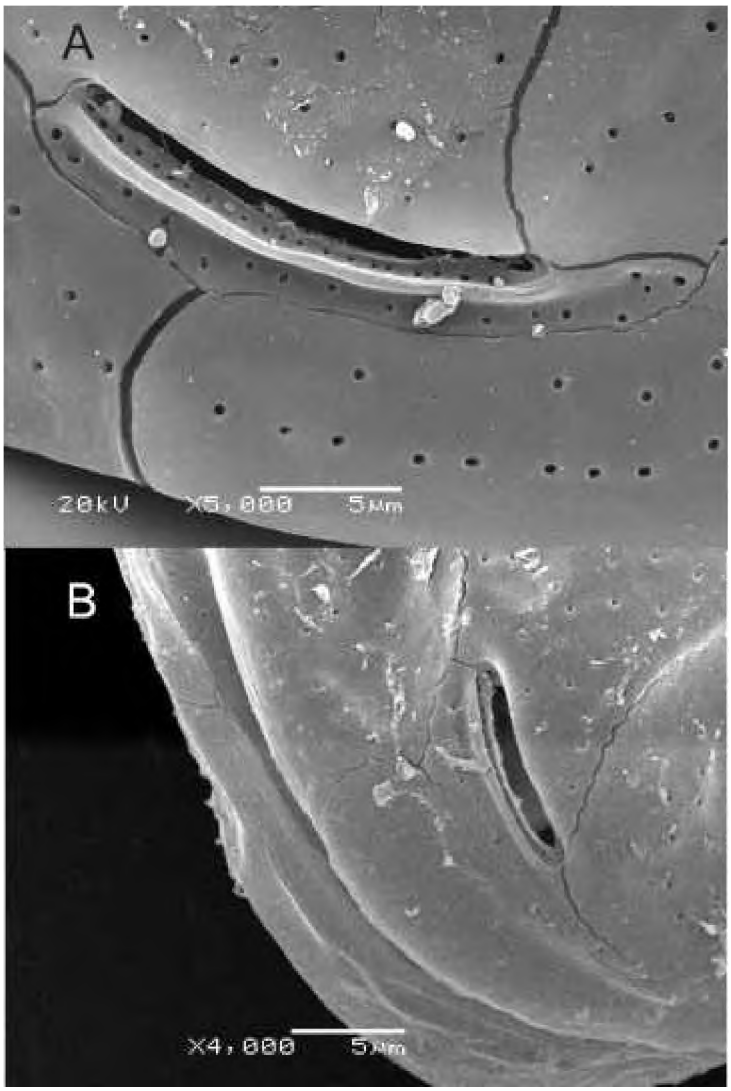

Fig. 3. The morphology of the Po plates: a) O. siamensis, b) O. ovata.

Acknowledgements

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• *Turkey*

Toxic and harmful algal species in the Izmit Bay, Marmara Sea

During the last 40 years, nutrient concentrations have obviously increased in many estuaries and coastal waters in the world due to the influence of human activities [1, 2]. This problem of marine eutrophication has been highlighted in recent years by the occurrence of increasingly severe toxic phytoplankton blooms in many nearshore waters worldwide. Toxic phytoplankton blooms in the sea are of even greater concern than in freshwater ecosystems [3]. Red tides and other toxic and/or noxious algal blooms mainly due to progressive eutrophication from terrestrial inputs have been observed by reporters and Turkish scientists [4]. Balkis and Aktan [5] reviewed plankton studies in the Marmara Sea (except Izmit Bay) continuing since the 1990s [6-10, 11], leading to the identification of 11 toxic phytoplanktonic species.

Izmit Bay, located in the north-eastern part of the Marmara Sea is one of the most polluted areas in the Marmara Sea, and an important centre for Turkish industry. The bay has been receiving more than 300 industries' effluents together with untreated domestic waste waters from the small cities, and from Izmit which is the most populous city of the region. Pollution problems have gradually become more serious since 1960. Previous studies showed that the bay waters have become eutrophic [1, 12] and red tides due to *Prorocentrum* species, and fish mortalities, have been observed in some periods (unpublished data).

This paper focuses on the toxic and/or harmful phytoplanktonic and benthic microalgal species recorded in Izmit Bay. In March 1999 and September 2000, phytoplankton, epiphytic, and epipelic algae were collected from sampling sites.

Fourteen toxic and harmful species; dinophytes *Ceratium furca* (Ehrenberg) Claparéde & Lachmann, *Dinophysis acuminata* Claparéde & Lachmann, *Dinophysis acuta* Ehrenberg, *Dinophysis caudata* Saville-Kent, *Dinophysis sacculus* Stein, *Gymnodinium sanguineum* Hirasaka, *Lingulodinium polyedrum* (Stein) Dodge, *Noctiluca scintillans* (Macartney) Kofoid & Swezy, *Phalacroma rotundatum* (Claparéde & Lachmann) Kofid & Michener, *Prorocentrum micans* Ehrenberg, *Prorocentrum minimum* Schiller; and diatoms *Pseudo-nitzschia delicatissima* (P.T. Cleve) Heiden in Heiden & Kolbe, *Pseudo-nitzschia* sp. (*Nitzschia seriata* (Hasle) were recorded in the bay. In addition to these species, *Prorocentrum lima* (Ehrenberg) Dodge was recorded as epipelic and epiphytic (on *Bryopsis hypnoides* Lamour, *Codium fragile* (Sur) Hariot, *Cystoseira barbata* C. Agardh var. *barbata, Cymadocea nodosa* (Uchria) Ascherson and *Zostera noltii* (Horneman)) in the littoral zone. *Prorocentrum lima* is a benthic dinoflagellate usually found attached to or associated with macrophytes, floating detritus, debris or other substrates and less commonly in plankton. These species were common in Izmit Bay, but toxic and noxious algal blooms were not recorded during the sampling period.

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• *Mexico*

First record of *Chattonella marina* in Bahía de la Paz, Gulf of California

Harmful algal blooms are common events in Bahía de La Paz, a coastal lagoon located on the southwest side of the Gulf of Calfornia [1]. A massive bloom of *Chattonella marina* was observed from April 24 to May 9, 2005 in Bahía de La Paz (Fig. 1). Live cells of *C. marina* were identified in unfixed samples. For cell enumeration water samples were fixed using acid lugol (1%) and analyzed with the standard Utermöhl technique [2]. Fixed cells were deformed and shrank making it difficult to identify without observing live cells (Fig. 2). Cell densities fluctuated between 1.9 and 3.5 x 10^6 cells L^{-1}. Cysts of *C. marina* were not observed in these samples, which suggests that environmental conditions were good for this species. Sea surface temperature during the bloom was 22°C. During monitoring on May 4th at a different collection site, *C. marina* cells were also observed but in lower concentrations

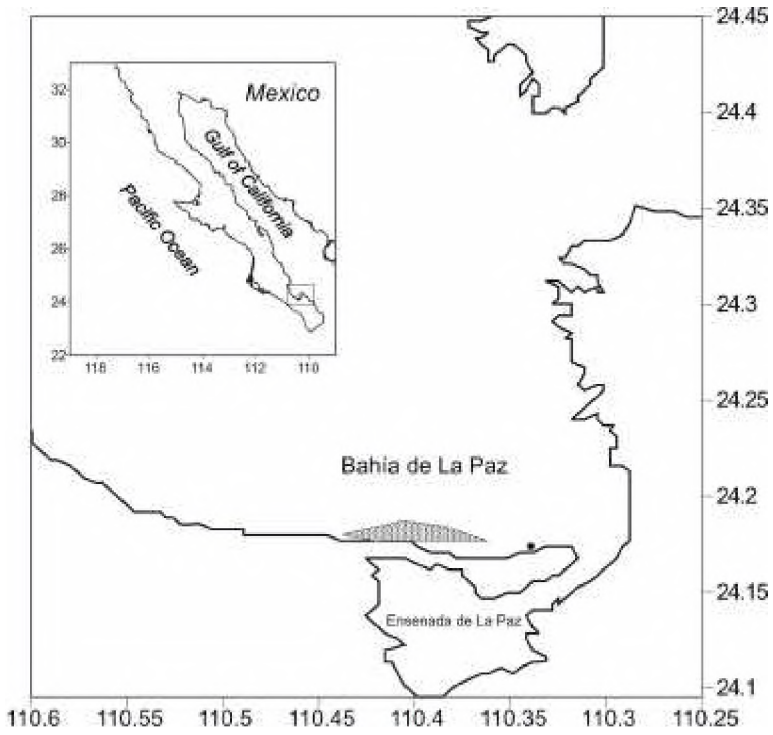

Fig. 1. Collection site showing location and size of Chattonella marina *bloom (shaded area) in Bahía de La Paz. * Indicates the site of additional sample.*

which ranged from 11 to 23 x 10^3 cells L^{-1}. As in other areas, different cell morphologies were observed (Fig. 2). However, in the samples collected on May 4th, round cells resembling temporary cysts were observed.

The presence of the ichthyotoxic phytoflagellates *C. marina*, *C.* cf *ovata*, *Fibrocapsa japonica*, and *Heterosigma akashiwo* (Raphidophyceae) have been reported in various localities on the Mexican Pacific coast as well as in the Gulf of Mexico [3, 4, 5], including a previous bloom report on the east coast of the Gulf of California [6]. However this is the first report of a bloom of *C. marina* for Bahía de La Paz. The highest abundances estimated for this event were an order of magnitude higher than previous reports [6].

Most marine Raphidophyceae are ichthyotoxin producers, and have caused severe fish mortalities with significant damage to the aquaculture economy in several countries. In Mexico loss of marine resources was associated with the presence of *C. marina* and *C.* cf *ovata* in Kun Kaak Bay, Sonora [6]. In Bahía de La Paz no fish or shellfish mortalities were observed, however the magnitude of this event is of special concern for fisheries and aquaculture activities in this area.

It is possible that these species have been undetected in most Mexican waters due to traditional methods used for sampling and preserving phytoplankton. Raphidophyceans like other flagellates, are very delicate, and deform or disintegrate in formalin solution; samples preserved with lugol have different morphology but can be recognized by a well-trained eye. We recommend that during the monitoring of harmful algae, non-preserved samples should be taken to observe the presence of delicate phytoplankton forms. During the bloom, clonal cultures were isolated. These will be used in the near future to confirm toxicity.

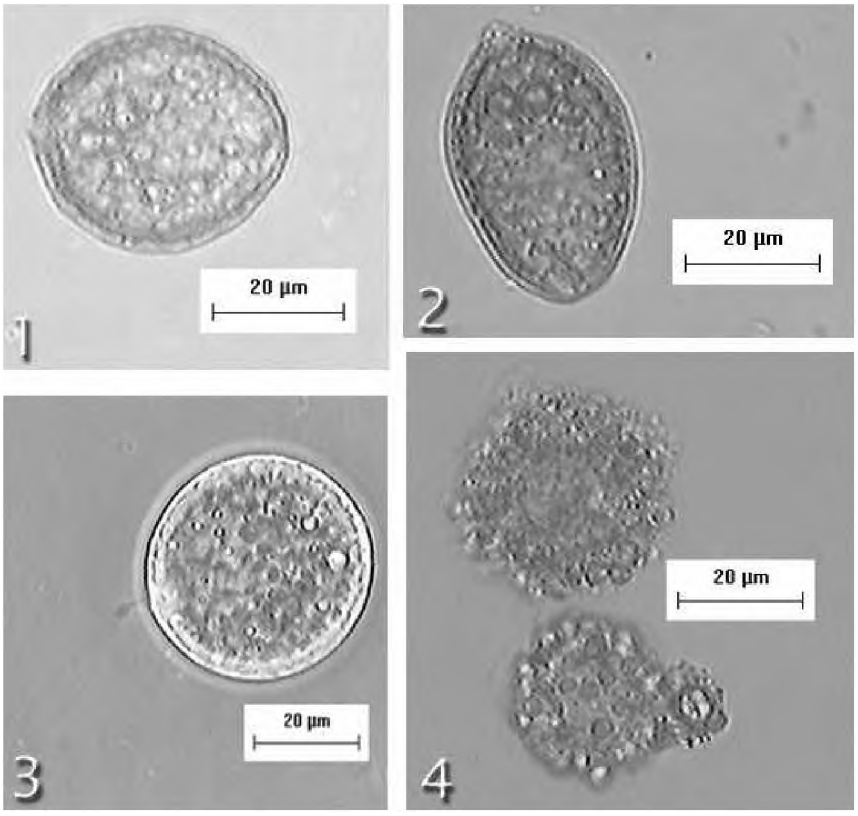

Fig. 2. Light photomicrographs of Chattonella marina *collected in Bahía de La Paz. Live cells (1-3), acid lugol fixed cells (4), and spherical cell showing flagella (3).*

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• *Italy*

First report of *Cylindrospermopsis raciborskii* in Italy

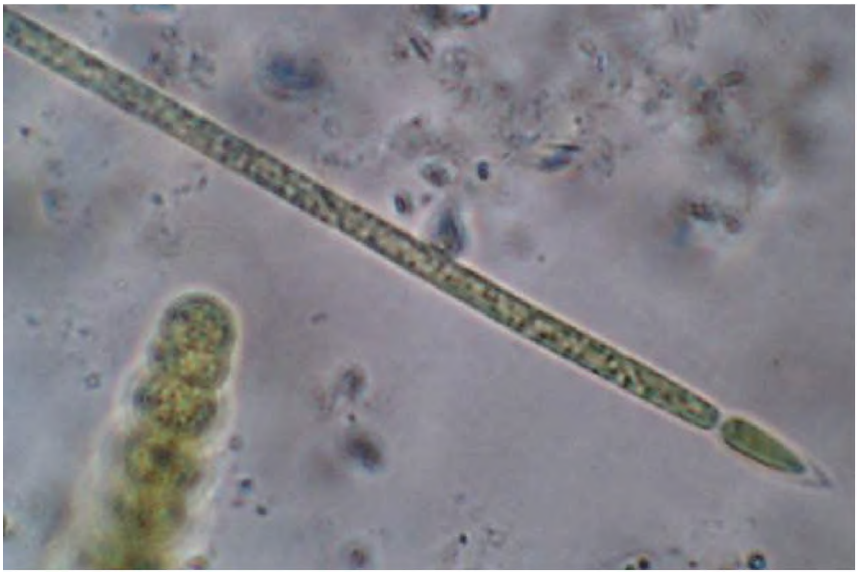

Fig. 1. Cylindrospermopsis raciborskii (600x).

Abstract

Cylindrospermopsis raciborskii is a freshwater cyanobacterium of tropical origin, also found in temperate regions. It can produce toxins like cylindrospermopsin (CYN) and saxitoxins (STX, NEO). In 2004, it was detected in three Italian lakes (Albano Lake, Trasimeno Lake and Cedrino Lake). Two of the three examined cases were toxic to a liquid chromatography coupled with tandem spectrometry apparatus (LC-MS/MS), and analysis demonstrated the presence of cylindrospermopsin (CYN).

Formerly *C. raciborskii* blooms were detected in 1995 (Trasimeno Lake), in 2002 (Albano Lake) and in 2003 (Cedrino Lake), but in those cases no toxicity analysis were performed due to the lack of a standard toxin.

Introduction

Cylindrospermopsis raciborskii (Wolos.) Seenaya et Subba Raju (Fig. 1) is a cyanobacterium found principally in freshwaters, although it has been detected also in the Caspian Sea [1].

C. raciborskii is filamentous, variable in length and 1-5 mm wide. The extremities of its trichomes are sharp, due to the shape of the terminal heterocysts [2].

C. raciborskii releases, during senescence or as a consequence of environmental stresses (such as thermal shocks or water purifying processes), a dangerous toxin, cylindrospermopsin, but saxitoxins (STX, NEO) can also be produced [3], the toxin affects the liver, kidneys, thymus and heart. Additionally, a carcinogenic potential can be observed *in vitro* [4].

The first episode of cylindrospermopsin human poisoning, caused by contaminated drinking water, was recorded in Palm Island (Australia) in November 1979, and led to an outbreak of hepatoenteritis [6].

C. raciborskii, originally known from central African and Australian lakes, is now to also recorded in temperate regions such as Germany [7], Portugal [8], and France [9].

Massive blooms of *C. raciborskii* were first noted in Italy in summer (June to September) 1995, in the lakes of Albano and Trasimeno. Moreover, from September to November 2003 trichomes of this species were observed in raw waters of a drinking water treatment plant in Sardinia (up to 15 x 10^6 cells L^{-1}), which treats water extracted from Cedrino Lake.

As the blooms of this cyanobacterium do not appear as floating scums and only slightly modify water colour, it is difficult for human eyes to spot the hidden danger. It is necessary to prevent access to contaminated waters and to conduct steady and exhaustive controls on endangered waters, and to improve public awareness of this issue.

Methods

Samples were collected from Albano Lake (July 2004) and Trasimeno Lake (Sept 2004) in central Italy, and from Cedrino Lake (Oct 2004) in central Sardinia.

For toxin analyses, 1 L water surface samples were collected in the central areas of the three lakes.

The microscopic observations of phytoplankton were carried out on Lugol fixed samples, using an inverted microscope. Cell density was determined by the Utermöhl technique. For the chemical analysis 0.2 ml of the sample (water) was directly injected into a liquid chromatography coupled with tandem mass-spectrometry apparatus (LC-MS/MS). Quantitation of cylindrospermopsin in samples was assessed by comparing its peak area resulting from the total ion current to that obtained by injecting a standard solution. The limit of quantification (LOQ) of the method was 0.22 ng/ml. The liquid chromatograph consisted of a Waters pump (Model 1525µ, Milford, USA), inline filter (2 µm pore size), Alltima 5-µm C-18 guard (7.5 x 4.6 mm i.d.) and analytical (250 mm x 4.6 mm i.d.) columns (Alltech) and was interfaced to a Micromass 4 MICRO API, Waters. For fractionating the analytes, phase A was acetonitrile and phase B was water, both 10 mmol/L formic acid. The mobile phase gradient profile was as follows (t in min.): t_0, A= 0%; t_8, A= 16%; t_9, A= 100%; t_{12}, A= 100%; t_{13}, A= 0%; t_{22}, A= 0%.

Results

The phytoplankton analysis revealed that the maximum density of *C. raciborskii* in Albano Lake (July 2004) was 6 x 10^6 cells L^{-1}, in Trasimeno Lake (Sept. 2004) was 9 x 10^6 cells L^{-1}, in Cedrino Lake (Oct. 2004) was 81 x 10^6 cells L^{-1}.

The chemical analysis revealed a concentration of cylindrospermopsin in

Albano Lake of 15 ng ml^{-1}, toxin trace in Trasimeno Lake (0.46 ng ml^{-1}) and no toxicity was found in Cedrino Lake.

Discussion

Cylindrospermopsin is an unusual alkaloid (m.w. 415 Da) and consists of a trycilic guanidine moiety combined with hydroxymethyluracil [10]. Apart from liver toxicity, mutagenicity [11] was shown *in vitro* and evidence also exists for its carcinogenicity *in vivo* [12]. The LD_{50} (i.p.) in mice is cells/kg 2,1 mg/kg over 24 h [13]. The occurrence of *C. raciborskii* on the three Italian lakes is correlated with a toxic presence of cylindrospermopsin only in two cases. The maximum density of *C. raciborskii* was found in the Cedrino Lake but the maximum toxin concentration was registered in Albano Lake: this demonstrates that wild populations of cyanobacteria species, catalogued as toxic, not necessarily or always, are implicated in toxin production.

Few years ago, the presence of *C. raciborskii* was revealed in Europe, only as far as Germany [6] Portugal [7], France [8] and now the presence in Italy is confirmed by observations over a ten-year period. The extending geographical distribution of this species could be linked to the migration of water birds like gulls, as they are important agents of dispersal for many aquatic organisms, by carrying resistant forms within their intestinal tract. The dispersal of freshwater species not capable of active overland transport and lacking resistant forms, while not so well documented has largely been attributed to transport via external surfaces of birds [14]. Comparative studies on ecological conditions and on phylogenetic relations of European wild populations of *C. raciborskii* could help to explain their difference in toxin production, too.

Anyway, these data show for the first time that *C. raciborskii* can also be found in Italian water bodies, also for drinking use, and that monitoring this cyanobacterium and associated cylindrospermopsin is a real emergent necessity.

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IOC Training Course on Qualitative and Quantitative Determination of Algal Toxins, Germany, 22 February to 2 March 2005

The IOC-BMU-AWI-DZMB-FSU JENA Training Course on Qualitative and Quantitative Determination of Algal Toxins was held at the Wattenmeerstation Sylt, AWI in List/ Sylt, Germany from 22nd February to 2nd March 2005. The course was the first in a series of five cooperative training courses implemented by IOC and Forschungsinstitut Senckenberg (DZMB), Friedrich-Schiller-Universität Jena (FSU) und Stiftung Alfred-Wegener-Institut für Polar- und Meeresforschung in der Helmholtz-Gesellschaft (AWI). It was financed by IOC and Germany: Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit (BMU); Applied Biosystems Darmstadt and Mr J. Gosch, Gosch-Seafood Tourist Service GMBH, List/Sylt, supported the course.



The course was hosted at the Wattenmeerstation Sylt of AWI and or-

ganized by Dr. Malte Elbrächter (FIS) Germany and in cooperation with Dr. Henrik Enevoldsen, IOC, Paris, Prof. Dr. Allen Cembella, AWI-Bremerhaven, Germany and Prof. Bernd Luckas, FSU-Jena, Germany with his team. Applications arrived from 96 persons from 46 nations. Due to financial and space limitations only 12 participants originating from 11 countries could attend the training course.

The goal of the course was to provide participants with an overview of the state of the art of different analytical methods for qualitative and quantitative determination of phycotoxins. Lectures and demonstrations on all relevant toxins were performed, and the participants were encouraged to establish scientific collaboration.

Qualitative and quantitative determination of algal toxins using different analytical methods (HPLC with selective detection, HPLC/MS coupling and LC-MS) were made on samples of algae and mussels provided mainly by the participants. The laboratory experiments focused on the analysis of Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP), and cyanobacterial toxins by application of chromatographic methods.

The next training course is planned for October 2006.

Marine and Freshwater Toxins Analysis First Joint Symposium and AOAC, Baiona, Spain, 11 - 14 April 05



Task Force Meeting was held April 11-14, 2005, in Baiona, Spain. The conference was convened under blue skies at the Hotel Talaso Atlantico just above the rugged coastline of the prolific mussel-producing Galicia region. The meeting was well attended by a global mix of academics, government regulators, seafood and analytical industries and industry associations. Over 120 participants pre-registered and over 150 attended in total.

The Minister of Fisheries of the Galician Government as well as the former Minister of Health of Galicia, who was instrumental to success in his supportive role working closely with the University of Vigo since the first efforts to manage the threat of marine toxins in Galicia, opened the conference. Also present was the Vice Rector of Institutional Affairs. Symposium Chair Ana Gago Martinez, and Task Force Chair James Hungerford spoke briefly and next warm recollections were given by Jose Antonio Rodriguez Vasquez, chair of the Analytical and Food Chemistry Dept., University of Vigo, of exciting and productive collaborations of the University with visiting researchers from Japan and Canada. Other keynote presentations followed on the current state of marine biotoxin monitoring in Galicia and an overview and progress report of the Marine and Freshwater Toxins Task Force.

Many excellent keynote and contributed presentations were given throughout the meeting. A unique aspect of the conference in Baiona was the integration of the Symposium and Task Force meetings, with the symposium presentations (72 total, 35 oral) preceding subgroup discussions in relevant toxin areas. This allowed for discussion of method applicability, current validation efforts, future directions, and a variety of other topics relating to toxin analysis methods and their validation. Discussion threads from the new AOAC on-line forums of the Task Force sup-

plemented these in-person subgroup meetings and discussion periods. Subgroup areas included in the Baiona program included assays for saxitoxins, LC methodology for saxitoxins and domoic acids, okadaiates and azaspiracids, yessotoxins, and ciguatoxins. Benjamin Suarez-Isla, James Lawrence, Ana Gago Martinez, Aurelia Tubaro, and co-chair Robert Dickey (for ciguatoxin chair Richard Lewis) chaired these sessions. Two other areas discussed were cyanobacterial toxins and LC-MS methods for multiple toxin groups. These very active areas are new to the Task Force and will soon become formal subgroups when chairs are appointed this year.

Benjamin Suarez-Isla, the subgroup chair, led discussion of saxitoxin assays with the primary focus on an IAEA-funded initiative to validate a receptor-binding assay (RBA) for the saxitoxins using radiolabeled saxitoxin. He presented results of a Single Laboratory Validation (SLV) study submitted by Fran Van Dolah, the RBA assay project leader. Participants also suggested pursuit of receptor binding assays using alternative (non radioactive) labels. James Lawrence led subgroup discussion on LC methods for saxitoxins and domoic acids. A positive vote by the Task Force last year has moved a pre-column oxidation LC method for the saxitoxins into review by AOAC for official method status This method is now in the final stages of First Action approval following minor revisions. Still, post-column methods for saxitoxins also remain an area of interest, with recent advances made by Japanese and Canadian research groups.

Several presentations on the cyanobacterial toxins were also given and the availability of toxin test kits was discussed, building on responses obtained following a request for this information in the on-line forum. Participants shared some of their experiences with the kits and additional kits were brought into the discussion, as was the need for cyanobacterial reference standards. The ciguatoxin subgroup session began with a proposal put forward in the ciguatoxin on-line forum by Robert Dickey to establish a reference collection of ciguatoxic fish tissues. The fish tissues would cover a range of ciguatoxicity and tissue origin and would be characterized by a combination of cytotoxicity assay and LC-MS methods. Practical issues including stability, storage requirements, costs, and issues regarding tissue sample transport were discussed.

Okadaic acids, their congeners and also azaspiracids were discussed in a subgroup chaired by Ana Gago Martinez and this addressed a range of topics. These included both PP2A assay marketed in Japan based on enzyme inhibition and a Biosense method based on competitive binding assay, also using PP2A. Also the relative merits of these two functional assays versus LC-MS-MS became the subject of a lively discussion. At the conclusion of this session was a statement that validation of both classes of methods, those based on assays and also LC-MS, should be pursued without competition between the two. At this stage each group, consisting of the LC-MS enthusiasts and those working on functional assays like PP2A, should reach a consensus on extraction conditions, which congeners to include, etc. Then validation plans should continue to move ahead as the overall process can be time consuming. The toxin sessions concluded with presentations on yessotoxin toxicology and yessotoxin detection methods followed by a yessotoxin subgroup meeting chaired by Aurelia Tubaro of Italy. Existing methodology and validation plans were discussed from the standpoint of single laboratory validation, and the issue of which YTX forms to include in methods was also discussed. The symposium was concluded with presentations by European government, industry, and industry association stakeholders. In these talks the need for validated methods was stressed, as was the need to make them stakeholder-friendly.

Comments from those who attended this four-day combined symposium and Task Force meeting were very positive and discussions and plans made at the conference rekindled hopes in the validated of additional methods.

The on-line forums will be used to review and carry forward what was discussed in Spain and to engage in further discussions both the Baiona participants and all interested in development, validation and application of methods for the toxins. As another step in our continuous path towards validated methods, a meeting will be held June 22-24, 2005 in Seattle, Washington, USA, in connection with the Pacific Northwest Regional AOAC Meeting. In Orlando, Florida, USA, a full Task Force meeting consisting of several toxin subgroups, will meet as part of our second symposium-Task Force conference at the AOAC Annual Meeting September 11-15, 2005. Two symposia, including a full day devoted to *Marine and Freshwater Toxins Analysis: Quality Methods for Public Health and International Trade*, and also a half-day session to honor Wiley Award winner Mike Quilliam, will precede these Task Force meetings. As with all our meetings, we encourage both researchers and stakeholders (and potential stakeholders) to attend.



SEPTEMBER 2006

12th INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

September 4-8, 2006. Copenhagen, Denmark.

Call for submission of abstracts and registration form: February 1, 2006.

Deadlines:

- Abstracts reception: May 1, 2006
- Early registration: June 1, 2006
- Late registration: July 15, 2006

Further information can be found at: www.issha.org.

Future events

DECEMBER 2005

GEOHAB: OPEN SCIENCE MEETING. HABS AND STRATIFICATION

December 5-8, 2005. UNESCO Headquarters, Paris, France.

The conference topics include:

- Physical processes relevant to stratification
- Maintenance of HAB populations in thin layers
- Selection of assemblages by different turbulent regimes
- Changes in small scale physical properties caused by the phytoplankton community
- Implications for sampling, monitoring and operational oceanography
- Detection systems

Important dates:

- Abstract deadline: October 1, 2005.
- Early registration deadline: November 1, 2005.

Further information can be found at: www.geohab.info.

GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

Harmful Algae News

Previous issues of HAN and newsletters of the IOC HAB Programme can be downloaded at http://ioc.unesco.org/hab/news.htm

Requests for subscription

Subscription to HAN is made by sending a request with a complete address to Ms V. Bonnet (v.bonnet@unesco.org).

ISSHA
International Society for the Study of Harmful Algae

ISSHA President's Corner

News from the 11th HAB meeting in Capetown, South Africa

Announcement of election of officers for the next 2 years include Patricia Tester as President, Karen Steidinger as acting past President, Gustaff Hallegraeff and Beatriz Reguera as Vice President, Tracy Villareal as Secretary and Nina Lundholm as Treasurer.

ISSHA's emphasis during the next two years will be to update the web site. Tracy Villareal is working with Stephen Bates, former ISSHA Secretary, to accomplish this. Take a look at www.issha.org and see how that is coming. ISSHA is committed to increasing its visibility and will be featured in a poster during the Third Symposium on Harmful Algae in the U.S., 2-7 October 2005 (www.visitasilomar.com). Also, ISSHA is a co-host for the summer ASLO (American Society of Limnology and Oceanography) meeting in Victoria 6-9 June 2006. Increasing our membership base is key to our success as an organization and we welcome new members, especially, student members and say thank you to our lifetime members for their strong show of support.

Maureen Keller Awards

Five Maureen Keller Student Awards were presented by ISSHA at the 11th Conference on Harmful Algae held in Cape Town, South Africa. Please join me in congratulating these young researchers for their excellent oral presentations and posters. Also, thanks go to the other students who presented 67 posters and 19 oral presentations and whose work helped spark many discussions during the meeting. The 32 judges who generously agreed to help evaluate the efforts of this diverse body of student work were recognized individually at the banquet. However, I would like to say again how much I appreciate their assistance to the ISSHA awards committee.

Though no biography is present, mention should also be given to the fifth awardee, M-L Chateau-Degat for her presentation on Neurological Signs of Ciguatera Disease.

Pat Tester, ISSHA President.



Members of the ISSHA Council during the IV General Assembly of the Society (Cape Town, South Africa, 18 Nov. 2004). From left to right: Beatriz Reguera, Gustaaf Hallegraeff, Don Anderson, Adriana Zingone, Øjvind Moestrup, Edna Granéli, Allan Cembella, Max Taylor and Pat Tester.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

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• Costa Rica

Alexandrium monilatum (Howell) Balech bloom in the Gulf of Nicoya, Puntarenas

The Gulf of Nicoya, an estuary on the pacific coast of Costa Rica, extends 80 Km, from the Tempisque River to the Pacific, with a maximum width of 50 Km at the mouth, and an area of 1550 Km^2. Hydrography of the Gulf depends on rainfall and wind patterns. Nutrient advection into the Gulf, during the rainy season (May to November) carried by the Grande de Tárcoles and Tempisque Rivers [1], have a stimulant effect on phytoplankton abundance [1-3] and composition [4].

The Gulf of Nicoya is a very important fishing ground on the Pacific coast. About 800 families make their living from shellfish extraction in the Gulf. The Gulf waters and the surrounding mangroves are also important nursery areas for larvae and juveniles of commercially important species, and many beach areas have intense tourist activity. Therefore, harmful algal blooms pose a threat to the Gulf resources, fisheries, consumer health, the incipient aquaculture activity and tourism.

Phytoplankton blooms have been documented for the Gulf since 1954. However, the identification of the causative organisms started in 1980 when the organism responsible for a red bloom that affected the entire Pacific coast of Costa Rica was found to be *Cochlodinium catenatum* Okamura [5]. Red tides dominated by *C. catenatum* have been common in the Gulf, along with blooms of *Mesodinium rubrum* Lohmann. Other potentially harmful species of dino-

(Cont'd on p.2)



Fig. 1. Antapical view of A. monilatum, *showing the characteristic pore plate with a large pore an radiating striae. From the September bloom in the Gulf of Nicoya, Costa Rica.*

Newly formed Korean Harmful Algal Bloom Research Group (KORHAB)

Huge damage to fisheries by HABs occurs every year in Korea. In 2001, Korean researchers met during the 2nd international HAB meeting at the National Fisheries Research & Development Institute (NFRDI) in Busan, Korea to discuss, and to form the Korean Harmful Algal Bloom Research Group (KORHAB). Eventually, KORHAB was organized at the first official HAB meeting in Hanyang University on June 17, 2005, with the participation of over 100 researchers from Universities, Research Institutes and relevant agencies. The ac-

(Cont'd on p. 3)



The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Institute of Biology, University of Copenhagen.

(Cont'd from p.1)



Fig. 2. Long chains and solitary cells of A. monilatum *(light microphotographs of Lugol's stained cells) from the September bloom in the Gulf of Nicoya. Costa Rica.*

flagellates reported for the Gulf were *Pyrodinium bahamense* var. *compressum*, *Gymnodinium catenatum*, *Alexandrium catenella*, and *A. monilatum* [6].

Alexandrium monilatum has been found associated with other bloom organisms in the Gulf but, even when it reached high concentrations, was never previously the dominant organism [6]. But since August 27, 2005, a greenish coloured bloom has been developing, and *A. monilatum* is the most abundant organism present. Solitary cells as well as chains up to 108 cells long were observed (Fig. 2). Water samples collected off the Puntarenas peninsula, on August 31st, at depths of 1, 5 and 10 m, showed concentrations of 4.95 x 10^5 cells/L (99.5% *A. monilatum*), 2.6 x 10^5 cells/L (98.1% of *A. monilatum*) and 7.2 x 10^3 cells/L (55.4% of *A. monilatum*) respectively. Other dinoflagellates accompanying this bloom were species from the genus *Ceratium* and *Prorocentrum*. Surface water samples from Isla Jesucita showed maximum concentrations of 4.3 x 10^5 cells/L.

Table 1. Dinoflagellate concentration (cells/L and % of total number), at the Puntarenas dock, (9° 58' 025'' N and 84° 49' 769'' W), Gulf of Nicoya. Costa Rica, 02 September 2005.

Species	Depth					
	1m		5m		8m	
	cells/L	%	cells/L	%	cells/L	%
A. monilatum	2,053,000	98.4	325,000	82.3	96,500	95
P. bahamense var. compressum	17,000	0.81	3,500	0.9	0	0
G. catenatum	3,000	0.14	61,000	15.5	1,000	1
G. splendens	6,000	0.30	2,500	0.6	500	0.5
C. catenatum	1,000	0.05	0	0	500	0.5

Water sampling was repeated on September 2nd at the Puntarenas dock and *A. monilatum* continued to be the most abundant species. However, other toxic dinoflagellates were observed as well (Table 1).

Surface water samples collected on September 09, south of the Puntarenas peninsula (09°55'78''; 84°48'08''), showed a maximum concentration of 3.2 x 10^6 cells/L.

During the sampling periods, salinity ranged from 29 to 30‰ and temperature from 30 to 31.5°C.

In areas where concentrations were below 5 x 10^5 cells/L, water was greenish-grey in colour but, when concentrations were higher, water colour turned olive green. This bloom produced cream or beige colour foam at the tidal break line. Skin contact with the water during sampling in bloom areas produced intense itching, far more intense than the itching produced during sampling of *C. catenatum* blooms.

Alexandrium monilatum produces ichthyotoxin that has not been found to accumulate in filter feeding shellfish [7, 8], but is however lethal to many animals [9]. It is a warm water species that has been reported from the Gulf of México, the Caribbean, Venezuela and the Pacific coast of Ecuador [8, 10]. Juhl [9] indicates that it requires a high N cell quota which can help explain the rareness of the blooms in the Gulf of Nicoya since phytoplankton in the Gulf is N-limited during the rainy season [2, 4] when growth conditions are optimal in terms of water temperature and salinity.

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GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

The **GEOHAB Core Research Project: HABs in Upwelling Systems** is available at:
http://ioc.unesco.org/hab/GEOHAB.htm

(Cont'd from p. 1)
companying photo shows the first KORHAB organizing committee elected. Official meetings will be convened twice a year regularly with three special lectures by invited speakers and presentations by KORHAB members.

The main purpose of this group is to provide a unique atmosphere in collaboration and in sharing information between Korean researchers in various HAB related studies in biology, oceanography, fisheries, and limnology. To take advantage of the existing international HAB related societies, such as GEOHAB, SCOR, APEC and ICES, KORHAB will actively cooperate to develop local and international HAB related research projects. In 2006, KORHAB will coordinate an "International Workshop for *Cochlodinium* Researches" to be held in Korea, sponsored by the Korean Ministry of Maritime Affairs & Fisheries (MOMAF) and NOAA of USA. KORHAB will also provide information and counsel on HAB related matters to local and international communities.

The Korean government will give full support to KORHAB activities. Currently the KORHAB office is located in NFRDI, Busan, Korea.

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• Egypt

Toxic phytoplankton species linked to massive invertebrate and fish mortality in the Eastern Harbour of Alexandria

The coastal area of Alexandria is highly eutrophic due to direct discharges of wastewater, mainly from two land-based sources, estimated as about 7 x 10^6 m^3d^{-1} of agricultural drainage water mixed with the overflow from Lake Mariout (main basin outfalls: about 500 x $10^3m^3d^{-1}$ primary treated and 300 x $10^3m^3d^{-1}$ untreated municipal waste water) into Mex Bay, west of Alexandria, and about 2 x $10^6m^3d^{-1}$ of industrial and agricultural waste water into Abu Qir Bay to the east.

The Eastern Harbour (E.H.) of Alexandria is located in the central part of the city. It is a sheltered, relatively shallow semi-enclosed marine basin. It was a recipient of huge amounts of municipal wastewater during the last three decades. Yet, it is encouraging in that remedial actions have being taken by the Alexandria Governorate to close the E.H. outfalls, as well as the main sewer of Alexandria (Kayet Bey) at its western vicinity, and their share of waste water had been diverted to the Lake after primary treatment. However, due to water circulation, the harbour is still affected by the water discharge, mainly from Mex Bay (8 Km long).

During August 2004 and July-August 2005, incidents of fish and invertebrate mortality occurred accompanying long duration of visible water discoloration. According to information received from marine protection and diving organizations, the authority of Alexandria Governorate and the environmental authority, "marine life on the bottom of the E.H. were severely destroyed. Dead fish and stunned demersal and pelagic fishes losing their equilibrium, gasping at the surface, swimming either on their sides or upside down, suffering from disorientation and unresponsiveness to human presence, and the presence of hundreds of small crabs found dead on the sand beach or seen migrating towards the beach at dawn or found dead in fishermens nets indicating signs of toxicity". Symptoms of anoxic conditions were also observed in dead fish, including yellowish coloration of the body and gills.

The last cases of massive invertebrate and fish mortality occurred in the harbour during October 1994 [1], caused by *Alexandrium minutum* with evidence of toxicity, and during 1998-2000 attributed to the first occurrence of *Gymnodinium mikimotoi* (0.93 x 10^6 cell l^{-1} on 11 August 1999, 1.8 x10^6 cell l^{-1} on 30 May 2000, and *Chattonella antiqua* (0.85 x 10^6 cell l^{-1} on 27 July 1999, 1.14x10^6 cell l^{-1} on 21 May 2000 [2]. The blooms in 1999-2000 were transferred to the harbour from Mex Bay (a highly eutrophic bay), through the idealized pattern of a biological spreading, neighborhood diffusion, since hydrographic processes must be responsible. However, the present blooms during August 2004 and July-August 2005 originated from the Eastern Harbour. Twentyfour species, monospecific or in combination, gradually increased in the neritic waters of Alexandria, and their intensity and geographical extent seem to be on rise; the phenomenon is now of regular occurrence in the warm seasons. Ten of them were recorded toxic [3].

During these blooms, surface water temperatures were from 27.5°C to >30°C, and salinity values low (34.6-37.5 psu), affected by discharged water from the west. Reduced salinity seems to contribute to the development of *C. antiqua* and *G. mikimotoi* in the harbour [4]. A stable water column is a permanent characteristic feature. Nutrient concentrations are generally relatively low, while the reverse is the case for the organic matter.

G. catenatum Graham became noticeable on 3 August 2004 (0.15x10^6 cell l^{-1}), under relatively high nutrient concentrations (1.68 µM PO_4^{3-}, 3.65 µM NO_3^-, 3.8 µM NH_4^+), and low organic matters (1.46 mg l^{-1}), and was followed by its major peak on 7 August, and less so on the next day. Phosphate and nitrate concentration were reduced to a

Table 1. Species linked to massive invertebrate and fish mortality in the Eastern Harbour of Alexandria during 2004-2005

Species	Year/Maximum Density (M. D)			
	2004	M. D	2005	M.D
Alexandrium ostenfeldii	9 August 14-15 21	$0.11x10^6$ about $0.32x10^6$ $0.51x\ 10^6$	 23 – 24-25 July 1 August	 $0.13x10^6$, $0.14x10^6$ and $0.52x10^6$, respectively $0.082x10^6$
Chattonella antiqua	21 August	$1.26x10^6$	25 July 13-14 August	$0.16x10^6$ $0.33x10^6 – 0.49x10^6$
Gymnodinium catenatum	7-8 August 15 August 17 21	$2.73x10^6$, $1.53x10^6$, respectively $1.6x10^6$ $0.99x10^6$ $1.34x10^6$	21 July 28 30 3 August 8 10 14	$0.38x10^6$ $0.33x10^6$ $0.56x10^6$ $0.58x10^6$ $0.62x10^6$ $0.78x10^6$ $0.62x10^6$
Gymnodinium mikimotoi	13 August	$0.16x10^6$	30 July 10-11 August 14	$0.06x10^6$ $1.17x10^6 – 1.92x10^6$ $0.73x10^6$

third on 8 August (0.18 µM PO_4^{3-} and 0.55 µM NO_3^-), ammonia was almost unchanged (3.81 µM and 3.47 µM, for the two days, respectively). These two peaks accompanied high levels of organic matter concentrations (7.5 and 8 mg l^{-1}). *G. catenatum* was found in Alexandria waters in 1992 [5, 6].

Alexandrium ostenfeldii (Paulsen) Balech *et* Tangen was first found west of Alexandria (Mex Bay) during 1992 [7], and in the Eastern Harbour during 1998 [2]. Its maximum density on 25 July 2005, in combination with other species, raised Chl. *a* content to 59.5 µg l^{-1}. Phosphate was at 0.62 µM, nitrate 1.62 µM and ammonia 4.18 µM. *Gymnodinium mikimotoi* Miya et Kominami ex Oda was previously recorded in the E.H. of Alexandria during September 1998 at 22.5-27.2°C and salinity of 34-36.5 psu [2]. Densities between $0.13x10^6$ and $1.66x10^6$ cell l^1 were found from late September–early October 2000 [8]. *Chattonella antiqua* (Hada) Ono attained increased density on 19 August, reaching a peak two days later, accompanied Chl. *a* at 65 µg l^{-1}. Its densities during 2005 were significantly lower than those recorded in the same period during 2004. *C. antiqua* was first described and recently founded in the Eastern Harbour to be most frequently at 24.5-26°C and 34.5-35 psu, and it shared a role in the 3 killing blooms during 2001 [4].

It is concluded that:

1- The water characteristics were deeply affected by water discharge from the west. A reduction in the discharged water is urgently required to improve the situation, not only in the harbour, but also along the whole coast of Alexandria.

2- The severe damage to marine life in the Eastern Harbour of Alexandria during August 2004 and July-August 2005 was a direct impact of the occurrence of 6 toxic phytoplankton species.

3- The long duration of the bloom during the whole of August could have amplified this dramatic event.

4- The sever consumption of inorganic nutrients signals their importance. However, high organic matter concentrations could help maintenance of the blooms.

5- Recurrent blooms are expected in the following years.

6- The rare occurrence of undesirable species such as *Alexandrium minutum*, *Pseudo-nitzschia pungens* and *Heterosigma* sp. could inflict more damage in the near future.

7- A monitoring programme must be carried out along the Egyptian Mediterranean coast to evaluate the real situation. Human health is placed at risk, ecosystems are altered, fishing and aquaculture suffer economic losses.

This communication is part of the monitoring program carried out in Alexandria waters for 143 days between July 2004 and October 2005.

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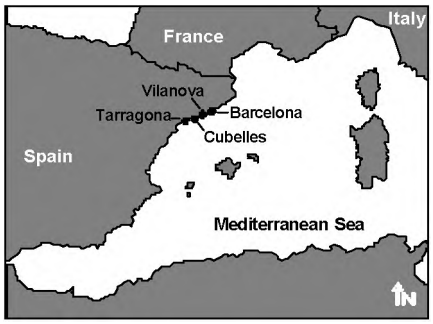
• Spain

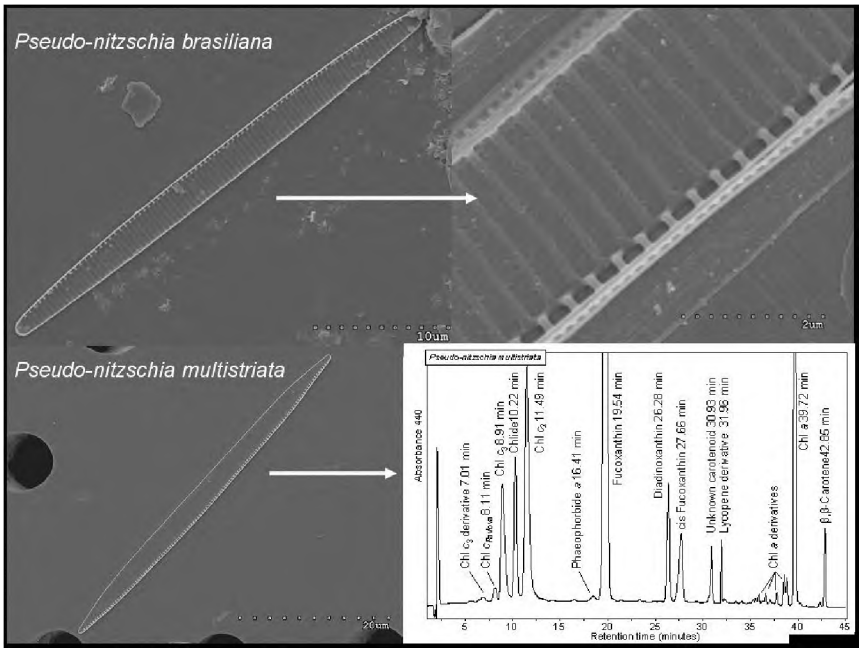
First detection of diatom *Pseudo-nitzschia brasiliana* (non toxic) and its relative *P. multistriata* (presumably toxic) in the NW Mediterranean Sea



Diatoms of the genus *Pseudo-nitzschia* are widely distributed in seas and oceans. They attract world wide interest since they were found to produce the lethal neurotoxin domoic acid (DA). Back in 1987, a member of this genus (*P. multiseries*) caused amnesic shellfish poisoning (ASP) in eastern Canada [1]. Production of DA is reported for at least ten species of *Pseudo-nitzschia* [2], and their blooms result in marked toxin accumulation through the food chains, with subsequent impacts on individual marine organisms, total ecosystems, humans, and even the economy [3]. In the Mediterranean, both *Pseudo-nitzschia galaxiae* and *P. multistriata* were shown to be toxic [4].

Since 1995, a continuous phytoplankton monitoring programme is carried out along the NE coast of the Spanish province of Catalonia. The region has a 400 km coastline, is densely populated, and contains many harbours (on average one per 10 km). These artificial structures provide suitable niches for organisms whose growth depends on nutrient-rich (semi-)confined conditions. During 2004 bucket samples were taken from 16 of these harbours and aliquots (150 ml) were preserved with Lugol's iodine solution. Samples were analysed with SEM and TEM techniques allowing identification of the genus *Pseudo-nitzschia* at the species level using their known morphological characteristics.

The most abundant *Pseudo-nitzschia* species recorded were *P. delicatissima*, *P. calliantha*, *P. multistriata* and recently *P. brasiliana*. The latter was first recorded off Cubelles beach (September 2004), with cell densities up to (2.8 x 10^5 cells L^{-1}). The species was, however, not dominant (30% of total *Pseudo-nitzschia* population) and co-existed with *P. calliantha* (30%), *P. delicatissima* (30%), and *P. fraudulenta* (10%). In September 2005, *P. brasiliana* was found to dominate (95.2%) the *Pseudo-nitzschia* population in Vilanova harbour (2.5 x 10^5 cells L^{-1}). To our knowledge, this species has not previously been reported from the Mediterranean Sea. Our measurements revealed an apical axis length from 23.41 to 39.4 µm (n=30), and a transapical axis length from 2.34 to 3.79 µm. These values coincide with those of the original description [5]. The raphe is extremely eccentric and not interrupted by a central nodule. The fibulae and the interstriae are regularly spaced, 20-26 in 10 µm. The interstriae contain two rows of poroids with a tendency for three. On 1 September 2005, *P. multistriata* was dominant (100%) in Tarragona port, with densities up to 4 x 10^6 cells L^{-1}. Cells of this species were isolated from this bloom and HPLC pigment analyses of the clonal culture obtained revealed Chl *a*, four polar chlorophyll *c* pigments, 6 carotenes and several degradation compounds.



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• Mexico

Is *Pseudo-nitzschia pseudodelicatissima* toxin the principal cause of sardines, dolphins, sea lions and pelicans mortality in 2004 in Mexico?

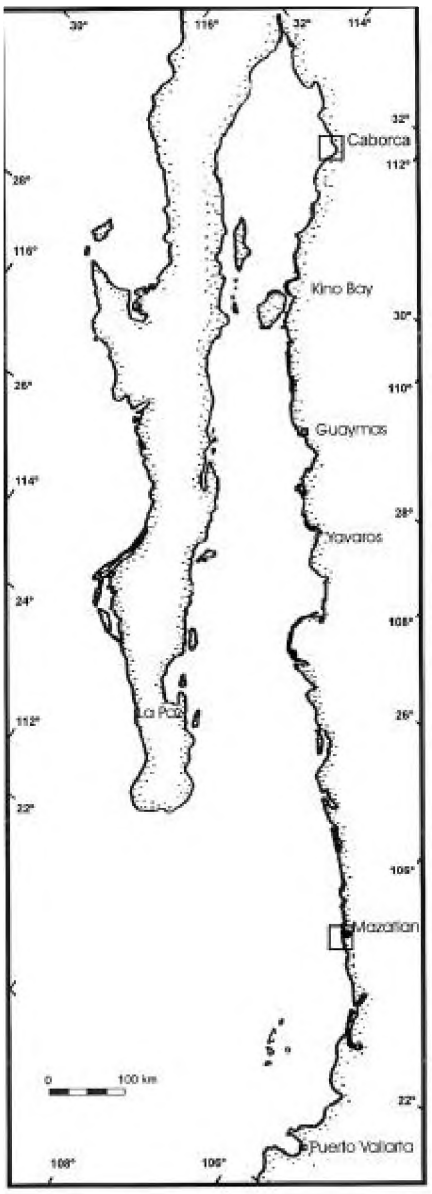

Fig.1. Map with localization of events reported here: Caborca, Sonora in January 2004 and Mazatlan, Sinaloa in July 2004.

On 13 January, 2004, 79 long beaked dolphins (*Delphinus capensis*) stranded at Bahía La Salada, in San Jorge Bay, Caborca, Sonora (31°03'54''N, 113°07'51''W) [1]. Fishermen reported several tons of sardines floating dead on the open sea during the first half of January (Fig. 1). The total counts of affected animals included 112 dolphins (103 long beaked *Delphinus capensis*, 9 common dolphins *Delphinus delphis*), 195 sea lions (*Zalophus californianus*) and 9 grey pelicans (*Pelecanus occidentals*), plus 20 tons of sardines (*Sardinops* ssp.) [2]. LCMS analyses of blood samples collected from live or recently dead stranded dolphins detected domoic acid (311-312 *m/z*) in three of the four samples tested. LCMS-MS of ions captured at 311-312 *m/z* and fragmented, produced daughter ions of 266-267 *m/z* in all four samples, a characteristic degradation product of domoic acid (Fig. 2). Unfortunately, no plankton samples were taken or analyzed at the time of the event. Climatological data presented the same image found during previous *Pseudo-nitzchia australis* blooms in the area and depicts a recurrent pattern of events that may help to predict occurrences (to be published elsewhere).

Table 1. Phytoplankton composition of samples taken in Mazatlán Bay (July 8, 2004).

Species	Cell number / ml	% total
P. pseudodelicatissima	3921	67.0
P. subfraudulenta	929	15.9
Gymnodinium sp.	825	14.1
Protoperidinium quinquecorne	138	2.4
Skeletonema costatum	17	0.3
Coscinodiscus sp.	10	0.2
Total	5840	100

Discolorations were observed in Mazatlan Bay, Sinaloa, Mexico (23°13'N, 106°2'W) during July 5-11, 2004. Samples collected the 8th and 13th were observed by inverted LM [3]. Results indicated the dominance of the genus *Pseudo-nitzschia* (Table 1), although the color was caused by an unidentified gymnodinoid and *Protoperidinium quinquecorne* (Fig. 3). Even when discoloration vanished on the 11th, samples from day 13th had 4850 cells/ml of *Pseudo-nitzschia* spp. as isolated cells and in pairs. *Pseudo-nitzschia* spp. cells present in the sample were analyzed by SEM and TEM and the most abundant species was identified as *P. pseudodelicatissima* (67% dominance of the total phytoplankton) (Fig. 4a-f). Although characteristics observed corresponded to *P. pseudodelicatissima* (Table 2), some differences were noted. There is a thickening of the middle portion (Fig. 4d) and the poroids present a membrane less fragmented than that shown on ICES leaflets [4], but similar to that shown by Lundholm *et al.*, [5], divided in two main portions; also, in the central part, there are two smaller poroids (Fig. 4e-f). *Pseudo-nitzchia* cf. *americana* (Fig. 4g-h), was infrequently observed and was not quantified. In the last species, the observed poroids showed non-fragmented hymens (Fig. 4h), but in the larger ones it is possible

Table 2. Morphometrical characteristics of the taxa discussed in the text.

Features	*P. pseudodelicatissima*	*P. subfraudulenta*	*P. cf. americana*
Valve view	Linear	spline shaped	linear
Chain overlap	1/6	¼	?
Apical axis in µ	64 – 94	52-72	20
Trans-apical axis in µ	1.5-2.0	4.2-5.4	1.7-2.5
Striae in 10 µ	38-47	36-	30-40
Interstriae in 10 µ	40	19-20	14
Rows of poroids	1	2	2
Poroids in 1 µ	4	5-6	5
Poroids fragmentation	2	2-5	0
Fibulae in 10 µ	22-26	15-20	22-30
Central interspace	+	+	-

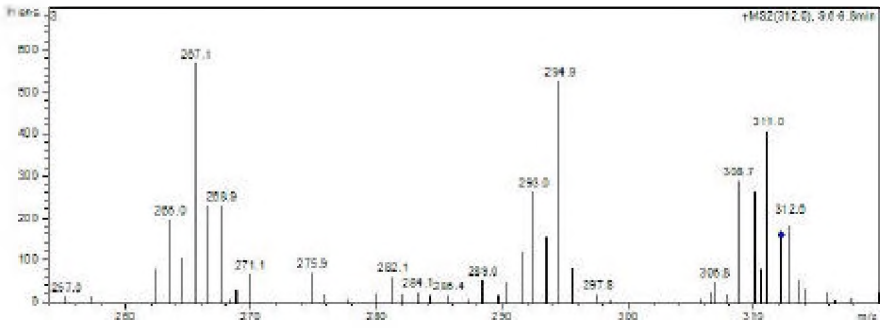

Fig.2. Graphs of LCMS-MS of blood samples collected from stranded dolphins (January 2004). Samples were cleaned-up by SPEC before HPLC analyses.

to appreciate a poly-fragmentation, suggesting that the size of the pore determines the fragmentation of the hymen (see white arrowheads on Fig. 4g). *P. subfraudulenta* (16% dominance of the total phytoplankton) was also found (Fig. 5a-f). This species shows wide variations of the fragmentation present on the cingular band, evidence of contiguous poroids (Fig. 5e-e´).

Previous *Pseudonitzschia* sp. blooms have not been detected due to the fact that they did not discolor the waters, even though Mazatlán Bay has been monitored frequently since 1979 [6]. Nevertheless, domoic acid has been detected and reported previously in the Gulf of California [7, 8] and *P. australis* mentioned as responsible. The correct identification of *Pseudo-nitzchia* species in the Mexican Pacific has not been deeply pursued in recent years. Hernández-Becerril identified 5 species and among them, reported the toxic species *P. australis* outside the Gulf of California [9], while inside the Gulf its presence is uncertain. This is the first documented report of the presence of the toxic species *P. pseudodelicatissima* in the Gulf of California. Previously, Gomez-Aguirre *et al*. [10] mentioned the species but the poly-fragmented hymen shown on their figures (6 a-c) is closer to *P. calliantha* [5], showed to segregate as a completely different clade by molecular analysis of its ITS1, 5.8S, and ITS" of the nuclear encoded rRNA [5]. Nevertheless, identifications based on the size, structure and fragmentation of the hymen may require further review in that, as shown here, the poroids present on the cingular band of *P. subfraudulenta* show wide variations of the fragmentation pattern: 2-3-4-6 fragments (Fig. 5e-e´).

In Mazatlán, ten dead brown pelicans (*Pelecanus occidentalis californiensis*) as well as fish from several species were found on July 17th, and the number of affected animal increased during the following days. Health Authorities looked for the causes but toxin analyses were not carried out. The toxin analysis of a bottle sample of the bloom revealed a toxin profile similar to that found in the blood of dolphins stranded in previous months (Fig. 2) [1]. Nevertheless, the lag time between both events (Caborca, Son. and Mazatlan, Sin.) and the differences between the samples obtained during them, do not allow us to establish the cause of death of the animals found nearby Mazatlan which were not analyzed for toxins. The evident lack of coordination of actions developed to

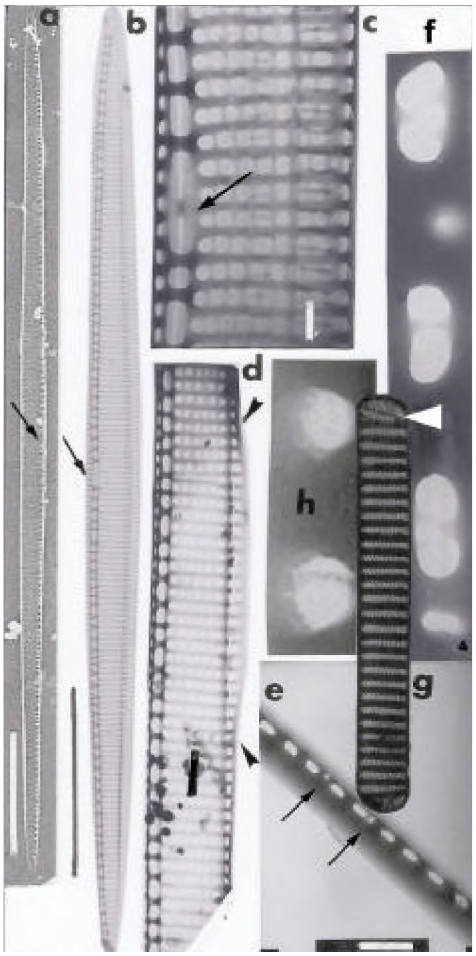

Fig.4. Pseudo-nitzschia pseudodelicatissima *and* P. americana. *a-b)* P. pseudodelicatissima *cells, valve view, SEM (a), TEM (b), (scale 10µm). c) Central inter-space, TEM (scale 0.5 µm). d) Widened middle region (arrowheads), TEM. (scale 1µm). e-f) Poroids in girdle band showing bifragmented hymen, TEM (scale 1 µm). g)* P. americana *cells in valve view, major pore showing poly-fragmentation (white arrowhead). h) Detail of poroids showing non-fragmented and bi-fragmented ones.*

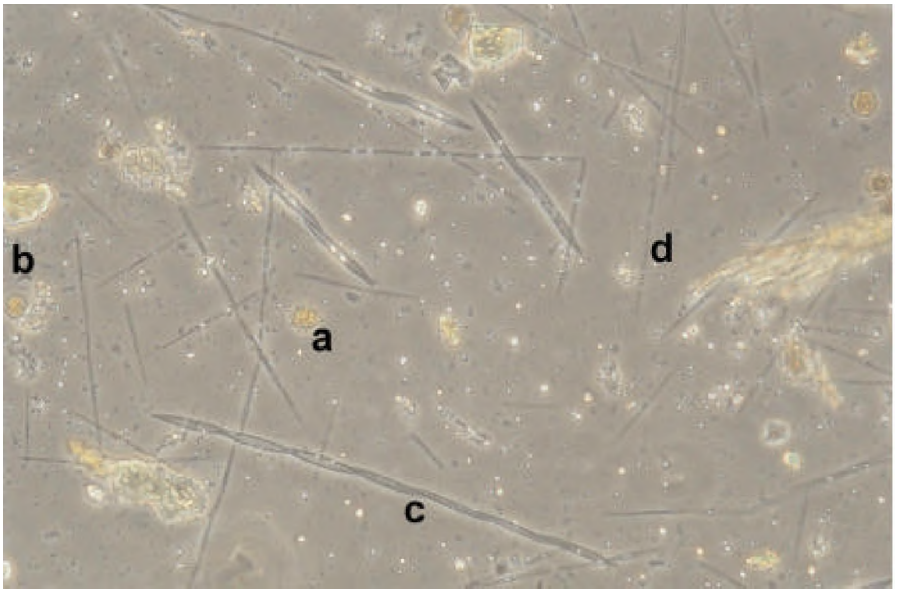

Fig.3. Plankton species found in water samples collected July 8th in Mazatlán Bay: a) Peridinoid; b) Gymnodinium *sp; c)* P. subfraudulenta*; d)* P. pseudodelicatissima.

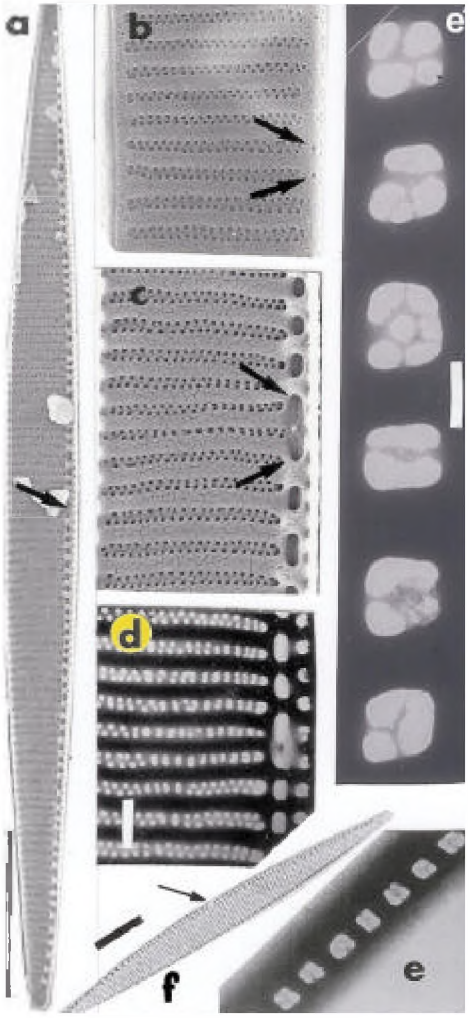

Fig.5. Pseudo-nitzschia subfraudulenta *a) Valve view, SEM (scale 10 µm). b) Central inter-space, external side, SEM. c) Central interspace, internal side, SEM. d) Central inter-space, internal side, TEM (scale for b, c & d 1µm). e-e') Poroids in girdle band showing different fragmentation patterns (scale 0.2 µm). f) Valve view, TEM (scale 10 µm).*

attend these events is responsible for the absence of strong arguments about the causes and frequency of these events, which can pass undetected or unexplained even when the coastal fauna is severely affected.

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• Denmark

Domoic acid in Danish blue mussels due to a bloom of *Pseudo-nitzschia seriata*

In March and April 2005, a bloom of the pennate diatom *Pseudo-nitzschia seriata* resulted in accumulation of domoic acid (DA) in blue mussels (*Mytilus edulis*) to levels above the EU-regulatory limit of 20 mg kg^{-1}. Domoic acid is a potent neurotoxic amino acid that has previously caused Amnesic Shellfish Poisoning (ASP) in humans after consumption of contaminated mussels from Atlantic Canada [1]. In the recent Danish incident, no human illnesses were reported, but as a precaution the mussel fishery was either conditionally open with intensified surveillance or closed for five weeks at various sites in Denmark. The Danish bloom probably originated north of

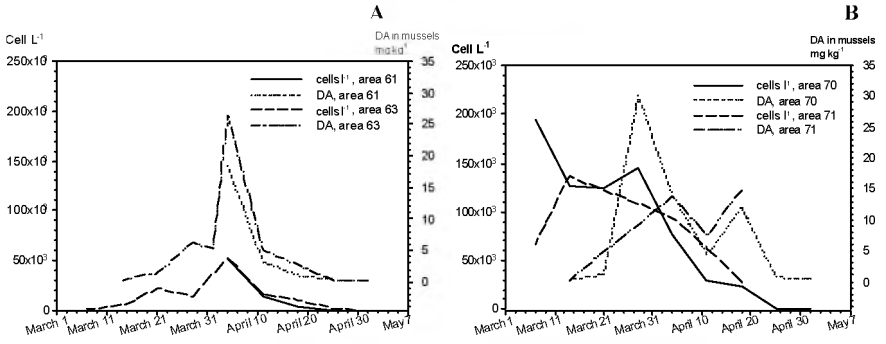

Fig. 1. Concentration of P. seriata *cells in the water column and of domoic acid in blue mussels during spring 2005 in two areas in Danish coastal waters. A. East of Jutland. B. North of Funen.*

Funen, a desirable area for mussel fishermen to collect mussels from natural shellfish banks. The highest concentration of *Pseudo-nitzschia seriata* observed during the episode was 195,000 cells l^{-1} and was detected on March 7th.

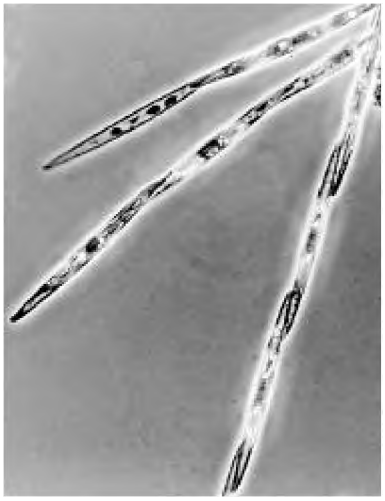

Fig. 2. Light micrograph of Pseudo-nitzschia seriata *chains.*

At that time, DA was not measured, but a week later DA concentrations were 0.4 mg kg^{-1} wet weight of mussels (Fig. 1). The *Pseudo-nitzschia* cell numbers decreased slightly until March 28 when DA in the mussels peaked at a concentration of 30 mg kg^{-1}. Two lots of blue mussels (each 27 tons) collected from a harvest area north of Funen on March 29th and 30th contained concentrations of 30 and 32 mg kg^{-1}, respectively, and were therefore discarded (Fig. 1). The bloom then declined in the waters north of Funen, but peaked further north on the east coast of Jutland. The peak of the bloom in that area was observed on April 3 at only 53,000 cells l^{-1}, but DA concentrations in the mussels reached 26 mg kg^{-1}.

During April, DA concentrations and cell numbers of *P. seriata* decreased, but on April 18th, mussels north of Funen still contained 12 mg DA kg^{-1} and *P. seriata* concentrations were 23,000 cells l^{-1}. As determined by LC-MS, the total DA concentration in the water at that time was 0.49 ng ml^{-1}, corresponding to a concentration of 21 pg DA $cell^{-1}$ of *P. seriata*. This is a rather high cellular concentration but is within the range (0.31 to 33.6 pg DA $cell^{-1}$) found in laboratory growth experiments conducted on Danish strains [2]. Similar concentrations have been measured from Scottish and Canadian strains, which yielded DA concentrations of up to 14.7 pg $cell^{-1}$ and 7 pg $cell^{-1}$, respectively [3, 4].

In areas on the east coast of Jutland, the *P. seriata* bloom corresponded with the peak of DA in the mussels, whereas north of Funen, cell numbers seemed to peak before DA accumulated in the mussels. The reasons for these apparent differences have not yet been determined, but might be due to the sampling procedure. The water samples for plankton analysis are aggregates of three sub-samples taken at different depths in the water column (surface, mid-water column, 1 m above bottom) in the harvest area. This aggregated sample may not be strictly representative of the actual plankton composition and concentration at the bottom of the water column where the benthic mussels were feeding, because the water column is often highly stratified in the harvest areas.

Relating the DA concentration to the concentration of *Pseudo-nitzschia* cells in the water column indicates that the action level of 200,000 *Pseudo-nitzschia* cells l^{-1} that is currently used within the Danish monitoring program is too high for *P. seriata* under these circumstances (Fig. 1). Along the east coast of Jutland, cell concentrations increased to around 50,000 cells l^{-1} simultaneously with DA concentrations rising to above the EU regulatory limit. A similar pattern was found in Atlantic Canada for this *Pseudo-nitzschia* species, where concentrations of 62,000 cells l^{-1} occurred when DA in the mussels was above the regulatory limit [4]. An action limit below 50,000 cells l^{-1} of *P. seriata* may also be valid for other countries where *Pseudo-nitzschia* cell concentrations

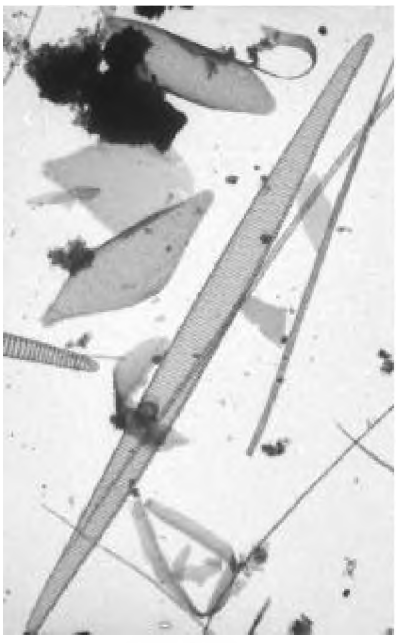

Fig. 3. Pseudo-nitzschia seriata, *TEM micrograph showing the asymmetrical valve.*

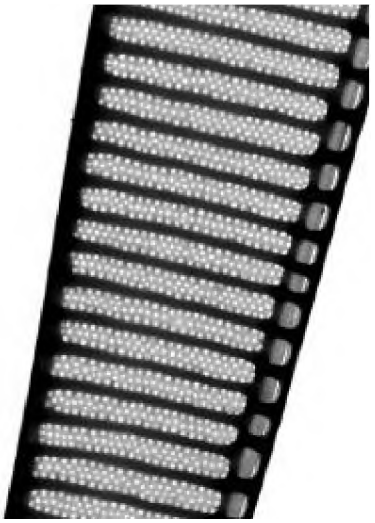

Fig. 4. Pseudo-nitzschia seriata, *TEM micrograph of valve showing fibulae, interstriae and the special poroid arrangement.*

serve as an early warning on whether or not DA monitoring is necessary. In any case, the interpretation of cell counts of *Pseudo-nitzschia* spp. as an index of impending toxicity in shellfish must be done cautiously. Certain strains of *P. seriata* from eastern Canada have been found to be non-toxic (Bates *et al.* 1989) and co-occurrence of toxic and non-toxic strains have also been recorded for many other *Pseudo-nitzschia* species.

The identification of *P. seriata* as the diatom responsible for the DA toxicity was confirmed by light and electron microscopy. *Pseudo-nitzschia seriata* is morphologically close to *P. australis* and *P. seriata* f. *obtusa*, and in contrast to many other *Pseudo-nitzschia* species, all three taxa are more or less asymmetrical (Figs. 2, 3). *Pseudo-nitzschia seriata* can be discriminated by the pointed tips in girdle view and by the poroid structure, with two rows of larger poroids, and one or two rows of smaller poroids in the middle (Fig. 4).

Pseudo-nitzschia seriata is a cold-water species that has been recorded from temperate and Arctic regions in the North Atlantic, but it is apparently absent from the north Pacific as well as from the southern hemisphere [5]. It is common in Scandinavian coastal waters, especially during spring. As far as we are aware, DA has only been detected in blue mussels at levels above the EU regulatory limit once before in Scandinavia - a sample from the west coast of Norway (Molde) in 2003 contained 23

mg kg^{-1} (T. Aune, personal communication). We know that Danish cultures of *P. seriata* are able to produce DA, as cultures isolated from 1992 and later have been found toxic [2]. Why therefore have we not detected DA due to *P. seriata* in mussels before in Denmark? Monitoring results dating back to 1990 show that only one previous bloom of *Pseudo-nitzschia* was observed in spring (Year 2000), whereas blooms of other *Pseudo-nitzschia* species occur during summer in 8 of 13 years. Thus the bloom of *P. seriata* during spring 2005 can be characterized as an exceptional bloom. But note that domoic acid in mussels is measured only if mussel fishermen wish to harvest mussels and if cells of *Pseudo-nitzschia* spp. are observed; hence data on DA occurrence during spring is limited. We do not know why *P. seriata* was able to reach such high concentrations that it caused DA accumulation in blue mussels. However, low water temperatures during February and March may have limited the growth of grazers (such as copepods) resulting in a lower grazing of *P. seriata*. This effect, combined with a rainy January and February, and much sunnier weather in March and April (up to 70% more than average) may have provided optimal bloom conditions for *P. seriata*.

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• Algeria

Detection of toxic *Gymnodinium catenatum* (Graham, 1943) in Algerian waters (SW Mediterranean Sea)

Gymnodinium catenatum is a naked chain and resting cyst forming dinoflagellate, known to produce toxins involved in PSP (Paralytic Shellfish Poisoning) events. *G. catenatum* was first described for the Gulf of California [1], but numerous studies in recent decades reveal a worldwide distribution of this species. In Eastern Atlantic waters, it was first recorded in 1976 in samples from the NW coast of Spain [2]. In 1987, *G. catenatum* was identified as the causative species of an important PSP event along the Mediterranean coast of southern Spain [3] (Fig. 2). A continuous monitoring program, initiated in 1997, revealed the regular presence of *G. catenatum* in this area [4]. *G. catenatum* has also been detected along North Moroccan coasts [5], in the Algerian Basin, and in offshore waters near the Sardinia Channel [6] (Table 1). We present here the first report of a toxic *Gymnodinium catenatum* in Algerian coastal waters, detected in samples collected during several cruises in 1986 and 2001-2002. Sampling area and frequency are presented in Figure 3 and Table 1, respectively.

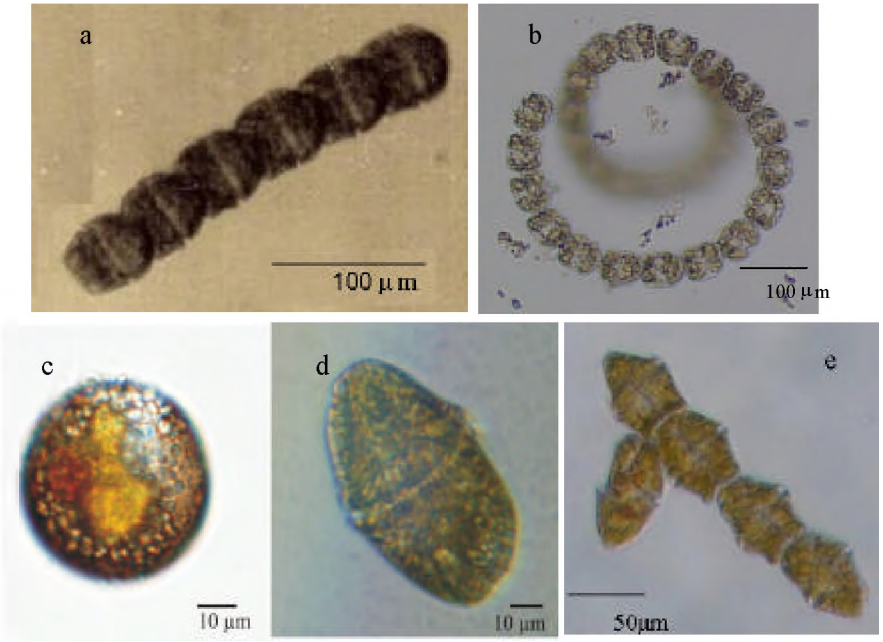

Fig. 1. Gymnodinium catenatum*: Light microscopic photographies of vegetative chains (a, b) and cyst (c) in field samples. Vegetative cell in culture (d). Vegetative chain in culture (e).*

During the annual cycle of El Djamila Bay between September 2001 and August 2002 (water temperatures 16.5 to 23.5 °C, salinity 36.5 to 36.9 psu, nitrate 0.08 to 0.59 µmole l^{-1}, phosphate 0.11-0.24 µmole l^{-1}, ammonium 0.01 to

Table 1. Occurrences of Gymnodinium catenatum *in the Mediterranean Sea: maximum concentrations, detection of toxicity and temporal distribution.*

Max. Conc. Cells^{-1}	Detection of toxicity	Month year	Location	Distance from the coast	Sampled periods	References
200		**Sept. 1986**	**Cap Caxine**	**1.5-21 (miles)**	**Autumn-Spring 1986-1987**	**Present study**
3000	Toxicity in shellfish	Febr.1989	Andalucia, Spain	Coastal waters	1987-1989	Bravo et al. 1990
10.000	Toxicity in shellfish	June 1995	Tetouan Region, Morocco		1992-1996 Monitoring	Tahri Joutei 1997
		Sept. 1999	Alboran Sea, Algerian Bassin Sardinia Strait	off-shore	1999 cruise	Gomez and Claustre 2001
34.000		July 2001	Andalucia, Spain	Coastal waters	1999-2002 Monitoring	Mamán et al. 2003
440		**Sept. 2001**	**El-Djamila Bay**	**0.5**	**2001-2002 annual cycle**	
2920	**Toxicity in a strain culture**	**Oct. 2001**	**Algiers Bay**	**1**	**2001-2002 seasonal cruises**	**Present study**
3200		**Oct. 2001**	**Bou-Ismaïl Bay**	**1 (miles)**	**2001-2002 seasonal cruises**	

0.04 µmole l^{-1}), *G. catenatum* was only detected in autumn, and showed low concentrations (<450 cells l^{-1}) at all times (Fig. 4). During the October 2001 cruise (water temperatures 18.5 to 23.5°C, salinity 36.7 to 37 psu, nitrate 0.27 to 1.16 µmole l^{-1}, phosphate 0.13-0.65 µmole l^{-1}, ammonium 0.01 to 0.07 µmole l^{-1}) high densities (maximum 3200 cells l^{-1}; Table 1, Fig. 5) were detected in Algiers and Bou-Ismaïl Bays. Viable *G. catenatum* cysts (Fig 1) were isolated from sediment samples collected in January 2002 (50 m depth) in Bou-Ismaïl Bay. These cysts were employed to obtain clonal cultures of *G. catenatum*. HPLC analyses revealed a peridinin-containing pigment composition (Fig. 7a). These results closely resemble the *Alexandrium*-like composition [7] reported for *Gymnodinium aureolum* [8]. However, it strongly differed from those reported for the 19'-hexanoyloxyfucoxanthin and gyroxanthin-containing ichthiotoxic relatives *Gymnodinium* sp., *G. mikomitoi*, *G. breve* and *G. galatheanum* [8, 9, and papers cited there]. Additional HPLC analyses of the Algerian strain revealed a toxin composition (Fig. 7b) dominated by the relative low toxic C1 and C2 compounds (15 and 239 Mouse Units µMol^{-1}, respectively – see ref. 10). Only trace levels of the more toxic isomers GTX2 and GTX3 (892 and 1584 MU µMol^{-1}) were detected, and some unidentified compounds (possibly including dcGTX2/3). The strong dominance of the C-toxins was consistent with the absence of published records on *G. catenatum*-associated PSP events in Algerian waters. The toxin composition of the strain analyzed more closely resembled that of Mediterranean analogues isolated from Andalucía (S Spain), than that of the (STX-containing) Atlantic representatives obtained from NW Spain [11]. However, all strains employed by the authors contained doSTX, and usually synthesized dcSTX, but these compounds remained below detection levels in our Algerian culture. Samples collected off Cape Caxine in September and October 1986 revealed the presence of *Gymnodinium catenatum* with concentrations lower than 250 cells l^{-1} (Fig. 6). During both periods *G. catenatum* coexisted with other potentially harmful species, including *Alexandrium* sp (chain-forming cells), *Dinophysis acuminata*, *Prorocentrum micans* and *Prorocentrum balticum*. Ours results show that *Gymnodinium catenatum* was already present in South-western Mediterranean waters prior to its first record in the Alboran Sea in 1987 [3]. Samples collected in November 2001 revealed the simultaneous presence of *G. catenatum* in coastal waters of both Algeria and Andalucía [4]. The presence of *G. catenatum* in the Mediterranean is nowadays restricted to the South Western Basin and seems to be associated with the Algerian Current, which flows from W to E along the north African coast as a continuation of the Almería-Oran Jet [12]. Molecular studies might elucidate possible relationships between the Algerian population and strains form other biogeographic regions. Harmful phyto-

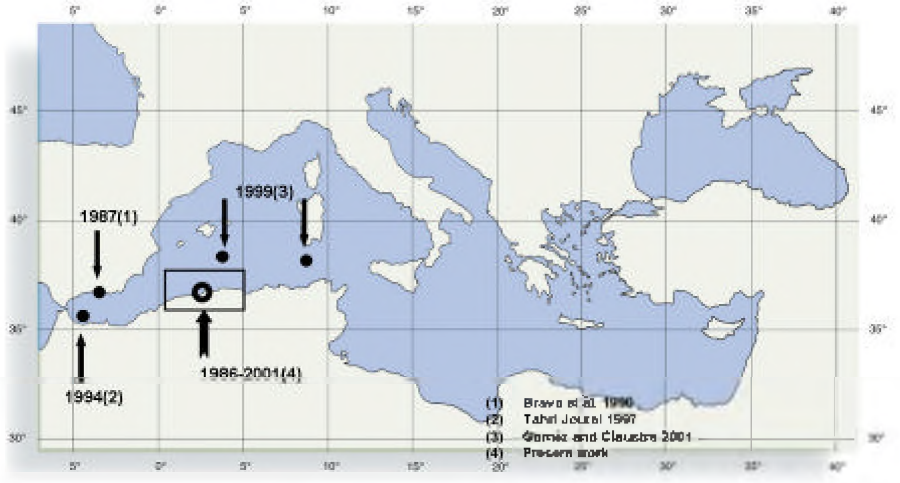

Fig. 2. First detection of Gymnodinium catenatum *in different regions of the Mediterranean Sea.*

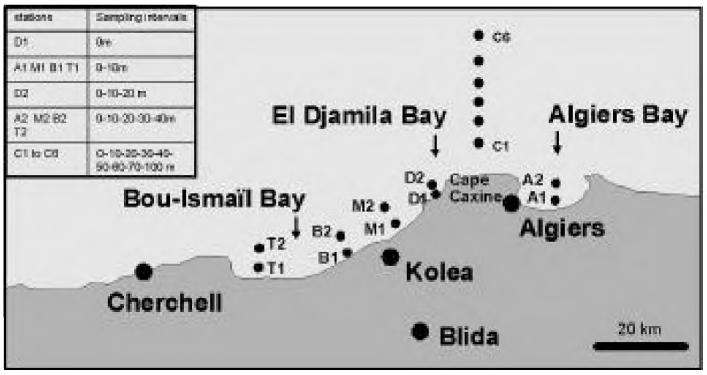

Fig. 3. Locations of the sampling stations in Algiers area.

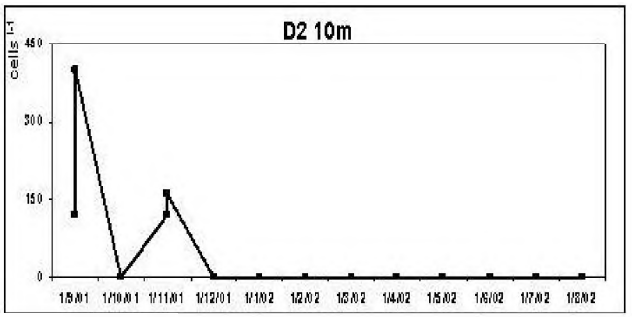

Fig. 4. Annual distribution of Gymnodinium catenatum *in El Djamila Bay at 10m.*

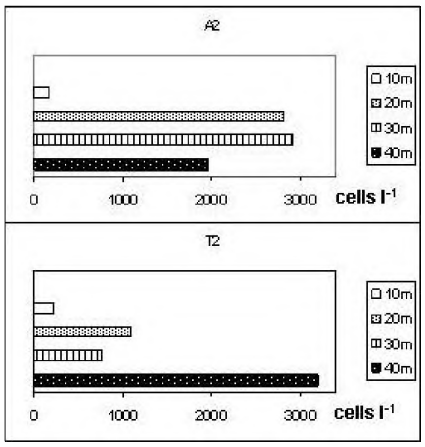

Fig. 5. Vertical distribution of Gymnodinium catenatum *in Algiers and Bou-Ismaïl Bays during October 2001 cruise.*

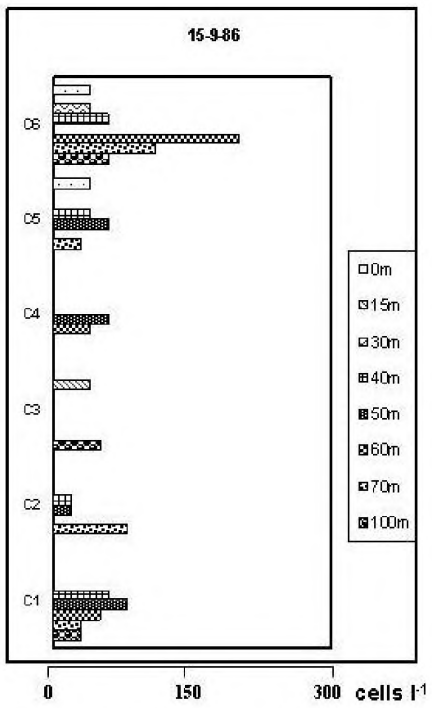

Fig. 6. Vertical distribution of Gymnodinium catenatum *off Cape Caxine September 15th 1986.*

plankton species are not yet continuously monitored in Algerian waters, even though associated intoxication events have been described for nearby regions [3, 5]. However, considering the regular presence of *G. catenatum* (and other harmful species) along the Algerian coast, its capability of synthesizing the toxic isomer pair GTX 2/3 and the wide range of additional compounds mentioned to occur in other strains, detailed studies on spatio- temporal distributions and their toxicity are most certainly recommended.

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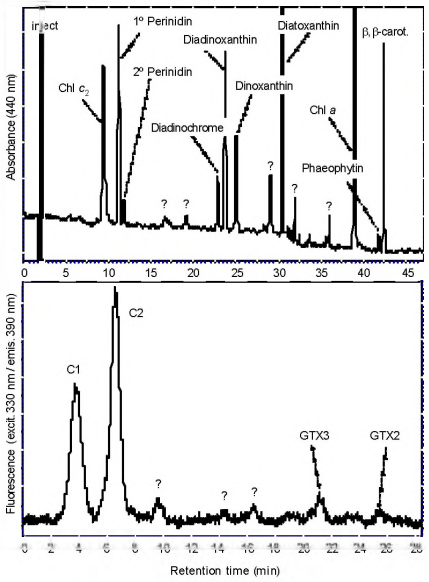

Fig. 7. Absorbance and fluorescence chromatograms obtained with HPLC analysis of photosynthetic pigments and toxins present in extracts of a Gymnodinium catenatum *strain isolated from Algerian coastal waters.*

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• The Philippines

Fish Kills Associated with Cochlodinium Blooms in Palawan, The "Last Frontier" of the Philippines

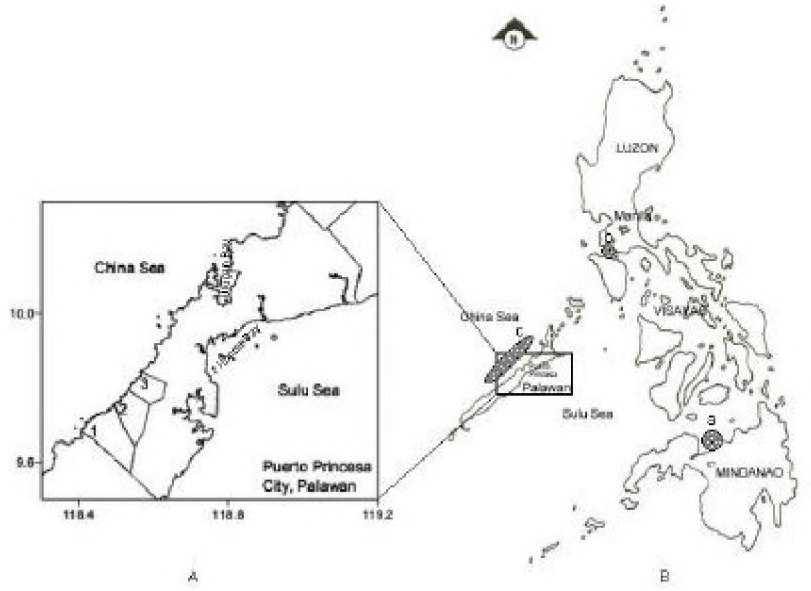

Fig. 1. A) Map of Puerto Princesa, Palawan showing sampling sites where fish kills were observed: 1) Napsan, 2) Bagon Bayan, 3) Simpocan. B) Map of the Philippines showing bloom occurrence of Cochlodinium polykrikoides*: 1) Iligan Bay, March 2002; 2) Balayan Bay, May 2002; 3) Puerto Princesa, March 2005.*

Puerto Princesa, Palawan is considered as the "last ecological frontier" in the Philippines due to its vast yet relatively unexplored natural wonders. It is one of the marine biodiversity hot spots of the world, and contributes to the unprecedented biodiversity of the Sulu-Sulawesi Ecoregion, which is home to 400 coral species, and 650 reef fish species, among others. Even though Palawan (Fig. 1) is protected by Republic Act 7611 which stipulates sustainable development of its natural resources, the growing demand for its resources make it vulnerable to threats of over-fishing, dynamite fishing, coastal development and pollution. Metro Manila (the Philippines capital) relies on Palawan to provide 60% of its fish requirements, while 55% of the country's total fish exports originate in Palawan.

A new threat, however, looms over Palawan's fisheries.

In response to a call for help from the Mayor of Puerto Princesa, Palawan, Edward Hagedorn on 16 March 2005, a research team led by Dr. Rhodora V. Azanza of the Marine Science Institute, University of the Philippines set out to investigate a fish kill in Puerto Princesa. Upon arrival in Puerto Princesa, a technical meeting with local government units was immediately held. The team, which included Jun Relox of the Central Bureau of Fisheries and Aquatic Resources (BFAR), without delay, proceeded to Barangay Bagongbayan, Palawan to collect water samples which revealed a high concentration of the naked dinoflagellate, *Cochlodinium polykrikoides*. Barangays Simpocan and Napsan were also visited where dead fish were evident on the shore (Fig. 1A).

Prior to the investigation, fishermen were at a loss as to what has been causing fish kills in Palawan since January. According to the report of Zosima Jampit (Community Environment and Natural Resources Officer-Quezon, Palawan) to the Regional Director of Environmental Management Bureau, Department of Environment and Natural Resource, Mimaropa Region, fish kills were first observed in the boundary between waters of Palawan and Sabah, Malaysia in January 2005. By February, fish kills reached Rizal, Palawan, and by March to Quezon, Palawan. Incidents were attributed to prevailing winds and currents. Fishermen sources revealed having witnessed a red discoloration off the Quezon waters with dense floating algae. Local fish affected were: flying fish, "*molmol*", "*sagisi*", "*isdang bato*" and "*solid*".

On 17 March 2005, a Press Conference was held to update the public concerning the fish kills in Palawan. It was with relief that the team of Mayor Edward Hagedorn announced to the public that fresh fish was safe for consumption and that the causative organism, *Cochlodinium polykrikoides*, has not been reported as toxic to humans, and only kills fish by depleting the waters of oxygen (Fig. 2). As of today, apparently, the bloom is moving farther to the North of Palawan, as we received reports of similar events in these areas, which however need to be confirmed.

A recent follow-up study included the monthly distribution of chlorophyll-a obtained from satellite images from MODIS Aqua Level 2 data with 1 km resolution. High chlorophyll content ($\geq$0.5 mg/m^3) were observed in February, 2005 in west and southwest Palawan and proceeded to spread northwards by March, 2005 which by then covered a large portion of the western coast (310 km in length and 80 km in width). The intensity of the bloom lessened by April, 2005 which was then concentrated in the northern tip of Palawan. Sabah, Malaysia being only a few kilometers away from Palawan showed corresponding waxing and waning of chlorophyll values. It is possible that this particular *Cochlodinium polykrikoides* bloom in Palawan could have originated from Malaysian waters following the circulation pattern in South China Sea during this season [1].

Background on *Cochlodinium polykrikoides*

Cochlodinium polykrikoides Margalef is an unarmored dinoflagellate species belonging to the Family Gymnodiniaceae. It forms chains characterized by having a conical epitheca and a bilobed hypotheca. Single cells are ellipsoidal with a displaced cingulum. It is cosmopolitan in nature but found more commonly in warm-temperate and tropical waters [2].

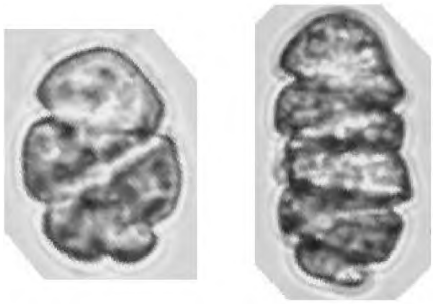

Fig. 2. Cochlodinium polykrikoides, *the non-toxic dinoflagellate observed to bloom during the fish kill in Puerto Princesa, Palawan on March 16, 2005.*

It has been causing fish kills in Asia, particularly annual blooms in Japan since the 1970's, Korea in the 1980's and China in the 1990's [3-5]. Extensive economic losses were reported, the highest, in Korea, of US$95.5M [6]. More recently, it has been blooming in Southeast Asia. Blooms of *Cochlodinium polykrikoides* were reported in Sabah, Malaysia in the latter part of 2003, and in Brunei in February, 2004 (Taha, pers. comm.). The bloom in Sabah recurred in January of this year [7].

In the Philippines, it caused fish kills in Iligan Bay, Southern Philippines and Balayan Bay, Batangas in March and June of 2002, respectively (Fig. 1B). In Iligan Bay, the bloom spanned 2000 m by 500 m wide killing demersal and pelagic fishes while in Batangas it covered 7000 m by 300 m spreading from Balayan Bay to Nasugbu killing mostly reef fishes [8, 9].

It could be theorized that blooms in Puerto Princesa were transported from Sabah where persistent blooms of this organism have been reported since January of this year. Summer months in the region are characterized by weak southwesterly winds. Such winds could have transported *Cochlodinium* cells from Sabah to Puerto Princesa.

The generally accepted mechanism on how *Cochlodinium polykrikoides* kills fish is by suffocation. Due to its dense concentration during a bloom, it competes with other marine organisms for oxygen. When *Cochlodinium* cells rupture and die, they produce a mucus-like substance that covers the gills of fish, causing damage which thereby enhances suffocation. The fish with damaged gills then abnormally produce mucus, furthering decrease of gas exchange in the gills [10].

Extensive experimentation on how to mitigate harmful algal blooms of this organism is being done in Korea, and include various water treatments: sophorolipid, loess, cocamidopropyl betaine (a surfactant) and clay [11]. Algicidal treatment include Bacillamide which was isolated from *Bacillus* sp. [12] and phlorotannins from the seaweed *Ecklonia kurome* [13].

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ISSHA President's Corner

NEWS

Happy New Year in 2006! Time is passing quickly as we make progress toward the 12th International Conference on Harmful Algae scheduled 4-8 September in Copenhagen. Check ISSHA's web site (http://www.issha.org/) for updates. In addition to the oral and poster sessions, break out groups and banquet, the Conference Organizers have added an auction to this year's meeting activities. From having attended the annual PSA (Phycological Society of America) auctions, I can assure you the ISSHA auction will become a very popular part of our meetings as well. It is an opportunity for you to bring algal related memorabilia, books, monographs, reprints or copies of your best micrographs or SEMs of toxic (or non-toxic) cells (or you fill in the blank) and share these with colleagues throughout the world. Notify gerth@bi.ku.dk with your contributions and look forward to an evening of fun at the ISSHA Auction.

PUBLICATIONS

Physiological Ecology of Harmful Algal Blooms (1998) edited by Anderson, Cembella and Hallegraeff are now available from ISSHA for $10 US (plus $10 handling and shipping; recipient is responsible for any duty charges). Please contact Nina Lundholm (NLundholm@bi.ku.dk) to order copies. Payment must be made in DK or US$ by cash or credit card (VISA or Master Card) only. The editors secured the

copyright to this volume and ISSHA is especially grateful for their generosity in allowing the Society to reproduce the original volume in a compact disc format. A quick check of the online availability and price of this volume listed 7 copies starting at prices over $800 per copy.

For those of you who were not able to join us for the Xth International Conference on Harmful Algae in St. Pete Beach, FL, the Proceedings are available through FWRI website (http://www.floridamarine.org/features/view_article.asp?id=12373). To access the Proceedings, click on Red Tide under Research, then HAB Information Links under Harmful Algal Bloom Facts and Information.

AWARDS

Maureen Keller Student Awards were presented by ISSHA at the 11th Conference on harmful Algae held in Cape Town, South Africa. Please join me in congratulating these young researchers for their excellent oral presentations and posters.

Karen Arthur was awarded Best Presentation for her talk on "The Potential Exposure of Green Turtles to Tumour Promoting Compounds from the Cyanobacterium *Lyngbya majuscule*". Collaborators for this project were G. Shaw, C. Limpus, G. Balaza and J. Udy. Karen is a PhD candidate at the Centre for Marine Studies at the University of Queensland, Australia. Karen's current work focuses on marine turtles and their interaction with harmful algae; such as, *Lyngbya majuscule*, an alga causing extensive blooms responsible for overtaking seagrass beds that provide shelter and forage for large populations of green turtle and dugong. Karen has been fortunate in working with a number of marine turtle experts such as Dr. Colin Limpus (Queensland Parks and Wildlife Service, Australia) and George Balazs (Marine Turtle Research Group, National Marine Fisheries Service, Pacific Islands Fisheries Science Center), and environmental toxicologist Dr. Glen Shaw (Centre for Environmental Toxicology, University of Queensland, Australia). Karen enjoys her research with marine turtles and believes "Turtles enchant all who come across them; they represent a link between peoples of the world as they migrate across international borders and yet are so susceptible to human impacts, living the large majority of their lives in a relatively localized inshore habitat".

Nicholas Touzet received Honorable Mention for his talk on "Inter and Intraspecific Variability in Morphogenetics and Toxin Composition of *Alexandrium* spp. in Irish Coastal Waters". Collaborators for his project include J.M. Franco and R. Raine. Nicholas obtained his degree in Marine Science from Universite de Bretagne Occidentale in Brest and his Masters in Toxic Interactions in Ecosystems and Toxin Biotechnologies from the Museum National d'Histoire Naturelle in Paris. He is currently focused on the morphogenetics and toxin composition of *Alexandrium* species in Irish coastal waters. He conducts his research in Ireland at the Martin Ryan Institute located at the National University of Ireland, Galway and is part of the research team led by Dr. Robin Raine. Nicholas' plans for the future include participating the in the EU 6th framework project SEED and eventually lecturing his research topic within third level institutions. Along with his academic achievements, Nicholas is also a sports fan and plays wing for the University rugby team. Congratulations Nicholas.

Pauliina Uronen received Best Student Poster for her work on "Transfer of Nodularin to the Copepod *Eurytemora affinis* Through the Microbial Loop". Collaborators for her project include P. Kuuppo, S. Sopanen, C. Svensen, C. Legrand, A. Ruhl, T. Tamminen, and E. Graneli. Paulina is earning her PhD in Finland at the Tvärminne Zoological Station at the University of Helsinki and the Finnish Environment Institute. She has studied species such as *Nodularia spumigena, Prymnesium parvum* and *Dinophysis* spp. Her current focus is on harmful algae and the transfer of algal toxins in the aquatic food web, specifically, the harmful species occurring in the Baltic Sea. Pauliina remarked that the Maureen Keller Student Poster Award encouraged her to continue studies with harmful algae. In addition to Pauliina's academic agenda, she can also be found sailing in the Baltic Sea, hiking in nearby forests and spending time with close friends and family.

Patricia Blair was awarded Honorable Mention Student Poster for her work on "Algicidal Bacteria Targeting the Toxic Dinoflagellate Karenia brevis: The Importance of Interactions within the Microbial Community". Collaborators for this project include M. Twiner, C. Mikulski, K. Jones and G.J. Doucette. She graduated Magna Cum Laude from West Virginia University, USA with a BA in Biology and a BS in Environmental Protection. She then taught coastal ecology at The Marine Science Consortium for three years. She is pursuing her Masters of Marine Biology at the Grice Marine Laboratory at the University of Charleston, SC. Her current research focuses on characterizing the interactions between algicidal bacteria and the Florida red tide species *Karneia brevis*. She conducts her work in the lab of Dr. Gregory Doucette of the NOAA Marine Biotoxins Program at the nearby Center for Coastal Environmental Health and Biomolecular Research. When out of the lab, Patricia enjoys rowing for her college crew team and playing volleyball. When she completes her degree program, Patricia hopes to continue working in the harmful algal bloom field.

Pat Tester, ISSHA President.
December, 2005.

Harmful Algae News

Previous issues of HAN and newsletters of the IOC HAB Programme can be downloaded at http://ioc.unesco.org/hab/news.htm

Requests for subscription

Subscription to HAN is made by sending a request with a complete address to Ms V. Bonnet (v.bonnet@unesco.org).

REMEMBER TO RENEW YOUR MEMBERSHIP IN ISSHA – NOW DUE EVERY SECOND YEAR ONLY!

From now on, payment is due every second year. Next payment is due from Jan 1, 2006 and is for the years 2006-2007.

Hence the fees in 2006 (January 1-December 31) are:

- Regular members 40$.
- Student members: 20$.
- Institutional members 80$.

If you want to avoid having to remember to pay the dues, please be aware that a Life Time Membership of ISSHA is possible for only $500.

Remember that you get a registration discount for the 12th International Conference on Harmful Algae in Copenhagen, September 2006 when being a member in 2006.

New applications for membership may be made at any time during the year. If paying in 2006, 2008, etc. payments are the regular rates listed above. Payment in the years in between (2007, 2009, etc.) will only be for one year as is hence half the price indicated above.

Please note that the Society revenue is primarily derived from annual contributions paid by membership dues.

To pay, please complete the form on the web-page (www.issha.org) and either renew or apply for membership.

PHYCOTOXINS

You can also get news on HAB research and events at the Phycotoxins-list at the internet.

The address of this list is: phycotoxins@www.agr.ca

To join the list, send "subscribe phycotoxins" to listserv@www.agr.ca

Archives are located at http://www.agr.ca/archives/phycotoxins.html



ISSHA AUCTION

At the 12th International HAB conference on Harmful Algae we will have an auction, the surplus of which will go to the International Society for the Study of Harmful Algae (ISSHA). But in order to become a success we need your help with donation of various items. Only your imagination sets the limit for what can be donated. It could be signed reprints, books, photos or craftsmanship (paintings, drawings, carvings etc.), homemade wine, beer or other specialities.

At www.issha.org you can alredy now see the items that have been donated for the auction. The auction catalogue will be updated regularly between now and the Conference.

If you want to make a donation please contact Gert Hansen (gerth@bi.ku.dk). We look forward to hearing from you.

The Organising Committee of the 12th International Conference on Harmful Algae.



Future events

FEBRUARY 2006

OCEAN SCIENCES MEETING

February 20-24, 2006. Honolulu, Hawaii.

Call for Abstracts Special HAB Session: Abstracts are requested for a special session on Harmful Algal Blooms: From Research to Management at the Ocean Sciences Meeting, convened by Gina Perovich (Perovich.Gina@epamail.epa.gov) and Quay Dortch (Quay.Dortch@noaa.gov). A description of the session and instructions for abstract submission are available at:

www.whoi.edu/redtide/announcements/special_session_OS080.html

JUNE 2006

ASLO SUMMER MEETING

June 4-9, 2006. Victoria, Canada.

The American Society for Limnology and Oceanography, the International Society for the Study of Harmful Algae, American Fisheries Society, Aquatic Plant Management Society and the Society for Canadian Limnologists invite you to attend the ASLO Summer meeting in Victoria, British Columbia , Canada on June 4-9, 2006. The theme of the meeting is "Global Challenges for Oceanography and Limnology".

Futher information at: www.aslo.org/forms/victoria2006.html

SEPTEMBER 2006

12th INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

September 4-8, 2006. Copenhagen, Denmark.

Call for submission of abstracts and registration form: February 1, 2006. Deadlines:

- Abstracts reception: May 1, 2006
- Early registration: June 1, 2006
- Late registration: July 15, 2006

Further information can be found at: www.bi.ku.dk/hab/

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

http://ioc.unesco.org/hab/news.htm No. 30

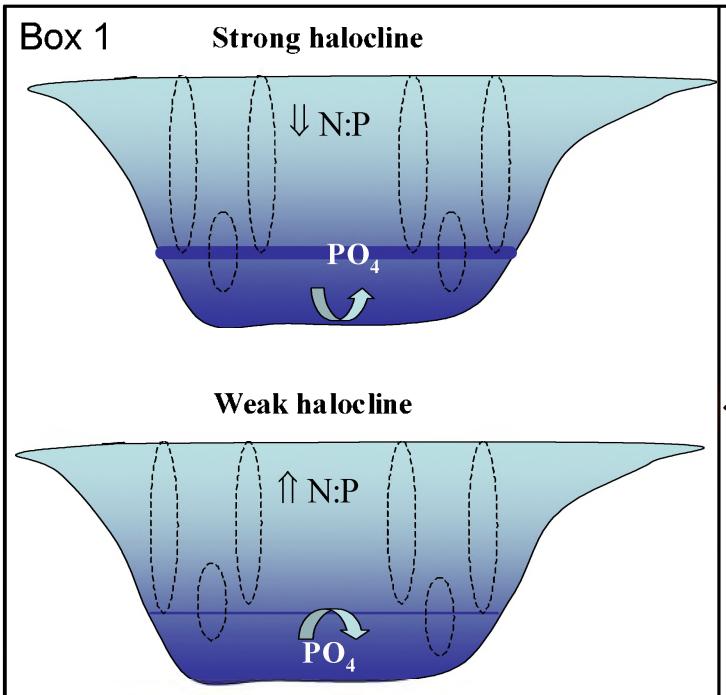
• GEOHAB

Old questions, new tools

In the Barents Sea, warm currents and cold currents intermingle. The Russian hydrographer Nikolai Knipowitsch and his successor Leonid Breitfuss attributed these patterns to insolation and advection respectively. A century later, this conflict is still not fully resolved, as deliberations at a meeting last year in Baiona (Spain) reveal [1]. An analogous biological problem was cause for dispute in the same decades. Winter herring had disappeared from the west Swedish coast about 1810, not to reappear until 1877. This "periodicity", traced through the whole last millenium by Axel Ljungmann, was attributed by Fredrik Laurentz Ekman (grandfather of Ekman transport) to variations in the strength of the low salinity Baltic outflow and preference of the winter herring for more saline "bank water" entering the Skagerrak from the North Sea. Per Teodor Cleve identified Arctic diatoms in the bank water, and Otto Pettersson sided with Ekman and Cleve, that herring, plankton, and water were advected together. But Hauken Hasberg Gran, supported by Johan Hjort, disagreed; they maintained that resting stages of Arctic plankton were present year round in the local sediments, and produced blooms when conditions became favourable. Contemporary students of the same problem have written that "In a hydrographically complex

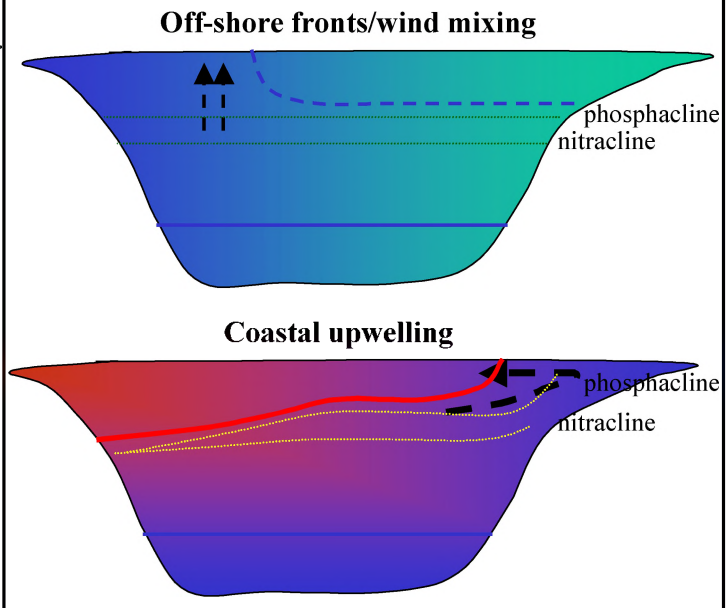
(Cont'd on p.2)

Box 1



A strong permanent halocline at 60-80 m creates poor oxygen conditions in the near bottom layer of the Gulf of Finland, Baltic Sea. With anoxic conditions, phosphates are released from the sediments and hence higher phosphorus concentrations occur in the upper water column after seasonal convective mixing (larger dashed circles) which reach the halocline. Consequent lowering of the N:P ratio in the upper water column creates favourable conditions for N-fixing cyanobacteria. With a weak halocline, phosphates bind to the sediments, and less is mixed to the upper water column. In an environment with high a N:P ratio, N-fixing cyanobacteria do not have a competitive advantage in phytoplankton succession, and hence the formation of intense blooms is unlikely.

I. Lips, Marine Systems Institute, Tallin University of Technology, Estonia.



During summer thermal stratification, upper water layer inorganic nutrients are depleted, and the main processes which bring nutrients from lower layers below the thermocline to the upper layers are wind induced mixing (especially in the frontal regions) and coastal upwelling. There is a quasi-permanent salinity front in the Gulf of Finland entrance area where inflowing saltier open Baltic Proper waters weaken the vertical salinity stratification and stronger wind impulses can bring nutrients from lower layers to the upper layer. As the phosphacline is located in the upper part of thermocine and the nitracline is about 5 m deeper, mostly phosphates are brought up during wind induced mixing and coastal upwelling processes. This will lower the N:P ratio in the upper layers and will create favourable conditions for cyanobacterial bloom development.

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Institute of Biology, University of Copenhagen.

(Cont'd from p. 1)

area such as the Skagerrak ... , a distinction between succession and sequence may be hard to perceive" [2]. Hydrographic complexity is not of course unique to the Skagerrak.

At the recent GEOHAB Open Science Meeting, HABs and Stratification (Paris, 5-8 December 2005), it was noted that blooms of harmful algae are often confined to "thin layers", raising questions like; are the dynamics of these populations controlled by biological or by physical mechanisms, or do they result from combinations of both? Further, what kind of evidence would we need to exclude different possibilities? These are hard questions, with echoes of the old dispute about density-dependent and density-independent population regulation which so exercized the minds of an earlier generation of ecologists. It is only quite recently that instruments have been designed which can collect the data we need to address some of these questions. The introduction to the conference abstracts reminds us that " ... thin layers (intermittent and less than 1m thick) pose problems for sampling and modelling of harmful algal populations. Coupling physical effects (turbulence, shear, advection) and biological behaviour (migration, physiological adaptation) holds the key to understanding vertical distributions, bloom dynamics, and patterns of toxicity." Thus questions like those asked in the late nineteenth century by some of the pioneers of marine science continue to puzzle us.

The coastal and inshore regions where HABs occur are hydrodynamically more diversified than offshore due to turbulent mixing by tides, and wind stress and runoff patterns. As well, HABs are often brief high amplitude events superimposed on seasonal cycles, so that Sverdrup's critical depth model cannot effectively capture the population dynamics [3]. Some field studies reported at this meeting detailed some of these complexities. *Alexandrium tamarense* blooms in the St. Lawrence Estuary are influenced by winds and runoff, by vertical migration, and perhaps also by humic substances (**Juliette Fauchet**). *Karenia mikimotoi* in the Western Approaches to the British Isles responds to insolation, salinity, and self-induced warming (**Liam Fernand**), and is advected from place to place by geostrophic jets related to cold pools (**Robin Raine**, and see [4]). Advection also seems to play a key rôle for *Dinophysis acuta* -in western Iberian waters; populations on the central Portuguese coast respond to downwelling and convergences, especially in a core area off Peniche-Aveiro. A northerly surface current, more intense in autumn, can advect populations to the Galician Rías Bajas (**Teresa Moita**), where it sometimes adds to the problems caused by the more persistent *Dinophysis acuminata* there (**Beatriz Reguera**; **Yolanda Pazos**). In the Gulf of Finland, a deep permanent halocline is sustained by intermittent advection from the North Sea, and renewal patterns determine the availability of phosphorus from year to year; cyanobacterial dynamics depend via vertical convection on this nutrient bank, as well as on the seasonal thermocline, so that the magnitude of blooms depends on both intra- and inter-annual events (see Box 1).

At HABXI in Cape Town, it was suggested that small gyres act as holding zones for *Dinophysis* populations in the Bay of Biscay. Many such gyres emerge in numerical experiments of the region; some of the mechanisms which may cause them to persist have been identified, and the characteristics which can lead to accumulation of *Dinophysis* explored (**Hongqin Xie**). The problem of identifying retentive structures of this kind in hydrodynamic simulations was tackled (**Marc Segond**) using Marco Dorigo's entrancing 'ant colony optimization (ACO) algorithm' (virtual ants), more familiar perhaps to oil shippers and trucking companies (see Box 2). This was applied to the output from the model used by Hongqin Xie, and shown to perform better than informal procedures. Its application to output from the Portuguese MOHID model (**Marcos Mateus**) [5] might provide new insights into the mesoscale ecology of *Dinophysis* in western Iberian waters already mentioned.

The Core Research Project [6] identifies eight key questions. Of these, five (A, B, F, G, H) are closely related, and were touched on to varying degrees, or at least implicit, during discussions at this meeting. These refer to (A) adaptive strategies, (B) seeding strategies,

Box 2

Ant based tool for retentive structure detection

Ant algorithms were introduced to computer science by Marco Dorigo. The main idea of such algorithms is to use ants' abilities to behave intelligently using only small interactions with their environment. Classical applications of such algorithms are finding the shortest path to a goal, digital network routing, etc. In our case, we adapted this algorithm to detect retentive structures in coastal waters. This kind of detection can be performed by some classical deterministic vector field analysis methods such as vorticity thresholding or streamline building, but these methods are not very efficient in coastal waters due to their high sensitivity to stream perturbations.

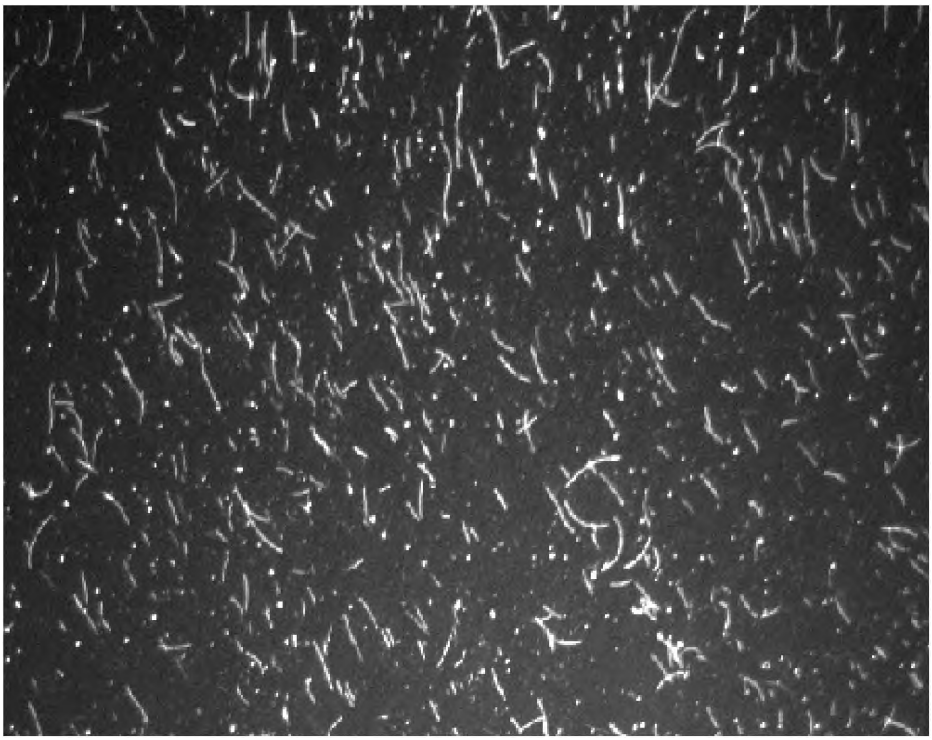
Our ant algorithm can perform retentive structures detection on any 2D vector field, taking in account stream strength and direction. It was first applied to detect structures from10km to 50km radius, to help to verify the hypothesis that anchovy demography is linked to the presence of these vortices.

Due to the very good flexibility of the algorithm, it can easily be applied to the detection of retentive structures on planktonic scales, depending on the scale and mesh of the vector field given to the algorithm. It is also very easy to make the algorithm take into account other parameters such as wind, salinity, bathymetry, ... , to perform special detection tasks. More precise references can be found in these articles:

- *Application d'un algorithme à colonies de fourmis à la détection de structures rétentives en eaux côtières (Marc Segond), Majestic'04, October 2004.*
- *Ant Algorithms for Detection in Coastal Waters (Marc Segond, Sébastien Mahler, Denis Robilliard, Cyril Fonlupt, Benjamin Planque, Pascal Lazure), EA'03, October 2003.*

More information can be found at http://lil.univ-littoral.fr/~segond/EN/Marsouin.html

M. Segond, Université du Littoral, Côte d'Opale, Laboratoire d'Informatique du Littoral, 50 rue Ferdinand Buisson BP719, 62228



Box 3

A 13.5 cm x11 cm section from a 32 cm x 32 cm image of chlorophyll a fluorescence, taken at about 35 m depth, 10 km off the coast of San Diego, CA, USA. A 6.5 mm thick sheet of green laser light was used to stimulate fluorescence, which was imaged at 680 nm with a very sensitive CCD camera, giving 0.3 mm x 0.3 mm resolution. The laser and camera system were mounted below a free-falling platform, giving undisturbed images of the 2D distributions of the phytoplankton. The long objects in the image are diatom chains, many from the genus Pseudo-nitzschia. *Some of the chains in the images were 2 cm long - much longer than chains obtained from bottle samples. Profiles of such images have shown ubiquitous fine-scale layering of the phytoplankton, even though the total biomass (bulk chlorophyll a fluorescence) varies smoothly through the water column. Such layering is presumably due to physical-biological interactions, including turbulence, vertical shear, swimming, sinking, and growth. Image courtesy of P.J.S. Franks and J.S. Jaffe, Scripps Institution of Oceanography, UCSD, La Jolla, CA, USA.*

(F) coastal morphology and bathymetry, (G) cross-shelf and longshore advection, and (H) climate indicators. The theme common to the five is retention (and dispersal), which has three components, behaviour, circulation, and topography.

One essential of life cycle closure is that some stage must be able to find its way 'home'. For example, in a hypothetical species with two mandatory stages, vegetative cells and cysts, some of the latter must end up in the parent seed banks which provide inocula for subsequent generations. If they do not, then the probability is that their progeny will inoculate different water masses, not those from which they were derived. Then the next growth phase may not encounter suitable conditions, and if it does, the next crop of cysts will be even less likely to find its way back to the parental seed bank. 'Losses' on this account may be advantageous in the sense of providing opportunities for dispersal and range expansion, essentials of long term survival, but will in most cases require to be underpinned by persistence in the core area. Life history strategies therefore require retention mechanisms, and seeding strategies are part of the same requirement (questions A & B). The timing of excystment, growth patterns, control of vertical distributions, and encystment are behavioural components of these strategies. Detailed accounts of these problems for *Alexandrium* in the Bay of Fundy and Gulf of Maine have been published [7].

Cross-shelf and longshore advection (G) are among the processes, given certain patterns of coastal morphology and bathymetry (F), which generate the hydrodynamic features which makes retention mechanisms possible. Vertical migration (VM) has long been thought to be in part a means to promote retention. If this is so, VM patterns need to be tailored to particular retention systems, and adaptable enough to respond to changes in the latter caused by climatic variations (H).

A variety of new instruments were described at this meeting, and it is now becoming possible to obtain more detailed views of the underwater environment at the small scales where individual phytoplankton colonies and cells interact with the microphysics. **Hidekatsu Yamazaki** maps biological and physical turbulent microstructure in freefall profiles using TurboMAP (Turbulent ocean Microstructure Acquisition Profiler) [8], and has demonstrated that phytoplankton tends to aggregate in regions of low turbulence. **Peter Franks** uses a sheet of laser light to stimulate chlorophyll fluorescence and views an undisturbed image plane with a CCD camera (Box 3). Particle tracking velocimetry (PTV) is being explored by **Alex Nimmo Smith**; this system uses four cameras to track suspended particles in three dimensions and provides views of fluid motion in a 20 cm cube [9]. These new tools, and the development of multidisciplinary projects (such as the EU FP & Harmful Algal Blooms in Thin Layers) focused on target species will hopefully shed new lights into the interactions between microphysical structures and microalgae growth and life history stages.

www.geohab.info

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Email: twyatt@iim.csic.es

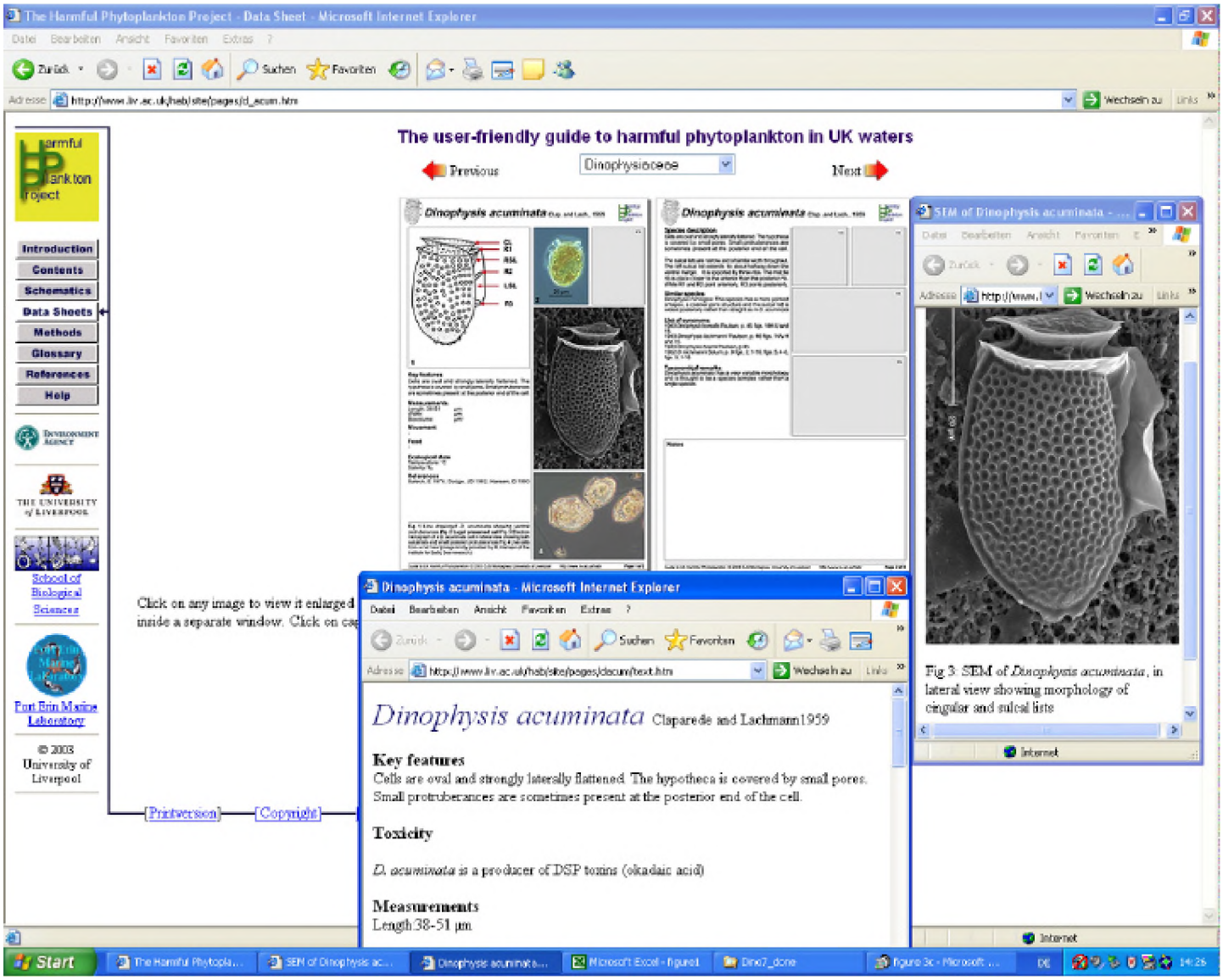
An Online Guide to UK Harmful Phytoplankton and its Future

Harmful algal blooms (HAB) and the occurrence of toxic species threaten the livelihood of coastal communities worldwide; they pose a risk to both human health and businesses, such as fisheries and aquaculture. Furthermore, it is clear that to prevent disasters, careful monitoring of harmful and toxic species is paramount. However, the identification and study of harmful species is complicated by the large number of phytoplankton groups that occur, even within a single sample (e.g., dinoflagellates, diatoms, raphidophytes and haptophytes). Unfortunately, the extensive and disparate nature of information on these taxa often impairs monitoring programmes, which are often staffed by non-specialists. Resources are, therefore, now needed to provide relevant and current information on HAB species in an easy-to-use, clearly structured, and condensed format.

To address this need in the UK, an online guide to harmful phytoplankton (http://www.liv.ac.uk/hab) has been established at the University of Liverpool with funding from the UK Environment Agency (EA). This resource not only communicates the taxonomic information normally found in scientific publications, but it is also of educational value and provides non-taxonomists with the information needed for the reliable identification of key harmful phytoplankton species.

The EA-funded site has a user-friendly navigation structure, resembling a cross between a field guide and a scientific paper. It starts with an *introduction* and *methods* section. Then, at the heart of the site are the individual species *data sheets* that contain data on the species, schematic drawings, and images of live and fixed cells. Individual species sheets can be accessed from a *contents page*, via drop down menus on each species sheet, or by simply browsing through all sheets.

The data sheets, when first accessed are essentially presented as thumbnails, providing a quick and informative overview. This would be similar to holding a page of a book at arm's length. Then, by clicking on different areas of the data sheet, either images or text, these areas can be viewed in new enlarged windows. In this way information can be arranged according to the needs of the user, and information on different spe-



cies can be compared. In addition, the site contains an illustrated *glossary*, a *schematics section*, and a *reference* list, all of which can be accessed from all data sheets. Finally, for those who do not have regular access to a computer, or prefer hard copies, all data sheets and other sections are available as pdf files for downloading and printing.

The HAB site is based on the well-tested format of its sister site (The user friendly guide to coastal planktonic ciliates, http://www.liv.ac.uk/ciliate). Both sites are proven tools, with considerable international educational value (the ciliate site now gets 2000 hits a day and serves over 70 countries, and the newer HAB site now receives 1000 hits per day). Both of these sites are technically very simple and can, therefore, be viewed on low power computers and with older browsers, which are still commonly used in many parts of the developing world.

Although the HAB site concentrates on species found in UK waters, it is still beneficial to a large number of users, as it combines detailed species descriptions with more general information on harmful genera and families found all over the world. In fact, the UK partners on this site are extremely keen to expanding it to serve the international community. Interested parties should contact David Montagnes (dmontag@liv.ac.uk).

Future plans

Although the HAB site described above has proven to be a useful resource, and received contributions from many researchers, it is still far from a comprehensive internet repository; it would be difficult for any one site be so. We are still faced with the problem that it is often difficult to access information that is spread over disparate databases, uses different formats, and is assembled using different software tools. In the study of harmful algae, even more so than in other areas of phytoplankton research, it is vital to rapidly verify identifications and communicate results on the basis of these IDs. There thus is a need to strengthen and formalise the connectivity of existing resources.

We suggest that accessing and sorting information from multiple resources will become a much easier task once existing online facilities are networked, and can be searched jointly. Such networks will be a more efficient and cost-effective way of dealing with taxonomic information. Concomitantly, this will lead to a greater chance of data remaining online in the long-term. In short, we need to pool our resources.

Our next step, therefore, is to link existing images, data, educational material, and analysis tools (e.g., taxonomic keys) to a true online database that will allow structured searches. We plan to achieve this through a collaboration with the recently funded EU project: PLANKTON*NET (coordinated by the Alfred Wegener Institute for Polar and Marine Research http://ebck.rd.awi-bemerhaven.de/protist/baypaul/microscope/general/page_01.htm). This project will first create a network of online plankton databases in partner institutes. Then, it will provide an opportunity to incorporate resources not belonging to the initial consortium into the network by assisting external partners in establishing their own PLANKTON*NET sites or hosting their data as image collections on existing PLANKTON*NET nodes. We, therefore, encourage collaboration with researchers holding taxonomic information about harmful phytoplankton. Interested parties should contact Alexandra Kraberg (akraberg@awi-bremerhaven.de).

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CYANONET: A Global Network for Cyanobacterial Bloom and Risk Management

Cyanobacteria harmful algal blooms (CyanoHABs) have been increasing globally in number, severity and duration, partly as a consequence of anthropogenic eutrophication. CyanoHABs are capable of producing a range of toxins which have been implicated in, and in some cases proximally responsible for, animal and human poisoning incidents throughout the world. However, recognition of the problems presented by cyanotoxins is variable, and significant geographical and institutional differences exist regarding the identification, monitoring, reporting and risk management of cyanobacterial blooms and toxins.

In order to increase CyanoHAB awareness, and to promote education and training, a global project (CYANONET) has been formed which takes into account regional and local characteristics, capabilities and requirements. The project (Co-ordinator, Geoffrey A. Codd, University of Dundee, UK) was created after the UNESCO International Hydrology Programme V Conference in Venice and is part of the UNESCO IHP-VI Action in Ecohydrology. CYANONET aims to provide a global situation assessment of the occurrence and impacts of cyanoHABs, cyanotoxins and risk management responses for the protection of water resources and health. Now at the end of Phase I of the project, CYANONET's Initial Situation Assessment and Recommendations have just been published.

This report* is available online at http://unesdoc.unesco.org/images/0014/001425/142557E.pdf and will shortly be available in printed format.

The CYANONET Report presents an initial situation assessment of the occurrence of cyanoHABs (blooms, scums, mats) and cyanotoxins in natural and controlled waters; adverse health

incidents (illness, poisonings, mortalities) of humans and animals associated with, or attributable to cyanobacteria and cyanotoxins; the existence and outcomes of systematic surveys and epidemiological studies of associations between cyanobacteria, cyanotoxins and human health; effects of cyanobacteria on water supply, waterbody-use and ecological status; the availability and implementation of management measures to reduce adverse effects of cyanobacteria and cyanotoxins; and the availability of educational, awareness-raising and training materials and practices.

Phase I of the CYANONET project has now been completed, and was the work of an International Steering Committee (ISC). Regional responsibilities for the promotion of CYANONET's aims and objectives are being addressed by: William R. Harding (Africa); Sandra M.F.O. Azevedo (South and Central America); Wayne W. Carmichael (North America); Suvendra N. Bagchi (Asia, Western sector); Kunimitsu Kaya (Asia, Eastern sector); Michael D. Burch (Austalasia and Oceania); and Geoffrey A. Codd and Hans C. Utkilen (Europe): plus a growing network of National Contacts. Approximately 70 countries in Africa, Asia, Australasia and Oceania, Europe, North and South America were included in the survey which showed that cyanotoxins occur, and have adverse effects on human and animal health throughout all regions for which data are available. Furthermore, a CYANONET website (www.cyanonet.org) with public access has been established (Website Manager, Tomasz Jurczak, University of Lodz, Poland) and is under development.

The Report has highlighted wide differences between countries regarding the information available about cyanoHABs and cyanotoxins, and in the availability of management strategies to monitor and control them.

Completion of Phase I of the CYANONET survey has enabled the ISC to identify needs and make recommendations for the next phase of CYANONET. These include: extension of the network of National Contacts, particularly in areas where little or no information has been obtained; to continue data collection, situation assessment and develop information sharing; and to develop guidance materials and management tools. By improving global communication and access to information about cyanoHABs and cyanotoxins, countries will be better equipped to make effective management decisions for the protection of water resources and human health.

* CYANONET: A Global Network for Cyanobacterial Bloom and Risk Management. Initial Situation Assessment and Recommendations. Codd, G.A. *et al*. International Hydrological Programme–VI, Technical Documents in Hydrology No. 76, 138 pp., UNESCO, Paris, 2005.

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The International Workshop on HAB Dynamics of Targeted Species in the East Asia Waters (EASTHAB II)

November 25-27, 2005, Qingdao China



Recurrent red tides appeared in the East Asia waters during the last decade and the potential harmful effects attracted concerns from the circumjacent countries. It has become a common interest to study the dynamics of the most important HAB species among these countries. To improve the mutual understanding of the most recent progresses on these studies, an international workshop on HAB dynamics of targeted species in the East Asia waters was held in Qingdao, China from Nov. 25 to 27, 2005. This was the second meeting organized under the framework of EASTHAB originally proposed in Jeju Island, Korea by scientists from Korea, Japan and China in Oct. 10-12, 2004. The meeting was hosted by Prof. Mingjiang Zhou in the Institute of Oceanology, Chinese Academy of Sciences and partially supported by the National Key Project of China, "The Ecology and Oceanography of Harmful Algal Blooms in China (CEOHAB, 2001CB4097)". 21 Scientists from Japan, Korea and China participated in the meeting with 16 oral and 1 post presentations. The meeting was focused on the dynamics of targeted species, especially *Cochlodinum polykridoides* and *Prorocentrum donghaiense* in East Asia waters. The infrastructure and potential role of EASTHAB were also discussed. It was decided that the next EASTHAB meeting would be held in Nagasaki, Japan in November of 2006.

• Chile

Green bloom of *Tetraselmis* sp. in Valparaiso Bay



Fig. 1

At the beginning of 2006, waters of beaches in Viña del Mar, Valparaiso Bay, Chile (32º57'S-71º33'W), (Fig. 1), appeared bright vegetal green, especially visible in the breaking waves (Fig. 2a). This unusual phenomenon alarmed inhabitants and tourists.

Surface phytoplankton samples (1L) were collected from January 2nd to January 6th 2006 at 4 sampling stations starting with the beach located at the mouth of Marga Marga river up to Montemar cove. Surface temperature and light attenuation (Secchi disc) were simultaneously measured. Samples were kept refrigerated in cool boxes with ice (6 ºC) and were transported to the Laboratories of Phycology, Marine Chemistry, Biochemistry, Electronic Microscopy and Quality Control of the Universidad de Valparaíso, as well as to the summer course "Spectral characterization of dissolved and particulate organic material from aquatic systems" held at the Faculty of Marine Sciences by Dr. Rodolfo Iturriaga to determine their spectral absorption.

Microscopic examination of the morphology and cell structure at the Phycology Laboratory confirmed the presence of the phytoflagellate *Tetraselmis* (Stein) (Chlorophyta, Prasinophyceae). The cells were extremely fragile and changed from motile and ellipsoidal to ovoid and non-motile (Fig. 2 b, c, d). Marga Marga beach showed the highest densities, with 3.0–5.0 x 10^5 cel/mL (haemocytometer 0.1 mm deep). Montemar cove was least affected and marginal to the phenomenon. During the two first days the green bloom was a monoculture, evolving at the 5th and 6th day to a culture with scarce presence of other phytoflagellates such as *Asteromonas gracilis* and *Chlamydomonas* sp., two species of dinoflagellates (one of them naked), and the diatoms *Chaetoceros* and *Skeletonema costatum* were observed.

The cells of *Tetraselmis* sp. isolated from the beaches of Viña del Mar do not resemble those of *T. suecica* and *T. tetrathele*, both recorded in Valparaíso Bay [1, 2]. Species of *Tetraselmis* are innocuous and several are often used as live food for marine aquaculture organisms in concentrations higher than 1.0 x 10^6 cel/mL.

Nutrient analysis of the samples showed an abrupt increase of the nitrate and phosphate concentrations between January 2nd and January 4th 2006. Nitrate increased from concentrations close to the limit of detection to values higher than 30 µmol/L. In the case of phosphate, the increase reached more than 20 µmol/L, with one of the localities approaching an unusual surface concentration of 46 µmol/L. The phosphate values decreased abruptly on January 5th to low values between 0,3 and 0.8 µmol/L. At the same time nitrate values fell below the detection limit, showing a close relationship in abundance with the green bloom.

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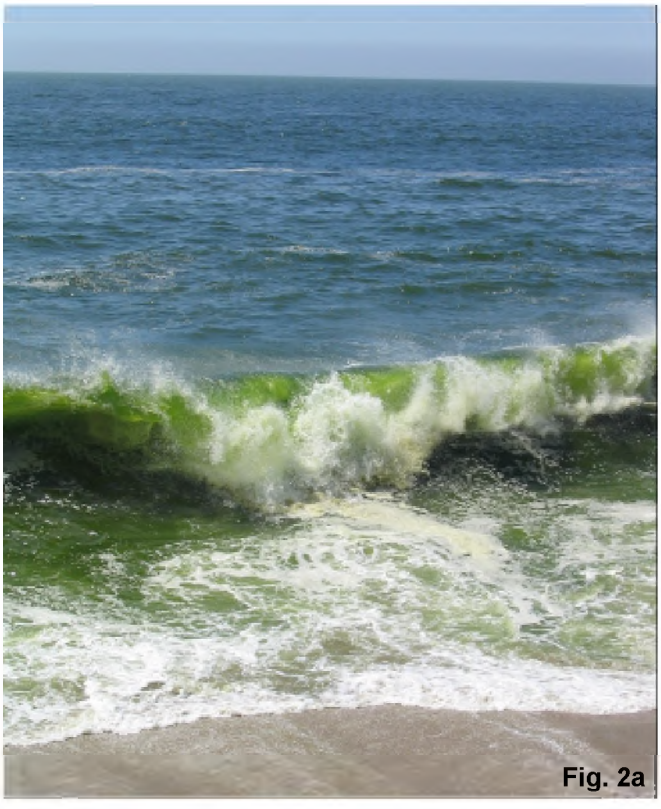

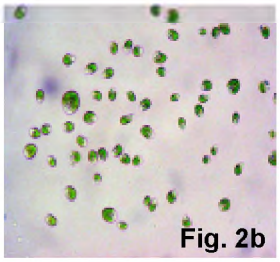

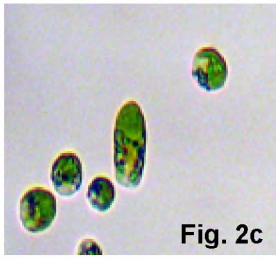

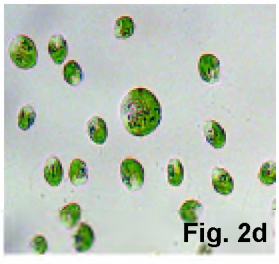

Fig. 2

• Tunisia

First detection of toxic *Pseudo-nitzschia calliantha* in Bizerte Lagoon, Tunisia



Fig. 1. Bizerte Lagoon: represented by a 130 km² surface area and an average depth of about 10 m, linked at the north to the Mediterranean Sea and at the west to Lake Ichkeul, through a narrow channel at Tinja.

Domoic acid toxicity was first reported in December 1987, on Prince Edward Island in Canada, when an outbreak of amnesic shellfish poisoning (ASP) occurred among humans who had eaten blue mussels [1]. It was subsequently reported along the Pacific coasts of California and Mexico, where mass mortalities of sea birds and sea lions were documented [2]. The domoic-acid-producing algae are certain members of the ubiquitous marine diatom genus *Pseudo-nitzschia* Peragallo that are widely distributed in the world's oceans, including coastal waters [3]. Their incidence in the Mediterranean Sea has been well documented [4].

The results of a monitoring program carried out in areas of bivalve mollusc production in Tunisia (Gulf of Gabs, Boughrara Sea, Lake Tunis and Bizerte Lagoon) showed that *Pseudo-nitzschia* species occur in the area [5]. The Bizerte Lagoon (Fig. 1) has zones of extensive aquaculture activity, where mussel production reaches 136,811 tons per year [6]. The presence of potentially toxic *Pseudo-nitzschia* spp. may be an impediment to further aquaculture development. For example, Sakka *et al.* [7] found that *Pseudo-nitzschia delicatissima* is a common member of the phytoplankton community.

Investigating the spatial and temporal distribution of potentially toxic *Pseudo-nitzschia* species in the Bizerte Lagoon is therefore crucial in order to understand the bloom dynamics in the area. For this purpose, monthly water sampling began in 2004, using Niskin bottles at 2 m below the surface, at four stations within the lagoon and one station in the Mediterranean Sea; results from one station (Fig. 1) are reported here. Members of the total phytoplankton community, including *Pseudo-nitzschia* species, were counted in Lugol's-preserved samples, using the Uthermöl sedimentation technique under an inverted microscope. Depending on whether the valve width is greater or smaller than 3 µm, and according to Hasle [3], *Pseudo-nitzschia* species were respectively ranked to the *Nitzschia delicatissima* or *Nitzschia seriata* group.

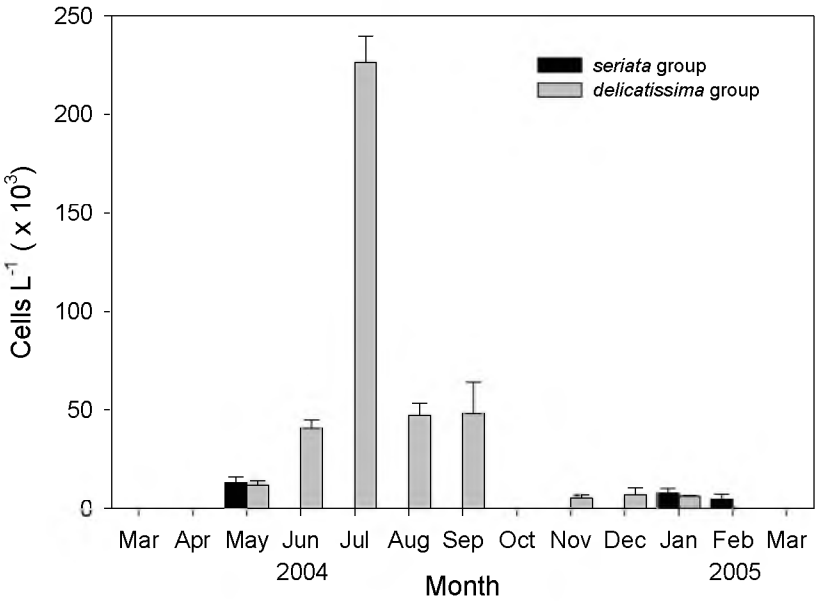

Fig. 2. Cell concentrations of Pseudo-nitzschia species within the Nitzschia delicatissima and Nitzschia seriata groups at the sampling location in the Bizerte Lagoon during March 2004 – March 2005.

As shown in Fig. 2, *delicatissima* group species were found to be dominant both numerically and throughout the year. Cells within the *seriata* group were present at much lower concentrations and only during May, January and February. The highest concentration of the genus *Pseudo-nitzschia* (226,390 cells L^{-1}) was recorded in July 2004, and was exclusively represented by *delicatissima* group species. A monoclonal culture (clone SI-5) was established by isolating a single cell from natural samples. Cells from the stationary phase (day 33) of this culture were analysed for domoic acid production by HPLC after FMOC derivatization [8]. The clone was confirmed to be toxic, with a domoic acid

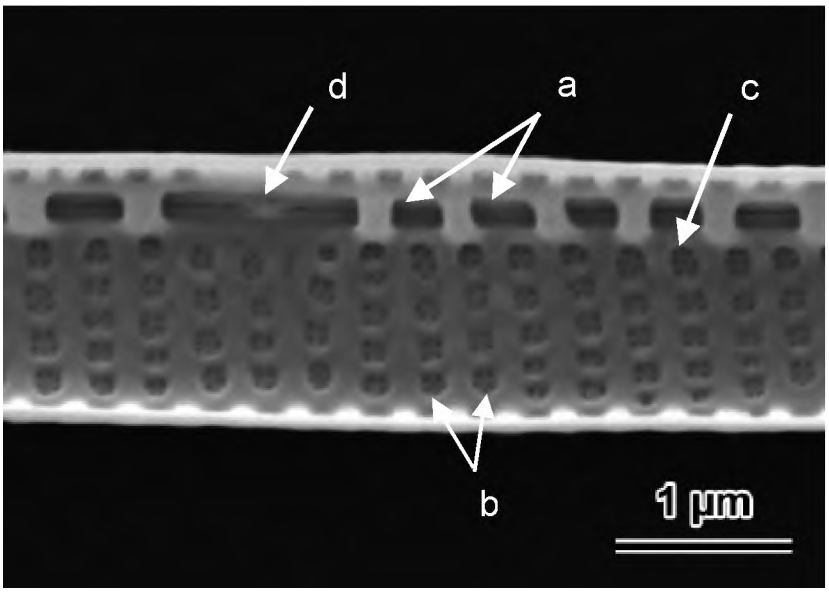

Fig. 3. Valve view of Pseudo-nitzschia calliantha collected in the Bizerte Lagoon. (a) Fibulae. (b) Striae. (c) Poroid. (d) Central interspace with the apical raphe ending. Scale = 1 µm.

concentration in the whole culture (cells plus medium) of 150 ng mL^{-1}.

For identification at the species level, acid-cleaned material collected on July 2004 was examined by SEM (Fig. 3). The valve width is 2.68 µm and the length is 35.1 µm. A central interspace is present, and fibulae are about 18 in 10 µm. Striae are more numerous than the fibulae, approximately 37 in 10 µm, and composed of a single row of poroids, with 4 poroids in 1 µm. The hymen of the valve poroids exhibit a flower pattern. These morphometrics are typical of *Pseudo-nitzschia calliantha* [9]. The toxic clone IS-5 was similarly identified as being *P. calliantha*.

This is the first time that the toxicity of any species of *Pseudo-nitzschia* has been demonstrated from Tunisian coastal waters, and the first for *P. calliantha* in the Bizerte Lagoon. It is still too early to fully describe the bloom dynamics and variability of *Pseudo-nitzschia* species in this lagoon. The continuation of this study should let us to know more about the distribution and abundance of *Pseudo-nitzschia* species in the area.

Acknowledgements

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• Peru

Training Course on Harmful Algal Blooms

3 - 5 November 2005, Pisco

The government of Perú through the Ministerial Agency (PCM) has signed an agreement with the International Bank of Reconstruction and Development (BIRF) for financial support and execution of the Project: Support for Improvement of Productivity and facilitation of Foreign Commerce. The main objective of this project is to sustain and increase Peruvian exports, through the Monitoring of Harmful Algal Blooms, to ensure the security and high quality of fishery products affected by HAB.

The Fishing Technology Institute of Perú – ITP, was created in 1979 as a governmental Institution depending on



the Production Ministry. Its main function is to promote scientific and technological research with the aim of optimal and rational use of aquatic resources. The ITP has a modern infrastructure with pilot plants for the manufacture and preservation of food products.

Since 2005, The Fishing Technology Institute of Perú is the governmental Institute in charge of establishing and improving National Programmes for the control of harmful algae blooms.

One of the tasks of the Project is training the professional staff involved in the sanitary control of fishery products. For that, a three day course-workshop was held in Pisco (ICA - Perú). The objective of the course was to provide participants with a broad perspective of the different aspects of harmful algae that need to be deal with in these programmes, and to train them and provide practice in the basic techniques which are applied in contingency plans for the occurrence of toxic/noxious algal events.

The course included theoretical classes, but special emphasis was given to practical workshops in which participants had the opportunity to collaborate in sampling activities. These included: (i) factors that favour the development of HABs, (ii) design elements in monitoring in different countries, (iii) the identification of noxious or toxic algae from their waters; and a practical class on board the IMARPE boat (Sea Institute of Perú), in Bahía de Paracas, Pisco, and phytoplankton sample analysis in IMARPE- Pisco laboratory.

A common situation in developing countries is that a few experts, sometimes in quite remote places, have to deal with varying aspects related to the occurrence of harmful algae events. So, several participants from other institutes attended the course, from Piura University (North Perú), University of San Luis Gonzaga-Ica (South Perú), the Marine Institute of Perú (IMARPE), and Non-Governmental Organizations like the Huayuná Institute and the Association of Mariculturists of Pisco.

The course was given by Lic. Graciela Ferrari from Montevideo-Uruguay.

• Spain

VII IOC-AECI-IEO Course on Toxic Microalgae and Marine Biotoxins



The IOC-IEO Science and Communication Centre on Harmful Algae, organized the VII Course on Toxic Microalgae and Marine Biotoxins that was held at the Instituto Español de Oceanografía in Vigo, Spain, from the 6th to the 24th February. As in previous years, it was sponsored by the IOC, the Agencia Española de Cooperación Internacional (AECI) and the IEO.

The course focused on "Monitoring programmes on Toxic Phytoplankton and Marine Phycotoxins according to European Regulations", and was intended for experts committed to the setting up or strengthening of their national or regional monitoring programmes on Harmful Marine Algae. 12 scientists from Latin America (Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Mexico and Peru) and Magreb (Tunis) participated in the course.

Contents included a general section on monitoring programmes and two specific workshops to be chosen by the participants: "Taxonomy and Ecology" or "Determination of Marine Biotoxins". It also included a sampling day on board the O.V. "J.M. Navaz" and the subsequent work at the laboratory with the samples taken to isolate and establish a culture.

Classes were given by experts from the IEO, Instituto de Investigacions Mariñas, European Union Reference Laboratory on Marine Biotoxins, Sanidad Exterior de Vigo, Instituto Tecnolóxico para o Control do Medio Mariño, Centro de Investigacións Mariñas and ANFACO-CECOPESCA. Participants had the opportunity to attend both theoretical and practical lectures in these research and monitoring centres. They also visited the facilities of an association of mussel growers, a molluscs depuration plant and the national association of canned seafood.

In general, this course experience was very positive both for the students and the participating experts. Fruitful contacts have been establish that will continue in the future.

ISSHA

International Society for the Study of Harmful Algae

ISSHA President's Corner

ISSHA WEBSITE UPDATE & 12TH INTERNATIONAL HA CONFERENCE

The ISSHA website (www.issha.org) has been upgraded and has a new look thanks to the efforts of Tracy Villareal (ISSHA Secretary) and Nina Lindholm (ISSHA Treasurer). Check it out and follow the link to the 12th International Conference on Harmful Algae which is just a few short months away (www.bi.ku.dk/hab) in 4-8- September 2006 in Copenhagen. This promises to be an exciting and productive meeting with some new activities including an auction and HAB Jam. If you would like to contribute something weird, wacky or wonderful to the auction please contact Gert Hansen (Gerth@bi.ku.dk). Per Hansen (PJHansen@bi.ku.dk) is interested in participants for a HAB Jam session, so don't be shy about sharing your musical talents.

Coming deadlines for the 12th HAB Conference include

- 1 June 2006 for the end of early registration
- 31 July for the end of late registration.

One of the things that will be addressed at the ISSHA General Meeting during the conference is the venue for the 2010 meeting. I would like to request that individuals, labs or groups interested in hosting the 14th HAB meeting, please contact me (Pat.Tester@noaa.gov) and plan to give a short presentation to the membership prior to the vote. As you recall the 13th HAB Conference will be in 2008 in Hong Kong, China.

2006-2007 MEMBERSHIP DUES

As a reminder to all ISSHA members, your 2006-2007 dues are now payable to Nina Lundholm. Conference participants can pay at the time they register but if you are not planning on attending the 12th HAB Conference, renew your membership to keep your voting privileges for the next slate of ISSHA officer (2007).

Books and CDs

ISSHA with IOC has the honor of co-publishing an illustrated photo guide to the

cyanobacteria authored by Gertrud Cronberg and Helene Annadotter. Hopefully this volume will be available in time for the 12th HA Conference. Watch for details on ISSHA's website. Also, remember that The Physiological Ecology of Harmful Algal Blooms is available on CD-ROM from the website as well.

Have a good summer and I'll see you in Copenhagen.

Warmest regards,

Pat Tester
ISSHA President



ISSHA AWARDS: CALL FOR NOMINATIONS

Current members of ISSHA are invited to submit nominations for the following awards:

The Yasumoto Lifetime Achievement Award

This award is offered in recognition of a long, and outstanding record of contribution to harmful algal research. Previous recipients: Dr. T. Yasumoto (2000), Dr. G.R. Hasle (2002), and Dr. T. Smayda (2002). Other senior scientists similarly honored by the harmful algal research community before ISSHA included Dr. L.Provasoli, Max Taylor, and Dr. E. Balech.

The Young Scientist Achievement Award

This award is offered in recognition of outstanding achievement in any aspect of harmful algal research by a scientist early in their career (i.e. within ten years of receiving their PhD). The award is intended for an exceptional or "break-through" contribution to an area of harmful alga research, rather than a cumu-lative record of achievement. Previous recipient: Dr. C. Scholin (2002).

Any ISSHA member may submit nominations for either achievement award, which should provide a complete, yet succinct description of the nominee's contribution (not more than one page). Nominations should be sent by e-mail to Barrie Dale (barrie.dale@geo.uio.no), Chair of the Committee on Achievement Awards, to be received by 28 April 2006. It should be noted that ISSHA does not necessarily give achievement awards at every International HAB Conference. Nominations received by 28 April 2006, will be considered by the ISSHA Council, and eventual awards will be presented at the XII HAB Conference in Copenhagen, 4-8 September 2006.

B. Dale, Chair of the Committee on Achievement Awards

Future events

JUNE 2006

TRAINING COURSE: PSP BY LC. AOAC METHOD 2005.6 - LAWRENCE *et al.*

June 19-21, 2006. Seattle, USA.

The AOAC International's Marine and Freshwater Toxins Task Force is offering, for the first time, a Training Course for OMA 2005.6 - Paralytic Shellfish Poisoning Toxins (saxitoxins) in Shellfish - Prechromatographic, Oxidation and Liquid Chromatography with Fluorescence Detection. Instruction for this new course will be by Health Canada, Foods Research Division and will be held June 20-21, 2006, at Washington State DOH lab with Lawrence method orientation and overview on the morning of June 19, 2006 at the Ramada Inn, Seattle Univ. District North.

The course will emphasize critical aspects for success using this method and is the first in a series of courses addressing the Task Force / OMA level official methods.

Futher information at: www.aoac.org/marine_toxins/task_force.htm

SEPTEMBER 2006

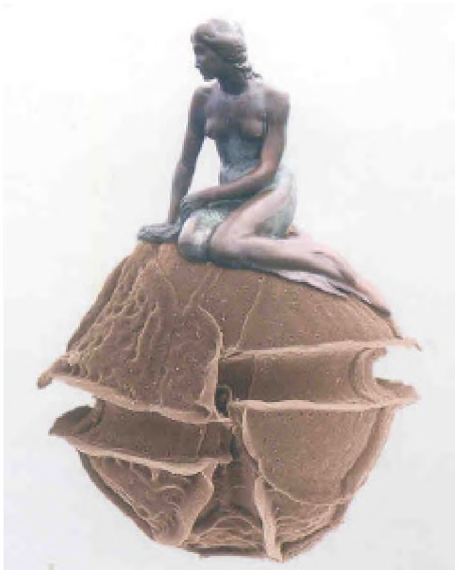
12th INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

September 4-8, 2006. Copenhagen, Denmark.

Call for submission of abstracts and registration form: February 1, 2006. Deadlines:

- Abstracts reception: May 15, 2006
- Early registration: June 1, 2006
- Late registration: July 15, 2006

Further information can be found at: www.bi.ku.dk/hab/





The other (far?) side of HAB's

Dear potential HAB performer,

You are invited to participate in a very special evening.

As a part of the social programme for the 12th International Conference on Harmful Algae in Copenhagen 2006 we invite you to perform and entertain the Conference participants in an informal and cosy atmosphere on Tuesday 5 September.

All contributions are welcome!

We expect the performances to be musical, poetic or "stand up", but we are open to all contributions.

Looking forward to your response at pa@bioconsult.dk / PJHansen@bi.ku.dk, the sooner the better!

The Conference Organizers
www.issha.org

MARCH 2007

ICMSS07

New Zealand is hosting the 6th International Conference Molluscan Shellfish Safety in Blenheim 18th-23rd March 2007. The conference is held every two years and brings together scientific, industry and regulatory people from all over the world. The conference agenda will allow the sharing of scientific research, management systems, debate on international trade issues and networking between stakeholders. More details can be found on www.nzfsa.govt.nz/icmss07.



HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

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• France

Identification of *Pseudo-nitzschia australis* and *P. multiseries* in the Bay of Seine. Was there a relation to presence of domoic acid in king scallops in autumn 2004?

In late 2004, the French phytoplankton monitoring network (REPHY of Ifremer) detected domoic acid (DA) above the EU-regulatory limit of 20 µg DA/g tissue in king scallops (*Pecten maximus*) from the Bay of Seine in the Channel (Fig. 1). Shellfish harvesting sites were closed for several months due to slow depuration of the scallops.

Accumulation of DA in French shellfish was previously correlated (as in June 1999) with blooms of the pennate diatom *Pseudo-nitzschia*, dominated most often by species of the «*pseudodelicatissima/cuspidata* complex» [1] or exceptionally by species of the «*seriata* complex». Scanning electron microscopy (SEM) analyses were performed and *P.*

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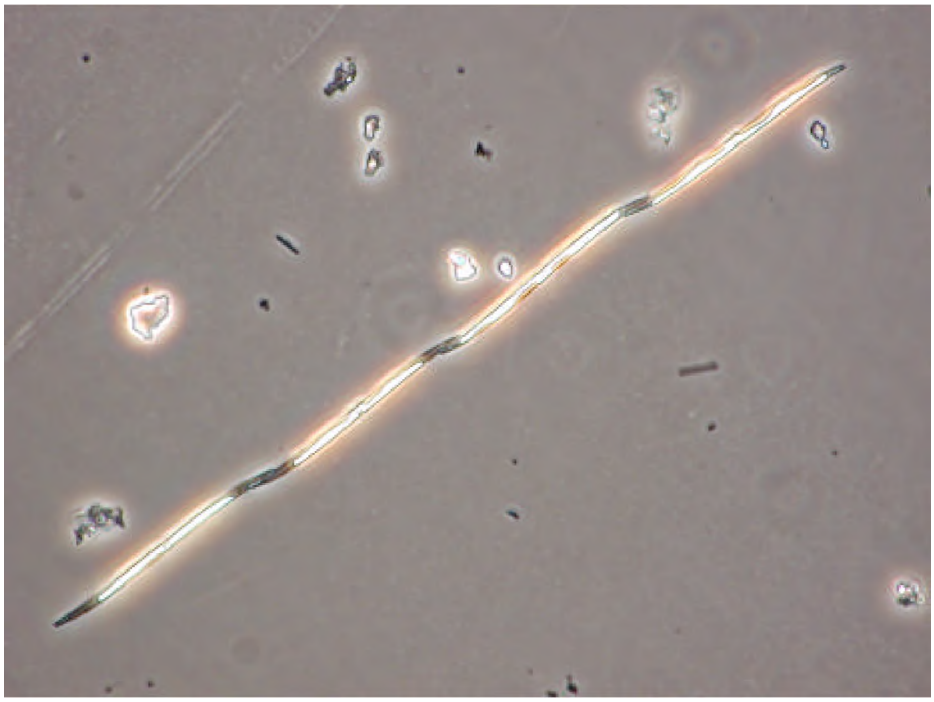

Fig. 2. Light micrograph of a chain of Pseudo-nitzschia *cf.* multistriata.

• Mexico

Bloom of *Chattonella subsalsa* in an impacted coastal lagoon in the Gulf of California

As in many countries, Mexico, with high population growth and increasing agriculture, mariculture, and tourism, has contributed to coastal eutrophication, providing conditions that favour development of harmful algae blooms (HABs). HABs are expected in areas with high nutrient input, and an increase in their occurrence in the last few years is often cited [1]. This has also been the case for Mexico [2]. An apparent increase of raphidophycean species (*Fibrocapsa japonica* and *Chattonella* spp.) along Asian and European coasts has been reported [3]. Their presence is of concern because of their toxins. Massive poisoning of economically important fishes has been reported to be caused by the small *F. japonica;* toxicity of *Chattonella* spp. has also been related to fish kills [4].

The presence of *Chattonella* species in a limited number of Mexican coastal areas [5, 6] is noted in two previous reports of blooms in the Gulf of California [7, 8], one of them associated with loss of marine resources [7]. However, the toxicity of these blooms has not been demonstrated in Mexican waters.

A bloom of *Chattonella subsalsa* was observed in Laguna Navachiste, Sinaloa on the eastern coast of the Gulf of California (Fig. 1). Laguna Navachiste is a shallow, semi-enclosed coastal lagoon characterized by harsh environmental conditions and severe

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pseudodelicatissima and *P. multiseries* were identified respectively [2]. More recently, the toxic species *P. calliantha* (which belongs to the «*pseudodelicatissima/cuspidata* complex») was shown to be also present in French coastal waters (Billard, unpublished data).

At the end of October 2004, just before the opening of the harvesting period, and when DA was detected in king scallops from the Bay of Seine, field samples were examined to look for the diatoms suspected to be responsible for this contamination. A first investigation by light microscopy (LM) of bottom seawater did not detectthe DA-producing benthic diatoms *Amphora coffaeformis* or *Nitzschia navis-varingica*. On the other hand, cells and empty frustules of at least four species of *Pseudo-nitzschia* were present in low numbers. The most abundant featured narrow cells with sigmoid ends (Fig. 2) as in *P. multistriata* or wide symmetrical valves as in *P. fraudulenta*, and the least abundant showed wide asymmetrical (type A) or narrowly fusiform valves with coarse fibulae and interstriae (type B). The usually toxic «*pseudodelicatissima/cuspidata* complex» was absent.

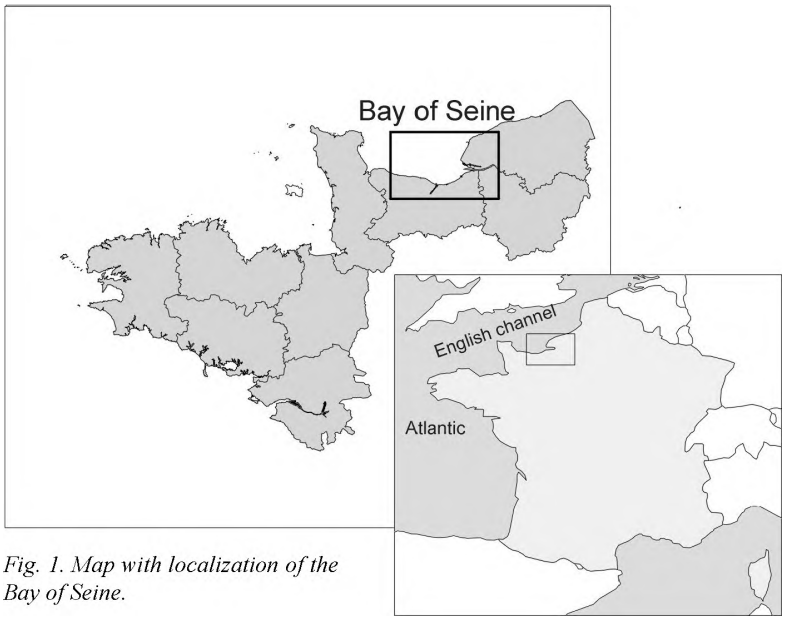

Fig. 1. Map with localization of the Bay of Seine.

During November 2004 a specific study was undertaken in several Bay of Seine sites and samples examined included: contents of scallop gastrointestinal tracts, sediment, bottom seawater and surface plankton (20 µm mesh size nets). Thorough LM analyses showed that only rare frustule fragments were present in scallops whereas rare sigmoid cells and a few empty frustules of the four species of *Pseudo-nitzschia* were recorded in the sediment samples; in bottom waters and surface nets, neither of the two morphotypes A and B was recorded. Three Bay sites sampled on November 29-30th for isolation of living cells and EM identification showed scarcity of *Pseudo-nitzschia*: two types only were recognized by LM, thin sigmoid cells and larger cells with symmetrical valves. The latter species, more abundant at the most coastal of the three sites, was isolated into unialgal culture and unambiguously identified by SEM as *P. fraudulenta* (Fig. 3).

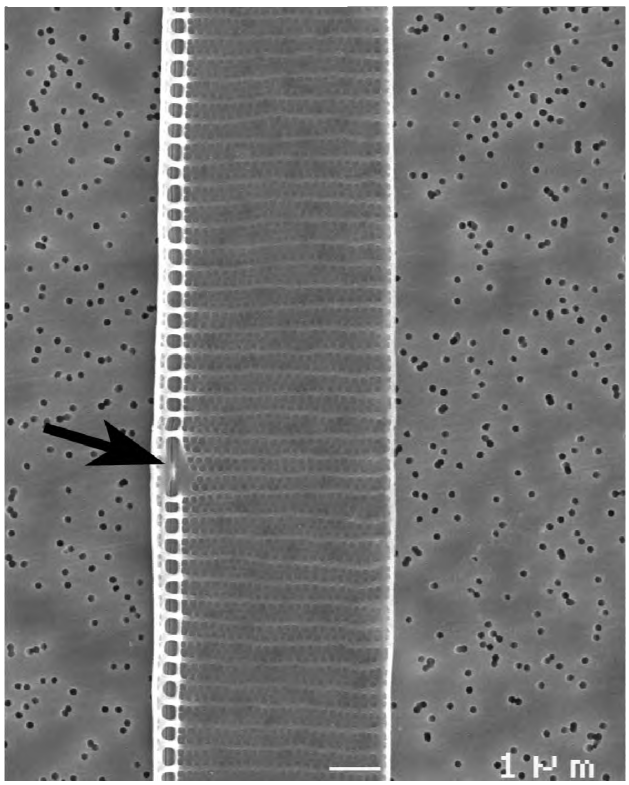

Fig. 3. *Pseudo-nitzschia fraudulenta*, SEM micrograph of valve showing central interspace, fibulae, interstriae and the typical poroid arrangement.

Since *P. fraudulenta* is generally considered non-toxic and other putative toxic *Pseudo-nitzschia* species were scarce in autumn of 2004, and in order to understand what had been responsible for the DA contamination, phytoplankton samples collected routinely in the Bay of Seine by the REPHY network during the previous summer were examined. A moderate *Pseudo-nitzschia* bloom was detected from mid-July to September, with a peak on September 14th, 2004 at 56,000 cells l^{-1}.

Light microscopy observations showed that the *Pseudo-nitzschia* summer assemblages included only three morphotypes: *P.* cf. *fraudulenta*, but also types A and B. Either type A or B was dominant during the period of mid-July to September as shown by estimation of cell densities of individual types. Type A was not only characterized by asymmetrical valves but also by 7.5-8 µm wide cells, with important overlapping ends (25-28% of cell length) as in *P. australis* or *P. seriata*; type B with narrowly fusiform (4.5-6 µm wide) and coarsely silicifed valves showed overlap of cell ends about 27-30% of length, as in *P. pungens* or *P. multiseries*.

Species identification by transmission electron microscopy (TEM) was carried out on acid-cleaned frustules following the method of Lundholm & Moestrup [3]. Examination of a subsample of the September 14th 2004 surface water collection allowed us to formally

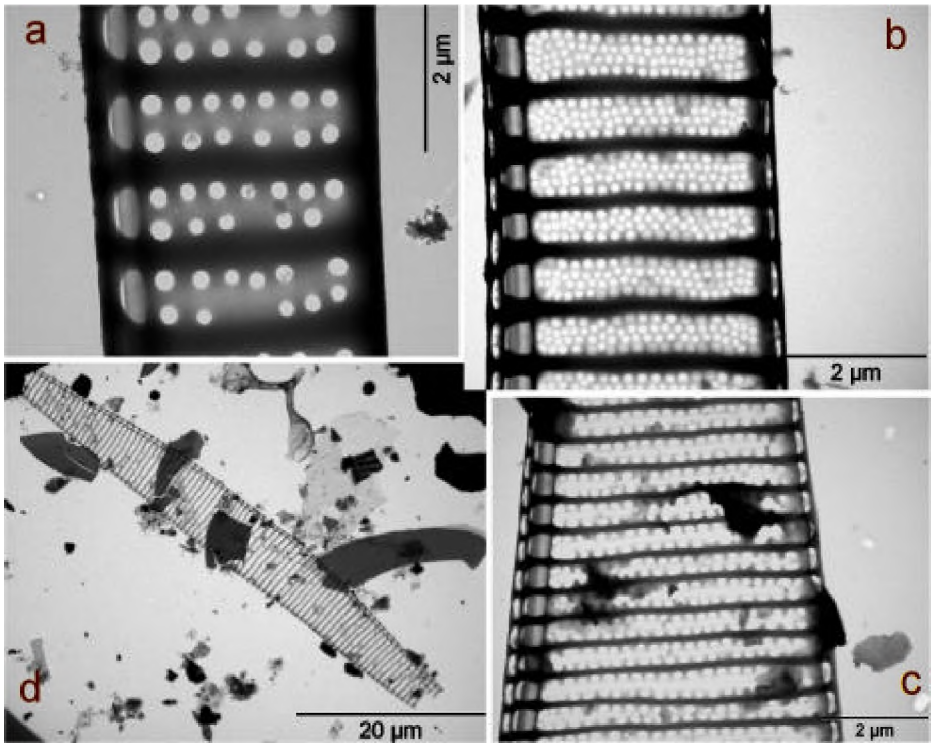

Fig. 4. TEM micrographs of valve views of Pseudo-nitzschia *spp. with arrangement of poroids. a)* P. pungens. *b)* P. multiseries. *c-d)* P. australis, *with d) showing asymmetrical outline of the valve.*

identify three species of P*seudo-nitzschia*: *P. pungens*, *P. multiseries* and *P. australis* (Fig. 4). The first two, which are roughly similar in shape, are easily distinguished by their ornamentation, P. pungens with 2 rows of poroids between thick interstriae and P. multiseries with striae showing 3-4 rows of tightly packed poroids. *P. australis*, with large asymmetrical valves, features thin interstriae (18 interstriae in 10 µm and approximately the same number of fibulae) and 2 rows of poroids per valve stria (4-5 pores in 1 µm). Following EM analyses, type A cells may therefore be assimilated to *P. australis*, while type B cells were either *P. pungens* or *P. multiseries*.

Individual chains of type A cells were isolated from a Lugol-fixed water sample collected in mid-August 2004 and processed for molecular analysis. The ITS1, 5.8S and ITS2 region and part of the 28S of the nuclear ribosomal DNA were sequenced following PCR amplifications. The resulting sequences were compared with known sequences of *Pseudo-nitzschia* species from other geographic regions available in databases (Fig. 5) and shown to be identical (100 % identity) with two sequences of Scottish strains of *P. australis* [4] and with other strains of *P. australis* from U.S.A [5, 6], New Zealand [U92260, Haywood, A.J., *et al.*, direct submission] and Denmark [AF417651, Lundholm, N. *et al.*, direct submission]. Results of the molecular analyses therefore confirm once more that type A cells were of *P. australis* and, furthermore that this species was present in mid-August in the Bay of Seine.

Pseudo-nitzschia multiseries had never been recorded before in the Bay of Seine, while this is the first report for the occurrence of *P. australis* in France. Both these species, known to be highly toxic, co-occurred during the summer bloom of 2004 in the Bay. They are suspected to be the main source of DA in the king scallops, while *P. fraudulenta* and the *P. multistriata*-like cells could be potential secondary sources of DA.

Fig. 5 . Pair-wise distance calculations using MEGA 3.1 (Neighbor joining, Kimura 2-parameters), accession numbers of the aligned sequences, and one of the two similar obtained matrices.

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
[1]								
[2]	0.000							
[3]	0.000	0.000						
[4]	0.000	0.000	0.000					
[5]	0.000	0.000	0.000	0.000				
[6]	0.000	0.000	0.000	0.000	0.000			
[7]	0.000	0.000	0.000	0.000	0.000	0.000		
[8]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

[1] P. australis Bay of Seine-28S partial, [2] AY452530-P.australis-28Spartial, [3] AY452529-P.australis-28Spartial, [4] AF440768-P.australis-28Spartial, [5] AF417651-P.australis-28Spartial, [6] U92260-P.australis-28Spartial, [7] U41393-P.australis-D1-D3, [8] U40850-P.australis-D1-D3.
[1] P. australis Bay of Seine-ITS1-2, [2] AY452528-P.australis-ITS1-2, [3] AY452527-P.australisITS1-2, [4] AY559850-P.cf.australis-18Spart-28Spart, [5] AY257842-P.australis-18Spart-28Spart

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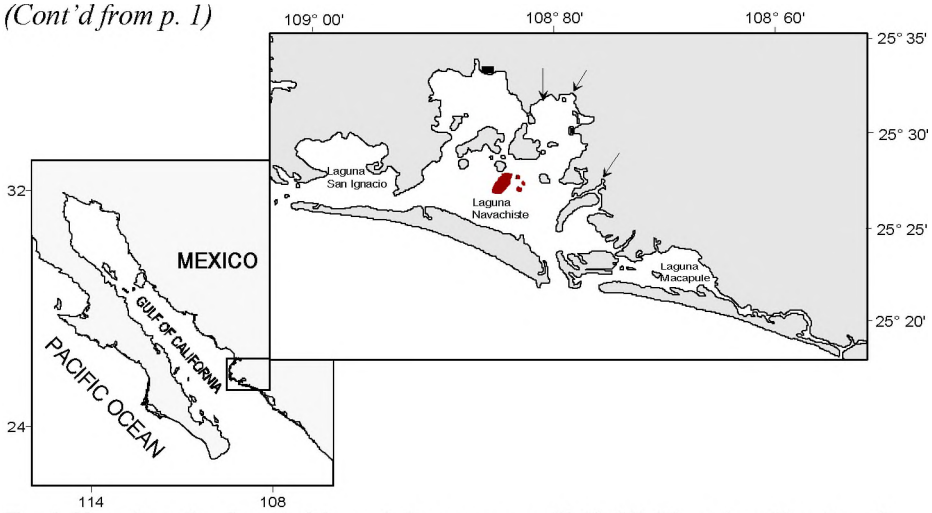

Fig. 1. Navachiste-San Ignacio-Macapule lagoon system (Gulf of California) and location of Chattonella subsalsa *patches (reddish-brown shaded area). Arrows indicate sewage drains.*

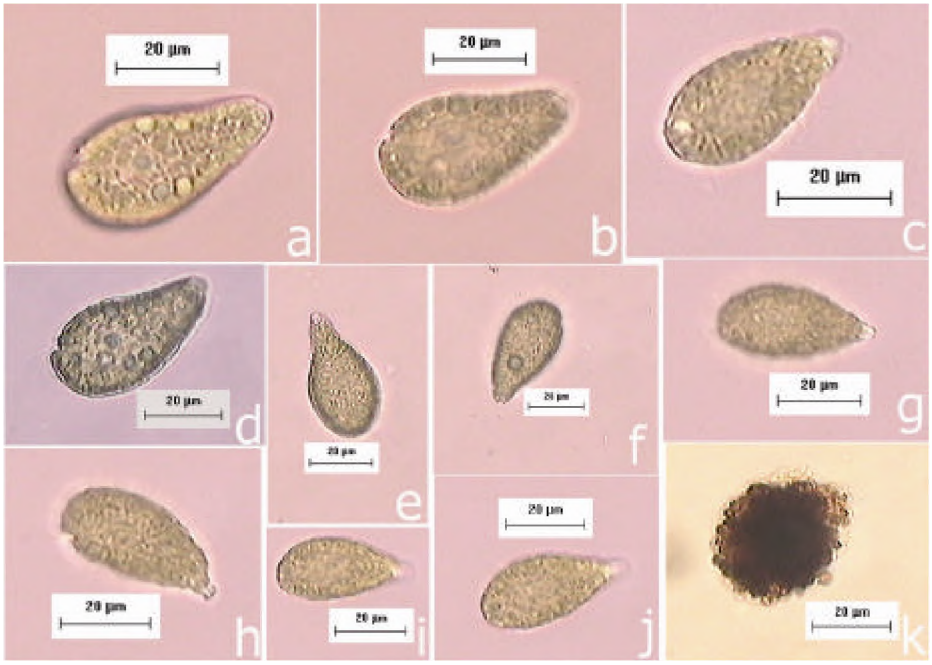

Fig. 2. a-k Chattonella subsalsa *collected at Laguna Navachiste. (a-j) live cells and (k) acid lugol fixed cells.*

anthropogenic stress [9, 10]. Water temperature varies from 15.4 to 32.7 °C and salinity ranges from 30 to 45 psu [10]. Superimposed on the extreme environmental conditions are a series of unique environmental pressures. Sewage dumped into the lagoon is considerable. Major point-source pollutant inputs include sewage outfalls from Guasave City and outfalls associated with runoff containing agricultural and aquacultural nutrients and waste. Discharges have been occurring for several decades [11, 10], and the long-term consequences of this situation are unknown.

This study includes quantification of a *C. subsalsa* bloom using 1% lugol fixed samples and the Utermöhl technique [12]. Identification of the species was achieved with unfixed samples (Fig. 2a-j). As in previous studies [6, 8], cells fixed with lugol deformed and shrank, making it difficult to identify without observing unfixed cells (Fig. 2k). The reddish-brown bloom, dominated by *C. subsalsa* (Table 1), was observed on September 3rd 2005 with concentrations between 500,000 to 960,000 cells L^{-1}. Similar concentrations were reported in April 2003 at Kun Kaan on the Sonora coast [7] and in April-May 2005 in Bahía de La Paz near La Paz, B.C.S. [8]. Physical and chemical conditions during the bloom (Table 2) suggest favorable conditions for bloom development. Size of cultured cells (n = 34) varied from 31.5 to 46.5 µm long and 17 to 24.5 µm wide. Cells are rounded in the anterior portion; whereas the posterior portion is small and obtuse; and an uncolored posterior tail is present. From a deeper pore,

Table 1. Identified taxa in the Chattonella subsalsa *bloom in Laguna Navachiste, Mexico.*

Species	cells L^{-1}
RAPHIDOPHYTES	
Chattonella subsalsa	952,059
DIATOMS	
Actinocyclus sp.	3,918
Cerataulina pelagica	7,836
Chaetoceros curvisetus	11,754
C. diversus	300
C. lorenzianus f. forceps	21,549
C. compressus	74,441
Coscinodiscus granii	3,918
Cylindrotheca closterium	15,672
Guinardia striata	15,672
Navicula sp.	3,918
Navicula sp. 2	27,426
Nitzschia longissima	121,456
Nitzschia sp.	11,754
Odontella sinensis	200
Pseudo-nitzschia sp.	11,754
Pseudo-nitzschia sp. 2	39,179
Rhizosolenia setigera	23,508
Skeletonema costatum	86,195
Thalassionema frauenfeldii	58,769
T. nitzchioides	39,179
Thalassiosira sp.	15,672
DINOFLAGELLATES	
Gonyaulax verior	3,918
Gymnodinium sp.	3,918
Gyrodinium spirale	3,918
Prorocentrum micans	7,836
P. triestinum	11,754
Protoperidinium sp.	7,836
CYANOPHYTES	
Cianophyceae (tricomes)	19,590
NANOFLAGELLATES	223,322
Total	1,828,218

Table 2. Average physical and chemical variables during Chattonella subsalsa *bloom in Laguna Navachiste, Mexico.*

Temp (° C)	pH	Salinity (psu)	OD (mg/L)	Secchi (m)	Cl *a* (mg/ m^3)	NO_2	NO_3	NH_3	PO_4
						(µM)			
33.1	7.9	38.5	6.8	0.5	8.7	0.28	0.64	4.4	0.46

two similar flagella emerge in opposite directions.

Blooms of *Chattonella* occurring during 2005 on both coasts of the Gulf of California indicate either that raphidophycean species have been overlooked for quite some time or that environmental conditions during recent years have become favorable for these algae. In future studies, cloned cultures will be used to determine toxicity and confirm identification of the species.

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• Italy

Blooms of *Mesodinium rubrum* in a coastal area of the Tyrrhenian Sea



Fig. 1 Gulf of Naples, Mediterranean Sea
** sampling station off Portici,*
▲ the long term study site MC.

Blooms of the photosynthetic ciliate *Mesodinium rubrum* have been among the first sea-water discolorations ever documented. In 1827 the phenomenon was reported from Valdivia by the German naturalist Pöpping [1], and seven years later by Darwin from the Bay of Concepción and Valparaíso [2]. These events preceded by almost a century the description of the species [3], yet the information provided by Darwin was detailed enough for Hart [4] to attribute them to *M. rubrum* blooms [5]. Since then, red-water blooms caused by *M. rubrum* have been reported from coastal waters of almost all oceans. Outbreaks exceeding 1000 cells/mL are a common feature in summer along the Atlantic coasts of Portugal, Spain and Africa [6-8] and in the UK [9, 10]. We report here information on blooms of *M. rubrum* in the coastal waters of the Southern Tyrrhenian Sea (Mediterranean Sea).

The most intense blooms were detected in the course of a national environmental monitoring program, along the coast of the Campania region, during which phytoplankton communities were investigated at several stations since June 2001. One monitored station is located 100 m off Portici (6 m depth), offshore a densely inhabited urban area (Fig. 1) which is characterised by nutrient enrichment due to urban sewage discharge and by continuous phytoplankton blooms (up to 16.6×10^4 cells/mL, 84% of diatoms) especially during periods of water column stratification (mainly May to September). On May 18, 2006, the surface waters off Portici showed a dark green-brownish colour and a considerable turbidity (Secchi disk depth 1 m). Utermöhl counts of a surface formaldehyde-fixed sample revealed a dense population of the mixotrophic ciliate *Mesodinium rubrum* (Fig. 2) (2.3×10^3 cells/mL). The accompanying phytoplankton population (4.2×10^4 cells/mL) was dominated by diatoms (84%), with *Skeletonema pseudocostatum* (39%) and small single-celled *Chaetoceros* spp. (36%) as the main taxa. *Mesodinium* concentrations on April 27 were 1.3×10^2 cells/mL while they dropped below detection in the following sampling on May 31.

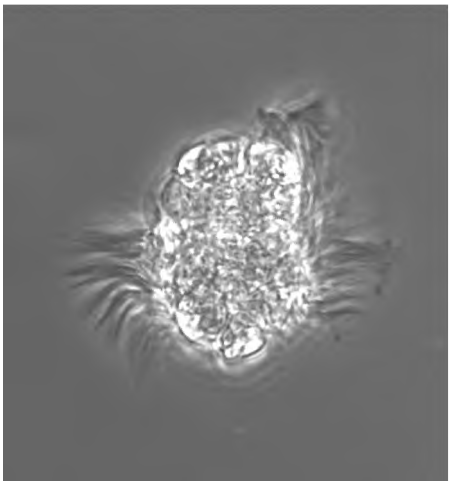

Fig. 2. Light micrograph of a formaldehyde-fixed cell of Mesodinium rubrum.

Despite the lower cell number, *Mesodinium rubrum* biomass was more than threefold that of the total phytoplankton (3.5 µgC /mL and 1.05 µgC/mL, respectively) due to the small size of the dominant taxa. The actual biomass value could have been underestimated, since *M. rubrum* is easily disrupted upon fixation with formaldehyde, which can cause about 60% loss as compared to Lugol's [11]. Correcting the cell counts for this theoretical loss the estimate would be 3.6×10^3 ind./mL, corresponding to 5.6 µgC/mL. Despite the considerable disproportion in biomass, the abundance of the rest of the population was probably high enough to mask the

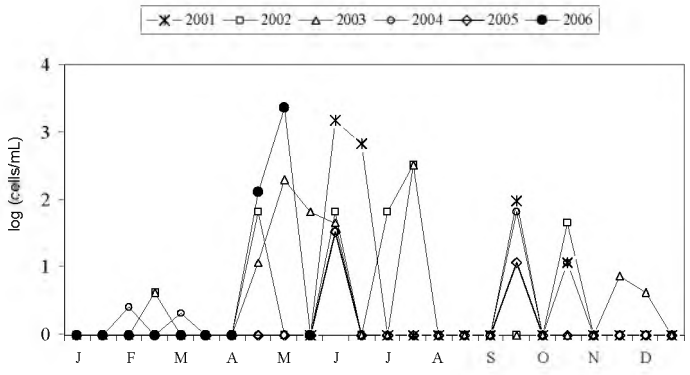

Fig. 3: Seasonal cycle of Mesodinium rubrum *at the monitoring station off Portici (2001-2006).*

red colour that is commonly reported for *M. rubrum* blooms [12].

As for the environmental conditions during the bloom, surface water temperature (21.16°C) was higher and salinity (36.40 psu) lower than the mean values of the month for the station (20 °C and 37.14 psu, respectively). Nutrients were extremely high (total nitrogen 46.5 µmol/L; total phosphorous 1.6 µmol/L and silicate 12.6 µmol/L) as often occurs in the area (mean values 28.6 µmol/L, 1.2 µmol/L, 5.9 µmol/L, respectively). Rapid heating of the surface water layer was recorded during the month of May, from 18.62°C on April 27 to 22.88°C on May 31 after which the surface temperature dropped to 20.44°C on June 13. The high temperature and low salinity values, coupled with high nutrient concentrations, are indicative of a very high retention rate which could have favoured the accumulation of the bloom. *Mesodinium rubrum* blooms are in fact not a rare event in the area. Comparably high concentrations (1.5 x 10^3 cells/mL) were also registered in June 2001 (Fig. 3) and the highest annual abundances of the species are usually recorded from May to August, which is also the period of maximum concentrations of the rest of the phytoplankton. It is conceivable that during these periods particular hydrographic/meteorological situations may lead to favourable conditions for more conspicuous outbreaks from time to time.

The blooms of *M. rubrum* seem to be confined to the very inner part of the Gulf of Naples, since much lower concentrations (below 100 cells/mL) were observed during a long term study at the site MC, 2 nm off Naples [13], which also correspond to the values reported for other sites of the Mediterranean Sea [14]. The only bloom of *M. rubrum* (3.9 µgC /mL) reported for this basin was observed in a highly eutrophic lagoon of the Po Delta area (Adriatic Sea) [15]. The peak abundance and biomass values of *M. rubrum* at the Portici station are comparable to those recorded during outbreaks at other sites; however even higher abundances [8, 16] have been recorded off Portugal and Mexico and up to 11 µgC/mL in the Kuril Islands (N. Pacific, Russia) [17]. Blooms of *M. rubrum* have frequently been reported associated with high numbers of dinoflagellates [6] or, as also observed in the Gulf of Naples, with diatoms [18]). Low salinity and high temperatures at surface in high nutrient areas are generally associated with the occurrence of *M. rubrum* blooms, which typically last only a few days [19].

Our observations in the Gulf of Naples show that *M. rubrum* can reach high concentrations in eutrophic Mediterranean coastal waters especially during the warmer season, when the recreational use of the sea is enhanced. Only a few other reports of discolorations caused by other species exist for the Tyrrhenian coasts [20-23], probably because these events may not be easily ascribed to phytoplankton blooms. However their impact on the aesthetic quality of the sea could seriously impinge on tourism and recreational activities that are among the major resources in many coastal Mediterranean areas.

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• Mexico

Fish mortality associated with *Chattonella marina* and *C.* cf. *ovata* (Raphidophyceae) blooms in Sinaloa (Mexico)



Fig.1. Foam associated to blooms of Chattonella *spp. Daily News Debate (May 9, 2006).*



Fig.2. Fish mortality location and study area.

From April to May, 2006, three red tide events occurred in Sinaloa, México. The red tide forming species were *Chattonella marina* and *C.* cf. *ovata*, the toxic dinoflagellate *Gymnodinium catenatum* was present during this period in low densities in Bahía de Mazatlán. The total number of red tide days was 33 days. The first bloom occurred on March 16-22, the species of highest abundance was *C. marina.* The second bloom was on April 10-14 and the third bloom occurred April 26 to May 12; both blooms were formed by *C. marina* and *C.* cf. *ovata*. The last bloom (from May 1-12, 2006) is detailed in this report. On the Sinaloa coast, strong discolorations in the sea were witnessed in the study period. The water was a yellow-brown colour; with abundant foam forming stripes parallel to the coast (Fig.1). On May 3-4, fish mortality occurred on some beaches of Sinaloa, the firsh off San Lorenzo River (Cosalá), the second in El Walamo (Mazatlán) and Las Cabras (Escuinapa) (Fig. 2). These mortalities could be associated with red tide events caused by *Chattonella* spp. This is an ichthyotoxic species, a producer of reactive oxygen species (ROS), and of haemolytic and neurotoxics substances. These microalgae affect fish gills and cause death. Initial inquiries suggested the fish mortality was associated with chemicals used cleaning a sugar refinery in the first case, and oxygen depletion (caused by discharged shrimp fishery waste) in the second case. Nevertheless, these fish mortalities occurred during this red tide period and could to contribute synergistically to fish mortality. There are no studies to prove this.but the great volume of dead fish in Las Cabras could not have been discharged from the two shrimp vessels. Besides, the seasonal prohibition for shrimp capture begins March 24. The dead organisms included benthic fish, mollusks and crustaceans, but most of the fish were a pelagic species ("palometas", *Trachinotus paitensis*), used for human and livestock consumption (Figs. 3. A, B).



Fig. 3. A, B. Fish mortality on Las Cabras beach. Daily News Noroeste (May 4, 2006).

Table 1. Count of Chattonella *spp. (*in vivo*) in Bahía de Mazatlán.*

Day-May	Cells ml^{-1}
3	750
5	95
7	228
10	74
12	35
15	0

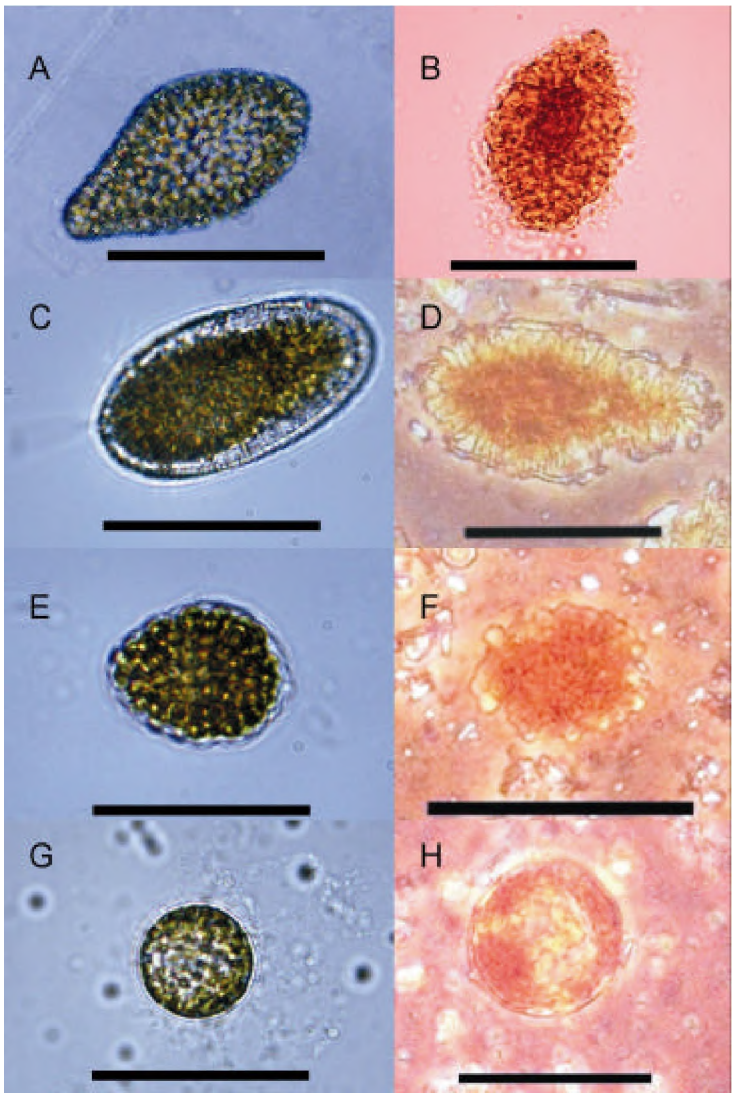

Fig. 4. Rhaphidophyceans in vivo*(blue) and fixed (rouse): (A-B)* Chattonella marina*, (C-D)* C. *cf.* ovata*, (E-F)* Chattonella marina *round form and (G-H) Temporary cyst form (scale = 50 µm).*

The yellow-brown discoloration of the sea water produced abundant brown foam along the Sinaloa coast. Sampling for this study were carried out in Bahía de Mazatlán. The physical-chemical conditions in the discoloration zone were: surface temperature 21.2-22.7ºC, salinity 34.5-34.7 psu, and dissolved oxygen 5.3 mg l^{-1}. Surface samples were collected daily in Bahía de Mazatlán. The identification, photography and quantification were carried out *in vivo* in light microscope with 40x objective. Cell abundance declined between May 1 and May 15 (Table 1). On May 5 surface samples were fixed, settled for 12 h and quantified with an inverted microscope [1]. The method indicated a phytoplankton abundance of 713,000 cells·l^{-1}. *Chattonella marina* and *C. ovata* were distinguished by their size and number (Fig. 4). The abundance of both species was 332 000 cells·l^{-1} and they constituted 46.5% of the phytoplankton composition (Table 2). This may be an underestimate since fixation destroys many cells. These cells were 30-70 µm long and 20-30 µm wide (Fig. 4), deformed, and some round like temporary cysts (Fig. 4. G, F), similar to those observed in Kun Kaak Bay [2]. Recently, some authors have reported the presence of Raphidophyceae in Mexican Pacific, blooms without fish mortality effects [4, 5]. This study represents the second case of a massive fish mortality associated with *Chattonella* spp. [2]. We not were able to identify species affected, but a conservative estimate is 12,000 to15,000 fish. or about 48-60 tons of fish, distributed on 3 km of beach from Las Cabras to El Palmito del Verde (taking in consideration 4-5 fishes, 3-5 kg per 1 m^2).

Acknowledgements

To E. Frausto S. and A. Núñez-Pasten for their technical support.

Table 2. Phytoplankton composition and abundance of Chattonella *spp. bloom in Bahía de Mazatlán (May 6, 2006).*

Species	Cells ml^{-1}	Abundance %	Toxin associated
Chattonella marina and *C.* cf. *ovata*	332	46.56	Ichtyotoxicity ROS, hemolysis
Peridiniaceae: *Peridinium* spp. and *Protoperidinium* spp.	172	24.12	
Gymnodinium catenatum	148	20.76	PSP
Pseudo-nitzschia sp.	15	2.10	ASP
Ceratium falcatum	10	1.40	
Prorocentrum gracile	9	1.26	
Gyrodinium sp.	5	0.70	
Thalassiosira sp.	4	0.56	
Scrippsiella trochoidea	4	0.56	
Gymnodinium sp.	4	0.56	
Ceratium balechii	3	0.42	
Dyctiocha fibula	3	0.42	
Ceratium furca var. *hircus*	3	0.42	
Prorocentrum micans	1	0.14	
Total	713	100.00	

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GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

GEOHAB Core Research Project – HABs in Upwelling Systems: Call for Participants

The GEOHAB report on *HABs in Upwelling Systems* (GEOHAB, 2005 http://ioc.unesco.org/hab/Upwelling%20IP-2006%20Final.pdf) specified that GEOHAB would form a subcommittee for the Upwelling Core Research Project. The Subcommittee began its work with a meeting in Villefranche, France on 18-21 January 2006. All four major eastern boundary current upwelling systems were represented: the Benguela Current (Grant Pitcher and Trevor Probyn), Iberian Current (Teresa Moita and Francisco Figueiras), California Current (Raphael Kudela and Vera Trainer), and Humboldt Current (Sonia Sánchez).

The Subcommittee identified six key questions related to HABs in upwelling systems for which some research is already ongoing and for which a core set of scientists for GEOHAB-related research could be identified. Full descriptions of the projects can be found at http://ioc.unesco.org/hab/Upwelling%20IP-2006%20Final.pdf, including the motivation for the projects, likely participating regions, a project coordinator and participants, project objectives, project approach and work plan, example projects, and expected project output. The intention is that this list will expand as more scientists worldwide link their research to these projects. We invite interested participants to contact the Project Coordinator for more details, or to indicate interest in these projects.

An immediate goal of the HABs in Upwelling Systems subcommittee is to summarize research to date in each of the project areas with the help of colleagues familiar with those areas of research. We envision a research publication detailing new work in each area of focus (review papers) specifically highlighting areas of need that will help to strengthen interest in collaborative studies. Please contact project coordinators to express your interest in contributing your expertise for research papers. We would also like to list a complete set of publications, including "gray" literature, in each research area for each upwelling region. This Core Research group will hold an evening discussion session on Wednesday, September 6, 2006 at the 12th International Conference on Harmful Algae in Copenhagen, Denmark to which all interested researchers are invited.

1. Project: Effects of nutrients on HAB population dynamics in upwelling systems

Project Coordinator: Trevor Probyn, Marine & Coastal Management, Cape Town, South Africa
Contact: Tprobyn@deat.gov.za
Project objectives

- Quantify the importance of upwelled nutrients to HABs as the f-ratio (N-based).
- Provide physiological understanding of HAB species' nutrition (N, P, Si) and biological supply mechanisms (regeneration).
- Determine the role of trace metals in HAB dynamics.
- Identify how nutrient availability and speciation may affect cellular toxicity (N, P, Si, trace metals).
- Provide input to HAB biogeochemical models.

Motivation

A defining attribute of upwelling systems is the periodic supply of nutrients from deeper waters that provides appreciable scope for phytoplankton growth and concomitant effects on higher trophic levels. Given the compelling evidence for a global increase in HABs, and their established negative impacts in coastal upwelling environments, it is important to understand how this potential production is channeled into HABs as opposed to, or as a successional stage of, the more benign/beneficial blooms that generally characterize these regions. In this regard, there appears to be a dearth of information in coastal upwelling ecosystems relating specifically to the nutritional aspects of HABs compared to the more typical non-HAB, diatom-dominated assemblages.

2. Project: Climate and HABs in upwelling systems

Project Coordinator: V. Trainer (NOAA Fisheries, USA)
Contact: Vera.L.Trainer@noaa.gov
Project Objectives

To investigate potential correlations between "long-term" HAB monitoring data (shellfish toxicity, toxigenic species, continuous plankton recordings) and local environmental data (e.g., nutrients, wind, temperature) as well as regional and global climate indicators (ENSO, PDO, NAO).

Motivation

Changes in upwelling intensity provide one of the substantiated lines of evidence for warming climatic trends, although the specific regional responses are still being established.

Several research groups have documented a correlation between HAB events (frequency and magnitude) and changing climate indicators. For example, HAB events are believed to be occurring more frequently during El Niño/Southern Oscillation (ENSO) events in the Humboldt Current system when low abundance of diatoms coincides with increasing numbers of new species (primarily dinoflagellates, including some toxigenic species) resulting in serious problems to the anchovy fishery, while ENSO events have also been correlated with toxic HAB events in Mexico. There is a need to compare interannual variability of HABs among upwelling systems as well as at different latitudes within a system. The study of regions in different hemispheres will allow comparisons to be made on the effects of the seasonal timing of arrival of climatic signals on HABs.

Influences of climate require well-documented time series of a sufficient duration to characterize the underlying mechanisms. Research needed to establish linkages between HABs and climate change includes data-mining efforts for short-term time series (examination of time series and satellite data), as well as projects involved in micro-paleontology (dating and characterizing sediment cores) for longer time series, and finally development of hindcast and forecasting models to help apply the knowledge gained to our understanding of HAB dynamics.

However, comprehensive, complete, long-term HAB datasets and corresponding environmental data are rarely available. Many HAB data are collected by managers only when necessary for the protection of public health, resulting in incomplete time series or inconsistent datasets. Effective time-series analysis requires very large data sets; an appropriate data policy conducive to data sharing (i.e., individual investigators will be willing to share data knowing that it will only be used for meta-analysis) will need to be established. Furthermore, care will need to be taken to differentiate climatic effects from other long-term changes such as urbanization and land-use practices, fresh-water diversion, etc.

3. Project: Genetic comparisons of HABs in upwelling systems

Project Coordinator: R. Kudela, University of California Santa Cruz (USA)
Contact: kudela@cats.ucsc.edu
Project Objectives

- Molecular characterization (e.g. sequence analysis for application of RNA/DNA molecular probes) for harmful algal species in each region
- Based on (1), determine whether HAB producing organisms are genetically distinct for the comparable regions
- Utilize differences in cell toxin quota of a given species in separate upwelling regions to allow characterization of genes and biochemical pathways important for toxin synthesis

Motivation

This project will address the role of genetic predisposition versus environmental conditions in toxin production in different upwelling systems within a given genus or species. It is well known that toxigenic HAB species exhibit variability in toxin production, both at the species and genus level, within a given upwelling system. Multiple environmental factors have been shown to influence HAB toxicity and ecology and it is possible that the entire range of observed spatial and cell-specific variability in toxin production is a response to subtle environmental cues. However, variability in genotypes is a common feature of phytoplankton. These genotypic differences are responsible for markedly different phenotypic expression in response to environmental cues within the same putative species. Therefore, the variability in toxin production is likely caused by a combination of genotype and environmental conditions. A genetic comparison of HAB organisms among regions is useful for differentiating between genetics and environment. Although there are several genetic comparisons that could be identified, here we focus on four potential targets that are found in all regions: *Pseudo-nitzschia* spp., *Protoceratium reticulatum, Lingulodinium polyedrum*, and *Alexandrium catenella*.

4. Project: Coastal morphology and its influence on HABs in upwelling systems

Project Coordinator: G. Pitcher (Marine & Coastal Management, Cape Town, S. Africa)
Contact: gpitcher@deat.gov.za
Project objectives

To establish the importance of coastline morphology and bathymetry in:

- creating local upper mixed layer conditions favourable for the selection and development of HABs and;
- determining bay circulation patterns favouring bloom development through retention.

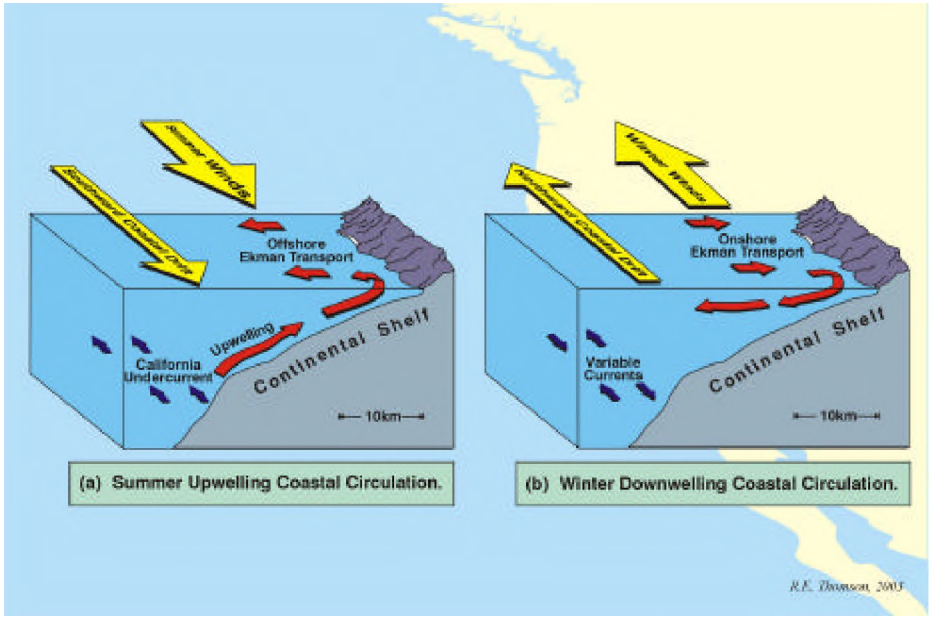
Motivation

Alongshore variability of coastal upwelling is mainly controlled by the interaction of the wind-forced shelf flow with coastline and bottom topography, resulting in the amplification and or reduction of upwelling-downwelling processes (Figueiras et al. in press). Consequent spatial variability in upper mixed layer characteristics and shelf flow related to coastline discontinuities, such as pronounced capes, coastal embayments or Rias (in northwestern Iberia), often favours HAB development by enhancing vertical stratification and by forming regions of retention. Comparative research within these regions will serve to assist in identifying the underlying physical processes responsible for the higher incidence of HABs within these regions.

5. Project: Seeding strategies within upwelling systems

Project Coordinator: A. Amorim (U. Lisbon, Portugal)
Contact: ajamorim@fc.ul.pt
Project Objectives

- To identify seed populations of particular HAB species in relation to oceanographic features important to their development.
- To describe sedimentary processes determining the location and accumulation of seedbeds (e.g. dinoflagellate cyst beds).
- To describe the seeding and overwintering strategies of particular HAB species.



- To determine the role of environmental parameters in species-specific seeding strategies (e.g., in establishing cyst dormancy, germination, growth, and the periodicity of blooms).
- To determine whether strategies of seeding between upwelling regions are similar.

Motivation

The identification of seed or over-wintering populations and the establishment of seeding strategies are important in understanding the development of HABs in upwelling systems, which are recurrent but not always predictable. To better understand and model HABs, species-specific life-history transitions, which may determine the initiation or termination of a bloom, must be considered as an important source of variance. In upwelling systems, advective processes are important in determining species-specific over-wintering and seeding strategies. For example, the encystment and excystment of dinoflagellates is often used to explain seasonal patterns, however very few studies have been conducted in the field. In areas where these studies have been preformed results indicate that closely related species differ markedly in their adaptive strategies despite their similarity in life-history transitions and habitat (e.g. Montresor *et al.* 1998). Dale and Amorim (2000) suggested three different seedbed strategies for HAB dinoflagellates: (1) those species without resting cysts, (2) those species heavily dependent on seedbeds, and (3) those species that produce large numbers of cysts but are seemingly independent of seedbeds. Thes strategies should be investigated for different dinoflagellate species within and between upwelling systems. Diatom populations such as *Pseudo-nitzschia* are often associated with thin layers both in the water column and near-bottom (e.g. Rines *et al.* 2002). Cells under these low light conditions are found to be viable, indicating that *Pseudo-nitzschia* may be present as "shade flora" in stratified water masses, which may function as seed-beds under favorable conditions. Other physical settings, including mesoscale eddies and frontal systems, have also been found to favour the accumulation of *Pseudo-nitzschia* populations. The existence of a *Pseudo-nitzschia* resting stage has been suggested, but no clear seeding strategy has been established.

Understanding and comparing species-specific seeding strategies in different upwelling systems will be useful in the development of models aimed at forecasting blooms and their impacts in coastal regions. A collaborative approach using standardized methods will benefit attempts to establish the sites of seedbeds and to determine the seeding strategies for HAB species in upwelling systems.

6. Project: The role of across-shelf and along-shore currents in the transport of HABs in upwelling systems

Project coordinator: F.G. Figueiras (IIM-CSIC, Spain)
Contact: paco@iim.csic.es

Project objectives

- To determine the relative importance of alongshore and across-shelf transport within the 4 regional upwelling systems in the initiation, accumulation, and transport of harmful algae to coastal environments.

Motivation

Upwelling systems are essentially heterogeneous with mesoscale structures such as eddies, fronts, filaments and river plumes interacting with alongshore and across-shelf currents. These currents are among the most important physical features of upwelling systems and frequently interact to influence HABs in upwelling regions (e.g. Fraga et al. 1988, Pitcher et al. 1998, Trainer et al. 2002). Along-shore currents are able to transport blooms from their sites of initiation while on-shore transport, associated with downwelling, often favours bloom accumulation in coastal waters where they impact fisheries and tourism. Offshore transport resulting from upwelling promotes their dispersion from coastal systems. Understanding the relative contribution of alongshore and across-shelf flow to the transport of harmful algae to and from the coast will be important in the development of accurate physical-biological models. Comparison among systems will aid to reinforce observations and refine models.

Contributed by GEOHAB Core Research Progam: HABs in Upwelling Systems Steering Committee members: Grant Pitcher, Chair; Francisco Figueiras, Raphael Kudela, Trevor Probyn, Teresa Moita, Sonia Sánchez, Vera Trainer, Ed Urban, and Henrik Enevoldsen

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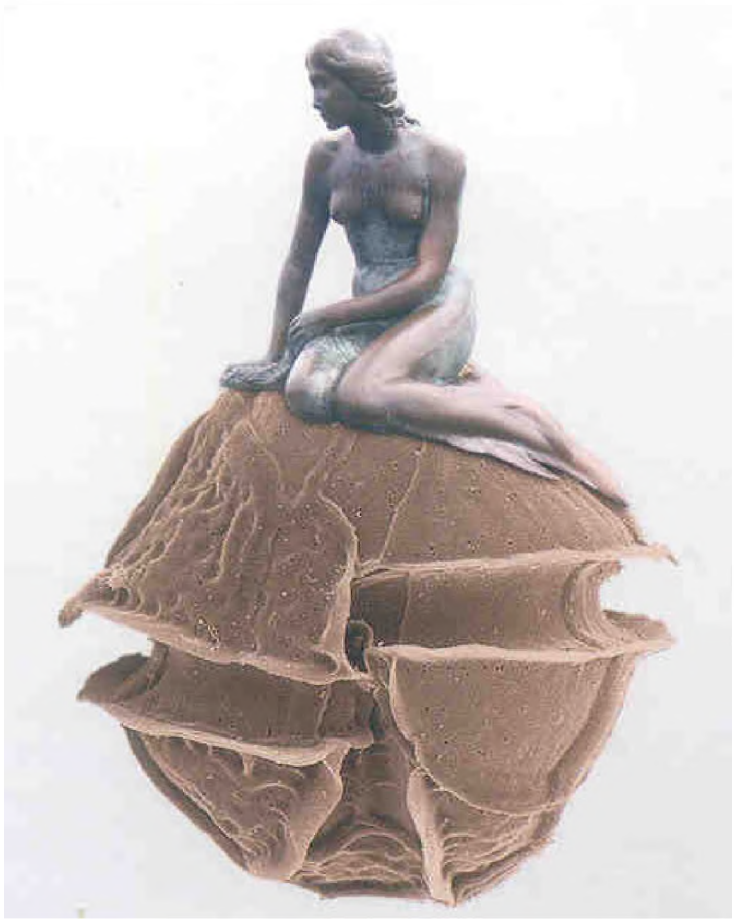

SEE YOU SOON AT THE COPENHAGEN 12TH INTERNATIONAL CONFERENCE

Future events

HABTech '07 Workshop

March 15-17, 2007. Nelson, New Zealand.

HABTech '07 is a workshop dedicated to new technologies for the detection of harmful algal blooms and toxins. The third HABTech workshop is being hosted by Cawthron Institute in Nelson, New Zealand, and is being held immediately prior to the International Conference on Molluscan Shellfish Safety to enable delegates to attend both events. For more information please visit: www.cawthron.org.nz/habtech07/habtech-07-workshop.html

ICMSS 07

March 18th- 23rd, 2007. Blenheim, New Zealand.

New Zealand is hosting the 6th International Conference Molluscan Shellfish Safety in Blenheim. The conference is held every two years and brings together scientific, industry and regulatory people from all over the world. The conference agenda will allow the sharing of scientific research, management systems, debate on international trade issues and networking between stakeholders.

More details can be found on www.nzfsa.govt.nz/icmss07.

HARMFUL ALGAE NEWS

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An IOC Newsletter on toxic algae and algal blooms

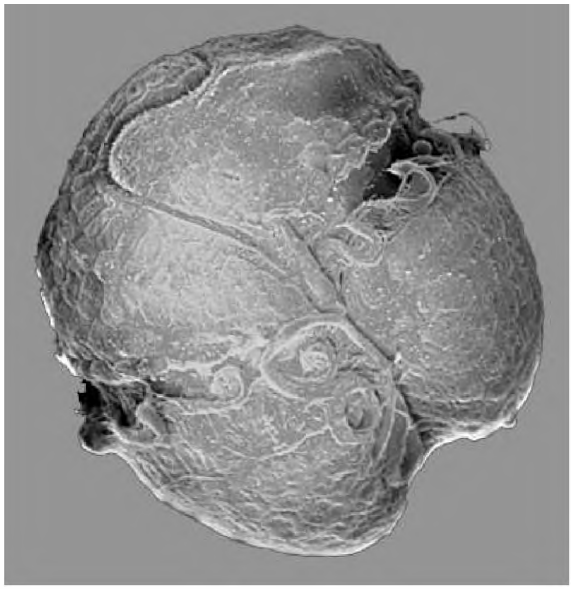
http://ioc.unesco.org/hab/news.htm *No. 32*

HAB XII - RESPONSES TO THE CONFERENCE

Taxonomy at the XIIth ICHA, Copenhagen 2006

The era of big taxonomic novelties for harmful species has apparently come to an end. Small but significant revolutions animated the taxonomic sessions at past HAB Conferences, ranging from the decision to use the genus name *Alexandrium* in place of other synonyms (Enrique Balech, Lund, 1989) to the finding of a harmful member in the so far inoffensive family Dictyochophyceae (Bente Edvardsen, Cape Town, 2004), and to the first cues of pseudo-cryptic species in *Pseudo-nitzschia* (Nina Lundholm, St. Pete's Beach, 2002). There were no big surprises this time but, as ever, a number of new species were presented, including a cyanobacterium (**Tuong Giang Nguyen Ngoc**), a raphidophyte (**Elif Demir**), several possible *Gambierdiscus* species (**Pat Tester**) and even a so far mysterious dinoflagellate (**Jennifer Wolny**), to remind us once more that our knowledge of microalgal diversity is still in progress.

It has become more and more evident over the years that species cannot always be delimited and identified with visual techniques, but also that other evidence is needed to support molecular information. The differences between *Karlodinium*



Scanning electron microscopy is not enough to tell which species of Takayama *is this.*

(Cont'd on p.2)

Dinophysis news



Light micrograph of a well-fed live Dinophysis acuminata *cell using the ciliate* Myrionecta rubra *as prey (Photo courtesy of Myung Gil Park)*

Any biologist accustomed to observe live populations of *Dinophysis* spp. has been entranced by the variability of their morphology (size and shape of their large hypothecal plates) and their cellular contents. Through the growing season of *Dinophysis acuminata*, the most ubiquitous and persistent species of the genus in temperate coastal waters, cells can appear thin and quite empty, or swollen with digestive vacuoles. The size composition of the population can range from homogeneous to a bimodal distribution of large and small cells or even to a complex array of small, intermediate and large cells. A large part of the size and shape complexity within a given locality can be explained by complex polymorphic life cycles [1], where large cells can go through a depauperating division *sensu* von Stosch [2], and produce two dimorphic offspring (with dissimilar halves) that further lead to the formation of small gamete-like cells.

(Cont'd on p. 3)

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Institute of Biology, University of Copenhagen.

(Cont'd from p. 1)

armiger and *K. veneficum*, which bloom together along the Spanish Mediterranean coasts, required an array of methods for accurate classification **(Esther Garcés)**. Even trickier is the case of the *Alexandrium tamarense / catenella / fundyense* species complex **(Santiago Fraga)**, where a mismatch exists between morphological and molecular information. Within this trend, several papers focused on new molecular approaches to detect cryptic, pseudo-cryptic or simply difficult to distinguish species **(Sonja Djerks, Mirelle Chinain, Laurence Myriam Elandoussi, Wayne Litaker, Sarah McDonald, Katherine Hubbard, Antonella Penna** and many others).

The opposite also seems to exist: shape may evolve faster than some molecular markers. Two autotrophic dinoflagellates blooming under the ice in northern areas, the freshwater *Peridinium aciculiferum* and the brackish *Scrippsiella hangoei*, differ in morphology, habitat, and physiology but share identical nuclear ribosomal DNA (SSU, ITS1- 2, 5.8S and LSU). Mitochondrial cytochrome b (COB) sequences indicate a recent evolutionary divergence **(Ramiro Logares)**. Both species are evolutionarily related to heterotrophic *Pfiesteria*-like species. Interestingly, *P. aciculiferum* produces allelopathic substances, and there is some evidence that *S. hangoei* produce toxins. It remains to be determined how the chloroplasts originated in the autotrophic species.

Despite the absence of big surprises, never before in this conference series did the discussion reach such peaks of pervasive disagreement and constructive criticism as in the workshop on taxonomy. The topic that raised the tone of the discussion is certainly not new: are there few or many microalgal species? Is phytoplankton genetic diversity meaningful or just 'philosophical dirt' [1]? Who is right, the splitters – those who go on separating known taxa into more species – or the lumpers – those who unify apparently distinct taxa under a same name? Is there any sense in establishing species that cannot be told apart in the light microscope - and at times not even in the electron microscope?

The first contribution to the debate came from **Tom Fenchel**, who provided an overview of his ideas, based on his scientific experience with ciliates but extrapolated to all aquatic microbes [2, and references therein]. Indeed for these organisms a limited number of species are found which are apparently ubiquitous in ecologically comparable systems. According to Fenchel, genetic diversity often does not correspond to any morphological or physiological peculiarity, being in this case the result of an accumulation of neutral mutations, whereby molecular differences between species only indicate how much time has passed since they diverged. In these views, the questions raised by evidence of cryptic and pseudo-cryptic diversity in aquatic microbes could be dismissed as much-ado-about-nothing.

An alternative perspective was presented by **Miguel De Salas**, who introduced the group of naked dinoflagellates belonging to the genera *Karenia*, *Karlodinium* and *Takayama*, once all known under the name *Gymnodinium*. These genera have not stopped 'expanding' since their establishment, but unfortunately differences between taxa are in most cases subtle and only revealed under certain observational conditions. The actual distribution of all these newly established species will hardly be revealed unless a whole array of techniques is applied, ranging from cultivation to molecular biology and electron microscopy. But does species identification really matter in such problematic cases? The opinions of the participants in the workshop were as different as the interdisciplinary nature of our conference allows. Most 'taxonomy-producers' emphasised the advantages of a better taxonomic resolution in terms of the definition of actual ecological and biogeographic patterns of diversity. Yet when a species is split into several almost indistinguishable species, life becomes very complicated for the 'taxonomy users', who need to identify phytoplankton in the light microscope in monitoring projects, or who have long used the 'old names' for organisms in physiological experiments, or who need to associate a given toxin with a certain species.

That splitting species introducing new names may actually turn out to be useful was largely supported by the examples provided by **David Mann** in his plenary talk. Differences in morphological details noticed by Dr. Lothar Geitler [3] a century ago formed the basis to name a wide set of 'varieties' within the benthic freshwater diatom *Cocconeis placentula*, of which only those illustrated by Hustedt [4] were subsequently reported, allowing information to be recorded that would have been lost without names to attach to the data. A host of new definitions of species crypticity was also clarified in this talk, among which the term semi-cryptic for those morphologically undistinguishable species that can be identified only when their provenance is known. The acknowledgement that the geographic origin matters is a good step forward, and an even better one will be to recognise the taxonomic importance of the ecological characteristics of different entities. It will finally return to the picture the relevance of the environment in the origin of species. We should certainly acknowledge that taxonomy is a science aimed at identifying meaningful natural groups and tracing evolutionary patterns, rather than a name-giving exercise. New species are scientific hypotheses that need to be tested with further research – that would be impossible without naming them – rather than to be reduced on the basis of 'horror diversitatis' (fear of diversity) which finds a contrasting analogy with the well known 'horror vacui' (fear of emptiness). (A simple and wise thought is that information attached to separate names can always be unified in the light of further knowledge, whereas the opposite is impossible).

These days taxonomy is facing questions that are much more challenging than those posed by the introduction of electron microscopy. The boost of information on the cell data-center, the DNA, is likely to make a difference in solving the taxonomic questions posed above. Information being acquired in this field is producing some exciting results

on genetic diversity patterns. This is likely to attract more people to the field, possibly allowing us to accumulate biological data in parallel with molecular data. Certainly, the assumption that genetic variation is meaningless and that it overestimates the actual diversity goes against this trend: why should students be attracted by meaningless diversity patterns? We should rather let these studies flourish, promoting research into cryptic and pseudocryptic diversity: in a few years the ground for this discussion will be much sounder, while rapidly developing technology will have solved all identification problems.

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(Cont'd from p. 1)

Variability is more obvious when live *Dinophysis* cells are examined by epifluorescence microscopy. The first notable difference between phototrophic *Dinophysis* spp. and other dinoflagellate genera is that they fluoresce orange. This is because - like small (10-15 µm) cryptophyte flagellates, cyanobacteria and *Myrionecta rubra* (=*Mesodinium rubrum*) - they have reddish accessory pigments called phycoerythrins. *Dinophysis* cells can exhibit an intense orange colour covering the whole cell, or only partially (preferentially in the peripheral part of the cell), or even have parts that fluoresce with a different colour. The distinctive fluorescence characteristics of phototropic *Dinophysis* spp. led to a study of their ultrastructure and description of their peculiar plastids with 2 membranes [3], distinct from the conventional peridinin-containing plastids of other dinoflagellates. The beauty of it all lies in the fact that in this morphological variability of *Dinophysis* spp., one can read a great deal about the previous history of the population, and about the physiological status of the individual cells [1, 4].

A key observation by Hansen [5] was that the heterotrophic *Dinophysis* (*Phalacroma*) *rotundata* can feed on the ciliate *Tiarina fusus* after piercing its lorica with a feeding peduncle and sucking its contents, a feeding mechanism known as "myzocytosis" [6]. It seemed logical to imagine that other species of *Dinophysis* could feed on other ciliates in a similar way, and a few year later, remains of ciliates were found in the digestive vacuoles of *D. acuminata* and *D. norvegica* [7]. But nobody had seen in nature what the potential prey of *Dinophysis* might be. During the last 15 years, many biologists were curious to find out what was the nutritional source of phototropic species of *Dinophysis*, and tried to grow them in all sorts of enriched media, with or without additions of small prey [8]. In the luckiest cases, picked cells, incubated in cell culture plates go through 4 or at most 6 divisions, and when transferred to fresh medium, small cells start to appear and the culture does not progress in a conventional way [9]. This is high-risk research, and nobody likes to have a Ph D student working for months on a topic that may produce inconclusive observations.

Advances in molecular biology were applied to *Dinophysis* issues. Janson [10] found that portions of the ribosomal DNA that code the plastids of *D. acuminata* are identical to those with the same function for the plastids of the cryptophyte *Teleaulax amphioxeia*. The hypothesis of kleptoplastidy came into conflict with the idea that *Dinophysis*' cryptophyte-like plastids are constitutive [11], i.e., the result of an evolutionary association between a "domesticated" cryptophyte prey and a eukaryotic cell.

Attention was diverted to the potential cryptophyte prey, but nobody managed to grow *Dinophysis* on them. Takahashi *et al.* [12] confirmed the cryptophyte-like sequence of the plastids of several DSP toxin-producing *Dinophysis* spp., and even developed molecular probes that, as a very innovative early warning system, could be bound to the cryptophytes with a plastid sequence like that of *Dinophysis* spp. plastids, and detect the prey before the build-up of *Dinophysis* populations.

On the first day of the XII International Conference on Harmful Algae (Copenhagen, 4-8 September 2006) the audience was astonished and delighted with the presentation of a Korean group (**Myung Gil Park**). Their video showed voracious *D. acuminata* cells attacking the ciliate *Myrionecta rubra* with their feeding peduncles. The cells of *Dinophysis* became fatter and fatter as myzocytosis proceeded, and empty cells of *M. rubra* were finally left with the appearance of unrecognizable bubbles. *M. rubra* was cultured with the addition of *Teleaulax* sp.

The findings of Park *et al.* [13] contitute a real breakthrough that opens new avenues for research on *Dinophysis* spp., so far hampered by the lack of established cultures. Far from being the final solution, their results have raised lots of new questions: Is *Myrionecta* the main (or the only) prey in natural populations of *Dinophysis* spp? Can *Dinophysis* survive on other nutritional sources when *Myrionecta* is not available? Do *Dinophysis* perform photosynthesis with stolen plastids from *Myrionecta*, or does the ciliate act only as an exogenous nutritional source? How does feeding on *Myrionecta* affect the toxin content of *Dinophysis* cells?. Inspiration to answer some of these questions can come from recent findings on the feeding behaviour of *Myrionecta* [14]: the ciliates do not use kleptoplastids from the *Teleaulax* cells they ingest, as suggested by Gustafson *et al.* [15], and they do have permanent endosymbionts. But cultures of *Myrionecta* could only be established when small amount of *Teleaulax* spp were provided.

Blooms of *Dinophysis* spp. and the subsequent detection of lipophilic toxins

(okadaic acid and derivatives, pectenotoxins) in shellfish above regulatory levels constitute the most persistent "pest" for shellfish growers in regions of Atlantic Europe, Japan, New Zealand, and Chile. *Dinophysis* is a cosmopolitan genus, and problems associated with their proliferation arise practically in any shellfish cultivation area that search for the cells and their toxins with the appropriate methods. During this conference, seven oral presentations and over fifty posters focused on or included aspects of *Dinophysis* spp. and their toxins.

Reports of the occurrence of *Dinophysis* spp. associated with DSP outbreaks were given for new regions in Southern Brasil (**Celia Villac**), the Atlantic coasts of Morocco (**Btissam Ennaffah**) and Croatia (**Zivana Nincevic**). The application of advanced analytical methods (LC-MS) is leading to the detection of pectenotoxins in most *Dinophysis* blooms where the presence of these toxins is explored (**Elizabeth Cañete**, **Zouher Amzil**, **Bengt Lundve**), and to the identification of new analogues (**Trine Torgersen**).

The use of resins that adsorb lipophilic toxins *in situ* (SPATT) in Canada (**Corinne Garnett**), Galicia (**Gemita Pizarro**) and Scotland (**Jean-Pierre Lacaze**) showed that they can act as early warning systems when concentrations of *Dinophysis* are extremely low, and that *Dinophysis*-related toxins can be present in the water column for weeks when cells are no longer detected. Crucial questions here will be to determine if the toxins are actively liberated by healthy *Dinophysis* cells, and in what state (free, attached to adsorbing particles) the water-borne toxins detected by SPATT occur. An important observation (**Kirsten Johansen**) is that a large proportion of toxins can be liberated by the cells during common (nets, pumps) concentration procedures.

Important questions on ecology and oceanography of harmful species, identified in the Science Plan and in the Open Science Meetings of the SCOR-IOC GEOHAB programme, were addressed in the Baltic, and in the Iberian Peninsula and South African upwelling systems.

The fine scale distribution of several *Dinophysis* spp. in relation to the physical structure of the water column was explored in the Baltic Sea (**Heidi Hällfors**), and the Galician Rías (**Beatriz Reguera**). The former confirmed that distributions are species-specific, and the latter showed the importance of considering the stage in population growth to interpret different aggregation pattern in the water column. Quantification of vacuolated cells was used also in the Baltic (**WF Carvalho**) and Galicia (**Sonsoles González-Gil**) as a proxy to identify conditions associated with mixotrophic behaviour. **Lourdes Velo** applied an artificial neural network approach to predict *D. acuminata* blooms in Southern Spain from weekly cell counts obtained from monitoring programmes.

The importance of cross-shelf and longshore transport of *Dinophysis* populations in the South African (**Grant Pitcher**) and Galician (**Laura Escalera**) upwelling systems was explored. **Sanna Sopanen** in the Baltic, and **Sobrino-Gonçalves** in Portugal tackled the difficult subject of species-specific selection and ingestion rates of *Dinophysis* by different copepod species.

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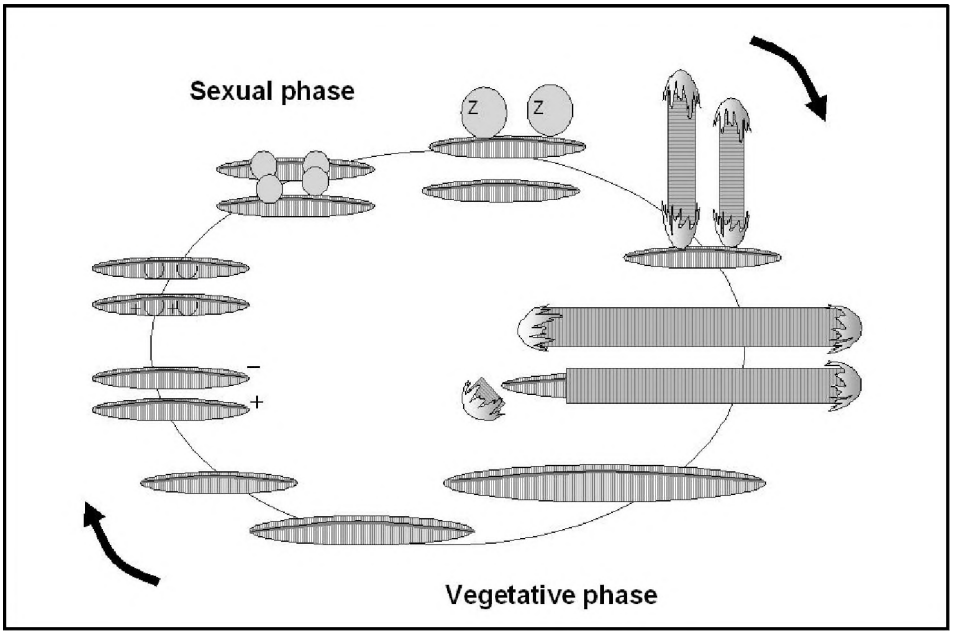
Phytoplankton life histories: a step forward

Understanding of evolutionary history, population structure and ecology of plants and animals is largely based on the knowledge of their life histories. However, what do we know about the life cycles, mating systems, formation of resting stages and other aspects of the life cycles of protists? A lively EC-funded workshop [1] provided an overview of our 'knowledge' and 'ignorance' on this topic. This workshop acted as catalyst for further research programmes (e.g. SEED) aimed at a better characterization of the life histories of marine phytoplankton organisms. Considering that toxic or harmful species are known in almost all phytoplankton classes, the Conference is an ideal forum for an update on new findings, innovative approaches and experimental studies on phytoplankton life cycles in general.

The very first approach for life cycle studies implies working in the laboratory with cultures. This might be considered as a limit – like studying an animal in the cage of a zoo – but it is the only way by which we can link together the different life stages in a coherent sequence of events. Up to now, the vast majority of studies have focused on phototrophic organisms: once they are provided with light and nutrients, they can – at least theoretically - grow. The cultivation of heterotrophic or mixotrophic protists is much more tricky, requiring the design and testing of a suitable diet that allows for their growth and life- cycle completion. **Kristin Gribble** presented for the first time detailed information on the sexual process – gamete formation, conjugation, production of a planozygote and a cyst – in a *Protoperidinium* species, *P. steidingerae*.

Protoperidinium are amongst the most abundant heterotrophic dinoflagellates and include a notable number of species capable of producing resting cysts. Elucidating the process of cyst formation and the morphology of the different life cycle stages will provide important information for a better refinement of the morpho-taxonomy of this genus. It will also open new avenues for experimental work aimed at understanding the triggers for encystment and excystment, which might be regulated by factors different from those active in phototrophic species.

When thinking about phytoplankton life histories, the switch between vegetative (capable of duplication) and resting stages immediately comes to mind. Those are indeed crucial points in an organism's life history, and have considerable implications for their ecology: the formation of resting stages might contribute to bloom termination, while vegetative cells germinating from spores or cysts are released in the water column and might provide the source for a subsequent bloom. Unfortunately, a proper quantification of these transition points is hampered by objective observational limits. Several posters addressed the role of resting stages in population dynamics (e.g. **Mitshiro Yoshida**, **Silvia Angles, Isabel Bravo, Cecilia Satta**). **Akira Ishikawa** has designed a 'plankton emergence trap' allowing the quantification of cyst germination, for which there are almost no field data; the authors found that germination of *Alexandrium catenella* cysts is continuous throughout the year, without any seasonal pattern, in contrast to the bimodal pattern recorded for the blooms. The formation of resting stages is not a prerogative of dinoflagellates, but is recorded in many other taxa. **Karin Rengefors** provided evidence for the formation of temporary and sexual cysts in the freshwater *Gonyostomum semen,* a raphidophyte responsible for skin irritation. **Shigeru Itakura** searched for differences in seeding mechanisms between diatoms and dinoflagellates: can different environmental conditions trigger the preferential germination of species belonging to one of the two groups?



The life cycle of a pennate diatom

No resting stages are known up to now for species of the diatom genus *Pseudo-nitzschia,* which nevertheless can produce regular and recurrent blooms in coastal areas. **Domenico D'Alelio** explored cell size variation in *Pseudo-nitzschia multistriata* over several years to find evidence for the occurrence of sexual reproduction. The occurrence of sexual events might be inferred by finding of auxospores and/or by the appearance of populations with a large cell size, which are produced following the sexual phase. Interesting questions can be addressed by the painstaking measurement of thousands of cells: does the bloom represent the occasion in which conjugation probabilities are higher? Is the bloom constituted by cells in the same size range? *P. multistriata* cell size gradually decreases over time and populations of different size succeed year after year, suggesting that sex in this species occurs every 1 or 2 years.

There other aspects of life cycles that are still poorly understood and/or quantified but interesting information is piling up. As an example, **Bente Edvardsen** showed that several species have haplo-diploid life cycles within the haptophyte genus *Chrysochromulina*. This means that both haploid and diploid stages are capable of growth. This life cycle is different from that recorded in diatoms, where cells are diploid and the haploid stage is represented by the gametes, or in dinoflagellates, where cells are haploid and the only diploid stage is the planozygote (and the cyst, if produced). What are the implications of these differences in the evolution of protists? What are the differences between the two stages in the haptophyte life cycle? At times they are surrounded by scales of different morphology, at times they look the same.

Protists might have mating systems (broadly speaking, the number of sexes) of different complexity. Again, this is an aspect with considerable implications, spanning from population genetics to evolutionary biology. A cell may require a mate of opposite mating type to perform conjugation, or it may be able to mate with cells of the same mating type; this makes a difference. In order to define the mating system of a certain species, multiple crossing experiments should be carried out to test if sexual reproduction can occur within a clonal strain, if strains of two opposite mating types have to be crossed, or if the system is even more complex. There were a few contributions in which crossing experiments were carried out (e.g. **Cátia Carreira**, **Setsuko Sakamoto, Domenico D'Alelio**). Obtaining a convincing answer might be tricky if we rely only on morphology. In dinoflagellates, the evidence for sexual reproduction can be provided by the formation of cysts (assuming that they are the product of sexual reproduction). However, we know that not all

planozygotes necessarily transform into cysts, and thus the lack of cysts does not imply the lack of conjugation. We might have conjugation followed by the formation of a planozygote that does not encyst, or our experimental settings might not be adequate to induce the sexual phase. We are faced with similar problems in diatoms, where the morphological evidence for sexual reproduction is provided by the formation of auxospores. Here again, the positive result is solid, but the negative one might be biased due to a sub-optimal experimental design, or to the fact that our strains are not in the right size window for sex. But do not get depressed: difficulties stimulate creative thinking!

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The Gundestrup Cauldron

The XII Conference on Harmful Algae convened in Copenhagen attracted 550 participants from 56 countries (EU with Norway and Croatia 230, USA 99, Japan 52, China 23; countries not represented in earlier conferences are Bangladesh, Kuwait, Madagascar, Namibia, Nigeria, Senegal, Turkey, and UAE)

Denmark has given us the Gundestrup cauldron, constructed from plates, like an armoured dinoflagellate – like other cauldrons of northern myth, it is a metaphor of the heavens, and, like these conferences, a source of inspiration. Denmark has also given us an epic poem, whose hero *Beowulf* may have used allelopathy to overcome the monster Grendel[1]; A list of famous Danes includes Tycho Brahe ("Ne frustra vixisse videar" – Will I not have lived in vain) – readers who attended the conference may have noticed his image as they entered the town hall reception – he rejected the heliocentric view of Copernicus, and, as a result, some Danes still hang their heads in shame; Ole Rømer, who devised an early temperature scale (in 1701, later improved by Fahrenheit in 1724, and still in use in North America); Eugenius Warming, a pioneer of community ecology and ecophysiology (he called it *epharmosis*), who offered the world's first ecology course[2]; Wilhelm Johannsen, who coined the word *gene* (in 1909), and focused attention on the genotype-phenotype duality. Denmark has also given us Shakespeare's *Hamlet*, Vikings, comedian Victor Borge, and *pølsevogn* (sausage wagons).

Barrie Dale frequently asks "What happened to the epidemic?" (right out loud). He refers to the persistent notion



that HABs are *spreading*. I think he means people are quieter about this now, that it is an idea whose time has passed, like that of the Vikings, who brought the clam *Mya arenaria* back across the Atlantic [1], and spread Baltic plankton to the Black Sea via Russian rivers [2]. Barrie was obviously too busy selling bric-a-brac to pay proper attention. Presented here were the first reports of toxic *Pseudo-nitzschia* in the Chesapeake (**Holly A. Bowers**; **Anne Thessen**), the first yessotoxins in Portuguese bivalves (**Susana S. Gomes**), the first bioluminescent *Alexandrium* bloom in Finnish waters (**Tore Lindholm**), the first records of *Gymnodinium catenatum* and *Pyrodinium bahamense* in Angolan waters (**Isabel Rangel**), the former also a recent arrival in Brazil (**Celia Villac**), the first azaspiracids in Moroccan waters (**Hamid Taleb**). Spreading is still alive and well.

"In recent years massive outbreaks of ... species ... have attracted much public atttention. However, since ... outbreaks are often sporadic and of short duration, they tend to catch the scientific community off guard and discourage systematic long term studies. ... Many "basic" questions remain a matter of debate. For instance the time/space scale of such episodes; the extent to which ... outbreaks are favoured by climatic triggers, physical forcing, or disequilibrium conditions linked with eutrophication and/or overfishing; the design of appropriate data sets and monitoring protocols. Among the many unresolved challenges confronting researchers, the tasks of tracking the complex life stages of these organisms, identifying their function in benthic-

planktonic coupling, and assessing their proper role and importance in food web models, are not the least."

All very familiar; the passage is from a CIESM report [3] about jellyfish. There have been frequent warnings in recent years that the future of the oceans belongs to jellyfish (jelly barrens, to match the urchin barrens of the seabed). There have been 'jelly tides' in the Black Sea (*Mneniopsis*), the Sea of Japan (*Nemopilema*), the Benguela Current (*Chrysaora*, *Aequorea*), the Gulf of Mexico (*Aurelia* and *Chrysaora*, Australian *Phyllorhiza*), the Mediterranean (*Pelagia* and *Rhizostoma*), Hawaian waters (*Carybdea* spp). Like so many ecological puzzles, these plagues are variously attributed to overfishing, global warming, eutrophication, in short to planetary mismanagement. In such scenarios, the toxins will certainly have a chance to be vectored by jellies **(Florence M. Boisson)**, and events like the turtle mortality in El Salvador **(Sergio Licea-Durán)** to increase in frequency.

Ruthless reductionists have modelled phytoplankton cells as spheres whose surfaces are porous, or perforated with pores, and which lead lives of nutrient sucking simplicity[3]. So, how many pores does a cell need to suck up its nitrate or phosphate rations? Surprisingly, the flux of nutrients into such cells by classical diffusion theory is proportional to the radii of the pores, not their surface areas, and only a very small fraction of surface is needed to maximize uptake rates[4]. "Thus many hundreds of different transport (or receptor) systems can be accomodated on the surface of the cell, each adsorbing particles of a specific kind with an efficiency approaching that of a cell whose entire surface is dedicated to one such task." [4]. So unless the rest of the surface is *simply* defensive (armour) or offensive (say trichocysts), there is plenty of space for other activities.

We are gradually learning how diverse these other activities are (allelopathy, quorum sensing, mating signals, ...). This diversity can be a "source of irritation" [5] because it forces one to abandon the relative safety of the physicochemical paradigm with its few free parameters, and enter the world of complex systems, to move from models of single populations to webs and networks; the ocean is not a guppy tank [6]. For others, the diversity (idiosyncrasies of taxonomy, natural history, and life cycles) provides a refuge from the irritations of reductionist models[5]! These opposing attitudes were apparent here in the conference *dialogue* between **Bill Sunda** and **Ted Smayda**. Parsimony is not in Smayda's tool-box, or at least was not deployed in this debate. Smayda's efforts to extend Margalef's mandala are evidence that he is not opposed to the search for patterns. Did HAB science grow as a result of Sunda's conjectures and Smayda's refutations? Bryan Magee once asked Karl Popper: "If science grows by conjectures and refutations, then why wasn't Newton's theory abandoned with Mercury's anomalous perihelion in 1820?" If you want to know Popper's reply, read Imry Lakatos' lectures[6], or write to the editor! We need feedback at the HAN office. Richard Levins stressed that however false, inadequate, and incomplete models are, they can provide qualitative and general insights; they are heuristic devices, and both mandalas and differential equations should find places in the ecologists' tool-box; both make trade-offs between generality, realism, and precision (Levins again).

Most of the ingredients which make HAB ecology difficult and fascinating were already broached at Takamatsu [7]. At that conference for example, Smayda was "not ready to embrace this view", that nutrients and water quality were of overwhelming importance in the generation of HABs, and he stressed that altered grazing patterns provoked by aquaculture and fishing, as well as climatological and geological cycles and the greenhouse effect, could enter the equation; those were trenchant comments at that time. So already 25 years ago the bottom-up vs top-down argument was on the simmer. Recently, emphasis has shifted from nutrient control to predator control. With increasing frequency, we hear views like "the bottom-up perspective has not been successful in predicting many patterns and features of pelagic communities, nor why specific organisms, morphologies, and life histories occur when and where they do" [8], or, "efforts to control activities that affect higher trophic levels (such as fishing) will have far larger impacts on community dynamics than efforts to control, for example, nutrient input, ... "[9]. What is at issue here is not whether eutrophication or overfishing or climate change or anything else *cause* HABs, but the "impoverished notion of causation that characterizes modern biological ideology" [10]. What is the cause of tuberculosis? Is it the tubercle bacillus, or unregulated industrial capitalism?

There were some novelties – we had sessions called "Ecophysiology & autoecology" – I am not too sure of the difference, but given the complexity of life in the real world which these conferences reveal, I wonder if either is of much relevance outside the guppy tank. Parallel sessions seem now inevitable, but we lose a great deal – claustrophobic poster sessions are also becoming the rule, but there is no obvious solution to the problem. One of the highlights of Lund was Max Taylor's summing up, which requires a rare talent, hard to emulate. He hit all the right notes. Victor Borge made people laugh by hitting carefully chosen wrong notes on the piano, immaculately timed. But offensive humour and rankling conceit do not achieve the same effect. "Ecology has no aims, but ecologists have" wrote Charles Adams[7]; some of those aims are too obvious to need advertisement.

Nearly six years ago, it took 32 hours to travel from Vigo to Hobart (great circle distance 18046 km) for HAB2000, about 564 km h^{-1} on a great circle route; this year, the travel time Vigo-Copenhagen (2140 km) for some of us was 34 hours, or 63 km h^{-1}. Luckily a strategically placed *pølsevogn* caught our attention on arrival. If the declining trend in velocity with time is real and linear, those of us who live in Vigo should consider setting out for Hong Kong about now - or are we already too late?

Footnotes

[1] Perhaps the psychoactive components of ergot infected barley.

[2] His influential textbook, *Plantesamfund* ..., was published in 1895. German and English editions followed.

[3] " … organisms envisioned as nutrient-requiring packets of chlorophyll, … " (Max Taylor 1990 at Lund).

[4] This is for spherical cells. For long thin cells like *Pseudo-nitzschia*, fluxes are more proportional to length.

[5] "Not all naturalists want to do science; many take refuge in nature's complexity in a justification to oppose any search for patterns." (Robert McArthur, *Geographical Ecology*).

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Photos by L. Velo & M. Lion



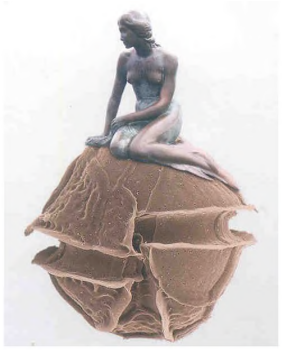














Yasumoto Lifetime Achievement Award to Dr. D.M. Anderson.



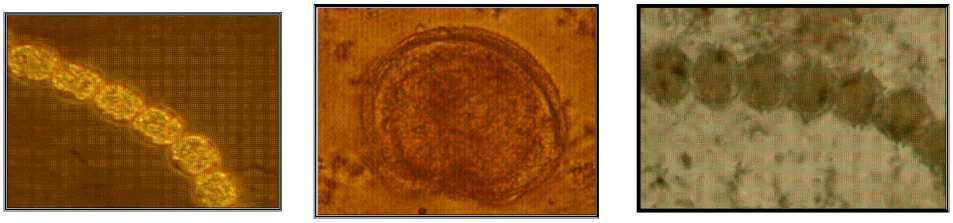
A present was given to Prof. T. Yasumoto on behalf of ISSHA.



Barry Dale in action during the ISSHA auction.

• Angola

First Records of *Gymnodinium catenatum*, *Gambierdiscus toxicus* and *Pyrodinium bahamense* on nothern Luanda coast, Angola



From left to right: Chain of Gymnodinium catenatum, Gambierdiscus toxicus *and chain of* Pyrodinium bahamense *(400x).*

Angola has two marine ecosystems, the cold Benguela Current and the warm Angola current. Only a few studies have been undertaken of the phytoplankton on the Angola coast. However, according to taxonomic assessments carried out by different authors between 1953 and 2002, *Prorocentrum balticum*, *Gyrodinium spirale*, *Alexandrium* spp. and *Chatonella* spp. have been detected on the Luanda coast, sometimes in association with mortality of resources [1-3]. There are great disparities in the information available about HABs within the southern Africa region, and though some harmful species are found throughout the region, others appear confined to particular areas.

From July 2003 to October 2004, 343 samples were collected at 4 stations on the northern Luanda coast from Fishing Harbor to Santiago Bay (Fig. 1).

The samples were collected at 4 different depths (5, 10, 15 and 20m) and then fixed with 2% formol. Thermometers and a portable refractometer (Atago S/Mill) were used to measure temperature and salinity. Counts were made by the Utermöhl method with phase contrast. A total of 206 phytoplankton species were identified. The most frequent were: *Prorocentrum micans, Thalassiossira rotula, Leptocylindrus danicus, Cylindrotheca closterium, Skeletonema costatum, Navicula distans, Thalassionema nitzschioides* and *Leptocylindrus minimus*. These species were most abundant at Station 2 (Cacuaco) and at 20m depth. Six harmful species were found amongst those identified, namely: *Alexandrium tamarense, Chattonella antiqua, Dinophysis acuminata, Gambierdiscus toxicus, Gymnodinium catenatum,* and *Pyrodinium bahamense*. Three of these were not recorded before on the Angola coast: *Gambierdiscus toxicus, Gymnodinium catenatum* and *Pyrodinium bahamense.*

The highest phytoplankton density were recorded in October, 2003 ($212 \cdot 10^5$ cell$\cdot$L^{-1}) and in September, 2004 ($132 \cdot 10^5$ cell$\cdot$L^{-1}) when the weather was changing from winter to summer. The lowest values were observed during the winter (May to September) (Fig. 2).

Both years, the high densities of harmful algae bloom occured in August at St1, following by St3, St4 and the lowest density at St2. In 2003 and 2004, *Pyrodinium bahamense* and *Gymnodinium catenatum* were dominant and caused blooms with maximum densities of the $349 \cdot 10^5$ cell$\cdot$L^{-1} and $212 \cdot 10^5$ cell$\cdot$L^{-1} respectively. *Gambierdiscus toxicus* occurred in 2004 at all stations. The highest density was $208 \cdot 10^5$ cell$\cdot$L^{-1} at St3. In August 2003 and 2004, the high density of harmful species, coincided with temperatures around 20ºC and salinity at or above 32. Minimum densities occured with temperatures above 22ºC and salinity below 32.

Gambierdiscus toxicus produces ciguatoxins. These are generally confined to tropical and subtropical waters and form part of the natural biota of coral reefs, [4]. Luanda is tropical, and the occurrence of this species can be considered normal. *Gymnodinium catenatum* can cause paralytic shellfish poisoning (PSP) occurs in Portuguese

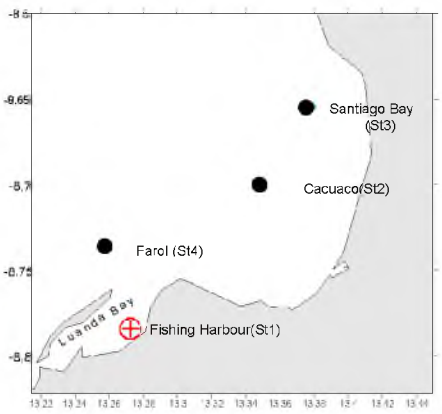


Fig. 1. Station positions (St1- 8º47´07´´S, 13º16´25´´E; St2- 8º42´23´´S, 13º20´07´´E; St3 8º39´73´´S , 13º20´50´´E; St4 – 8º44´30´´S, 13º15´56´´E).

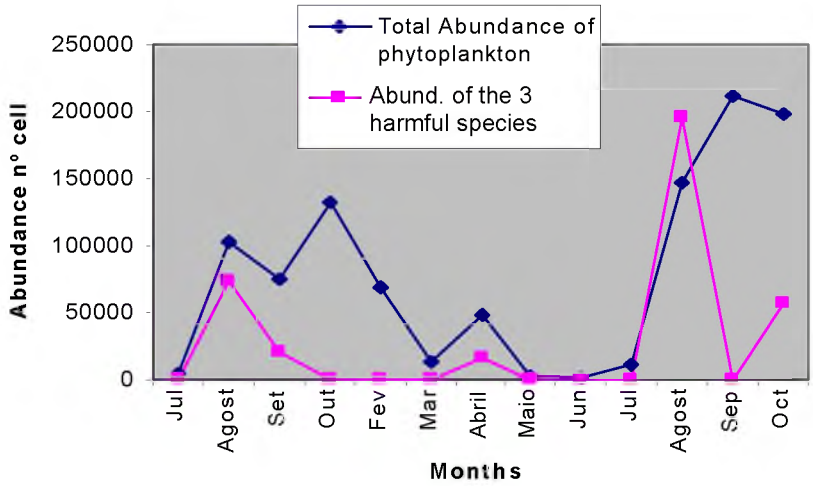


Fig. 2 Phytoplankton and harmful species density from July 2003 to October 2004 on northern Luanda coast.

waters where temperature and salinity average 15°C and 36, respectively. In Morocco, *G. catenatum* occurs at temperatures of 14.8 -17.2°C and salinity of 33.6-36.6 [5]. In the region, species of *Gymnodinium,* are known in South Africa, and cause mortalities of fish and sub- and intertidal fauna [6]. *Pyrodinium bahamense* causes PSP and fish mortality in several parts of the world. This species has bloomed in eastern Indonesian waters (Kao Bay) with high temperatures (30.9-31.5°C) and low salinity (29.2-30.1) [7]. Our data for temperature and salinity are quite different.

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• Italy

BENTOX-NET, a Research and management initiative on *Ostreopsis* spp. and other benthic microalgal blooms on the Italian coast

Ostreopsis ovata (Fig. 1), *O. siamensis* and other congeneric species are typical components of the 'ciguatera community', i.e. the association of benthic microalgae found along with *Gambierdiscus toxicus* s.l. in tropical waters. These dinoflagellates colonise benthic macroalgae and seagrasses or attach directly to the substrate, whereas only rarely are they found in the plankton.

The presence of *Ostreopsis ovata* was reported at Villefranche sur Mer (NW Mediterranean Sea) in the 1970s (Max Taylor, pers. comm.) but massive blooms of this species have recently become a threat at several sites along the Italian coasts (Fig. 2). Two distinct species, both producing palytoxin-like toxins, have so far been identified in the northwestern Mediterranean basin: *O. ovata* and *O.* cf. *siamensis* [1]. Blooms of *Ostreopsis* spp. were first recorded along Tuscany coasts in 1998, when thick mucilaginous layers hosting thousands of cells covered both biotic and abiotic substrates [2, 3, 4]. During blooms, mucilage flocs and cells may detach from substrates and float in the water column [5]. In the last 3 years, bloom events have been reported from several other sites in summer and autumn (Fig. 2).

The impact of these blooms on the ecosystem may be serious, with hypoxia, anoxia and benthic invertebrate kills. In addition, skin irritations, fever, respiratory affections and conjunctivitis have caused the hospitalisation of several hundred people. These symptoms have been explained with the presence of a palytoxin-like substance produced by *O. ovata* in both the water [6] and aerosol. In addition to *Ostreopsis* spp., at least two other potentially toxic species may abound on macrophytes, i.e. *Prorocentrum lima*, which produces okadaic acid, and *Coolia monotis*, which produces toxins whose effects on humans are unknown.

The dynamics and consequences of *Ostreopsis* blooms observed in Italian seas and recently along the Spanish coast [7] can hardly be compared to other HAB cases, and pose many questions as to their management during the high risk phases and over longer time scales. Many scientific questions need to be answered to clarify the phenomenon and its possible expansion in coming years, ranging from the exact identification of the species involved, their geographic provenance, physiology, ecology, bloom dynamics, possible role in the trophic web, to the toxins, their production, fate in the trophic web and in the medium. These researches require a multidisciplinary approach. In addition, given the relevance of these phenomena to human health and economic activities, it is crucial that research and management are conducted in a coordinated and integrated way.

To this end, the network BenTox-net (**BEN**thic potentially **TOX**ic microalgae **NET**work) has been

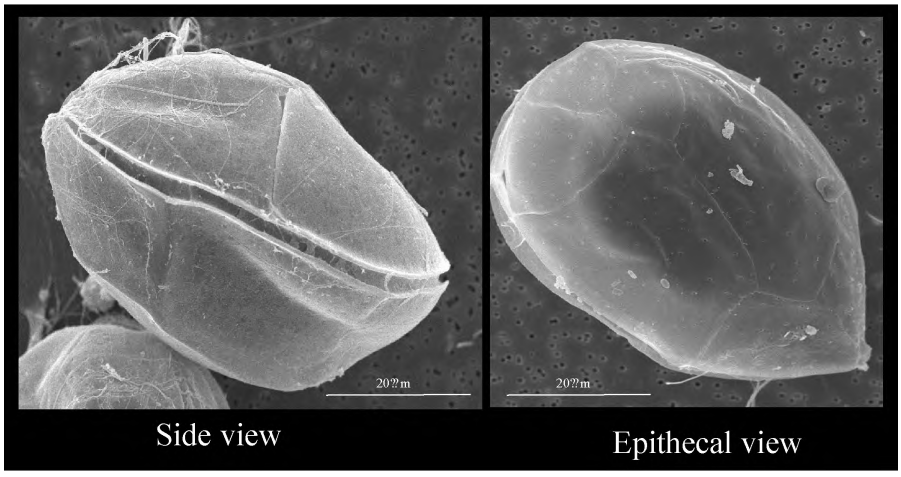

Fig. 1. Ostreopsis ovata *SEM, left: side view; right: apical view. Pictures taken by Maria Grazia Giacobbe and Magda Vila.*

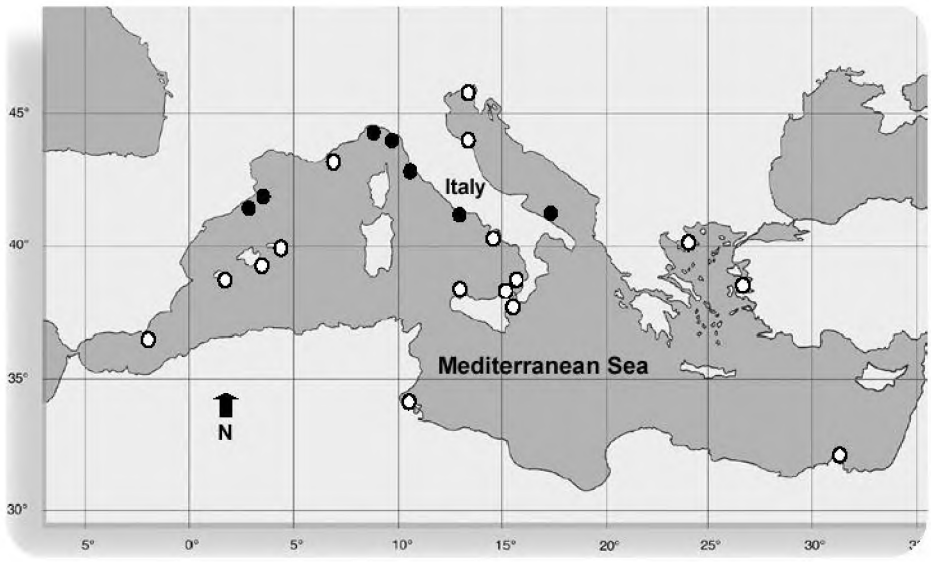

Figure 2. Map of Mediterranean localities where Ostreopsis *spp. have been detected. Full circles mark sites where problems have been reported.*

established among scientists from various Italian laboratories. Participants to the network cover a wide range of expertise including taxonomy, toxin chemistry, genetics and ecology of harmful and non-harmful microalgae. BenTox-net aims are the following:

- to establish communication among research groups working on potentially toxic benthic microalgae;
- to set up intercalibrated sampling and analytical procedures, allowing comparison of data gathered at different sites by distinct groups;
- to continue or establish monitoring activities in areas susceptible to benthic blooms;
- to create links with local agencies that are in charge of bloom management as for environmental quality, public health, seafood protection, tourism and recreational activities in coastal areas;
- to establish an alert strategy tailored on the needs and expertise available in different Italian regions;
- to refine communication strategy with the press and public, to avoid misinformation or unjustified alarms;
- to prepare and submit research projects and secure their funding by public institutions of different nature;
- to train local agencies personnel on methods for sampling and identification of potentially toxic micro-organisms;
- to produce scientific and reach-out papers to be published in national and international newsletters and scientific journals;
- to organise scientific and technical meetings with different participants and target audiences, to promote the delivery of information and research and management activities related to harmful benthic microalgal blooms.

BenTox-net is a bottom-up initiative driven by the need for integrated and effective action in response to the threat posed by this new type of harmful algal blooms. BenTox-net is established based on spontaneous and voluntary participation of the scientists and respective research groups in the list below. At present, no other funds and resources exist beyond those available at the individual Institutes. This hardly allows for basic activities to be set up as response to alert situations in case of blooms. However, the level of protection offered is very limited at this stage, considering the lack of scientific knowledge at local scale and in general. An optimised monitoring and management strategy requires preliminary investigations with extended and frequent sampling over long periods of the year, coupled with ecological, physiological, molecular and toxin chemistry studies. This can only be achieved with support and resources obtained through the search for funding, which will have high priority among the activities of the network.

The Principal Investigators of the 19 groups so far participating in the network are listed below. The network is open to further participation and will shortly seek links with Mediterranean partners.

Roberta Congestri, Università Tor Vergata, Roma.
Antonella Penna, Università degli Studi di Urbino, Pesaro.
Adriana Zingone, Stazione Zoologica A. Dohrn, Napoli.
Maria Grazia Giacobbe, IAMC-CNR, Messina.
Cecilia Totti, Università Politecnica delle Marche, Ancona.
Patrizia Ciminiello, Ernesto Fattorusso, Università degli Studi di Napoli Federico II, Napoli.
Antonella Luglié, Università degli Studi di Sassari, Sassari.
Antonella Bottalico, Università di Bari, Bari.
Michele Scardi, Università Tor Vergata, Roma.
Mauro Bastianini, ISMAR CNR,Venezia.
Claudio Grillo, ARPAL, La Spezia.
Roberto Poletti, Centro Ricerche Marine, Cesenatico.
Rossella Pistocchi, Università di Bologna.
Monica Casotti, ARPAT, Massa Carrara.
Carmela Caroppo, IAMC-CNR Taranto.
Rossella Barone, Università di Palermo.
Marina Cabrini, OGS Trieste.
Aurelia Tubaro, Università di Trieste.
Luisa Mangialajo, Università di Genova.

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R. Congestri, Università Tor Vergata, Roma, Italy.

A. Penna, Università degli Studi di Urbino, Pesaro, Italy.

A. Zingone, Stazione Zoologica A. Dohrn, Napoli, Italy.

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Stronger focus on sustained observations of harmful algae

The IOC has for more than a decade given attention to activities aimed at developing capacity in research, sustained observations and management of harmful microalgae through its Harmful Algal Bloom Programme and the Global Ocean Observing System. Recently, new initiatives have been taken to strengthen focus on operational marine observations for harmful algae and relevant parameters.

Rapid developments in technology and a strong focus in the international community on operational marine observation systems have made it relevant to publish a new manual on real-time coastal observing systems for marine ecosystem dynamics and harmful algal blooms. The manual is expected to be released by UNESCO Publishing early 2007. Editors are Marcel Babin, Colin Roesler, and John Cullen.

The WMO-IOC Joint Technical Commission for Oceanography and Marine Meteorology (JCOMM) has an expert team in the Services programme area focused on Marine Accident Emergency Support (MAES) - largely dealing with marine pollution emergency response and search and rescue operation support. JCOMM/MAES is now also exploring how they might be able to support operational harmful algal bloom warnings and what their team can offer, and what JCOMM observations are required. JCOMM will explore this topic at their next meeting 29-31 January 2007, in Angra dos Reis, Brazil.

The Scientific Steering Committee (SSC) for the IOC-SCOR science programme GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms, www.geohab.info) has opened a dialogue with the GOOS Regional Alliances to address the inclusion of, and approach to, observation systems to detect harmful algal blooms and associated environmental conditions in regional GOOS components.

The GEOHAB SSC wishes to facilitate GOOS in making measurements that could help in understanding and forecasting HABs. Also, the SSC seeks to encourage regional and national observation systems to deploy advanced species-specific sensors that can detect HAB organisms, as well as other environmental sensors that will help us understand HAB initiation, development, and collapse. Sensors in this latter category include remote sensing of parameters such as ocean colour, sea surface temperature, sea surface salinity, currents and fronts, etc., as well as in situ measurements of fluorescence, sub-surface temperature and salinity, water column structure, nutrients, and other parameters relevant to HABs.

In some national or regional observing systems HAB applications have been invoked as a major justification for such observing systems. However, in most cases it appears that little of the observing infrastructure is specific to HABs. Clearly, information about temperature and currents is extraordinarily useful for HAB applications, but insufficient to make accurate forecasts.

The GEOHAB SCC considers that information exchange between the HAB and GOOS communities is very important to interact actively in development of operational systems for routine HAB observations.

Harmful Algae News will keep you updated on developments within these new initiatives.

IOC Training Courses

Open for applications: IOC Identification Qualification in Harmful Marine Microalgae, University of Copenhagen, 2007

The IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Denmark has organized more than 20 international training courses in taxonomy and biology of harmful microalgae. The 2007 course includes an examination at the end of the course with an IOC Certificate of Proficiency in Identification of Harmful Algae issued to participants who pass the examination. The course includes 90 hours of teaching and is divided into two parts, each consisting of 45 hours of teaching. The first part of the course is an internet teaching programme, while the second part is a practical workshop in species identification (scheduled tentatively for 20-30 August 2007). The course is organised by the University of Copenhagen, Denmark. Details at: www.ioc.unesco.org/hab/train.htm

FANSA News



The 7th IOC Regional Science Planning Workshop on Harmful Algal Blooms in South America (FANSA) was held in Lima, Peru, between the 21st and 23rd, June, 2006, as a continuation of periodical meetings occurring since 1994. The IOC-FANSA meeting was supported by IOC (UNESCO) and hosted by Instituto del Mar del Perú (IMARPE) (local contact: Dr Sonia Sánchez). The Workshop was opened by the President of the Board of IMARPE, Rear Admiral Hugo Arévalo Escaró, who welcomed participants and thanked IOC for continued support of scientific and operational analyses of HABs in South America. He was followed by Dr. Leonardo Guzmán, Chairman of IOC—FANSA, who gave a brief overview of the working group, the expectations of previous workshops, and future objectives. The Minister of Production of Perú, Mr. David Lemor Bezdin, emphasized the importance of strengthening regional capacity to face HAB problems associated with serious problems in Public Health, fisheries resources and marine ecosystems.

Twenty six representatives from Argentine (1), Brazil (2), Chile (2), Ecuador (2), Uruguay (2) and Perú (17) attended the WS. Mónica Lion (IOC-IEO Science and Communication Centre on Harmful Algal, SCCHA, Vigo, Spain) acted as IOC observer. Additional time was dedicated to revision of the HAB-FANSA website by L. Guzmán, S. Méndez and L. Proenca, editors of this IOC initiative. The Workshop consisted of four sessions: 1) State of HAB problems within the region and novelties in participant countries; 2) State of the scientific knowledge, management of HABs and its effects within the region: comprehension level and operational actions; 3) Information networks, training activities, scientific information exchange, local development and actions for public education and information dissemination, 4) Workshop recommendations. The Workshop resulted in eighteen recommendations:

· The need for taxonomic training on different taxonomic groups to strengthen monitoring programmes.

· To organize a second UNESCO Knowledge Portal e-Learning Training Course on Taxonomy and Biology of Harmful Marine Microalgae.

· The HAB-FANSA website should include the scientific and operational capacities available within each FANSA country.

· The FANSA countries agreed to revise and complete the available information on HAB thematic maps prior to its official presentation.

· Members of the FANSA WG should stimulate other regional researchers to contribute with material and suggestions to the HAB website.

· An agreement to invite the IOC-ANCA group to integrate the HAB website, to establish mechanisms to favour integration, and for at least one representative of each WG to attend the ANCA/ FANSA meetings.

· Agreement to contribute validated data up to 2000, through the focal point of each FANSA country, to upgrade HAEDAT (once the new electronic format becomes available).

· Agreement to encourage cooperation among different experts on shared topics of interest, as in the case of *Alexandrium, Pseudo-nitzschia* and *Dinophysis* blooms.

· The IOC-IEO SCCHA from Vigo, Spain, should continue training courses to update and improve marine toxin determinations on shellfish for human consumption.

· Specific training courses on official techniques to determine biotoxins in food of marine origin, conducted by regional specialists, were suggested.

· To progress in the accreditation of Marine Toxin Analytical Laboratories (ISO 17025), IOC coordination and support to perform during 2007 an inter-calibration exercise for PSP determination (AOAC, 2000) between the EU Reference Laboratory on Marine Toxins and the Official Laboratories of FANSA countries was requested.

· The IOC Executive Secretary should inform each FANSA country of its capacity to maintain financial support prior to planning future actions of FANSA.

· Each national representative should inform its IOC National Focal point about IOC budget restrictions to be presented at the IPHAB Panel.

· Reinforce educational, training and dissemination campaigns on HABs, oriented to different population sectors.

· Developments of issues, and progress reached in specific topics by each country should be suggested for the preparation of the agenda.

· To explore the feasibility to organize a specific workshop on problems associated with *Alexandrium* in the region, prior to the next IOC FANSA WS.

· The next IOC FANSA WS will be held in Mar del Plata, Argentina, hosted by the Instituto de Investigación Pesquera (INIDEP).

Leonardo Guzmán,
Chair FANSA.

ISSHA President's Corner

The International Society for the Study of Harmful Algae (ISSHA) sponsored the 12th International Conference on Harmful Algae, held in Copenhagen (4-8 September 2006), and held there on 7th September its Fifth general assembly. The picture in the bottom shows the executive and attending council members. Over 200 ISSHA members attended this meeting.

Pat Tester (President) opened the meeting. The minutes from the previous assembly were approved and different council members summarized the activities and achievements of ISSHA in the last year. **Nina Lundholm** (Treasurer) presented the financial statement, covering the period November 2004 to August 2006. **Don Anderson** (Chair of the Travel Awards Committee) thanked a list of sponsors and the members of his committee that allowed the review of 60 applications and the partial support of 35 applicants from 16 countries to attend the conference.

Jane Lewis (Chair of the Publications Committee) announced a new ISSHA-IOC publication: "Manual on Aquatic Cyanobacteria" (by Gertrude Cronberg & Heléne Annadotter), recalled the affiliation of ISSHA to the IOC-UNESCO Newsletter Harmful Algae News, and recognized the efforts of Tracy Villareal (ISSHA Secretary) to maintain and revise the ISSHA website.

Barrie Dale (Chair of the Awards Committee) reviewed the rules for the Awards. Over 100 students were considered for the best poster and oral presentation at the XII ICHA. The awards were announced during the conference Mermaid Banquet. The Yasumoto Lifetime Achievement Award was given to **Dr. Donald M. Anderson** (Woods Hole Oceanographic Institution, USA), for a life successfully dedicated to the study of *Alexandrium* spp. (physiology, population dynamics) and a vigorous managerial and cooperative interaction with the HAB community, both at a national and international level (see p. 9). Awards to the best oral presentation was given to **Tilman Jens Alpermann** (Alfred Wegener Institute, Germany) and the first and second awards for the best poster to **Katherine Hubbard** (University of Washington, Seattle, USA) and **Jabulani Ray Gumbo** (University of Pretoria, South Africa) respectively.

Henrik Enevoldsen, on behalf of his Co-chairs (P. Tester and A. Zingone), gave an update on HAB-MAP, the ISSHA initiative to compile regional summaries of the occurrence of toxic and ichthyotoxic marine microalgae, for 11 areas of the world. Data are now available for the Mediterranean and South America.

Presentations were given in support of the venue for the 2010 International HAB Conference, by Yves Collos (for Montpelier, France), Jane Lewis (for Edinburgh, Scotland), and Kalliopi Pagou (Hersonissos, Crete). Members voted on the venue during the General Assembly. Crete was elected as the venue for 2010.

Once again, the President remimded ISSHA members of the advantages of society membership, invited them to contribute with suggestions for new initiatives, and thanked them for their participation.

For the first time in ISSHA's existence, an auction to raise funds for the society was organized the evening following the assembly. The auction was not only a financial success, but also great entertainment thanks to the great communication skills of Barrie Dale (see p. 9.).

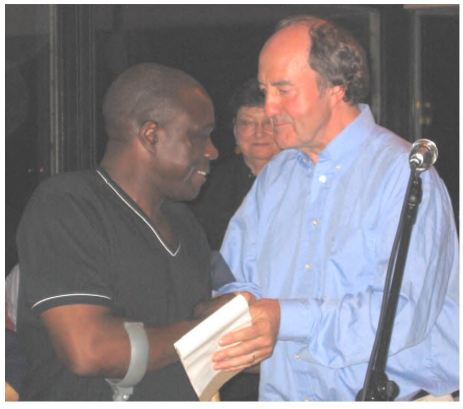
ISSHA
International Society for the Study of Harmful Algae



T.J. Alpermann, Award to best oral presentation.



K. Hubbard, Best poster presentation.



J.R. Gumbo, 2nd prize for poster presentations.



Photo by L. Velo

Eighth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB-VIII), UNESCO, Paris, 17-20 April 2007

The Panel (IPHAB) was formed in 1991 to set priorities for and ensure the financial resources for implementation of a global HAB Programme. A primary task is still to identify the required resources, so that the international community jointly can continue to implement activities on capacity building, international cooperative research, and communication networks. The membership of the IPHAB is open to Member States of IOC which have declared to the Secretary IOC their involvement or intention to participate in the development and implementation of the HAB Programme on a global, regional, or national scale. The Provisional Agenda and other documents for the Session is available at the IPHAB home page at http://ioc.unesco.org/hab/act2.htm.

The major focus of the Eighth Session of IPHAB is tentatively, capacity building, the GEOHAB Research Programme, HAB Databases, formulation/endorsement of specific objectives for regional activities, and HAB observations and their inclusion in components of the Global Ocean Observing System, GOOS. The Agenda is furthermore open to the priorities of Member States. Based on its discussions the IPHAB will recommend to the IOC Assembly a work plan for the IOC HAB Programme for 2008-2009.

Patricio Bernal, Assistant Director-General, UNESCO, Executive Secretary, IOC.

Future events

MARCH 2007

6th International Conference on Molluscan Shellfish Safety - ICMSS 07

March 18th- 23rd, 2007. Blenheim, New Zealand.

New Zealand is hosting the 6th International Conference on Molluscan Shellfish Safety in Blenheim.

An extensive programme is being planned, which promises a unique opportunity to share information on all aspects of molluscan shellfish safety from management of environmental contamination to innovation in laboratory methods. This conference brings together shellfish industry, regulators and research scientists from all points on the globe to provide a comprehensive programme on managing the safety of shellfish. The programme will comprise plenary lectures, scientific sessions, poster sessions, and field visits that reflect the latest trends in research and development in the shellfish sector.

More details can be found at: www.nzfsa.govt.nz/icmss07.



New Release

Manual on aquatic cyanobacteria

A photo guide and a synopsis of their toxicology, Gertrud Cronberg & Heléne Annadotter, Lund University, Sweden

Potentially harmful cyanobacteria occur widespread in the aquatic environment and this manual treats their taxonomy, identification, and toxicology across freshwater, brackish and marine environments.

Published August 2006, 110 pages
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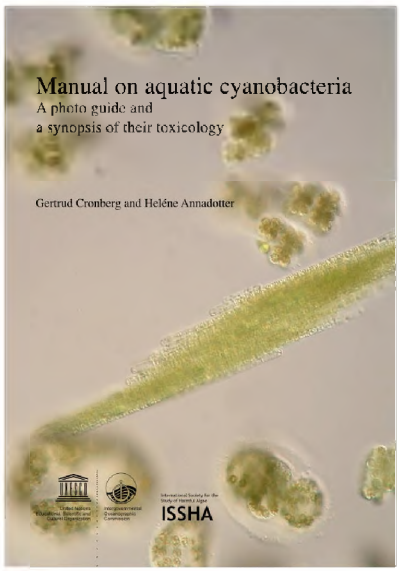

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

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• Italy

Behavioural trait of *Ostreopsis ovata* (Dinophyceae) in Mediterranean rock pools: the spider's strategy



Fig. 2. Mucilaginous networks.

Ostreopsis (Ostreopsidaceae, Gonyaulacales, Dinophyceae) is a thecate dinoflagellate occasionally planktonic but generally benthic, opportunistically attaching to different substrates (e.g. macroalgae, rocks, sediment or detritus aggregates) by forming a mucilaginous matrix within which the solitary cells can move [1].

The morphology of the genus is well known [2], yet the taxonomy is still ill-defined. Cells have a biconvex, drop-shaped theca, antero-posteriorly compressed and dorso-ventrally twisted as compared to typical dinokont cells. The life cycle includes the formation of a resting stage (cyst), probably a hypnozygote. From a nutritional standpoint *Ostreopsis* is a mixotroph, though its trophic behaviour is still poorly known. A peculiar protruding structure, the ventral pore, is thought to be involved both in prey capture in phagotrophic conditions and in mucilaginous fibril extrusion [3]. The genus is cosmopolitan in coastal or estuarine marine waters in temperate, subtropical and tropical areas, where species generally thrive in summer in environments characterised by low energy hydrodynamic conditions and high nutrient availability.

Over the last decade, people swimming or exposed to marine aerosols along the Tyrrhenian and Adriatic Coasts of the Mediterranean Sea have reported a series of symptoms (rhinorrhoea, bronchoconstriction, coughing, fever, dermatitis). These symptoms have been

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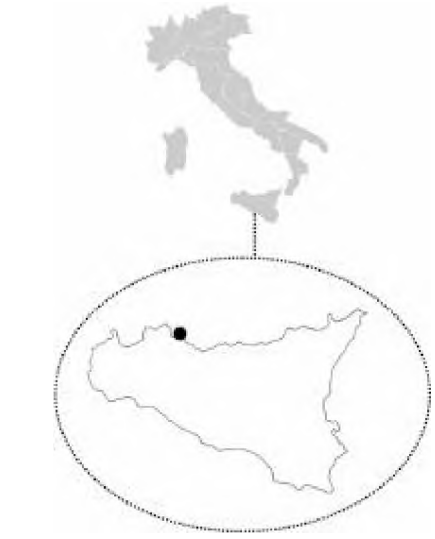

Fig. 1. Map of sampling station.

• Scotland

A prolonged *Karenia mikimotoi* bloom in Scottish waters in 2006

Karenia mikimotoi (Fig. 1) was first recorded in European waters in Norwegian fjords [1], and has subsequently been identified as a member of the phytoplankton community in various parts of North West Europe [2]. Most recently, in 2005, a major *K. mikimotoi* event occurred to the west of Ireland, with pelagic and benthic mortalities reported by Silke *et al.* [3].

Karenia mikimotoi has regularly been identified in Scottish waters, but with few major environmental consequences. However, a red tide in a number of sea lochs of the Firth of Clyde in 1980 was associated with fish deaths in Loch Fyne [4, 5]. Subsequently, *K. mikimotoi* assumed reduced

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associated with high densities of benthic microalgae belonging to the genus *Ostreopsis* (Dinophyceae), and mainly to the species *O. ovata* Fukuyo. Toxin analyses of the two species identified in the Mediterranean area, *Ostreopsis ovata* and *Ostreopsis* cf. *siamensis*, have shown the presence of putative palytoxin [4, 5]. Toxins could accumulate in the trophic web, thus representing a potential although so far undefined sanitary risk. Given the relevance of these phenomena, the network BenTox-net (**BEN**thic potentially **TOX**ic microalgae **NET**work) has been established among scientists from various Italian laboratories [6].

Along the north-western coasts of Sicily (Italy), similar symptoms were reported in summer 2005 and 2006. These cases were more widespread and intense in July and August 2006, when they had a significant impact on economic activities related to tourism. Cases were registered along the littoral of Palermo and Trapani. On those occasions, the Regional Agency for Environmental Protection (ARPA-Sicilia) recorded high densities of *Ostreopsis ovata* both on macroalgae and in the water column. Records of the presence of *Ostreopsis* spp. in Sicily date back to 2001 along the eastern coast (*Ostreopsis* cf. *siamensis* Schmidt) [7] and to 2003 (*Ostreopsis* sp.) along the western coasts (unpublished results).

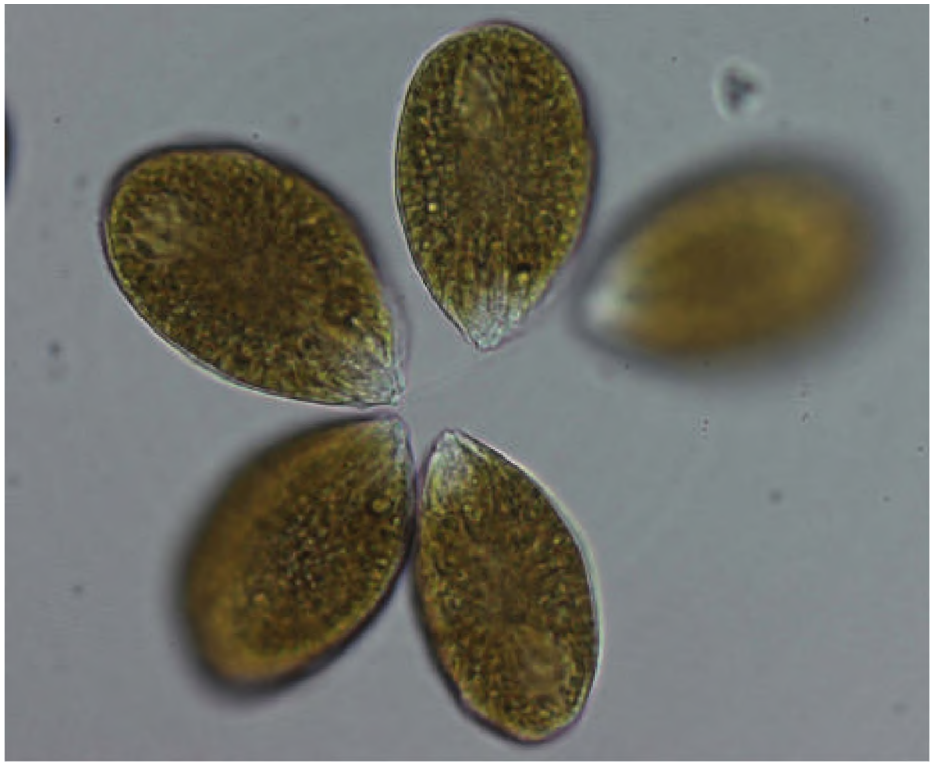

Fig. 3. Ostreopsis *cells extruding exopolymeric substances from the ventral pore.*

Research on the seasonal dynamics of *Ostreopsis ovata* started in autumn 2006 at a sampling station (38° 06' 23.3'' N, 13° 29' 58.9'' E) on the coast of Sicily (Fig. 1), where respiratory problems were reported in summer 2005 and 2006. The coastline is characterized by many shallow (5-10 cm) rock pools with a pebbly bottoms. Macroalgae dominating the pools were Chlorophytes (*Ulva rigida*, *Chaetomorpha linum*) and Rhodophytes (*Corallina elongata*, *Pterocladiella capillacea*). A number of potentially toxic dinoflagellates were identified on the macroalgal surfaces, including *Amphidinium carterae*, *Amphidinium operculatum*, *Coolia monotis*, *Ostreopsis ovata* and *Prorocentrum lima* [8].

During this study, *in situ* and laboratory observations on live samples led to the detection of a peculiar behavioural strategy of *Ostreopsis ovata* cells in relation with the production of the mucilaginous matrix [9]. *In situ*, in low turbulence conditions, an extended network of reddish mucilaginous strings bridging all the different kinds of available substrate floated in the morning probably due to oxygen bubbles trapped in it (Fig. 2). Microscopical analyses of the network showed a dense population of *Ostreopsis ovata* freely swimming in this mucilaginous matrix. Samples of living macroalgae collected during this event and placed in a large tank showed within a few minutes many reddish mucilaginous threads bridging drifting algae and the tank's walls. Light microscope observations showed *Ostreopsis* cells extruding exopolymeric substances from the ventral pore (Fig. 3) and cooperating in the rapid formation of the strings (Fig. 4). Small

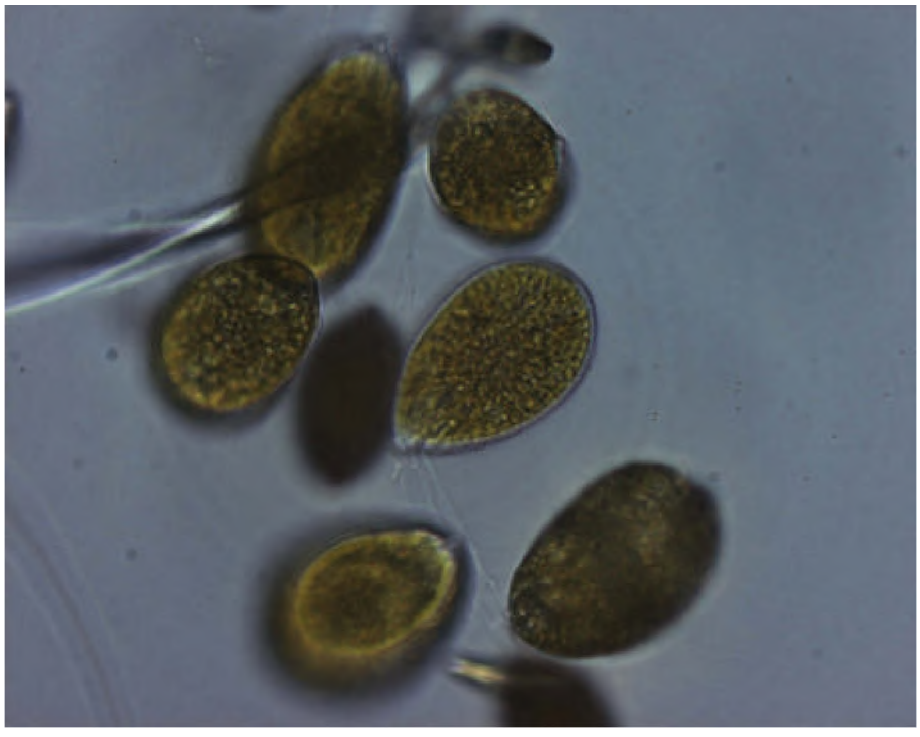

Fig. 4 Ostreopsis *cells cooperating in the formation of strings.*

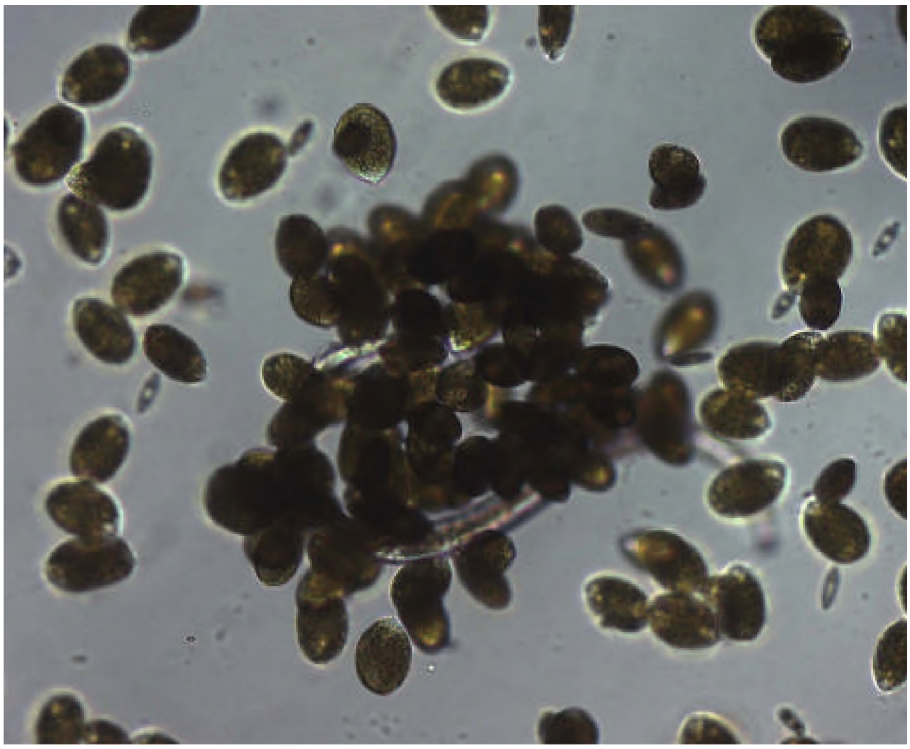

Fig. 5. Ostreopsis *cells jumping on a potential predator.*

organisms were seen trapped in the newly formed networks, including potential predators of *Ostreopsis* (e.g. nematodes). At once and amazingly, hundreds of *Ostreopsis* cells jumped on the partially immobilised organisms (Fig. 5). Two available videos reveal the useless efforts of attacked organisms (nematode and copepod) to rid themselves of *Ostreopsis*. This is the first record of the aggressive behaviour of *Ostreopsis ovata*. Similar actions are detected in many dinoflagellates, such as the heterotroph *Pfiesteria* [10]. A ventral "peduncle-like" sticky structure was observed in some specimens of *Ostreopsis* (Fig. 6) and could play a role in a hypothetical micropredation. However, *Ostreopsis* can also produce high toxin concentrations with allelopathic effects like the haptophyte *Prymnesium* [11]. Is the observed attack strategy of *Ostreopsis* a predation or a defence mechanism? Further studies are needed to answer this question. Grazing impact reduction and/or feeding strategy are therefore possible hypotheses to consider in addition to other suggestions (e.g. anchoring to the substrate, intercellular communication) on the complex role of the mucilaginous matrix produced by *Ostreopsis*.



Fig. 6. Ostreopsis with a ventral 'peduncle-like' sticky structure.

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Mucilaginous networks were described in *Ostreopsis heptagona* by Norris at the Saint Andrews conference (Norris DR *et al* 1985: *In*: *Toxic Dinoflagellates*. Anderson DM *et al* (Eds.), Elsevier: 39-44. See also Congestri R *et al* 2006. *Harmful Algal News* 32: 11.

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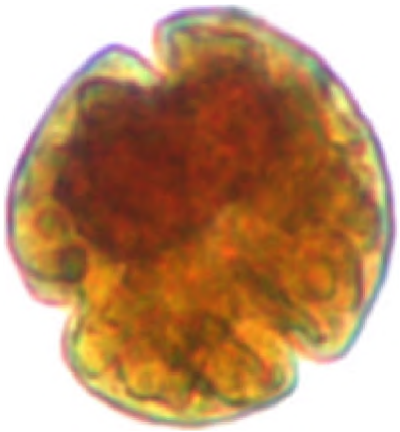

Fig. 1. Karenia mikimotoi *cell from SW Skye, collected mid June 2006.*

Table 1. Mortalities of marine organisms reported to SEPA and thought to be related to the K. mikimotoi *bloom.*

Date	Location	Organisms Affected
17/07/06	Lochaber	Crabs
04/08/06	South Uist	Heart urchins
05/08/06	South Uist, North Uist	Lugworms (*Arinicola marina*)
12/08/06	Orkney	Cockles and lugworms
		Diver reported mortalities of sea scorpions and conger eels in Scapa Flow
12/08/06	Lochaber	Crabs, mussels, cockles and lugworms
12/08/06	Wester Ross	Starfish and lugworms
12/08/06	NW Skye	Fish, crustaceans and invertebrates
12/08/06	Lewis/Harris	Crabs and lobsters (asphyxiated)
12/08/06	Orkney/Shetland	Fin fish kills
16/08/06	NW Skye	Fish deaths
18/08/06	Orkney	Flatfish, lugworm, cockles, *Echinocardium* sp., *Echinus* sp.
21/08/06	Orkney	Sea scorpions, conger eels, *Echinus* sp., sunstar, crabs, scallops
23/08/06	Golspie, E Sutherland	Lugworms and polychaetes
24/08/06	NW Sutherland	Divers report mortalities offish and scallops

significance in Scottish waters for nearly two decades, with the next recorded blooms of red tide proportions being in 1999 in Orkney and in 2003 in the Orkney and Shetland Islands. However, somewhat prophetically, Smayda [6] noted that *K. mikimotoi* must be recognised as posing a major threat to aquaculture in Scotland, despite its insignificant adverse impact to date.

Phytoplankton samples are currently collected on a regular basis from 31 sites around the Scottish coast as part of the monitoring programme for the presence of toxin-producing phytoplankton, funded by the Food Standards Agency Scotland, and operated by the Scottish Association for Marine Science (SAMS). The programme monitors species or genera of possible concern to shellfish safety. However, the operation of this programme also allows enumeration of other potentially harmful phytoplankton, including *K. mikimotoi*. Phytoplankton samples are also collected from six sites by Fisheries Research Services (FRS), Aberdeen, as part of their coastal ecosystem monitoring programme, where full community phytoplankton analysis is performed, generating long-term time series in order to examine the effects of climate change on the phytoplankton community. In both programmes, Lugol's iodine-fixed samples were enumerated using the Utermöhl technique.

In 2006, these monitoring programmes indicated the presence of a dense bloom of *K. mikimotoi* around the Scottish coast. The organism was identified at most of the SAMS biotoxin monitoring and FRS coastal ecosystem monitoring sites regularly surveyed. However, it was not recorded from the Firth of Clyde area in Loch Fyne or in Loch Ryan in the south west, and was present on only one occasion in Loch Striven (Clyde area). Densities markedly exceeded normal "background levels" for a prolonged period at many sites; the distribution is outlined in Fig 2. The peak bloom density was 3.7 x 10^6 cells L^{-1} in Scapa Flow (Orkneys) on the 14th August.

Elevated *K. mikimotoi* densities in nearshore waters were first evident in the north in Orkney in the first week of July (Fig 3). However, this early bloom remained relatively modest in density (peaking at 3 x 10^3 cells L^{-1}) and declined to low concentrations by mid July. A second and more prolonged increase in *K. mikimotoi* was evident on the Scottish west coast in mid July, with densities of 5.3 x 10^3 cells L^{-1} in Loch Scridain, Mull, and 7.6 x 10^4 cells L^{-1} in Loch Roag, Lewis on 17th July (Fig. 3). These observations coincided with elevated chlorophyll concentrations that had previously been evident offshore from satellite remote sensing (Fig. 4a), reaching the coast at these locations (Fig. 4b). These satellite data strongly suggest that the *K. mikimotoi* bloom developed offshore and was advected

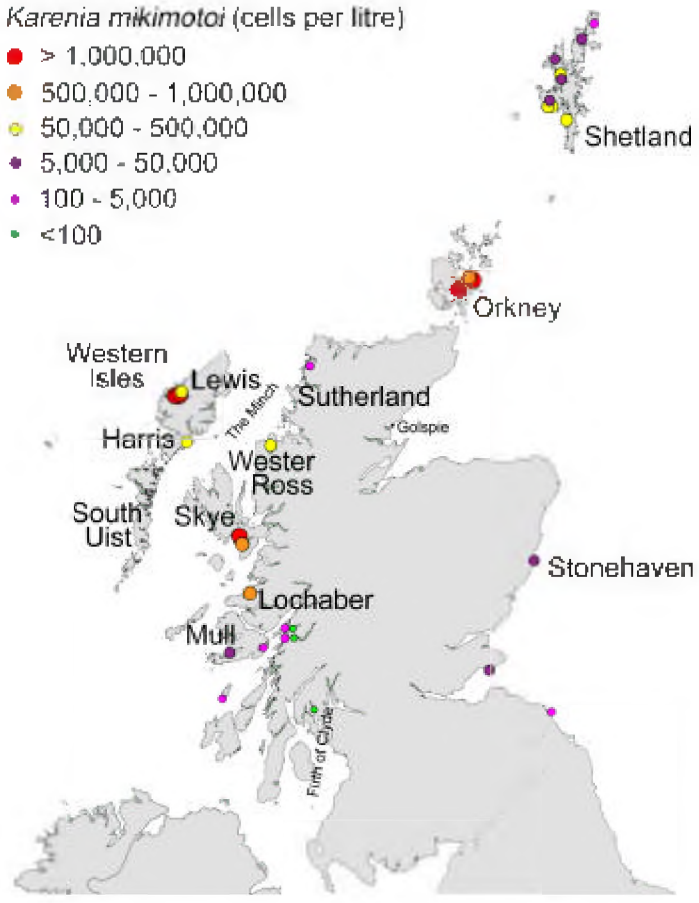

Fig. 2. Distribution of K. mikimotoi *around the Scottish Coast.*

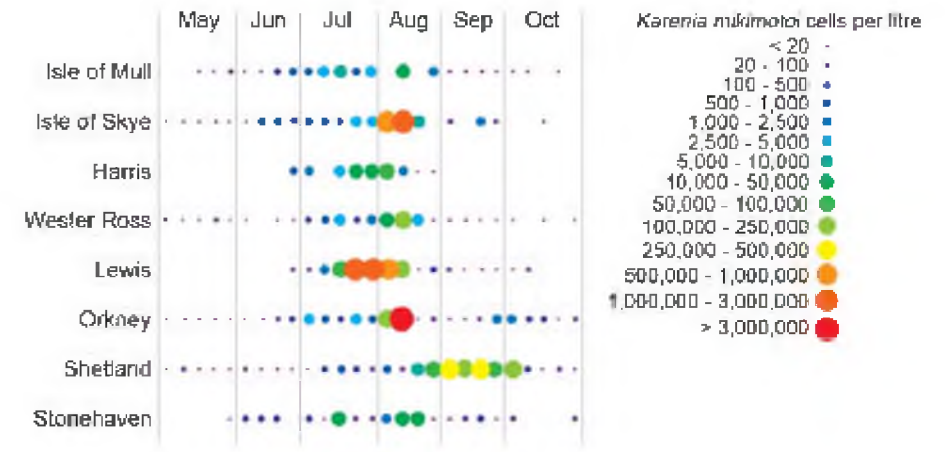

Fig. 3. Time series of K. mikimotoi *development at selected sites.*

towards the coast. Such a method of development and transport is consistent with a study of *K. mikimotoi* in Irish waters by Raine *et al.* [7].

K. mikimotoi densities showed only modest further increase in Mull but rapidly reached worryingly high densities in the Western Isles, with a value of 1.7 x 10^6 cells L^{-1} on 31st July. As the cell density declined in early August in the Western Isles, increases occurred in the Orkneys and Shetlands, and also on the north east coast at Golspie (Dornoch Forth) and Stonehaven during mid August. While the advective transport of phytoplankton in Scottish waters is relatively poorly understood in comparison with other areas [6], the progression of the bloom is consistent with cells being transported in the Scottish coastal current [8]. However, the rate of increase of cell numbers and the peak cell density at particular sampling sites (generally within sea lochs) will be related to the particular hydrological and chemical status of the site and are hence quite variable between locations.

The somewhat delayed development of the bloom at sites within the Minch, at sites in Wester Ross and Skye (Fig. 2), is at first glance somewhat at odds with this latitude based pattern. However, these observations can be related to the fact that much of the water of the coastal current fails to enter the Minch, but flows to the west of the Western Isles [9], suggesting that the seed population would have been reduced within the Minch.

The major impacts of the 2006 *K. mikimotoi* bloom were mortalities of benthic organisms due to anoxic conditions that followed the bloom. Table 1 details the faunal mortalities reported to the **Scottish Environment Protection Agency** (SEPA) and FRS during August 2006 and thought to be related to the *K. mikimotoi* bloom. The first report of mass mortalities of crustaceans was from Loch Shiel on 17th July 2006. No water sample was received and so no causative organism was identified. Public reports of mortalities of benthic organisms such as the lugworm (*Arenicola marina*), common cockle (*Cerastoderma edule*), blue mussel (*Mytilus edulis*), heart urchin (*Echinocardium* sp.), edible sea urchin (*Echinus esculentus*), common starfish (*Asterias rubens*), sun starfish (*Solaster papposus*) and king scallop (*Pecten maximus*) were common throughout August. Additionally, fishermen and divers reported mortalities of fish such as the sea scorpion (*Myoxecephalus scorpius*), conger eel (*Conger conger*) and flatfish (Pleuronectiformes) as well as mortalities of crab and lobster. Mortalities were reported from the west coast, the Western Isles of Lewis and Harris, the north coast, the Orkneys and Shetlands, and the Scottish east coast as far south as Golspie.

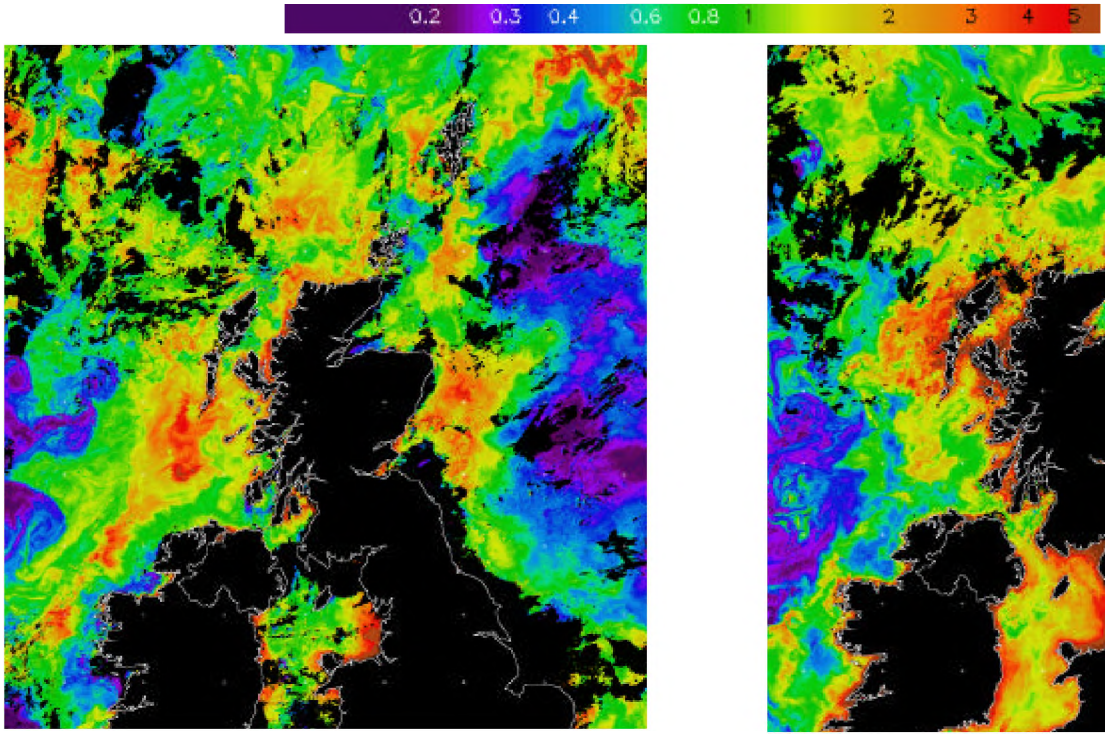

Figure 4a. 7-day composite of chlorophyll-a data from the MODIS Aqua sensor (week ending 22nd June 2006)

Figure 4b. 7-day composite of chlorophyll-a data from the MODIS Aqua sensor (week ending 13th July 2006)

Karenia mikimotoi also has the potential to negatively affect farmed fish through both the production of haemolytic cytotoxins [10, 11] and, if in sufficient density, hypoxia [12]. For some reason, the 2006 *K. mikimotoi* bloom was not associated with the same level of farmed fish mortalities as in 2003, where extensive fish mortalities (53,000 farmed fish) were reported from four sites in the Shetlands. However, aquaculture production is of great economic and social importance in Scottish waters with a value of £340 million, directly supporting 2,300 full-time equivalent jobs in the fragile rural economy of the Highlands and Islands. It is therefore important both environmentally and economically to better understand the dynamics of the 2006 bloom and thus allow early warning and mitigation of any future occurrences.

Presently, the reasons for the development of the 2006 bloom remain unclear. However, it is possible that remnants of the 2005 Irish *K. mikimotoi* bloom were able to over winter on the shelf in the manner suggested by Raine *et al.* [13] to provide the seed population. The unusually warm summer and resultant elevated sea temperatures and favourable winds may then have provided suitable conditions for bloom development and subsequent advection to the Scottish coast.

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• Mexico

Bloom of *Pseudo-nitzschia fraudulenta* in Bahía de La Paz, Gulf of California (June-July 2006)

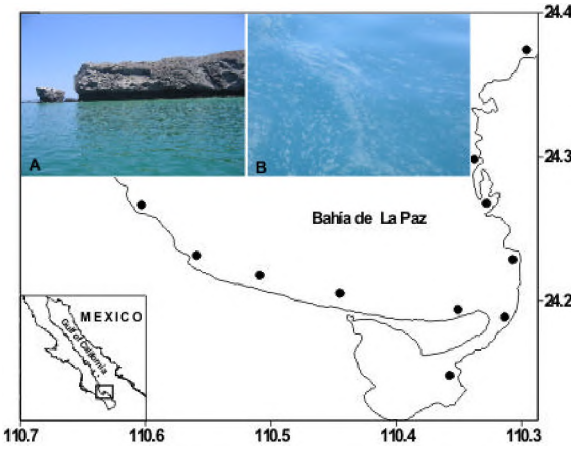

Fig 1. Sampling stations along the shore of Bahía de La Paz, Gulf of California (●); Inset A. General view of the bloom; Inset B. Close up of the surface during the Pseudonitzschia *bloom. Insets are bloom pictures taken from a boat.*

Records of harmful algae blooms (HABs) have increased in the past 15 years in the Gulf of California [1, 2]. Natural events, as well as human activities have contributed to this increase; however, some of the increase could be related to an increase of articles on this subject. The ciliate *Myrionecta rubra* and dinoflagellate *Gymnodinium catenatum* are the main blooming species in the Gulf of California. Bahía de La Paz is located on the southwestern coast of the Gulf of California, one of the most important bays to the local economy because of shellfish extraction and fisheries. HABs are frequent and periodic in Bahía de La Paz. More than 25 species are involved [2]. Recently, some diatoms and raphidophytes have been included in the list of blooming species in this area [2, 3].

From 10 June to 28 July 2006, green and green-brown patches appeared on beaches along Bahía de La Paz (24°062' -24°112' N, 110°192' -110°262' W). Water samples were collected in plastic bottles for testing nutrients (NH_4, NO_3, NO_2 PO_4, and SiO_4), pigments, and identifying and measuring abundance of phytoplankton species. Phytoplankton samples from the bloom areas were fixed and preserved with Lugol's solution for identification and cell counts. Cell counts were made in 5-ml settling chambers under an inverted phase contrast microscope. Net phytoplankton samples were taken using a net 20 µm mesh. A sub-sample of 30 ml was filtered (filters Whatman GF/F) to determine the presence of domoic acid. *Pseudo-nitzschia* species were identified to species level using scanning electron microscopy (SEM). Analyses of toxins were carried out with an HPLC-MS API 165 PE SCIEX according to the procedure in Hummert

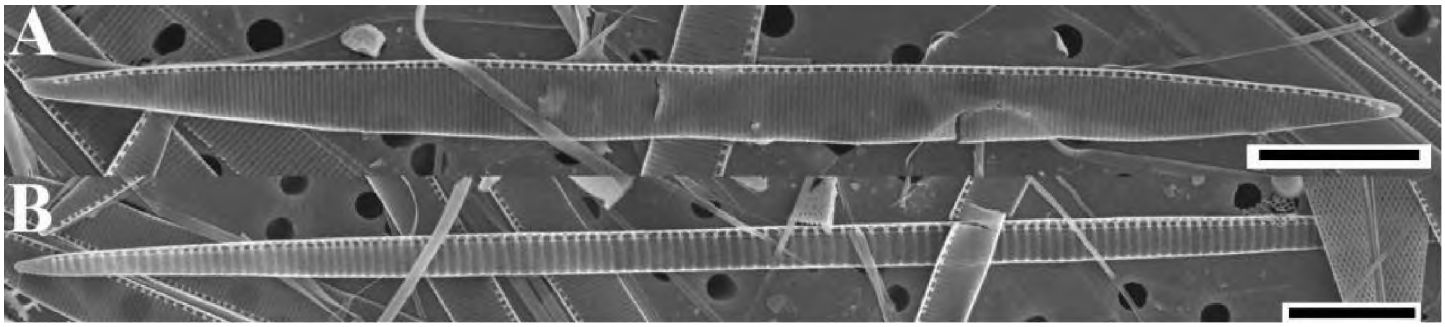

Fig. 2. A) Pseudo-nitzschia fraudulenta, *B),* Pseudo-nitzschia pungens *(images courtesy of Jim Ehrman, Digital Microscopy Facility, Mount Allison University, Sackville, New Brunswick). Scale bar 10 μm.*

et al. [4]. The detection limit of the method is 3 ng DA absolute.

This bloom was observed along the Bahía de La Paz shore where temperature ranged from 18.5 to 26.5ºC (Fig. 1). Nutrient concentrations during the bloom were (in μM): NH_4 (<0.5), NO_3 (0.1-2.71), NO_2 (0.1-0.183), PO_4 (0.073-1.44), SiO_2 (2.94-35.5). Dissolved oxygen varied from 6.60-8.42 mg/ml. Phytoplankton biomass (in mg/m^{-3}) and pigment composition was composed mainly of chlorophyll *a* (1.03 to 23.03) and fucoxantin (0.36 to 6.38). During the first week of this bloom, we measured the highest densities of *Thalassiosira* sp. ($1.24–1110\times10^3$ cells L^{-1}). Fish mortality occurred from obstruction of gills. Several tons of dead fish were removed from the beaches during the first and the second week. Simultaneous with the *Thalassiosira* bloom, *Pseudo-nitzschia* became abundant forming a new bloom. *Pseudo-nitzschia fraudulenta* was by far the most abundant species (Fig. 2A). Average valve measurements were(in μm): length (87.2), width (5.02), striae/10μm (2.46), fibulae/10μm (1.38), poroids/1μm, (5.26). *P. pungens* (Fig. 2B) and a *P. pseudodelicatissima–* complex were also present, but were scarce. Highest abundances of *Pseudo-nitzschia* spp. varied from 5 to 13×10^5 cells L^{-1}. *Pseudo-nitzschia fraudulenta* is considered an uncommon form in the Gulf of California [5]. The bloom occurred under upwelling conditions characteristic of this season [6], accompanied by low temperature (18.5 to 19°C) at the start of the bloom. These temperatures are low, compared with typical seasonal water temperatures of 26 to 28°C in June, which were measured at the end of the bloom. Chain-forming diatoms of medium and large size, such as *Pseudo-nitzschia* spp., and *Thalassiosira* spp. dominate spring and summer upwelling events in coastal waters [7]. This suggests that *P. fraudulenta* could be considered as a blooming-species characteristic of upwelling systems.

Other bloom-forming species collected during sampling were *Gymnodinium catenatum*, *Cochlodinium polykrikoides*, *Polykrikos kofoidii*, *Ceratium furca*, and *Peridinium quinquecorne*. Domoic acid was detected in net phytoplankton samples (24 to 52 ng/filter). *P. fraudulenta* has recently been demonstrated to produce domoic acid [8]. Domoic acid, the agent causing amnesic shellfish poisoning (ASP), occurs in at least eight species in this genus [8, 9]. Because of the high densities of *P. fraudulenta* in samples we believe this species was the main source of domoic acid in net phytoplankton samples. However toxicity of *P. fraudulenta* must be confirmed in laboratory strains because no domoic acid was detected in cultures of this species from the Gulf of Naples [10]. The highest concentration of DA in the chocolata clams (*Megapitaria squalida*) was only 0.55 mg kg^{-1}. *P. fraudulenta*, *P. pungens* and *P. pseudodelicatissima* have been reported to produce low levels of domoic acid [8, 9]. The action limit for the amount of DA allowed in molluscan shellfish was established at 20 mg kg^{-1}. During this *Pseudo-nitzschia* bloom, many schools of sardine were feeding on this diatom, and in turn, hundreds of brown pelicans were feeding on sardine. However, no die-offs of sardines or pelicans were observed. This is the first bloom of *P. fraudulenta* containing domoic acid recorded in this region. We continue to monitor *Pseudo-nitzschia* species in Bahía de La Paz and other embayments from the Gulf of California and collect different strains to determine their levels of toxicity.

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• Angola

Pseudo-nitzschia spp. *and Prorocentrum micans* blooms in Luanda Bay, Angola

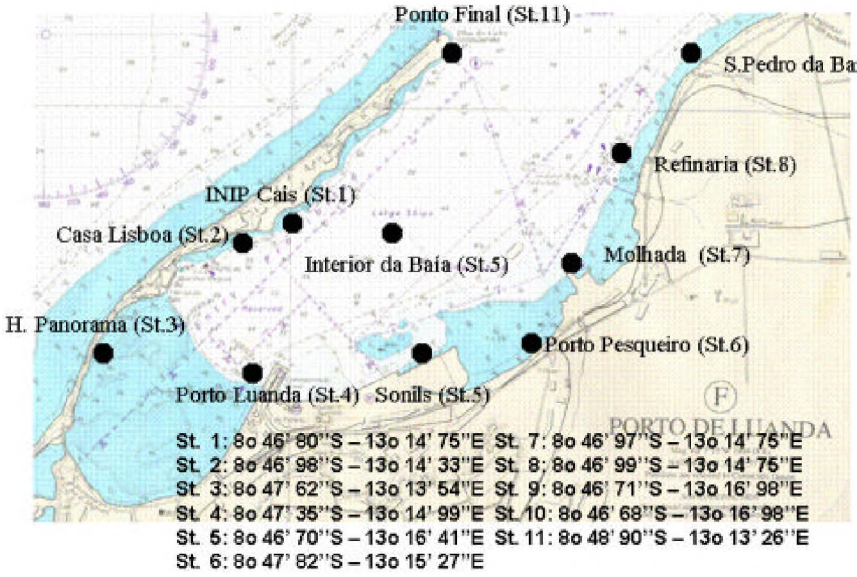

Fig. 1. Map of sampling location in Luanda Bay

In May-June 2006, two red tides with mass mortalities of fish, cephalopods, crabs and lobsters occurred in Luanda Bay. In the first bloom on 24 June, with sea temperature around 22ºC and salinity 27, *Pseudo-nitzschia* spp dominated, with maximum concentrations of the 670 x 10^3 cell L^{-1}. The second event was dominated by *Prorocentrum micans* with maximum concentrations of 5 x 10^6 cell L^{-1}. The latter bloom with brown color water, extended throughout the, Bay and for 2 days caused mortalities of pelagic and demersal fish, a cephalopod, crabs, and lobsters (Table 1).

Surface samples were collected at 11 stations in Luanda Bay on 24 May and 13 June 2006 (Fig. 1), fixed immediately in 2% formol, or kept alive for some hours to help species identification. The Utermöhl technique was followed, using an inverted microscope with phase contrast "Axiovert 200". Cells counts are shown in Table 2.

Some species of *Pseudo-nitzschia* produce domoic acid, an amino acid with high neurotoxic activity in humans [1-3]. In the Benguela current, *Pseudo-nitzschia* species occur, but the presence of domoic acid has not been observed [4]. On 13 and 14 June 2006, water temperature was about 25ºC and salinity 33. At this time, the dinoflagellate *Prorocentrum micans* dominated the bloom with mean concentrations of 2.8 x 10^6 cell L^{-1} and 0.57 x 10^6 cell L^{-1} on 13 and 14 June respectively (Fig. 2).

In this second event, the first dead organisms were detected on the morning of 13 June; the mortality intensified during the day in the whole Bay, and decreased on 14 June in the coastal areas (Fig. 3). This event caused panic amongst the

Table 1. Species identified in the mortality (13-14 June 2006).

Demersal fish
Hemiramphus brasiliensis
Citharichthys stampflii
Muraena melanotis
Dentex barnardi
Brachydeuterus auritus
Lycodontis mareei
Acanthurus monroviae
Cephalopholis sp.
Epinephelus sp.
Bodianus speciosus
Pomadasys incisus
Bothus podas
Neoniphon sp.
Stegastes sp.
Balistes capriscus
Conger conger
Scarus hoefleri
Pelagic fish
Sardinella maderensis
Trachurus trecae
Liza falcipinnis
Laeops sp.
Spicara nigricauda
Cephalopods
Sepia officinalis
Crustaceans
Panulirius regius
Callinetes marginatus
Callinetes amnicola
Callinetes pallidus

Table 2. Densities cell L^{-1} in different stations in Luanda Bay.

Station	*Pseudo-nitzschia* spp 10^3 cell L^{-1}	*Prorocentrum micans* 10^6 cell L^{-1}
1	0	2.35
2	1.1	0.14
3	0	0.37
4	667	1.66
5	72	2.87
6	251	0
7	0	0.13
8	0	3.28
9	0	5.0
10	0.7	0.10
11	317	0.04

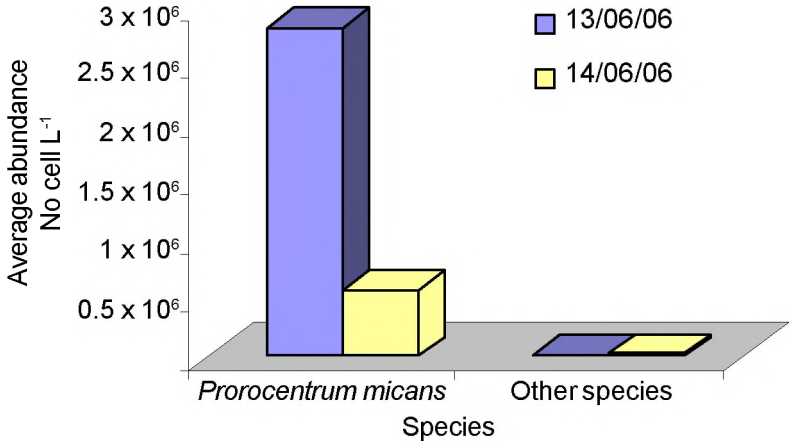

Fig. 2. Abundance of Prorocentrum micans *on 13 and 14 June, 2006*



Fig. 3. Dead fish floating.

fishermen as more species became involved (Table 1). *Prorocentrum* has previously been identified as a cause of fish mortalities in Angola [5]. *Prorocentrum micans* is not regarded as toxic, but in high concentrations can cause anoxia as bloom material decays. A similar event was recorded in St. Helena Bay, South Africa, in 1994, in which *Prorocentrum micans* associated with others species was associated with mortality of several fish species [6]. In this event the death of marine fauna was caused by anoxia or poisoning by hydrogen sulphide generated by anaerobic bacteria.

On 24 May and 13-14 June, *Prorocentrum micans* was widely distributed in the Bay, with a maximum concentration of $5x10^6$ cell L^{-1} at St. 9, followed by Stations 8, 5, 1, and 4. The The lowest concentration was at St. 11 with 38.9×10^3 cell L^{-1} (Table 2). The event of May, dominated by *Pseudo-nitzschia* spp was restricted to a smaller area. The largest concentration was at St. 4 with 66.7×10^3 cell L^{-1}, followed by St. 11 with 317×10^3 cell L^{-1} and St. 6 with 251×10^3 cell L^{-1} (Table 2).

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• Sri Lanka

Microcystin producing *Microcystis aeruginosa* in Lake Beira, Sri Lanka

Cyanobacteria or blue green algae are aquatic and photosynthetic prokaryotes which form harmful algal blooms (HAB) under optimal conditions. Of the more than 50 genera of freshwater cyanobacteria identified, 12 are capable of producing toxins [1]. Mass presence of these organisms in freshwaters is of increasing concern worldwide due to their production of a range of hepatotoxins and neurotoxins. *Microcystis* is the most common of these toxic cyanobactria. *Microcystis* occasionally forms dense aggregations of cells that float on the surface of the water forming a thick layer or 'mat'. These blooms potentially affect water quality as well as the health of human and natural resources. Decomposition of large blooms can lower the concentration of dissolved oxygen in the water resulting in death of fish in the water bodies.

Sri Lanka has thousands of man made 'tanks' or reservoirs. One of these, Lake Beira (6º 56' N & 79º 51'E),



Fig. 1. Lake Beira.

is a unique landmarks of Colombo and a historic relic from its colonial past. Today it covers 65 hectares with a catchment area of 448 hectares. Previous reports on water blooms have revealed the occurrence of toxic cyanobacteria in fresh water bodies of Sri Lanka, including Lake Beira [2].

In May 2006, heavy growth of algae and large numbers of fish deaths were recorded in Lake Beira. According to the National Aquatic Resources Agency (NARA), Sri Lanka, no epidemic was found among the fish, and oxygen deficiency, especially in the early hours of the day, caused the deaths.

Therefore, a study was carried out to determine whether any toxic cyanobacteria were present in the algal bloom. A molecular technique was used to identify cyanobactria present in the Lake waters. Water samples were collected aseptically in sterile brown glass containers on 24th May 2006. These samples were collected from different sites and cultures originated from the bloom analyzed. Presence of the gene tested (the *mcy*E gene, which encodes the glutamate activating adenylation domain of the microcystin synthetase gene cluster) in the samples indicated that the algal strains isolated have the potential to produce microcystins, and the species present was identified as *Microcystis aeruginosa* (EF051238 and EF051239). Thus the cause of the fish deaths could have been either cyanophyte toxins or oxygen depletion. By combining microscopy with molecular techniques, the presence of toxigenic cyanobacterial in raw water sources and recreational waters can easily be monitored.

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• Spain

Mucilage event associated with *Gonyaulax fragilis* in NW Mediterranean Sea

Table 1. Environmental parameters observed in surface water of some stations located in the area affected by mucilage (for beaches n=10 and offshore stations n=4).

	Beaches Min-Max (Avg)	**500 m offshore** Min-Max (Avg)
Date	19 and 26/07/06	12 and 13/07/06
Temperature	28-30.5 (29.02)	20.07-21.7 (20.99)
Salinity	37.6-38.2 (37.87)	37.32-37.99 (37.76)
PO4 (µM)	0.05-0.51 (0.13)	0.02-0.08 (0.05)
NH4 (µM)	0.06-1.48 (0.85)	0.51-0.79 (0.62)
NO2 (µM)	0.05-0.63 (0.15)	0.07-0.08 (0.08)
NO3 (µM)	0.46-17.55 (3.19)	0.24-0.25 (0.24)
H4SiO4 (µM)	0.04-5.82 (2.20)	0.41-0.86 (0.67)
Mean daily wind speed*	0.3-1.2 (0.6)	
Max. wind speed*	10.4	

* *Data set from two meteorological stations located near the affected area.*

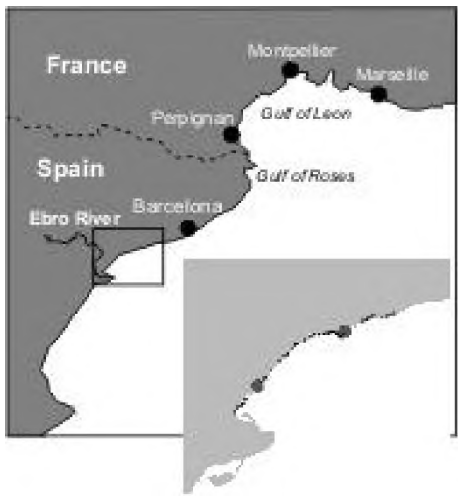

Fig 1. Area affected by mucilage (grey grid) along the Catalan coast. Dots in the lower map show the sampling sites; big dots= mucilage samples, small dots= water samples.

The accumulation of mucilage in coastal waters may have important consequences for local fishing and tourist industries. Sporadic episodes of mucilage production have been observed for many coastal waters worldwide, and at some locations, like the Adriatic basin (Mediterranean Sea) and Tasman Bay (New Zealand), such events have been recorded as recurrent. In the Adriatic Sea, the formation of mucilage has been historically attributed to extracellular organic matter produced mainly by diatoms [1, 2]. However, some studies suggested that the appearance of mucilage might also be related to the dinoflagellate *Gonyaulax fragilis* (Schütt) [3, 4]. Pompei *et al.* [4] observed the constant concomitant presence of *G. fragilis* and mucilage formation, and detected the capacity of this organism to produce large amounts of extracellular carbohydrates in culture. In addition, Pistocchi et al. [5] found similarities in the composition of monomeric carbohydrates of *G. fragilis* exudates in culture (especially when grown in f/2 medium) and those analyzed in mucilage derived from natural waters. In Tasman Bay, mucilage accumulation was attributed to the polysaccharide exudates of *Gonyaulax hyaline* (Ostenfeld et Schmidt) [6], which is very similar to *G. fragilis*. In the Western Mediterranean Sea, mucilage events associated with *G. hyaline* were reported for coastal waters of Andalusia (South Spain) during autumn 2002 [7]. Along the Catalan coast (NE Spain), the presence of mucilage was observed in many occasions, but its detection was never associated with a specific phytoplankton species.

Between 10 July and 4 August 2006 aerial monitoring of the Catalan coast (performed on a daily base during summer months) revealed several aggregates of mucilage along a 65 km section (Fig. 1). In general, this mucilage presented a green-brownish colour (Fig. 2) and its formation occurred after a long period of calm weather conditions associated with high atmospheric pressure. The environmental variables considered during this event are presented in Table 1.

In the context of the Harmful Algae Monitoring Programme (initiated in 1995), mucilage was collected from two

aggregates, separated by a distance of 25 Km, on 27 July. One of them was located near the coast (Arenal beach), whereas the other was located 500 m offshore of the Pineda beach (Fig. 1). Samples were fixed with Lugol's solution and examined under an inverted microscope equipped with epifluorescence detection. *G. fragilis* was identified using the epifluorescence mode after the addition of Calcofluor [8] directly into the sedimentation chamber (Fig. 3).

Microscopic analysis revealed that *G. fragilis* and *Pseudo-nitzschia calliantha* (identified using the SEM technique) were the two most abundant species in both mucilage samples collected (with maximum abundances of 2.27 x 10^6 and 23 x 10^6 cells L^{-1}, respectively). Other organisms were also abundant, including *Chaetoceros* spp. in the Arenal sample and *P. caciantha*, *Ostreopsis* sp. and some species of gymnodinioids in the Pineda sample. In the latter, many additional species were detected at low concentrations. Several of the *G. fragilis* cells present in the mucilage showed sticky material leaving from their apical part (Fig. 3).

High abundances of *Pseudo-nitzschia* spp. are commonly observed along the Catalan coast [9]. In contrast, *G. fragilis* has only been recorded in few cases, and always at very low densities [10, 11]. However, during the above mentioned mucilage event, *G. fragilis* occurred along 60% of the beaches monitored in the area (Fig. 1), with a maximum abundance of 2160 cells L^{-1}.

This is the first study reporting exceptionally high *G. fragilis* cell numbers along the Catalan coast. Moreover, it is the first record indicating a wide distribution of this species in the area. Based on these two observations, the formation of mucilage was most likely associated with *G. fragilis*. Additional studies are, however, needed to confirm this association and to evaluate possible contributions by species from other taxonomic classes simultaneously detected.



Fig. 2. Aerial and coastal photographs of mucilage (from ACA)

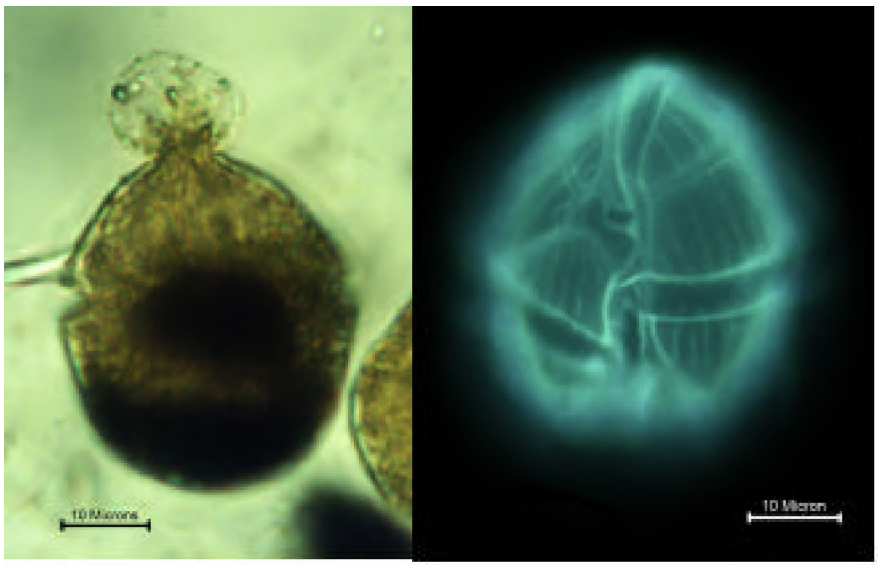

Fig. 3. Light and epifluorescence microscopy photographs of G. fragilis. *Note the sticky material leaving from their apical part.*

Acknowledgements

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• Italy

Bloom of *Ostreopsis ovata* on the Conero Riviera (NW Adriatic Sea)

At the end of September 2006, an unusual bloom of the benthic dinoflagellate *Ostreopsis ovata*, occurred on the Conero Riviera, a rocky promontory along the sandy coast of the NW Adriatic Sea. The bloom was observed as a brown, velvet-like mat, covering natural and artificial rocks, seaweed thalli, and mollusc shells. Due to the weak association of microalgal cells with the substrate, the mat was easily resuspended in the water column by waves and mechanical action. The bloom persisted for 30-40 days and by mid-November, only a sporadic presence of *O. ovata* was observed.

The occurrence of *O. ovata* in microalgal communities of western Italian coasts is known since the 1980s, when it was reported in the Tyrrhenian Sea [1, 2]. In the last decade, bloom events became more frequent, and their occurrence in summer in the Tyrrhenian and Ligurian Seas was associated with death of benthic organisms and/or human health problems such as respiratory difficulties and skin irritation [3-6]. *O. ovata* has been reported also on the Sicily coast (Giacobbe pers. comm.), [13] and in the southern Adriatic Sea, where it has been observed since 2001 [7]. This is the first report for the northern Adriatic basin. Moreover, in the same period, *O. ovata* has also been observed on the rocky coast of the Gulf of Trieste, NE Adriatic Sea (Cabrini & Monti, pers comm.).

O. ovata is a producer of palytoxin-like compounds [8]. Due to its appearance at the end of summer, when tourist affluence was decreasing, the bloom was apparently not associated with health problems, although unknown toxicity, not due to other common toxins of this area, such as YTX and DSP, was detected in mussels in the same period (Poletti, pers. comm.).

In the northern Adriatic Sea, microphytobenthic communities are commonly represented by diatoms and filamentous cyanobacteria, which exhibit typical seasonal behaviour.

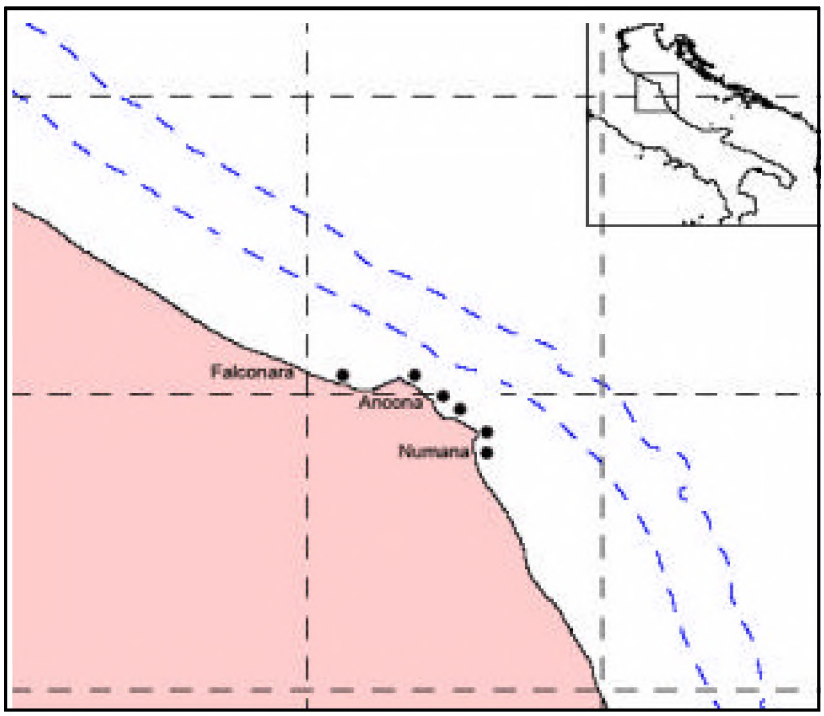

Fig. 1. Conero Riviera (Adriatic Sea) and location of sampling sites.

Benthic dinoflagellates such as *Prorocentrum lima* are rarely observed, always in summer, at low abundance, and only associated with soft sediments [9, 10].

The appearance of this bloom lead to intense sampling, from the sandy coast of Palombina to Numana harbour (Fig. 1): sampling was carried out in different areas and on different substrates and in the water column, to estimate microalgal abundance. Results show that *O. ovata* cells colonized a variety of substrates, such as macroalgae, rocks, mussel shells, and also benthic invertebrates (Fig. 2). *O. ovata* was not observed in samples collected on sandy beaches. Densities reached 6500 cells cm^{-2} on rocks and 20,000 cells g^{-1} fresh wt (corresponding to 124,000 cells g^{-1} dry wt) on seaweed samples. These values were lower than those observed in the Ligurian [5], Catalan [11], or Aegean Seas [12], but at the time of sampling the bloom was declining. Higher numbers were found on ramified filamentous (e.g. *Chondria*) and parenchymatic (e.g. *Dictyota*) algae than on laminar thalli (e.g. *Ulva*). The abundances of *O. ovata* in the water column amounted to about 2000 cells L^{-1}.

The identification and counting of *O. ovata* in the samples was carried out with an inverted microscope (Fig. 3) under epifluorescence using Calcofluor. However, due to the taxonomical uncertainties with *O. siamensis*, the diagnosis was confirmed by the molecular PCR based analysis.

Ostreopsis cells were isolated using a micropipette, and monospecific cultures were maintained in F/2 and F/4 media at 21 ± 1°C and a 14:10 h (light:dark) photoperiod. Illumination was provided by fluorescent tubes with a photon irradiance of 100 µmol photons m^{-2} s^{-1}.

Samples *O. ovata* from scraped mats and macroalgal thalli were processed for molecular analysis using the PCR based method. Genomic DNA and genus and species specific PCR assays were carried out as described by [13,14]. Genomic DNA was promptly amplified by PCR assay using genus and species-specific primers. Designed primers for the high variable

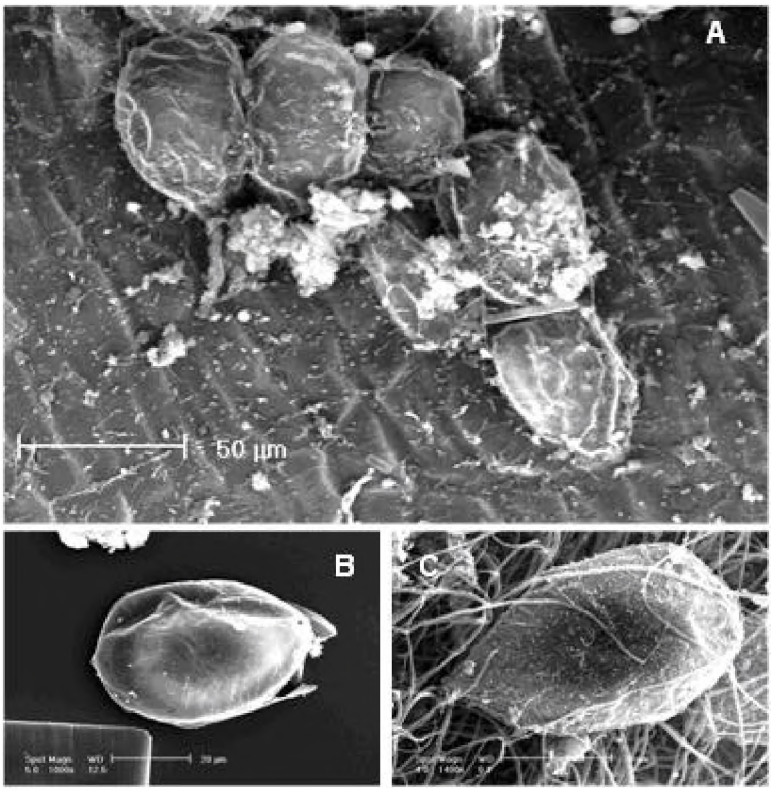

Fig. 2. SEM micrographs of Ostreopsis ovata *on different substrates: (A) Cells of* O. ovata *on brown alga* Dictyopteris polypodioides. *(B)* O. ovata *from a rocky substrate. (C) Cell of* O. ovata *on brown alga* Cystoseira compressa, *covered by filamentous cyanobacteria.*

and conserved ITS-5.8S rDNA regions for the genus *Ostreopsis* and species *O. ovata* and *O.* cf. *siamensis* gave PCR amplified products of appropriate size for the genus and species *O. ovata*: 92 bp and 210 bp, respectively. No amplified products specific for *O.* cf. *siamensis* (223 bp) were obtained. The primers showed high specificity with no other detectable bands observed, and did not amplify non-target genomic DNAs. The PCR based method applied to different kind of environmental samples proved to be a rapid and sensitive way to confirm the taxonomic identity of *O. ovata* at the species level.

Ostreopsis ovata is a common species in tropical areas. Its occurrence in Mediterranean microalgal assemblages, may be a result of a recent introduction to the Mediterranean from ciguatera areas [2]; alternatively, its presence in microphytobenthic communities may have been neglected in the past. This species seems to be expanding its range rapidly, even in colder northern areas. Its occurrence in coastal areas of the northern Adriatic Sea (Conero Riviera and Gulf of Trieste) has probably been favoured by the increased water temperatures of the last decade [15]. It clearly poses increasing risks for human health, and effects on benthic communities.

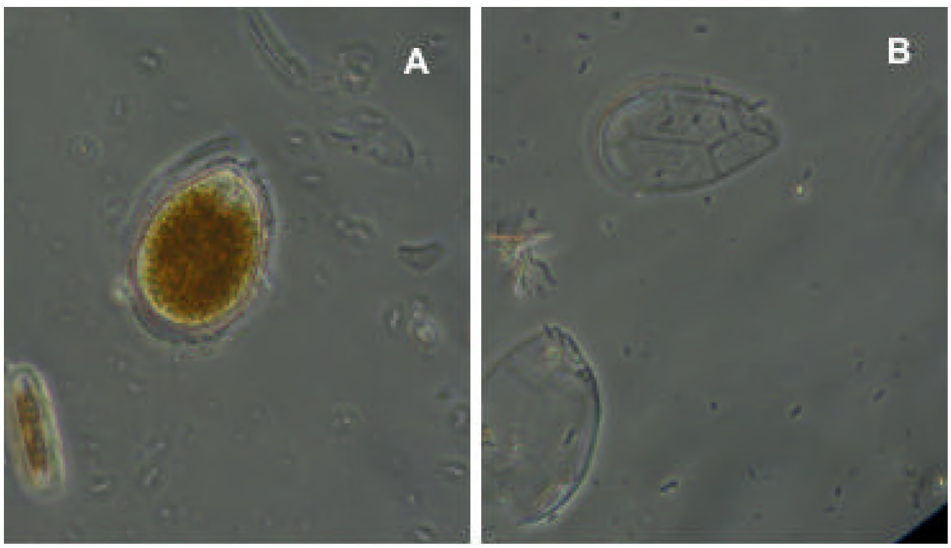

Fig. 3. Light micrographs of Ostreopsis ovata: *(A) formaldehyde fixed cell. (B) Empty cell showing thecal plates.*

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• Egypt

Toxic phytoplankton species link to invertebrate and fish mortality in the Eastern Harbour of Alexandria (Egypt) during July-August 2004-2005

The coastal area of Alexandria is subjected to direct discharge of wastewater, mainly from two land-based sources, estimated as about 7 x 10^6 m^3 d^{-1} of agricultural drainage water mixed with the overflow from Lake Mariout (main basin outfalls: about 500 x $10^3 m^3 d^{-1}$ primary treated and 300 x $10^3 m^3 d^{-1}$ untreated municipal waste water) into Mex Bay, west of Alexandria, and about 2 x 10^6 m^3 d^{-1} of industrial and agricultural waste water into Abu Qir Bay, east, which renders the sea water highly eutrophic.

The Eastern Harbour (E.H.) of Alexandria is located in the central part of the city. It is a sheltered, relatively shallow semi-enclosed marine basin. It was a recipient of huge amounts of municipal wastewater during the last three decades. Yet, there is an encouraging fact in that remedial actions have been taken by the Alexandria Governorate to close the E.H. outfalls, as well as the main sewer of Alexandria (Kayet Bey) at its western vicinity, and their share of waste water had been diverted to the Lake after primary treatment. However, due to water circulation, the harbour is still affected by the water discharge, mainly from Mex Bay (8 Km long).

During August 2004 and July-August 2005 incidents of fish and invertebrate mortality occurred accompanying long duration of visible water discoloration. According to information received from marine protection and diving organizations, the Alexandria Governorate and the environmental authority, that "marine life on the bottom of the E.H. were severely destroyed. Dead fish and stunned demersal and pelagic fishes losing their equilibrium, gasping at the surface, swimming either on their sides or upside down, suffering from disorientation and unresponsiveness to human presence, and the presence of hundreds of small crabs found dead on the sand beach or seeing migrating towards the beach at dawn or found dead in fishermen nets indicating signs of toxicity". Symptoms of anoxic condition were also observed in dead fish include yellowish coloration of the body and gills. The last cases of massive invertebrate and fish mortality occurred in the harbour during October 1994 [1], caused by *Alexandrium minutum*, with some evidences of toxicity, and during 1998-2000 attributed to the first occurrence of *Gymnodinium mikimotoi* (0.93 x 10^6 cell L^{-1} on 11 August 99, 1.8 x 10^6 cell L^{-1} on 30 May 2000, and *Chattonella antique* (0.85 x 10^6 cell L^{-1} on 27 July 99, 1.14 x 10^6 cell L^{-1} on 21 May 2000 [2]. The blooms in 1999-2000 were transferred to the harbour from Mex Bay (a highly eutrophic bay), through the idealized pattern of biological spreading, neighborhood diffusion, since hydrographic processes must be responsible. However, the present blooms during August 2004 and July-August 2005 originated from the Eastern Harbour. Now, it has become clear that 24 species monospecifically or in combination), gradually increased in the neritic waters of Alexandria, and their intensity and geographical extent seems to be on the rise; 10 of these species are recorded as toxic [3].

The present red tides maintained surface water temperature above 27.5°C to >30°C, and low salinity (34.6-37.5 psu), affected by discharged water from the west. Reduced salinity seems contributing a part to explain the development of *C. antiqua* and *G. mikimotoi* in the harbour [4]. Water column stability is a permanent feature. Generally, nutrient concentrations were relatively low, while the reverse was true for organic matter.

G. catenatum Graham became noticeable on 3 August 2004 (0.15 x 10^6 cell L^{-1}), under relatively high nutrient concentrations (1.68 µM PO_4, 3.65 µM NO_3, 3.8 µM NH_4), and low organic matter (1.46 mg L^{-1}), followed by its major peak on 7 August, declining the next day. Phosphate and nitrate concentration were reduced to a third on 8 August (0.18 µM PO_4 and 0.55 µM NO_3), ammonia was almost unchanged (3.81 µM and 3.47 µM, for the two days, respectively). These two peaks accompanied high levels of organic matter (7.5 and 8 mg L^{-1}). *G. catenatum* was first found in Alexandria waters in 1992 [5, 6].

Table 1. Species linked to invertebrate and fish mortality in the Eastern Harbour of Alexandria during 2004-2005.

Species	Year/Maximum Density (MD, cells L^{-1})			
	2004	MD	2005	MD
Alexandrium ostenfeldii	9 Aug 14-15 Aug 21 Aug	$0.11x10^6$ about $0.32x10^6$ $0.51x10^6$	 23,24, 25July 1 Aug	 $0.13x10^6$, $0.14x10^6$ and $0.52x10^6$, respectively $0.082x10^6$
Chattonella antiqua	21 August	$1.26x10^6$	25 July 13-14 Aug	$0.16x10^6$ $0.33x10^6 – 0.49x10^6$
Gymnodinium catenatum	7-8 Aug 15 Aug 17 Aug 21 Aug	$2.73x10^6$, $1.53x10^6$, respectively $1.6x10^6$ $0.99x10^6$ $1.34x10^6$	21 July 28 July 30 July 3 Aug 8 Aug 10 Aug 14 Aug	$0.38x10^6$ $0.33x10^6$ $0.56x10^6$ $0.58x10^6$ $0.62x10^6$ $0.78x10^6$ $0.62x10^6$
Gymnodinium mikimotoi	13 August	$0.16x10^6$	30 July 10-11July 14 Aug	$0.06x10^6$ $1.17x10^6 – 1.92x10^6$ $0.73x10^6$

Alexandrium ostenfeldii (Paulsen) Balech *et* Tangen was first found west of Alexandria (Mex Bay) during 1992 [7], and in the Eastern Harbour during 1998 [2]. Its maximum density on 25 July 2005, in combination with other species raised Chl. *a* content to 59.5 μg l^{-1}. Phosphate was at 0.62 μM, nitrate 1.62 μM and ammonia 4.18 μM.

Gymnodinium mikimotoi Miya et Kominami ex Oda was previously recorded in the E.H. of Alexandria in September 1998 at 22.5-27.2°C and salinity of 34-36.5 psu [2]. Densities between 0.13 x 10^6 and 1.66 x 10^6 cell L^{-1} were found from late September–early October 2000 [8].

Chattonella antiqua (Hada) Ono attained increased density on 19 August, reaching a peak two days later, accompanied Chl. *a* at 65 μg L^{-1}. Its densities during 2005 were significantly lower than that recorded in the same period during 2004. *C. antiqua* was first described and recently found in the Eastern Harbour to be most frequently at 24.5-26°C and 34.5-35 psu, and it shared a role in blooms causing mortalityduring 2001 [4].

It is concluded that:

1. The water characteristics were deeply affected by water discharge from the west. A reduction in the discharge rate is urgently needed to improve the situation, not only in the harbour, but also along the whole coast of Alexandria.
2. The sever damage of marine life in the Eastern Harbour of Alexandria during August 2004 and July-August 2005 was a direct impact of the occurrence of 6 toxic phytoplankton species.
3. The present blooms originated from the E.H.
4. The long duration of the bloom during all August could help this dramatic event.
5. Increased water temperature could be a crucial triggering factor for the rapid occurrence and development of *G. catenatum*.
6. Water stabilization was a constant ecological factor.
7. Nutrient concentrations were always low, yet nitrate and ammonia showed moderate values at times. The reverse was true for those of dissolved organic matter.
8. The consumption of inorganic nutrients signals their importance. However, high organic matter concentrations could help maintenance of the blooms.
9. The species succession and degree of dominance varied with time.
10. Toxic red tide species are on the rise in Alexandria, *A. ostenfeldii* became a red tide bloom species in The Eastern Harbour. The phenomenon has become of regular occurrence in the warm season (April - October).
11. Recurrent blooms are expected in the future.
12. The rare occurrence of undesirable species such as *Alexandrium minutum*, *Pseudo-nitzschia pungens* and *Heterosigma* sp. could inflict more damage in the near future.
13. A monitoring program must be carried out along the Egyptian Mediterranean coast to evaluate the real situation. Human health is placed at risk, ecosystems are altered, fishing and aquaculture suffer from economic losses.

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• Guatemala

Cochlodinium catenatum on Guatemala coast (2004 & 2007)

Cochlodinium catenatum (Kofoid & Swezy 1921) was first detected May 28th 2004 in Guatemala coastal waters. Satellite images from June 5, 2004 (Fig. 1A), show southern waters partially occupied by high density patches of chlorophyll (>20 mg/m^3). Although there is an area of lower density, the whole coastline of Honduras, El Salvador and the Golfo de Fonseca was completely invaded by high densities of chlorophyll (10-30 mg/m^3). This event lasted for approximately 32 days, until June 27. To confirm the presence of the species, phytoplankton samples were taken from 4 sites on June 23 (Fig. 2). Those samples confirmed dominance of *C. catenatum* with a maximum of 964,000 cells L^{-1}, and forming chains of 2-4 cells (Figs. 3 A, B). The hydrological characteristics are summarized in Table 1 and reveal high nutrient levels, possibly due to the rainy season. There are high nutrient concentrations in the continental waters that flow into the Pacific.

From January 8 until January 29, 2007, there was also a red tide event caused by the same species. Unfortunately, communication of the event was late and no hydrological data could be taken. A sample of water from the dock contained 6,970,000 cells L^{-1}. The dominating chains were formed by 4-8 cells (Fig. 3 C, D). Temperature was 28° C. The satellite image showed a bloom in the Guatemalan Pacific Region (Fig. 1B). It is pertinent to clarify that *C. catenatum* has frequently been mistaken with *C. polykrikoides*. According to Chinese and Japanese studies [1], three morphotypes are recognized: *C. convolutum*, *C. polykrikoides* and *C. sp. (=C. catenatum*). Those morphotypes are

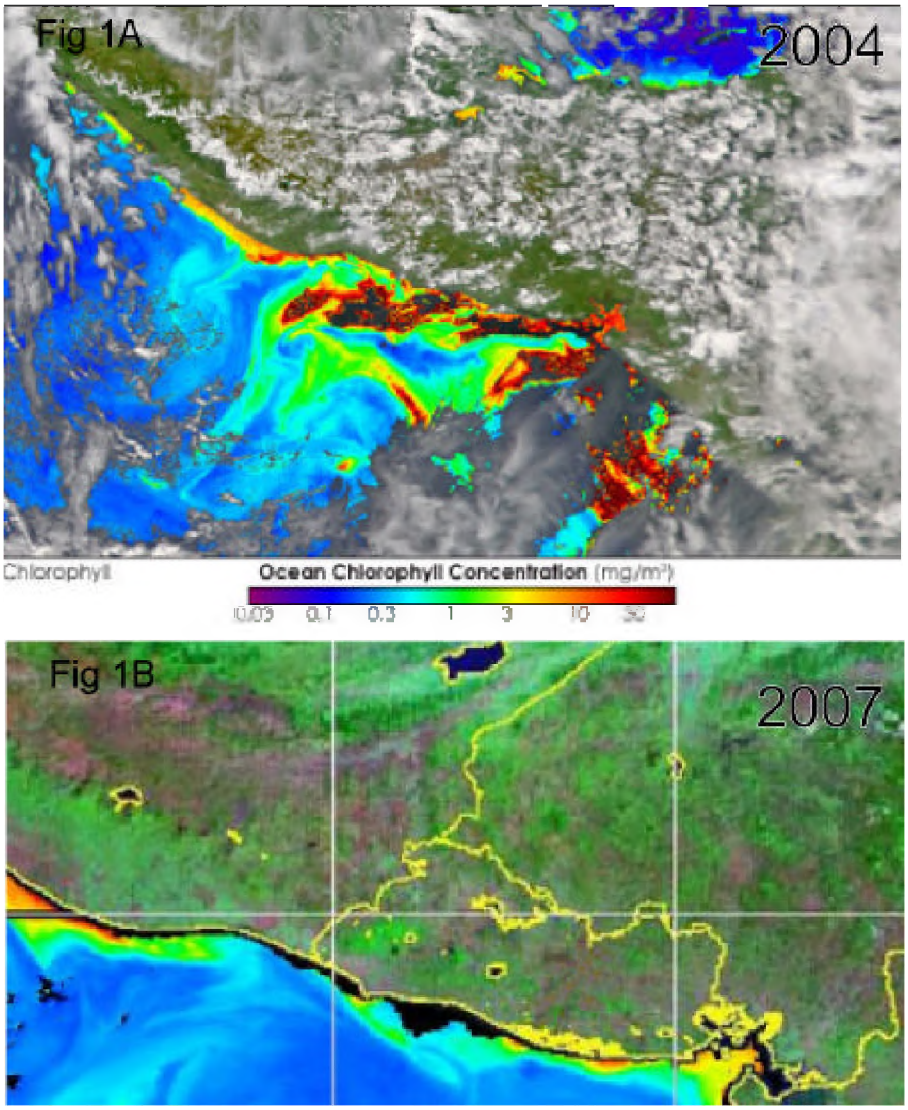

Fig. 1 A-B. SeaWiFS images on June 5, 2004 and January 24, 2007. Image courtesy of SeaWiFS Project, NASA/Goddard Space Flight Center, and ORBIMAGE.

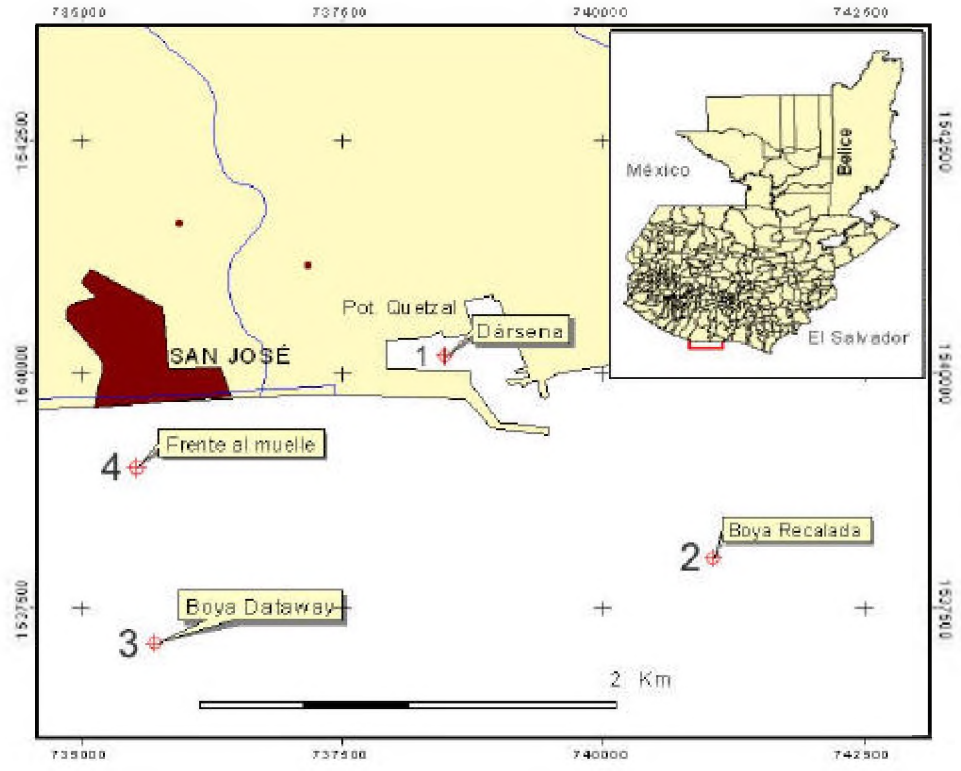

Fig. 2. Chart of sampling sites on June 5, 2004.

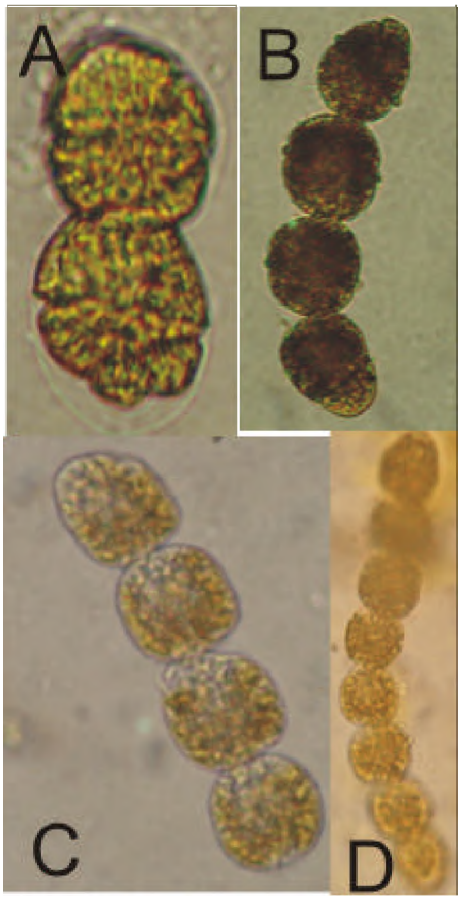

Fig. 3 A-D. Morphology of Cochlodinium catenatum. *A) Two-cell chain. Note the refringent granules (chloroplasts); B) four-cell chain; C) four-cell chain; D) eight-cell chain.*

recognized by the type of chloroplasts they possess. While *C. convolutum* has reticulated chloroplasts, *C. polykrikoides* has rod-like longitudinally aligned chloroplasts. *C. catenatum*, the one found in these episodes, has granulated chloroplasts (Fig. 2A). It is possible however, that both species coexist on the Pacific coast of Central America and Mexico, as they do in China and Japan. This coincides with the descriptions of *C. catenatum* from Bahia Banderas, Mexico [2]. It also coincides with the descriptions of *C. polykrikoides* found in La Paz, Baja California Sur, Mexico [3], because the photographs show granulated chloroplasts and a truncated epicone, not round as in the case of *C. polykrikoides* [4]. Additionally the densities found in La Paz are very similar to the ones reported in this case. There was a fish mortality, but it was not possible to determine its extent nor the species affected.

The satellite images indicate that the blooms are typically coastal, linked to upwelling that starts in January and with

Table 1. Hydrological characteristics and cell concentrations of Cochlodinium catenatum *(cells mL^{-1}).*

Sampling sites	*Cells mL^{-1} (surface)	*Cells mL^{-1} (5 m)	Temp. (°C)	Salinity (ppt)	pH	P (mg L^{-1})	PO_4 (mg L^{-1})	NO_3 (mg L^{-1})	Sechii disk (m)
Site 1	964	120	30.3	32.8	7.8	0.54	1.64	5.3	1
Site 2	848	60	32.7	32.7	8.1	0.04	0.11	4.2	9
Site 3	132	40	31.2	32.2	8.3	0.04	0.14	5.2	11
Site 4	68	36	31	32.1	8.2	0.07	0.2	3.6	5

*Sample from June 23rd, CEMA OBIMAR. Sampling points: * Counts cells mL^{-1}. Site 1: Dársena. Site 1: Dársena Puerto Quetzal. Site 2: Boya Recalada. Site 3: Boya Dataway. Site 4: Frente al muelle.*

time move along the entire Central American coast, until mid-year when they reach the Golfo de Fonseca. That was the cause of the large fish mortality of in the southern part of the Golfo de Nicoya, Costa Rica, caused by the same species of alga (personal communication, M. Vargas-Montero). The dominant species may vary according to the intensity of the currents that move the din cysts towards the surface, as well as the prevailing environmental conditions.

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• IOC UNESCO



Eighth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB)

The IPHAB is a mechanism to focus and cooperate on research, capacity enhancement and networks on harmful algae and their effects. The Panel has existed since 1991 and has initiated this newsletter, GEOHAB, manuals and guides, training courses etc.

At its meeting held at UNESCO Headquarters, Paris, from 17 to 20 April 2007, the Panel reviewed the actions completed during 2005-2006. The Panel noted that Denmark, Spain, Japan and the USA had maintained their significant financial support for the IOC HAB Programme. However, the Panel stressed that the IOC HAB Programme will only be able to develop and implement at the present rate in 2008-2009 if there is such financial support from Member States to fund programme staff and activities. The major achievements reported and discussed included: (i) development of the regional activities ANCA, FANSA, HANA and WESTPAC/HAB; (ii) the developments within GEOHAB including an Open Science Meeting on HABs in Stratified Systems, and publication with SCOR of the GEOHAB Research Plans for the Core Research Projects in Upwelling and Eutrophic Systems; (iii) the continued development of the IOC-ICES-PICES Harmful Algal Event Database; (iv) the implementation of eight training courses and several training-through-research projects; (v) the continued publication of the IOC Harmful Algae News; (vi) the results of the ICES-IOC WGHABD; (vii) the IOC co-sponsorship of several international HAB conferences; and (viii) provision of HAB literature to developing countries. The Session included a thorough self assessment of IPHAB mandate and impact. Dr. Leonardo Guzman (Chile) was elected new Chair and Dr. Phil Busby (New Zealand) was re-elected as Vice-Chair.

The major decisions of the Panel concern (i) the formulation and communication of an IPHAB strategy for assisting Member States in the mitigation of harmful algal events; (ii) the implementation of HAB monitoring within the Global Ocean Observing System GOOS; (iii) strengthened biotoxin monitoring, management and regulations in cooperation with FAO and WHO, (iv) a strengthened regional HAB Programme development; (v) development of a Harmful Algal Information System within IOC' International Ocean Data Exchange Programme IODE; (vi) the continuation of providing a taxonomic reference list of HAB species. The planned activities were summarized into a Work Plan and budget for the IOC HAB Programme 2008-2009.

At www.ioc.unesco.org/hab/IPHABVIII.htm you will find detailed background documents on IPHAB, its Work Plan, and its membership.

• France

First record of diatom *Pseudo-nitzschia americana* in French waters

Since 2004, the *Pseudo-nitzschia americana* complex has been recorded along French coasts of the North Sea, Channel and Atlantic Ocean. It has been observed either epiphytic on setae of *Chaetoceros* or planktonic in stepped chains (Fig. 1). Samples have been analysed with light microscopy (LM). The apical axis was 21-45 µm long and the transapical axis was 2.2-3.8 µm wide (n=12). Cell overlap in chains was 7-10 % of the length. But these measurements were not enough to identify specimens of this complex at the species level.

In late October 2006, a short chain of 4 cells was isolated for cultivation from a seawater sample collected in Bay of Douarnenez (Fig. 2). After the culture was established, a subsample was studied using scanning electronic microscopy (SEM) to supplement the morphological characteristics (Fig. 3). Cells had no larger interspace between the two central fibulae and the raphe was not interrupted by a central nodule. There were 18-20 fibulae per 10 µm and 25-31 interstriae in 10 µm. The structure of the striae, parallel to the transapical

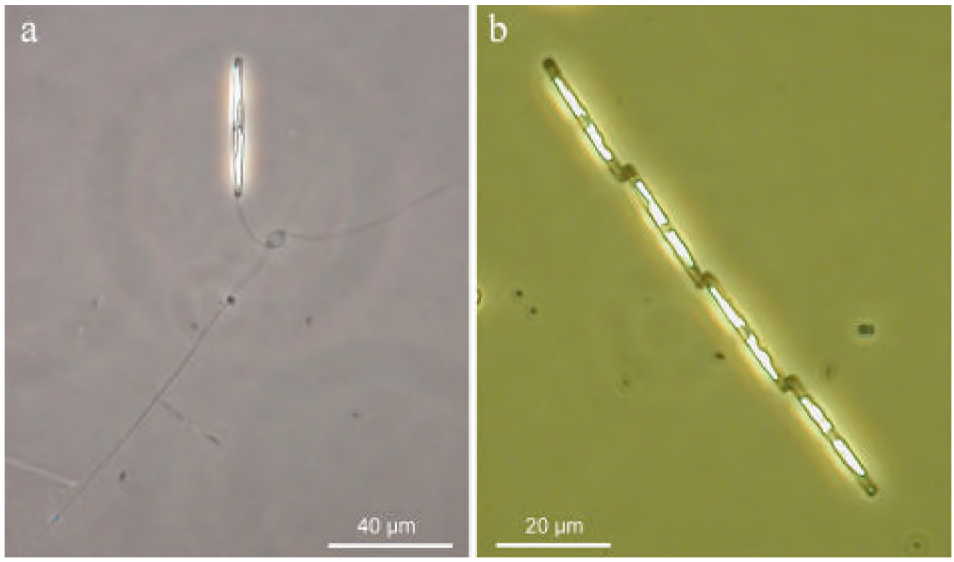

Fig. 1. Light micrographs showing an epiphytic cell on a setae of Chaetoceros *(a), a short stepped chain of 4 cells (b).*

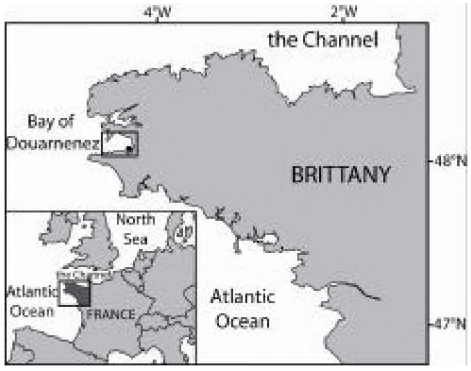

Fig. 2. Map of Bay of Douarnenez and the sampling site.

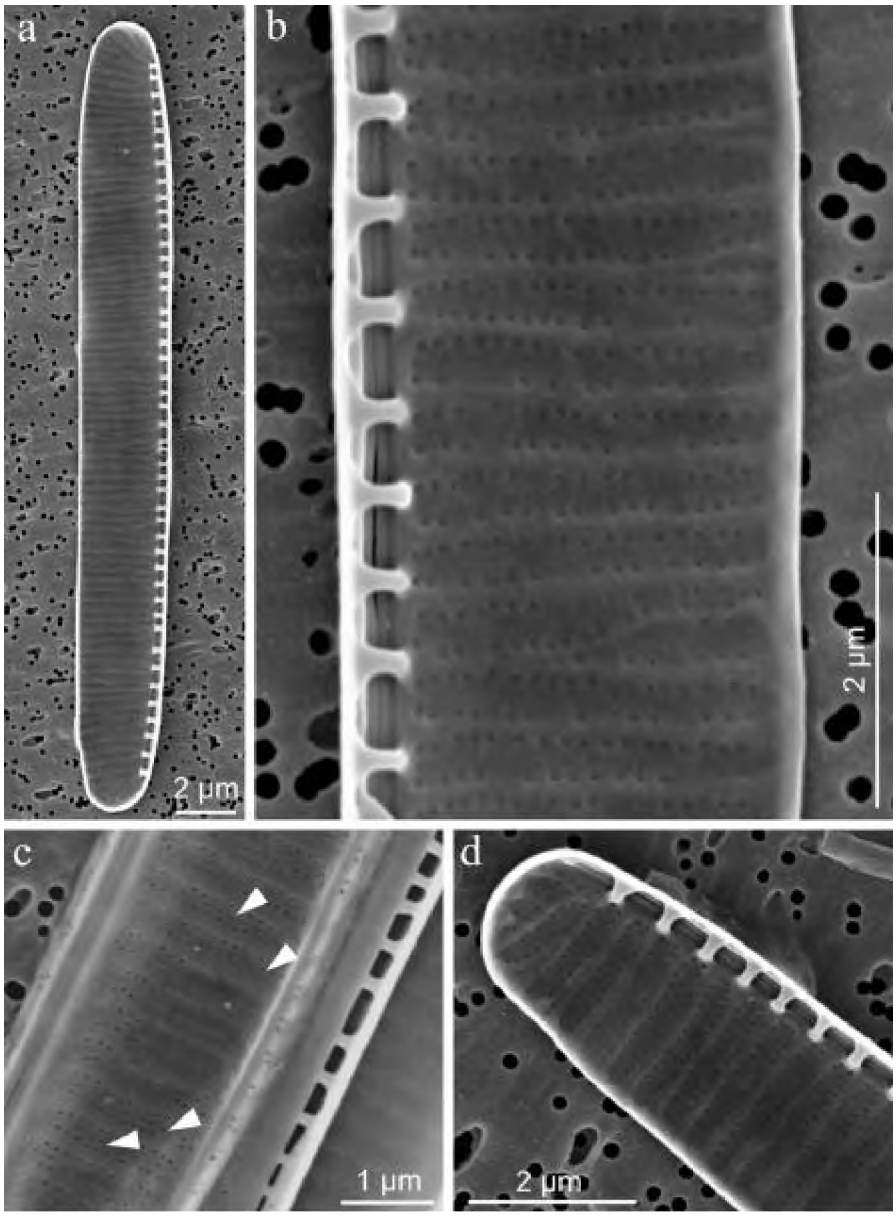

Fig. 3. Pseudo-nitzschia americana*, SEM micrographs of valve views showing an outline of the valve (a), an internal view of the middle portion (b); note the absence of the central interspace and the stria pattern, an external view (c); note the tendency for three rows of poroids (arrows) and an inside view of the apex (d); note the arrangement of the striae.*

Table 1. Comparative data on the morphology of species within Pseudo-nitzschia americana *complex.*

Species	Length (µm)	Width (µm)	Cell overlap (%)	Central interspace	Fibulae (/10 µm)	Interstriae (/10 µm)	Rows of poroids	Poroids (/1 µm)	Source
P. linea	13.4-26.6	1.8-2.2	Unknown	No	18-22	38-42	2-3	8-10	Lundholm, 2002
P. brasiliana	12-65	1.8-3	Unknown	No	20-26	20-26	2-3	7-10	Lundholm, 2002
P. americana	16-42	2.5-4	Unknown	No	18-24	26-31	2-3	8-10	Lundholm, 2002
P. americana	16-40	2.5-3	Unknown	No	18-20	27-30	2-3	8-9	Hallegraeff, 1994
P. americana	18-26	3.5-4	Unknown	No	20-21	28-30	2-3	Unknown	Hernandez-Becerril, 1998
P. americana	24-32	4	Unknown	No	20-21	26-28	2-3	6-7	Orlova, 2002
P. americana	12-39	2.5-3.7	10	No	18-23	24-38	2	7-10	Kaczmarska, 2005
P. americana	21-45	2.2-3.8	7-10	No	18-20	25-31	2-3	8-9	Our data

axis except at the poles, comprised mainly two rows of poroids, but a rudimentary third one was occasionally observed. The number of poroids per 1 µm was 8 or 9.

Compared with data on the 3 species of this complex, our cells were too wide to correspond to *P. linea* [1] and the number of interstriae per 10 µm, intermediate between that described for *P. brasiliana* (20-26) and *P. linea* (38-42), falls in the range mentioned for *P. americana* [1-5] (Table 1). Although *P. americana* is considered a cosmopolitan species, it has not been previously reported in France. Because of its low abundance in field samples, this species could not be identified without isolation followed by an examination using electronic microscopy.

Our results are in agreement with a recent Canadian publication [5] and corroborate the ability of *P. americana* to occur not only as single cells but also in short stepped colonies, as reported for its sister taxa from warmer waters, *P. brasiliana* and *P. linea.*

Acknowlegments

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• Brazil

Diarrhoetic shellfish poisoning (DSP) outbreak in Subtropical Southwest Atlantic

Dinophysis cf. *acuminata* is currently found in samples from the southern coast of Brazil and has been identified as a possible threat to the flourishing mussel culture in this part of the country [1]. The production of okadaic acid, a cause of DSP, by *D.* cf. *acuminata* in the region was earlier characterized, and cultured mussel samples tested positive by mouse bioassay [2].

In January 17th, 2007, during monitoring by mussel and oyster producers in association with our laboratory, *alert* counts for *D.* cf. *acuminata* (i.e. 400 cell L^{-1}) were found at Cedro's beach at Palhoça, South Bay of Florianópolis, Santa Catarina. The following day, a sample from the other side of the bight, at Ribeirão da Ilha, was collected. The counts were higher, and a sample from a *Perna perna* tested positive with the mouse assay (acetone extract of digestive glands).

These results were communicated to the recently formed National Committee for Mussel Sanitation Control - CNCMB. The committee closed the North and South Bays of Florianópolis for mussel collecting and commercialization. The closure was then followed by a more extensive sampling programme to cover a larger region to include other mussel culture areas.

Given the absence of previous epidemiological data and the need to protect consumers, the ban was extended to oysters, clams and scallops, all regularly consumed by the population either from aquaculture or extracted from natural beds.

As the causes and characteristics of the ban started to be fully covered by the media, cases of human intoxication started to be reported, including former cases.

In Florianópolis, positive mouse bioassays persisted for 10 days. The ban was suspended on January 27 when cell counts reached lower values (i.e. 150 cell L^{-1}). During this episode, only *Perna perna* was contaminated, considering 2 out of 3 mice deaths in 24 hours. No contamination of other species was observed.



Fig. 1. Dinophysis *cf.* acuminata

On January 30th, a few days after the first positive in the South Bay of Florianópolis, 130 people were attended at the health clinic in Bombinhas city, about 70 km to the north with gastrointestinal distress. Due to media exposition, these cases were quickly related to DSP by local health authorities and later confirmed by mouse bioassay. A second ban was set throughout Tijucas Bay. In this area, counting of *D.* cf. *acuminata* reached the highest value, 5.2 x 10^4 cell L^{-1}, recorded for Brazilian waters.

The bloom at Tijucas Bay persisted for a longer time, but in contrast to Florianópolis, oysters were also positive by mouse bioassays. The area was free from DSP only after 23 days, when the ban was suspended and mussels again collected and commercialized.

During this episode, the health authorities recorded more than 150 cases of DSP. But the number of cases not notified was also high, indicating that this was a large scale event. This human intoxication scenario might have been even worse if prompt action had not been taken. Santa Catarina is a summer tourist destination. Tourists mainly from Brazil and Latin America, come to enjoy the beaches, but also to consume the nationally famous seafood. The ban was imposed during the high season. While consumers were protected, producers and restaurant owners suffered economic losses. The economic consequences have not been fully assessed, but apart from larger producers, small artisanal growers who depend on mussel culture were also affected.

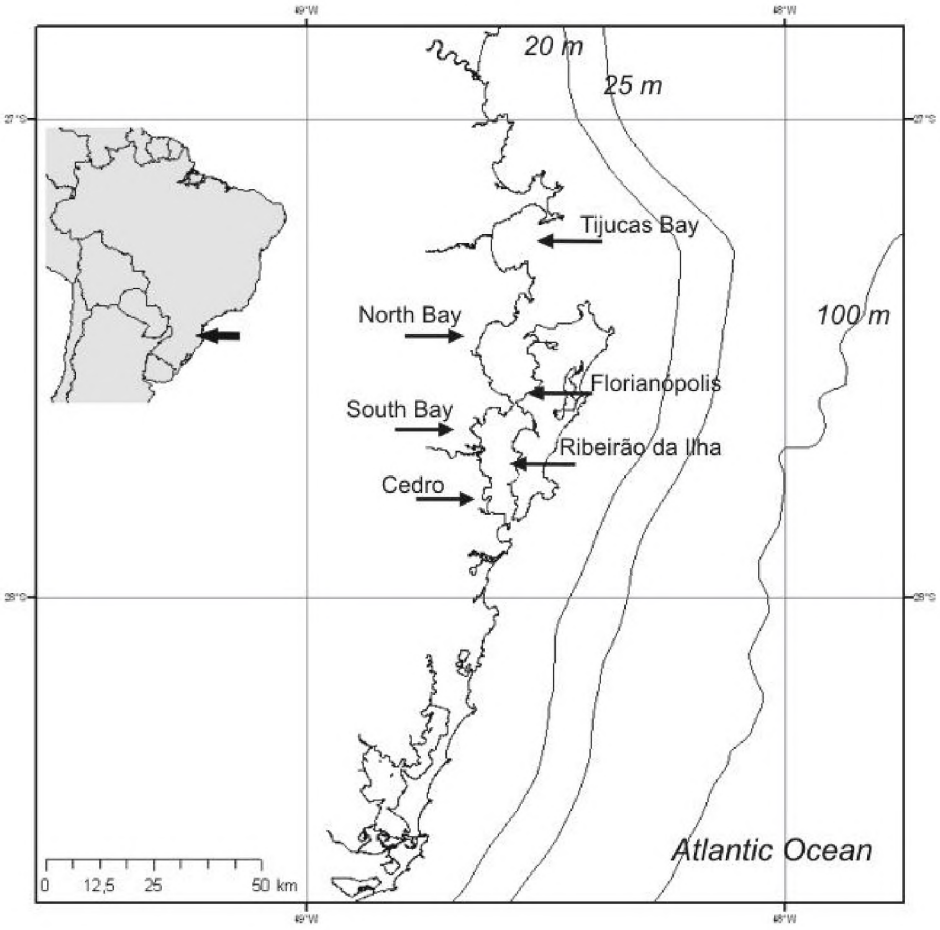

Fig. 2. Santa Catarina Coast, Brazil, showing the sites affected by the DSP outbreak.

The data has still being processed to provide guidelines to set up a full HAB monitoring programme. This programme will by more comprehensive as apart from DSP toxins, we have already characterized PSP caused by *Gymnodium catenatum* [3, 4] and ASP by *Pseudo-nizschia* spp. (data not published).

This was the first fully characterized outbreak of mussel contamination with human intoxication in Brazil. It was also the first time a contingency plan was put into practice. The action involved the collaboration of mussel producers and municipal and federal government agents.

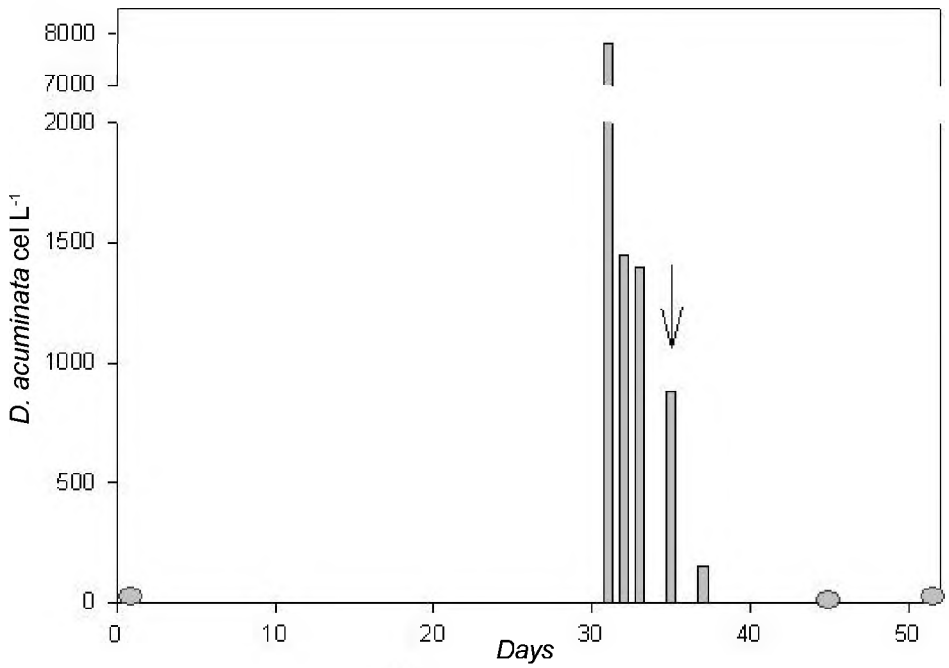

Fig. 3. D. cf. acuminata counts at Ribeirão da Ilha, the largest oyster producing area in the country, from December 19th to February 9th. Circles shows sampling days and the arrow the negative bioassay.

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A Europe – Chile cooperation on *Alexandrium* blooms

Puerto Montt 19th December 2006

Chilean scientists from the 10th region (Los Lagos) and French scientists from Finistère (Western Brittany) met for ten days for a workshop organised and financially supported by the "French department of Finistère", in Puerto Montt and in Chiloe Island, Chile.

The goal of these meetings was to initiate scientific cooperation between scientists from both areas belonging to various organisations: the Austral University of Chile, University los Lagos (I-mar Research Center), University of Chile (Marine toxins laboratory), IFOP los Lagos, the French National Research Council (phytoplankton laboratory CNRS), University of Western Brittany (LUMAQ), and the French marine research institute (IFREMER).

Discussions focused on the identification of key points for the improvement of HAB management. One of the main HAB problems experienced by both countries in recent years was caused by recurrent blooms of *Alexandrium* spp. first detected in the Magellan Straits in 1972, *A. catenella* now regularly affects the 3 most southern regions of Chile, from 52°S to 42°S (a bloom was expanding from the South of Chiloe Island during this meeting in December 2006). The massive occurrence of *Alexandrium* along French coasts is also recent. *A. minutum* has bloomed regularly since 1988 in several estuaries of northern Brittany, whereas *A. catenella* has been abundant since 1995 in Thau Lagoon (South of France).

From these meetings, nine topics were identified for a multidisciplinary working programme focused on the study of *Alexandrium* spp: their origins, the dynamics and monitoring of the blooms, environmental impacts, and the possibility to reduce their harmful effects.

The first subject, "*origin, dynamics and monitoring*" aims to improve the early detection, dynamic forecasting and understanding of the mechanisms that support bloom development:

1. Long-term regulations of blooms by parasitic infections. lguillou@sb-roscoff.fr, mseguel@uach.cl, dvarela@ulagos.cl.
2. Population dynamics of invasive *Alexandrium spp.* migrating along the Chilean coast. lguillou@sb-roscoff.fr, mseguel@uach.cl and dvarela@ulagos.cl.
3. Genetic cartography of the *Alexandrium* cysts from sediments by genetic tools. lguillou@sb-roscoff.fr, mseguel@uach.cl and dvarela@ulagos.cl.
4. Evaluation of hyperspectral remote sensing application for the assessment of *Alexandrium* blooms. Denis.de-labroise@univ-brest.fr, golivares@mail.ulagos.cl, lguzman@ifop.cl and alexcle@telsur.cl.



The workshop group at I-Mar, Puerto Montt (Chile) 18th December 2006 .

The second subject "*environmental impact and reduction of effects*" aims to characterise *Alexandrium* interactions with the environment:

5. Impact of *Alexandrium* blooms on the natural macrofauna. caranda@ulagos.cl, alexcle@telsur.cl and French partner to be identified.
6. Impact of *Alexandrium* blooms on fishes in aquaculture. genevieve.arzul@ifremer.fr and alexcle@telsur.cl.
7. Kinetics and mechanisms of post-bloom toxin reduction. Denis.de-labroise@univ-brest.fr and caranda@ulagos.cl.
8. Comparison of *Alexandrium* sensitivity to toxic compounds (metals, pesticides) in relation to their geographical origin. genevieve.arzul@ifremer.fr, francoise.quiniou@ifremer.fr, alexcle@telsur.cl and dvarela@ulagos.cl.
9. Use of cystein in salmon farming as a tool against mucus overproduction in HAB impacted salmon. genevieve.arzul@ifremer.fr and gvidal@ifop.cl.

Actions will start in 2007 with scientific and student exchanges, and ideally should serve as the basis for a future collaboration under the next European FP7 calls. Thus, in this context, we are interested to develop collaborative projects that may concern these nine actions together with other EU members.

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HAB XII – Responses to the Conference

Clay flocculation, and other Mitigation topics at the XIIth ICHA, Copenhagen 2006

One might suppose that one of the principle preoccupations at a conference on Harmful Algal Blooms would be mitigation of the harm they do. Yet, based on my perusal of the Book of Abstracts, mitigation of HABs was the subject of 0 out of 112 oral presentations (0%) and 19.5 (joint principle subject counted as 0.5) out of 374 posters (5.2%), giving only 4.0% overall. Corresponding percentages for presentations on Monitoring, Management and Forecasting were 22.5, 1.2 and 2.5% respectively. In mitigation, the above figures do not take into account that 1 out of the 5 mini-symposia that took place on Wednesday was devoted to the mitigation topic, "HABs and clay flocculation: Some species, some places, but not a silver bullet".

This mini-symposium, led by **Mario Sengco** and **Kevin Sellner**, was a lively and dynamic event, and comprised oral presentations, no posters, and plenty of discussion; the room was packed. As preliminary information on the mini-symposium pointed out, the use of natural clays to control HABs has been investigated for over 10 years in Japan, China, and South Korea, to minimise their impacts on aquaculture, and in Australia to treat blooms of the cyanobacterium *Microcystis* in fresh water. In the USA, clays have been used effectively against marine and brackish water species, but the chief research effort is concentrated on *Karenia brevis* blooms around Florida.

Sengco gave an overview of the use of clays in the USA, particularly against *K. brevis*. While clay use over large areas of the coastal ocean would be prohibitively expensive, it may be economically feasible in limited areas of high amenity value. Phosphatic clays generally give the best results. However, there are concerns that toxins in cells thus sedimented to the bottom could remain to intoxicate benthic organisms, and **Kevin Sellner**, **Monica Bricelj** and **Anne-Gaelle Haubois** presented careful work on this problem, showing that the benthic layer of sedimented clay and flocculated cells can indeed transfer a relatively small amount of brevetoxin and its derivatives to deposit-feeding clams.

An important research effort, including large-scale trials in aquaculture areas, is being conducted in South Korea, and **Chang-Kyu Lee** presented aspects of this. The material favoured is yellow clay, because it works and is readily available locally. A suspension of clay in seawater is sprayed at high pressure onto the sea surface from barges. It has been found quite successful in reducing large-scale blooms of *Cochlodinium polykrikoides*. **Zhiming Yu** explained how clays have been used successfully, particularly when modified with quaternary ammonium compounds, to eliminate *Heterosigma* and *Prorocentrum*, with no apparent impact on co-cultured oysters. Yu also explained how various clays are used to eliminate dense, wind-concentrated floating mats of material from decaying *Microcystis* blooms, which disfigured an arm of Xuanwu Lake, where the 10th Chinese National Games were to be held. This technique completely eliminated the problem in time for the games, but required considerable socio-economic commitment. Nevertheless, in freshwater, clays have flocculent properties different from those in seawater, and so are less efficient at HAB removal. To replace the need to truck in huge quantities of clay, Yu's Chinese colleagues have been working with modified local soils (MLS). Binding material, such as chitosan, is combined with local earth, and flocculates *Microcystis* well. The authors have even been adding macrophyte seeds to the MLS that subsequently germinate, and can help turn a lake from a turbid plankton-dominated ecosystem into one of clear water dominated by benthic plants.

In the Philippines, the efficiency of clays has been investigated in removing the toxic *Pyrodinium bahamense*, as well as *Amphidinium* and *Chattonella*. Local ball clay was found the most effective, with >99% removal **(Larry Padilla)**.

Although not concerned with clays, I thank the organisers for allowing me to give an impromptu presentation about the potential role of cysteine in mitigating the harm done by HABs. In the laboratory, cysteine has been shown to fluidify potentially suffocating slimes produced by cultures of *Karenia* and *Gymnodinium*, to reduce haemolytic action due to their production of active oxygen, and to increase fish survival. Cysteine, also by fluidifing mucus, can in addition protect fish against suffocation following inhalation of the diatom *Chaetoceros*. Studies *in situ* are still required to show what protection cysteine may afford to aquaculture installations.

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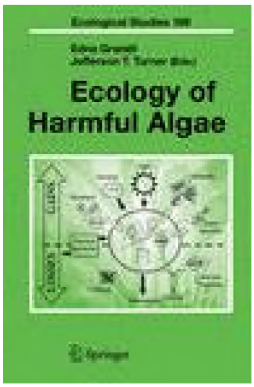
Book Review

Granéli E & Turner JT 2006. ***Ecology of Harmful Algae*** (Springer), xxvi + 439 pages, 45 figures, 15 tables, hardcover.
Price: EUR 129,95; GBP 100,00; USD 169,00
ISBN: 978-3-540-32209-2

This book (GT), claim the editors (p6), is intended to provide an 'updated synthesis of HA ecology' *vis a vis* the *Bermuda* volume (ACH) [1]; the latter was based on the proceedings of a NATO Advanced Study Institute held at the Bermuda Biological Station in 1996, and also published by Springer. GT contains thirty chapters in about 360 pages (excluding front and back matter), so each chapter is only about 12 pages long including bibliography. This brevity may be the reason for the rather telegraphic style adopted by some contributors. How much can really be said about the molecular biology of



diatoms (p17) in one paragraph, about bivalves as toxin vectors in little over a page (p286-7), or about dinoflagellate biogeography in two pages (p26-7)? This constraint has led to chapters which are short overviews rather than in-depth accounts of the different themes treated. As such, several of the chapters succeed individually, and provide useful updates on ACH to the extent that these themes were addressed in Bermuda, but since there is no clear overall argument, the synthesis promised by the title and the introduction fails to emerge.

Some more detailed comments follow. GT takes no hard looks at population dynamics, contains only slight reference to ecological theory other than the ordination models of Margalef (the famous mandala), Reynolds (life form intaglio), and Smayda (habitat templates). MacArthur's distinction between *r*– and *K*– strategies, derived from the logistic equation, and closely related to the mandala, is dealt with in some detail in chapter 11, more briefly elsewhere (pp61, 81, 132). Some contemporary topics of research in HA ecology are only mentioned briefly, like cell cycles (only p219), cyst bed dynamics, thin layers (p86), cold pools, retention mechanisms (pp86, 128, 131). The virtual absence of any oceanographic component (only chapter 10 in GT; three dedicated chapters in ACH, as well as integral to many of the others there) is a serious omission, since regional hydrodynamics are of major importance in bloom dynamics, essential components of the impact of nutrient enrichment on coastal waters, and necessary background to any prognoses of the impacts of coastal engineering, climate trends, and so on in predictive models.

Cembella wrote (ACH, p649) "... it could be argued that progress in defining the critical parameters regulating bloom dynamics ... has been disappointing." This view, echoed in GT (p60), is no longer tenable. Big steps forward have been made in modelling in the last decade; there are some excellent papers published in a special volume of *Deep-Sea Research* [2]. Although the focus there is on the Gulf of Maine, the volume has lessons which can be transferred to all regional seas. In any case, the kind of "coupled physical-biological model" implied (p60) is not the only kind of model worth exploring. The updating intent of the editors has failed on this score.

Chapter 18 introduces the ideas of "up-shock" and "down-shock", the first of which I take to be synonymous with 'shift-up', a term borrowed from bacterial chemistry, and introduced to phytoplankton ecology by MacIsaac [3]. This chapter tells us that "The only nutrient levels and ratios that ultimately matter are internal, not external" (p237), which reminds us of Droop's cell quota model, and recalls for example the finding that oceanic *Rhizosolenia* mats have internal nitrate pools 10^6 times higher than the nM external concentrations [4]. What rate of change in external nitrate concentration would be needed to "up-shock" (p229) these *Rhizosolenia* populations? What possible processes in nature, other than those of the cells themselves, could *remove* nitrate in such a manner as to cause "down-shock"? It is almost a relief to be told that the experimental results "may be of little or no ecological consequence" (p237). Also, "changes in toxin content are associated with disturbed (unbalanced) physiology ...", which I take to mean pathological. Fogg [5] listed some elementary cytological signs of pathology due to nutrient problems in algae. Such signs have been observed in nature, but are rare; that being so, could we claim that eutrophication is not a problem, at least for microalgae? A distinct view of the relation between nutrient limitation and toxin production is summarized on p218.

We do not seem to be any closer to a resolution of the bacterial conundrum than we were ten or even twenty years ago (chapter 19), despite the now long-standing Silva-Kodama-Doucette prokaryotic hypothesis. The idea that bacteria play an important rôle in toxin synthesis still seems to be making heavy weather of it, so this is perhaps not a topic that a wise supervisor would want to point a graduate student at.

Grazing is dealt with in chapter 20, but only by zooplankton, amongst which are copepods. It fails to refer to an apparently important point made in chapter 27 (by the same author!), that copepods do not eat HA directly! Grazing by bivalves, which after all are the most important vectors of algal toxins to humans, is not mentioned in chapter 20, but gets a showing chapter 22, and is identified as an important control of brown tides in chapter 9. Chapter 21 is about pathogens of HA, and includes parasites. The interesting idea that HA activity might be regulated by the bacteria on seaweeds (p275) provokes the question, are some HABs due to sea urchins? I refer of course to the urchin barrens from which seaweeds have been removed by overgrazing. More of this sort of approach, and the old fashioned 'natural' ecosystems which are being so badly mismanaged in many regions will call for their own reconstruction.

Major (*Pyrodinium*, a significant human killer) and minor (*Noctiluca*) players in the HA field, both treated fully in ACH, are hardly mentioned here (the former gets a sentence, no more, on p61, the latter is merely listed, p6), neither is in the index. Several new toxin groups were discovered in the mid-1990s, but are not dealt with in this volume, such as azaspiracids (only mentioned once, p59) and spirolides (not mentioned at all).

The *opus magnum* of Justus von Liebig [6] (not Leibig, p304) has been described as "... a mixture of lasting truths, exaggerated conclusions, and plainly erroneous assumptions", clearly a successful recipe, since this highly influential book went through nearly fifty editions in various languages, nineteen in North America alone, and increased in length by about a 1000 pages in the process. Liebig is often maligned, since he explicitly linked his argument about limiting nutrients to agricultural systems, and was careful to exclude 'natural' ecosystems, for which he stressed the mutual dependence of plants and animals. "A long-standing paradigm" (p343) does not have to stand forever.

For some decades, cultural eutrophication of fresh waters and coastal waters has been almost universally regarded as a bad thing, while the same process is a virtue on agricultural land and in algal cultures. The Greek root of the word after all gives us the sense of healthy, well-nourished. But this conventional wisdom is now being questioned; in the Seto Inland Sea for example, mandatory *reduction* of phosphorus inputs has been linked to an *increase* in toxin-producing dinoflagellate abundance and reduction in fishery yields [7]. Yet here (p346), an *increase* in phosphorus inputs in Tolo Harbour is linked to an *increase* in dinoflagellates. Growth of brown tide species *Aureococcus* and *Aureoumbra* is depressed by inorganic nitrate additions (chapter 9).Thus new questions emerge, how much eutrophication is desirable, and at what stage does it become undesirable.

Lord Taverne's *March of Unreason* (2005) decries those who "use evidence selectively and unscrupulously to bolster prejudice, and who go through the motions of inquiry only to demonstrate some foregone conclusion". This is exactly what Taverne himself does in pursuit of his own ideological agenda. The same tendency can be recognized here. For example, fig 26.4 (p346) purports to demonstrate a "broad relationship between nitrogen loading and HAB proliferation" (p345); it does nothing of the kind, as even a cursory glance reveals, and as is admitted by the authors on the next page. The apparently simple idea that the size of a population is regulated by the capacity of its environment to supply it with resources is remarkably difficult to confirm [8]. But however weak these arguments are scientifically, their obvious concern is well placed; there is a broad consensus that we cannot continue overexploiting nature much longer if we are to survive. "Our total inheritance took billions of years to assemble; it is being squandered in decades" [9]. One way forward is to estimate how resilient HA 'infected' coastal waters are; there is a large literature on ecological resilience. It is not the *amount* of nitrogen, say, that causes problems, but the ways in which its production and consumption are uncoupled [10]. Ideology appears elsewhere too. The mantra "HABs are increasing ..." appears in its canonical form (p159), but mercifully only once. That *Phaeocystis* "disturbs the food-web" (p159) can also be viewed as dogma, as a major contributor to primary production, it perhaps structures it.

The various known toxins are frequently thought to play allelopathic roles (not allopathic as on p189, 197, which refers to harsh medical practices), in the sense that one plant or algal species suppresses another. This view is raised in the introduction (p4), and again in later chapters (12, 15, 17). So far, evidence in favour has proved elusive, and convincing experiments are difficult to accomplish. *Chrysochromulina* may be toxic to a bryozoan "when given as the only food source" (p178), so too are hamburgers to our children if eaten to the exclusion of other food. Nor is there strong support for the view that the known toxins are grazer deterrents (chapter 20). A rôle in either iron uptake or copper detoxification is proposed for domoic acid (p209). There are several reasons to doubt that any of these views is a strong candidate for a general hypothesis. For example, if simple aldehydes really are synthesized by diatoms for allelopathic ends, why go to the trouble of constructing the much more complex polyethers like ciguatoxins, brevetoxins, or yessotoxins, or the expensive (in terms of nitrogen) members of the saxitoxin family. The functions of secondary metabolites are likely to be as varied as their chemical structures. As pointed out by Wright & Cembella in ACH, various kinds of communication (e.g., quorum sensing) *are* strong candidates for the more elaborated 'toxins', because accurate communication has to ensure against ambiguities, and that does have high costs. But other rôles are likely too, and some toxins may aid predation (summary on p183).

The editors claim that every chapter was reviewed by themselves as well as one to three referees, so three, four, or five reviews in each case. How then did such expressions escape their attention, like "a planktonic phytoplankton bloom" (p39), "In marine and coastal waters, nitrogen and phosphorus are seldom in high enough concentrations to sustain the growth of the full array of phytoplankton species co-existing in time and space." (p198), "borrow" chloroplasts (p164)? Do they give them back afterwards? "This limited functionality, essentially being selection-neutral ..." (p239). Whatever it means, how do we know that? What is the difference between natural and bacterial degradation (p249)? The discoloured water witnessed by Darwin during the *Beagle* survey off Chile is generally agreed to have been due to *Mesodinium*, not to a dinoflagellate as stated here (p3); Darwin even provided a recognizable sketch. Readers of this newsletter may remember that the same organism had been described by the German naturalist Pöpper in 1827, seven years before Darwin's visit, in the same region [11]. Surely at least one referee should have noticed this small detail? In summarizing experiments, there is no reason to avoid providing numerical data if it is available; thus, "*Karenia mikimotoi* was **somewhat** reduced ...", "... the ciliates **appear to have been extensively** consumed by copepods ..." (p361); somewhat? Appear to have been? "... zooplanktivorous fish **appeared to** decrease copepod biomass ..." (p362), well did they or didn't they?

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GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

GEOHAB – Core Research Project on Stratified Environments. A first application in the Bay of Biscay



Scientists from IFREMER (France), MRI (University of Ireland), IEO-Vigo (Spain), CEFAS (U.K.) and John Hopkins University (USA) during the HABIT 06 cruise onboard RV Thalassa.

Our current understanding of oceanic ecosystems and particularly of phytoplankton population dynamics largely depends on the ability to observe distributions and processes at different scales. To this effect, a 3-week field trip of the R/V *Thalassa* (Ifremer) in the Bay of Biscay (France) was partly funded by the European Commission and US-NSF under the programme HABIT (Harmful Algal Blooms in Thin Layers). A team of 25 scientists from the different partner institutions (MRI, IFREMER, IEO, CEFAS and Johns Hopkins University) deployed a wide variety of innovative instrumentation and methodology over the continental shelf.

The Scanfish and an Autonomous Underwater Vehicle (AUV) allowed us to describe the chlorophyll maximum located in the pycnocline, and its continuity at different scales from 1 to 100 km. The stability of the AUV allowed measurements of the velocity field around the pycnocline at a resolution of 20 cm. The in-house Ifremer particle-size profiler was equipped with a new in situ 2D-imaging fluoromicroscope: it can resolve very thin-layers (<20 cm) containing monospecific populations of *Chrysochromulina* sp., and it provided samples at the same resolution on the vertical axis. One of the important outcomes of this cruise was the detection *en route* of phytoplankton accumulations with a specific configuration of the Simrad ER-60 echo sounder. It revealed internal waves breaking on the shelf and the propagation of solitons generated at the shelf break. The zone where waves break is likely to have a great impact on populations distributions.

Three members of the GEOHAB Core Research Programme on Stratified Environments (P. Gentien, R. Raine, B. Reguera) participated in the cruise, together with L. Fernand from CEFAS and E. Malkiel (Johns Hopkins University)

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GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Regional Meeting on Future Focus and Cooperation in HAB Research

2nd Asian GEOHAB Meeting organized by the GEOHAB SSC in cooperation with IOC/WESTPAC and the IOC HABViet Project

Nha Trang Vietnam, 28 January-1 February 2008

The Scientific Steering Committee for GEOHAB (GEOHAB SSC) and WESTPAC/HAB is pleased to announce that a regional meeting on future focus and cooperation in HAB research, a Second Asian GEOHAB Meeting, will be held 28 January-1 February 2008 in Nha Trang, Vietnam. The local host will be Institute of Oceanography Nha Trang and the IOC HABViet Project.

Scientific Programme:

The First Asian GEOHAB meeting was held in Tokyo 15-16 March 2007. The Second Asian GEOHAB Meeting will provide an opportunity to develop further ideas and outlines of regional and national research relevant to GEOHAB, establish partnerships and identify research components that may merge into the GEOHAB Core Research Projects.

The Meeting will consist of two parts.

· The first part will be a two-day seminar with broad participation and presentations on various aspects of harmful algae. This part will also serve as a forum for exchange of new regional research results and ideas between researchers, industry, government and local users, and other interested parties in Vietnam and regionally.

· The second part will be a two-day workshop for presentation and discussion of ongoing and planned research relevant to GEOHAB. The workshop will consist of plenary and subgroup discussions on development of specific new research projects or coordination and cooperation among ongoing projects. It is the objective to draft a plan for the development of Asian GEOHAB.

Organizing Committee:

· Ken Furuya, Japan (GEOHAB SSC and WESTPAC/HAB)
· Ming-Jiang Zhou, China (GEOHAB SSC)
· Nguyen Ngoc Lam, Vietnam (Local host)
· Doan Nhu Hai, Vietnam (Local host)
· Robin Raine, Ireland (Chair GEOHAB SSC)
· Patricia Glibert, USA (Chair GEOHAB Core Research Project of HABs in Eutrophied Systems)
· Jacob Larsen, Denmark (HABViet Project IOC Science and Communication Centre on Harmful Algae)
· Henrik Enevoldsen (IOC)

Important Dates:

Call for Submission of Abstracts: 1 May 2007.

Final Date for Receipt of Abstracts: 1 January 2008.

Registration deadline: 21 January 2008.

Registration:

There is no registration fee to attend the meeting.

Financial support:

There will be possibilities for financial support to invited participants and people applying for support. Please submit applications for support by 1 November 2007 to h.enevoldsen@unesco.org. Please indicate in applications which part of the cost can be covered from own sources.

Venue:

The meeting will be held at Institute of Oceanography, Nha Trang, Cau Da 01, Nha Trang , Khan Hoa Province, Vietnam. Nha Trang has an international airport as well as frequent domestic connections to Hanoi and Ho Chi

Please use the web site www.geohab.info to register and to keep up to date on meeting developments.

Harmful Algae News

Previous issues of HAN and newsletters of the IOC HAB Programme can be downloaded at http://ioc.unesco.org/hab/news.htm

Requests for subscription

Subscription to HAN is made by sending a request with a complete address to Ms V. Bonnet: v.bonnet@unesco.org.

ISSHA

International Society for the Study of Harmful Algae

ISSHA President's Corner

Plans are underway for the 13th International Conference on Harmful Algae scheduled for 3-7 November 2008 in Hong Kong. Professors K.C. Ho from the Open University of Hong Kong and Mingjiang Zhou of the Institute of Oceanography, Chinese Academy of Sciences in Qingdao will Co-Host the Conference. Members of the ISSHA Organizing Committee and the Local Organizing Committee will meet on the 16th of June 2007 to select the themes of the conference and discuss candidates for plenary speakers. The venue for the 2008 meeting will be the Hong Kong Disneyland Hotel. Watch the ISSHA website (www.issha.org) for updates on the Conference preparations.

In the coming year ISSHA will prepare a slate of candidates all offices for an election to be held prior to the 13th Conference. The Executive Committee Members will be renewed and our standing committees can always use new talent. If you are interested in serving the Society, please contact an officer or Council member or send your nomination to Karen Steidinger (Karen.Steidinger@MyFWC.com). Remember that you must be an ISSHA member at the time you make the nomination and your nominee must also be an ISSHA member. Top check on your membership status see the ISSHA website or contact Tracy Vallareal (tracy@utmsi.utexas.edu). To become an ISSHA member or renew your membership, please contact Nina Lundholm (nlundholm@bi.ku.dk).

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Future events

OCTOBER 2007

AGU Chapman Conference: Long Time-Series Observations in Coastal Ecosystems: Comparative Analyses of Phytoplankton Dynamics on Regional to Global Scales

8-12 October 2007. Rovinj, Croatia.

Abstract Deadline: 7 May 2007.

This Chapman Conference will compare phytoplankton dynamics in coastal marine ecosystems where perturbations from terrestrial, atmospheric, oceanic sources and human activities converge to cause changes that ramify across local and global scales. The objective is to assemble and synthesize multi-decade observations toward quantitative and descriptive depictions of phytoplankton variability as an indicator of environmental change across the full diversity of coastal ecosystem types. The visions are a global phenology of phytoplankton at the land-sea margin and a conceptual model from which coastal ocean observing systems can be built. Detailed information, including instructions for abstract submission, is available at www.agu.org/meetings/chapman/2007/bcall/

OCTOBER 2008

Ciguatera and related biotoxins

21-31 October 2008. Nouméa, New Caledonia.

A workshop on the latest developments in ciguatera research, covering environmental influences, causative organisms and socio-economic and medical aspects:

Tentative programme:

- Toxinogenesis.
- Environmental influences.
- Chemistry of involved ciguatoxins.
- Clinical treatment and folk remedies.
- Toxicology and mechanisms of action. Ciguateric fish species.
- Socio-economic impacts.
- Collection and use of clinical and epidemiological data.
- Risk assessment and management.

The workshop will take place at the IRD Noumea Centre and the SPC symposium facilities, Noumea, New Caledonia.

For additional information: www.ird.nc/ciguatera



NOVEMBER 2008

The 13th International Conference on Harmful Algae 2008

3-7 November 2008. Hong Kong-China.

The International Society for the Study of Harmful Algae (ISSHA) is pleased to announce that the 13th International Conference on Harmful Algae will take place in Hong Kong on 3-7 November, 2008. Supporting organizations include School of Science & Technology, the Open University of Hong Kong and the Association of Harmful Algal Bloom of South China Sea (AoHABSCS).

The Conference will address all issues related to the causes and effects of Marine and Freshwater harmful microalgae and, to serve as a forum for the exchange of the new research results and relevant ideas among researchers, industries, government and interested groups.

The Conference will address the following areas of interest:

- Allelopathy.
- Climate change- impact on harmful algae.
- Ecophysiology and autecology.
- Ciguatoxin.
- Cyanobacteria associated event.
- Genetic diversity, biogeography and dispersal vectors.
- Genomics.
- HAB population dynamics.
- Increased occurrence of harmful algae following natural or man made disasters in the sea and freshwater ecosystem.
- Monitoring for harmful algae and observing systems.
- Public health and economic impacts.
- Taxonomy and phylogeny.
- Toxin synthesis and chemical structure of toxins.
- Toxin analysis and detection methods.
- Toxicology.
- Regional events.

More information at: www.hab2008.hk



HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

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• Australia

First record of dinoflagellate *Gymnodinium catenatum* Graham (1943) from Lakes Entrance, Gippsland Lakes, Australia

Gymnodinium catenatum Graham (1943) was first recorded in Australia from Tasmanian waters in 1980 [1]. Dating of cysts in sediments from the Huon River suggests an introduction date to Tasmania of around 1973 [2]. Vegetative cells and/or cysts have since been recorded from several mainland sites (Fig. 1) [3, 4]. The new finding brings the number of affected Australian bioregions to 12. This is the only algal species on the Australian Quarantine and Inspection Service (AQIS) Marine Target list recorded in the Gippsland Lakes [5].

The Gippsland Lakes (~270 km east of Melbourne) comprise a system of shallow, estuarine lagoons, occupying a former coastal embayment now separated from the Tasman Sea (east of Bass Strait) by long, narrow sandy barriers that form part of the Ninety Mile Beach. They have a surface area of approximately 360 km^2 and comprise three main lakes: Wellington, Victoria and King. The catchment drains an area of 20,600 km^2, about 12% of Victoria's land mass, through five major river systems. A permanent entrance to the sea was established in 1889 at the eastern end of the lakes, at the town of Lakes Entrance (Figs. 1 and 3).

Monthly sampling for dinoflagellates began in the Gippsland Lakes in 2001, as part of an RMIT University PhD project on HAB species. Weekly sampling began in February 2006 for the Environment Protection Agency (EPA) and the Department of Sustainability and Environment (DSE) as part of an expanded Cyanobacteria and HAB monitoring program. In January, 2007, a live (non-preserved) two-cell chain resembling *G. catenatum* (Fig. 2) was analysed by light microscopy from an area of Lakes Entrance known as the Warm Holes (Fig. 3). Cell width is ~38 µm. The surface is covered by amphiesmal vesicles. The deep girdle groove has a displacement of 1/3 – 1/4

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• New Zealand

Massive *Karenia mikimotoi* bloom in Northland, New Zealand: Use of traditional and molecular techniques for rapid identification of HAB species

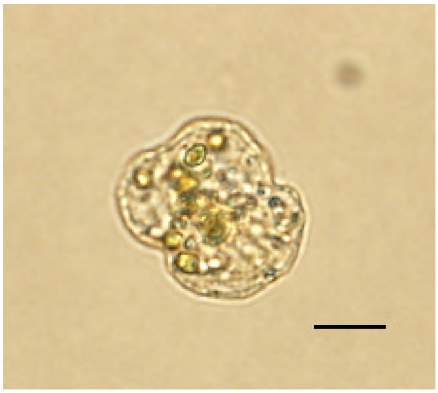

Fig. 1. Lugol's preserved K. mikimotoi *cell from Te Tii (Northland, NZ). Scale bar = 5 µm*

In April 2007 water sampling was initiated by Northland Health Protection Officers after complaints by the public of red water discolouration, and fish and eel deaths in the water around Te Tii in the Te Puna Inlet near Kerikeri (Fig. 2). At the time of sampling (17 April 2007) the red discolouration could no longer be seen, but preserved (Lugol's solution) samples were sent to the Cawthron Institute's Microalgae Laboratory (Nelson) for analysis. *Karenia* cf. *mikimotoi* was identified under the light microscope as the organism producing the bloom, with cell counts over 7.6 x 10 cells L^{-1} (Fig. 2). Species in the genus *Karenia* can be difficult to distinguish from each other under the light microscope [1] and are identified as *Karenia* cf. *mikimotoi* for the New Zealand Non-Commercial Marine Biotoxin Monitoring Programme [2]. This term encompasses the following species: *Karenia bidigitata, K. brevisulcata, K. mikimotoi, K. papilionacea* and *K. selliformis.*

Preserved samples were analysed

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The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

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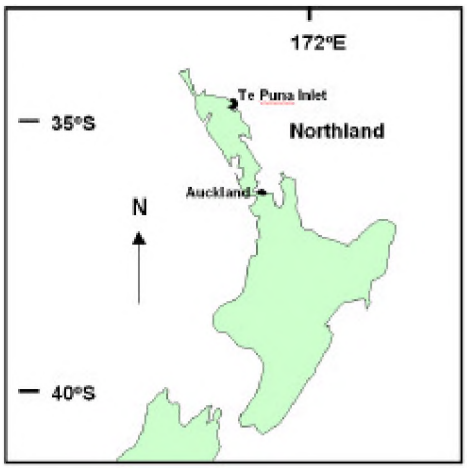

Fig. 2. Map of North Island, New Zealand showing bloom location, Te Puna Inlet, Northland region.

by real-time PCR and fluorescent *in situ* hybridisation (FISH) for a range of species from the *Karenia* genus. Real-time PCR assays were used to detect for *K. mikimotoi, K. brevisulcata, K. umbella, K. papilionacea* and *Takayama tasmanica*, and FISH assays to detect for *K. mikimotoi, K. brevisulcata, K. brevis, K. umbella, K. papilionacea, K. selliformis, Gymnodinium aureolum, Takayama* spp. (genus), and *T. tasmanica*. Samples analysed by real-time PCR showed that the sample was dominated by *Karenia mikimotoi*, with *T. tasmanica* present at very low levels. The preserved samples were successfully amplified in this real-time PCR assay and results were available the same day as sample receipt.

The FISH assay also confirmed the dominant organism as *K. mikimotoi*, however the results were more difficult to interpret as the Lugol's solution interfered with the fluorescent signal despite the addition of a decolourant. *Karenia* cells are also easily destroyed during the FISH process. The FISH assay could not detect the low numbers of *Takayama tasmanica* and analysis time was much longer than for the real-time PCR assay.

Further samples were collected on 19 April 2007 within Te Puna Inlet to determine the extent of the bloom. Live samples were analysed using *K. mikimotoi* probes with real-time PCR, FISH and sandwich hybridisation assay (SHA) formats. All produced positive results with analysis time for real-time PCR and SHA much less than for FISH. At present we have not standardised and quantified the real-time PCR assay for *K. mikimotoi*. However, a standard curve relating optical density readings to cell counts using cultures of New Zealand isolates of *Karenia mikimotoi* has been calculated for SHA, enabling semi-quantification of results. The SHA targets ribosomal RNA (rRNA) and the concentration of rRNA per cell seems to be higher in field samples compared with cultures. From samples from the 2007 Northland bloom we were able to adjust our standard curve to more accurately reflect field samples.

Oyster and water samples taken from Te Puna Inlet were analysed by LC-MS/MS for brevetoxins (PbTx2 and PbTx3). No PbTx2 or PbTx3 was detected in the oyster or water sample by either method. The limit of detection for the LC-MS/MS method was <0.01 mg kg^{-1} for the oyster flesh and <1ng/L for the water sample.

The bloom covered a moderately sized area as additional samples taken on the 19 April throughout the inlet were all positive for *K. mikimotoi* with counts in the millions of cells L^{-1}. Samples taken from a nearby site, Tapeka Point, had lower numbers of *K. mikimotoi* present (21,000 cells L^{-1}). Almost a month after the first visual sighting of the bloom, low numbers *K.* cf. *mikimotoi* cells (ca. 200 cells L^{-1}) were still being detected by light microscopy in samples collected at Kerikeri Inlet, near Te Puna. Specimens from the bloom were isolated and are now held in the Cawthron Microalgae Culture Collection.

The Northland area of New Zealand has previously experienced severe *K. mikimotoi* blooms. In 1992/93, 180 cases of illness that fit the symptoms of neurotoxic shellfish poisoning (NSP) occurred along the coast of Northland [3-5]. Brevetoxins and brevetoxin analogues were detected in shellfish samples causing the total closure of all bivalve industries in New Zealand [4]. The causative organism was initially tentatively identified as *K.* cf. *brevis* (previously *Gymnodinium* cf. *breve*), a species not detected in New Zealand previously. It is now known that *K. mikimotoi* was the dominant bloom species, and *K. brevis* remains undetected in New Zealand waters to date [1].

In addition to brevetoxins, *K. mikimotoi* is known to produce hemolytic glycolipids [1] (that cause gill damage in fish and which have been linked to fish kills in Japan and Norway), and gymnocins A and B [6, 7], which are thought to be ichthyotoxic. The cause of the fish kills associated with the *K. mikimotoi* bloom in the Te Puna Inlet is not known. Oyster and water biotoxin screens by LC-MS were negative for brevetoxins and brevetoxin analogues.

Microalgal blooms can be associated with fish kills due to anoxia, a process that is intensified when a waterbody is stratified. Stratification can be caused by freshwater inputs to estuaries. Two weeks prior to the Te Puna bloom, Northland experienced extreme flooding which increased freshwater and nutrient inputs into the inlet. This is similar to the weather conditions preceding a large *K. mikimotoi* bloom in Irish waters in 2005 [8]. Rainfall averages for the month of the 2005 Irish bloom was almost double the 30-year average. High rainfall levels may have created favourable conditions for the formation of the 2007 Northland bloom and increased the rate of anoxia in the waterbody, resulting in associated fish kills.

By combining the use of traditional (light microscopy) and molecular methods, in particular real-time PCR and SHA, we were able to quickly identify that the bloom causing species was a potential neurotoxin producer and reliably confirm the identification to species level using a combination of molecular tools. The molecular methods were relatively quick and results were available the same day as sample receipt. Microscopy and molecular methods combine well to obtain a definitive species identification, avoiding a possible misclassification of *Karenia* species as occurred with the Northland 1992/93 bloom.

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(Cont'd from p. 1)

of the cell length and the distinctive sulcus extends well into the epicone. Identification was confirmed from this image in August, 2007, by G. M. Hallegraeff and M. de Salas (UTAS, Hobart).

The Warm Holes (Fig. 3) are a series of five shallow pools, remnants of part of the original natural channel to the sea and now a central water-feature of the Lakes Entrance golf course. The navigable section of the original channel is known as Cunninghame Arm, which became a backwater following construction of the permanent entrance. The new entrance is located ~ 5-7 km west of the original natural opening. These ponds are similar in function to the retention ponds constructed as pollutant traps in housing – golf course developments along the South Carolina (USA) coast [6]. This functional similarity results in similar biological outcomes. The tidally restricted, nutrient-enriched ponds provide niche habitat for potential HAB species including *Karlodinium veneficum*, *Karenia mikimotoi* and the cyanobacterium *Nodularia spumigena*.

Water quality at the time of sampling (*Table 1*) shows low dissolved oxygen (DO) and relatively high (although common at this site) chlorophyll *a* levels.

Table 1. Warm Holes water quality Jan 03/07

Depth (m)	°C	PSU	Chl *a* µg/L	DO%	pH
0.4	21.72	34	20	39.7	7.41

Salinity can approach ocean levels in 4 of the ponds and temperatures (16 ºC mid-winter, 2007 Hole 4) are often higher than those found in the broader lake system (8-9 ºC typical mid-winter). The Warm Holes are a significant spawning area for black bream (*Acanthopagrus butcheri*) an important commercial and recreational fish species. Occasional fish-kills are commonly ascribed to the effects of reduced DO levels, but investigative pathology has been limited.

Cunninghame Arm forms the main harbour for a large commercial fishing fleet that operates outside the lakes in the south-east finfish trawl fishery. Prawns (*Melicertus plebejus*, *Metapanaeus macleayi*) are popular recreational species and are also taken commercially offshore. Mussels (*Mytilus edulis*) are harvested non-commercially for human consumption and fish-bait, from the main channel area outside Cunninghame Arm.

Two primary questions arise from the finding of *G. catenatum* in the estuary: (1) Will it be able to establish? (2) How did it get here – offshore ballast-water discharge, fishing boat bilge/ ballast-water, fishing gear, coastal water currents, other?

The species has become well established in Tasmania [7], but mainland populations are ephemeral and vegetative cells and cysts are rarely reported. For instance, extensive cyst beds recorded at Lorne (south-west Victoria) in 1993 were not found again in a subsequent survey [8]. There are no reports of mainland events similar to the extensive Tasmanian blooms in the Huon and Derwent Estuaries (1986, 1991, 1993) that closed the shellfish industry for several months. Intriguingly, dating of Port Lincoln sediments [9] estimates introduction to this mainland site at around 1960 (± 20 yrs) or at least ~25 years ago, yet this population has also failed to thrive.

The reasons for the differences in population dynamics across Bass Strait are unclear. All Australasian isolates (and an isolate from the Seto Inland Sea, Japan) are considered genetically identical [7]. The consistent inability of *G. catenatum* to fully establish viable populations across a broad range of mainland habitats, including sheltered creeks, estuaries, bays and open coast, suggests more than simply a stochastic combination of adverse physical/

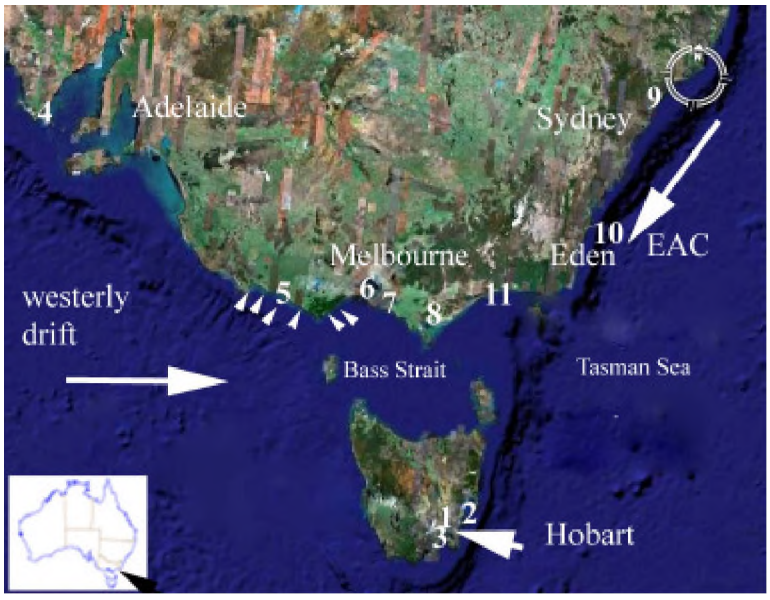

Fig. 1. G. catenatum *sites: (1) Derwent River; (2) Triabunna; (3) Huon River; (4) Port Lincoln; (5) south-west Victoria six sites; (6) Port Philip Bay; (7) Bass River; (8) Port Welshpool; (9) Cowan Creek; (10) Bega coast; (11) Lakes Entrance. East Australian Current (EAC). (Images: Google Earth; NASA; TerraMetrics; Geoscience Australia)*

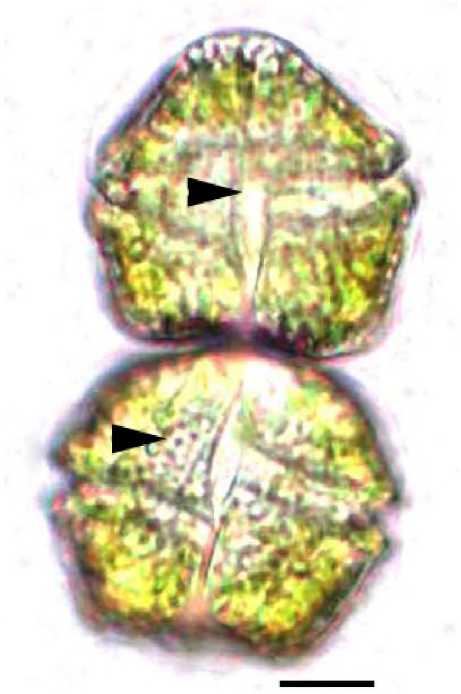

Fig. 2. G. catenatum. *Distinctive sulcus (arrow top) Amphiesmal vesicles (arrow bottom). Scale bar = 10 μm.*

chemical conditions.

Propagule pressure has been identified as a major determinant of species' invasion success [10]. Small populations are more likely to become extinct than larger ones and small populations are more vulnerable to environmental variation. Furthermore, declining populations can be fortified through repeated immigration and sub-populations spread across a number of habitats can mitigate the effects of adverse conditions on the whole population. Does this help explain the establishment pattern of *G. catenatum* in Australia?

The port of Triabunna (Tasmania) was opened for woodchip exports to Japan in 1971, around the estimated time of *G. catenatum* introduction (1973). It has operated more or less continuously since that time. Currently 800,000 tonnes of woodchips are exported annually through the port, equal to about 10 Cape size bulk carriers, each with a 50,000 tonne ballast-water potential. Mandatory ballast-water management was introduced in Australia in 2001, but even triple-flushing of ballast tanks at sea can leave around 5% of the original water, potentially equal to 2.75 x 10^9 cysts/ship [11] (and can create further problems depending on species present at the exchange site). Propagule pressure from 30 years of ballast water import at 10 or more visits per year (depending on ship size) would have been considerable.

Conversely, secondary invasion of mainland sites via ballast-water from Tasmania would have been restricted by: 1) size/type of vessels; 2) frequency of visits e.g. Drake & Lodge [12] conclude that reducing the number of ship visits is more effective in reducing the spread of species by ballast-water, than eliminating key "hotspot" ports; 3) need for carrying ballast i.e. goods are carried both ways; 4) probability of picking up live cells/cysts. Therefore the failure to fully establish on the mainland may be explained in part by low propagule pressure.

However, woodchips have been exported to Japan for over 35 years from the Port of Eden (Fig. 1) yet there is no record of *G. catenatum* from the Eden sediments or water column. Different ballast-water management may explain this apparent anomaly, as deballasting was routinely conducted at sea prior to entering Eden harbour [13], thereby eliminating any propagule pressure.

Proximity of the Warm Holes to the ocean entrance and the boat harbour suggests 2 potential transport vectors: 1) enhanced coastal current intrusion during extended periods of low river-flow; 2) in-harbour vessel bilge/ballast water. The first hypothesis requires either a coastally-entrained inoculum/bloom, or an infected offshore ballast-water dump. Both are difficult to determine, although the East Australian Current (EAC) may play a role in transporting microalgae south from the mainland to Tasmania, and possibly westward to Lakes Entrance (e.g. the appearance of *K. papilionacea* in the eastern lakes in 2003). The directions of current flow and wind through Bass Strait alternate seasonally - west to east in winter; reverse in summer (typically, south-west fronts can appear at any time of the year). Therefore cysts or vegetative cells could potentially be transported from either direction. The second hypothesis may be tested to a limited extent by cyst/sediment surveys at wharf areas and tracking of vessel movements.

On the basis of propagule pressure, risk of establishment in the Gippsland Lakes would appear to be low, although the source of the cells is unknown and repetitive introductions remain possible. Even without the known history of activity at mainland sites, it is unlikely that *G. catenatum* would flourish throughout the lakes, due to the highly volatile salinity regime. However, cyst formation is a life-history adaptation that could facilitate establishment and opportunistic blooming, particularly in sheltered depositional areas around Lakes Entrance where cysts could accumulate, e.g. Warm Holes, Cunninghame Arm and North Arm (Fig. 3). If the species became established, other similar areas within the lakes could provide refugia for cyst accumulation and potential germination and should be monitored.

The finding of *G. catenatum* is a

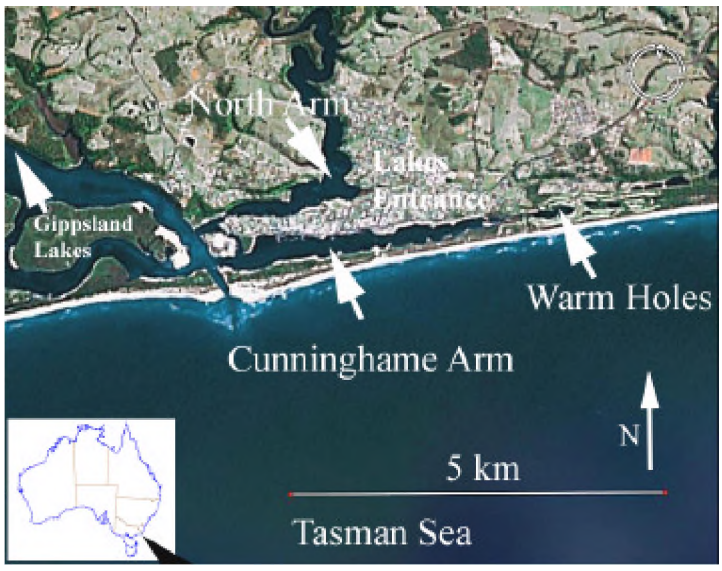

Fig. 3. Warm Holes, Lakes Entrance (Lat. 37° 52' 18"S - Long. 148° 01' 33"E) Gippsland Lakes, Australia (Images: Google Earth; Geoscience Australia)

result of the RMIT University research project and the expanded EPA monitoring program, as no regular monitoring for phytoplankton species was previously conducted in the area.

Major flooding in June, 2007, brought about a reversal of a 10 year dry period, which had seen a steady increase in lake salinity (~0.2 PSU/year at eastern Lake King). However, saline water remains at depth throughout the eastern lakes and stratified conditions can favour dinoflagellate growth through vertical migration. The cycle of drought and flood is a characteristic of this climate and may become more extreme under global warming predictions. It remains to be seen how adaptable this species will be to local climate variation and climate change in general.

Acknowledgements

Thanks to Professor Gustaaf Hallegraeff for comments and advice and Dr. Miguel de Salas for confirmation of species identification; Professor J. S. Buckeridge RMIT University; Dr. Warren Davies EPA Victoria.

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• Israel

Fish kill by *Prymnesium parvum* in the Arava Valley, Southern Israel

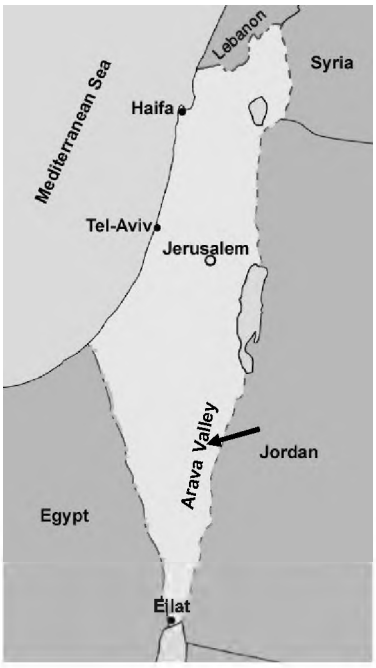

Fig. 1. Location where Prymnesium parvum *bloom occurred (arrowed).*

A bloom of the haptophyte *Prymnesium parvum* occurred in January 2007 in the Arava Valley, a desert area located in the south of Israel (Fig. 1), causing significant mortalities in an ornamental fish farm.

Continuous, 'creeping' mortalities in molly, *Poecilia* sp., and koi ('Japanese carp'), *Cyprinus carpio* ponds in this region started in 2003, when the system which uses water from local aquifers, was switched from flow-through to closed circulation. The fish are grown in a variety of ponds (concrete, plastic-lined and earthen). Water temperature at the farm is maintained at 25±1.5°C and salinity of the inlet water fluctuates between 300 and 500 mg/l of chloride. Effluent water flows through a sedimentation pond and is then recirculated into the fish ponds. Whenever the salinity of the ponds rises to 700-1100 mg/l of chloride (1.3‰ - 2‰) due to evaporation, fresh well water is added.

Gross pathology, parasitological and histopathological analyses in a sample of about 20 molly and koi did not reveal the presence of pathogens or any particular abnormalities, as was also reported in similar cases by other authors [1, 2]. A microscopic examination of the water, however, revealed relative abundance (exceeding 1 x 10^5 cells/ml) of the haptophyte *Prymnesium parvum* (length 13.5 µm ± 1.5, width 9 µm ± 1) (Fig. 2). A bloom of the green microalga *Kirchneriella lunaris* was also observed, while the presence of diatom species of *Chaetoceros* and *Skeletonema*, and microflagellate species of *Cryptomonas* and *Chlamydomonas* was also detected. No mortality occurred in ten fish transferred into an aquarium with fresh, uncontaminated water and kept under observation for over a month, indicating that the fish were in an initial and still reversible stage of intoxication, a feature already noted by other authors [3].

The system was treated with 10 ppm ammonium sulfate according to the recommendations of Reich and Aschner [1], and fish mortality ceased.

Outbreaks of this flagellate in Israel were first reported in the fall of 1945 [1]. Carp-stocked ponds in the Beisan (Beit She'an) Valley in the northern part of the country were affected. The condition gradually spread south and in the following winter reached the ponds of Beit–Ha'arava, a settlement north of the Dead Sea. This occurrence was not surprising, as although situated approximately 100 km apart, these two localities shared the same water supply.

Deterioration of water quality in aquaculture often creates conditions suitable to *P. parvum* blooming and consequent fish poisoning [2, 4, 5].

Aquifers in the southernmost part of Israel and mainly desert Arava Valley often yield brackish water, free of pollutants, that can be used to raise warm-water fish.

P. parvum propagation in this recent incident was quite clearly triggered by the up to three-fold increase in salinity, and favored by the moderately warm water of the fish pond and general

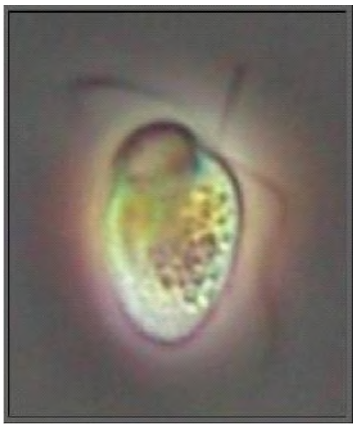

Fig. 2. Prymnesium parvum.

deterioration of recirculated water quality.

The renewed occurrence of *P. parvum* in this region is an unwelcome incident, which may require new management strategies to control its spreading.

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• Mexico

A bloom of *Noctiluca scintillans* and its possible cysts in the Gulf of California, Mexico

Marked discolorations were observed on 12-13 February 2007 at stations 13, 14 and 15, during Cruise Talud X in the central Gulf of California. The dominant taxon was *Noctiluca scintillans*, followed by *Pseudoeunotia doliolus*, *Chaetoceros* spp. and *Gymnodinium catenatum*. *Noctiluca* was counted in 50 ml surface samples settled for 24 hours [1]. Results (Fig. 1) were similar to those reported elsewhere (range 60 to 3720 cells L^{-1}) [2]. Surface temperature (17 to 18.9 °C) and salinity (35.1 to 35.3 PSU) data collected by CTD were within normal values for the season.

Most *Noctiluca* cells measured 200 to 800 µm diameter, corresponding to trophonts (Fig. 2A-B). Nevertheless, some cells resembling a gametogenic event were also observed (Fig. 2C). The life cycle of *Noctiluca* consists of a vegetative stage (trophont) and a zoospore forming stage [3, 4]. Until recently, the fate of zoospores was unknown, but Fukuda and Endoh [5] describe the complete life cycle, showing the isogamic, homothallic nature of this process; there may also be anisogamy, if the zoospores are considered as microgametes. Some vegetative cells look like macrogametes [6], a view sustained by an intermediate stage observed here (Figs. 2B, C, E); other specimens recall *Leptodiscus* sp. (Fig. 2D; Fig. 3B), identified for the first time in this region.

In some samples of this *Noctiluca* bloom, a structure resembling a cyst of great size was observed, spherical, 1.14 mm diameter, with triangles of 80 µm on each side all over the surface. On

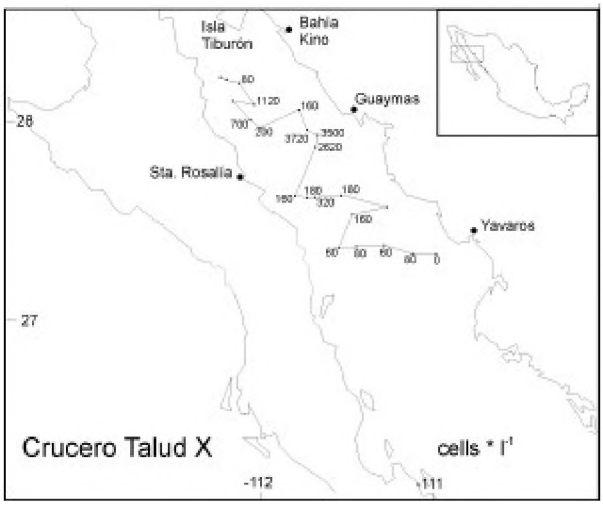
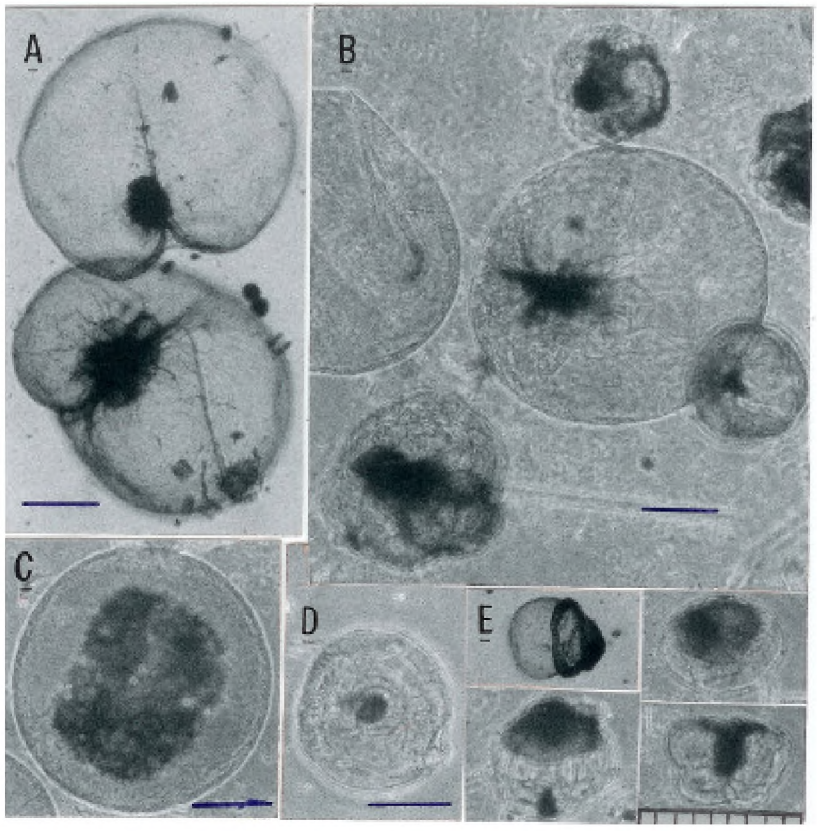


Fig. 1. Abundance (cells L^{-1}) of Noctiluca scintillans *in surface samples collected in Gulf of California, 10th to 15th February, 2007.*



Figs. 2. Noctiluca scintillans. *A) Two cells in vegetative stage (trophont). B) Gametocyte mother surrounded by small cells. C) Nucleated stage? D) Possible Leptodiscacean. E) Four different small specimens, scale bars 50 µm. Other scale bars 200 µm.*

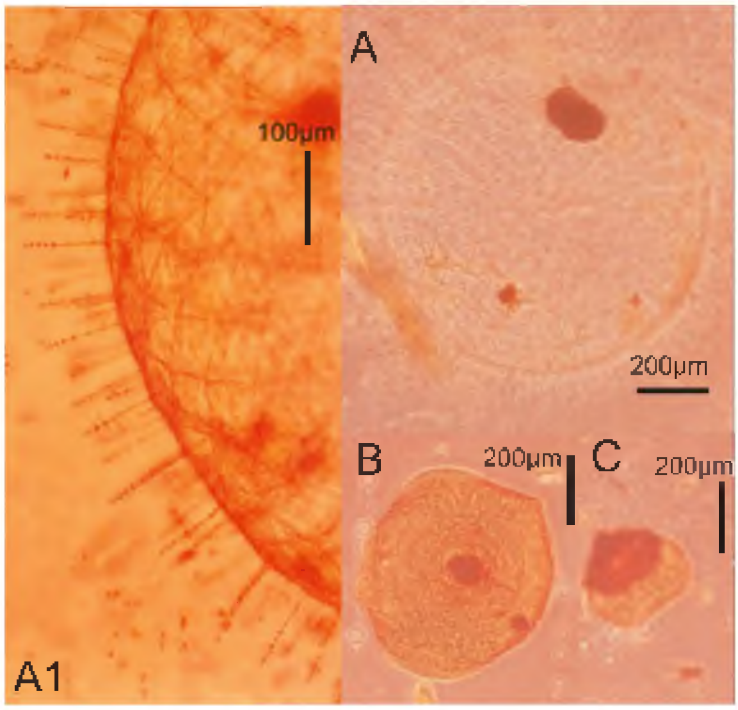


Fig. 3. Possible cyst of Noctiluca. *A) Spherical structure ornamented with small triangles of 80 µm on each side and projecting spines on vertices. A1) Detail of projections, which bear small spines near their ends. B) Possible* Leptodiscus. *C) Small trophont.*

the vertices of each triangle, a process projects outside bearing small spines distally (Figs. 3A-A1). To discard the possibility that we were dealing with a copepod egg, a thesis dealing with 16 species was reviewed and none showed a similar structure [7]. Furthermore, copepods eggs are smaller than 250 µm in diameter. However, we cannot yet confirm that it this is a cyst of *Noctiluca*.

Acknowledgements

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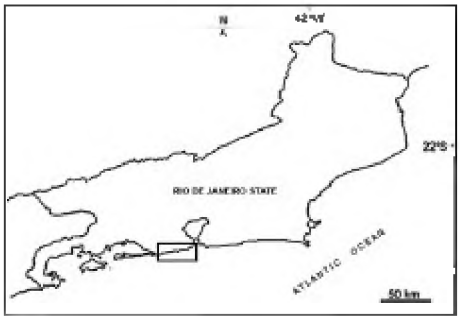
• Brazil

Bloom of *Alexandrium minutum* Halim on Rio de Janeiro coast: occurrence and toxicity

For the first time in the region, a major bloom of *Alexandrium minutum* Halim was observed off Rio de Janeiro Coast, 23 April 2007 (Fig. 1). Water discoloration was detected from 21st to 26th April, covering an extensive area including several beaches. The red to brownish water was fully evident to beach goers, and reported in the newspapers.

Phytoplankton samples were collected on the 25th and later on 27th, when a surface sand sample was also taken from Leblon beach (Fig. 1). The cells from 250 L of the bloom water samples were concentrated in a 10 µm



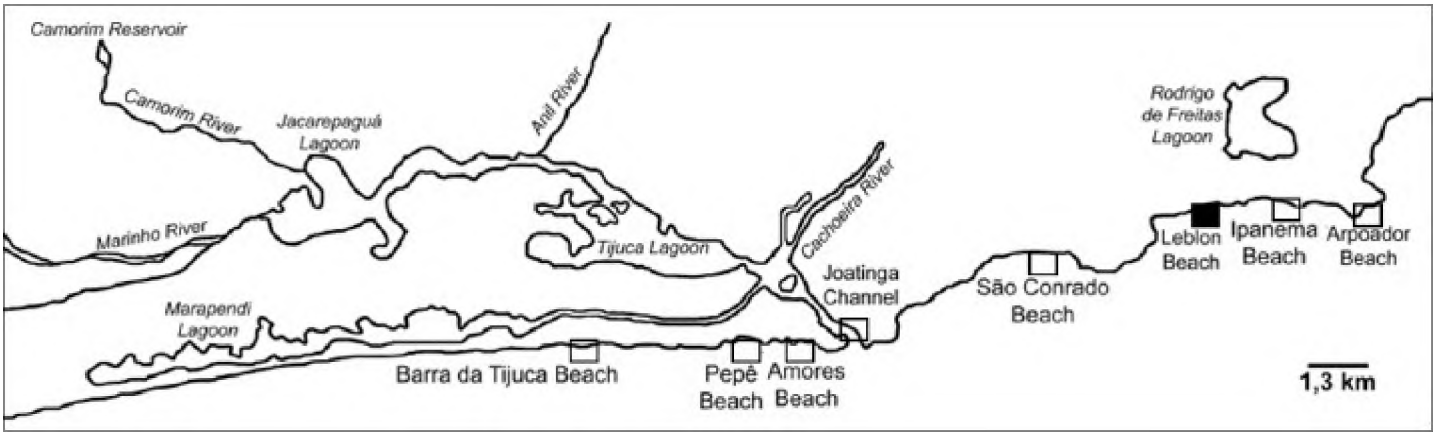
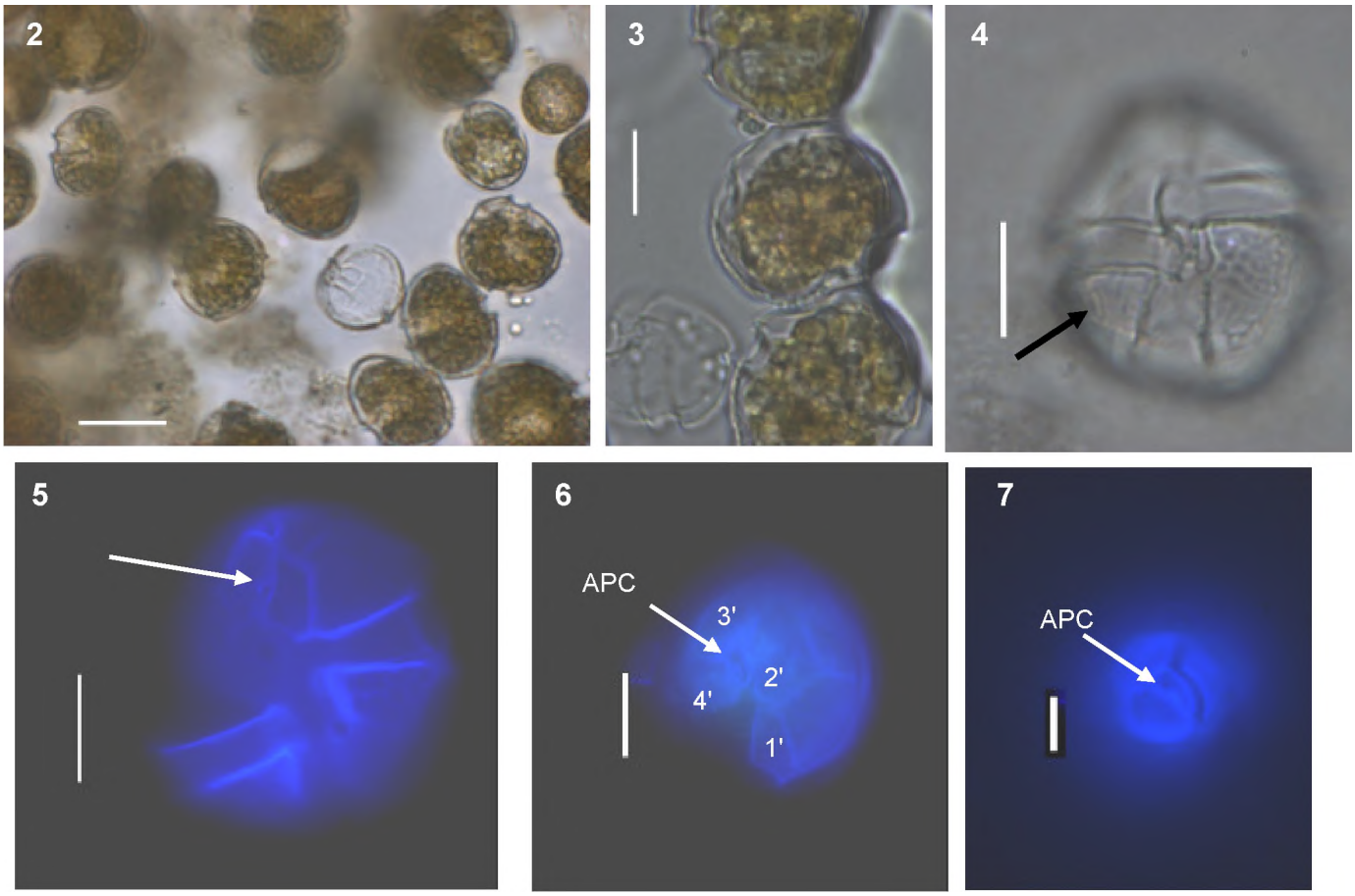


Fig. 1. Map of Rio de Janeiro Coast showing the bloom area (open rectangles) and sample site (closed rectangle)



Figs 2-7. General morphology of A. minutum *cells under light microscopy (bright field): (2) isolated live cells; (3) chains; (4) ventral view of clarified cell showing the reticulated pattern of hypotheca (arrow). Figs 5-7. Calcofluor stained cells under fluorescence microscopy: (5) ventral view of cell showing a conspicuous ventral pore (arrow); (6, 7) apical view of cell showing the apical pore complex (APC) (arrow) and the four apical plates (').*
Scale bars = 20 µm (Fig. 2); 10 µm (Figs 3-7).

net to 500 ml. Part of the sample was fixed for identification and enumeration under microscopy and the remainder further concentrated and stored for toxin analysis. For the analysis of sand, 20 g was removed from a total of 1 K of collected sediment. From this, two aliquots of 1 mL were extracted, made up to 10 mL with filtered seawater, homogenized and filtered through 80 µm and 20 µm sieves, respectively. The slurry from each aliquot was made up with filtered seawater to a total of 10 mL. One aliquot was used for counting and the other for morphological analysis.

Table 1. Toxin profile obtained by post column HPLC from A. minutum *bloom collected on Rio de Janeiro Coast, 25/04/2007.*

Toxin	Extract concentration ng/ml	Concentration in STX equivalent (%)
GTX 1	0	0
GTX 2	31.0	7.79
GTX 3	0	0
GTX 4	8.8	4.31
GTX 5	0	0
dc-GTX2	0	0
dc-GTX3	0	0
Neo-STX	130.1	83.47
dc-STX	0	0
STX	6.3	4.43

The dissociated and clarified thecal plates were stained by Calcofluor® and observed under light and fluorescence microscope. The quantitative analysis was carried using settling chambers under an inverted microscope. A small pellet containing bloom cells was extracted with 0.1 N HCl under sonication and promptly analyzed for toxins, using the HPLC post column derivatization method of Oshima [1]. The peaks were compared to 10 available standards purchased from NRC/Canada.

Alexandrium minutum cells were found in the water and also in the sand samples. The samples showed the common phenotypic plasticity of vegetative morphological characters generally observed in natural populations [2-5]: cells usually solitary, or sometimes forming short chains of 2-4 cells; oval shaped to irregularly elliptic in dorsal view, with (17.1-) 19 - 31.2 (-32.3) µm long; 17.1 -32.2 (-32.3) µm width (n = 100) (Figs. 2-7). The thecal formula is Po, 4', 6", 6C, 8S, 5", 2"", characteristic of *A. minutum*. The Po plate is directly connected through a channel to the pore less plate 1. The foramen is drop shaped and a ventral pore is clearly observed on plate 1'. The margins of the hypothecal sulcus generally present expansions resembling spines, and the posterior sulcal plate is without a pore. Most individuals (75%) showed a thick reticulate pattern with hypothecal *areolae*. Some cells showed irregular epithecal spacing similar to *striae*, and rare reticulate spacing.

Cell abundance ranged from 4.3 x 10^5 to 1 x 10^2 cell L^{-1} on April 25th and 27th respectively. Vegetative division was observed in samples from April 25th, while sexual reproduction, with consequent cyst formation was observed in water and sand samples collected on 27th. The sand sample contained 1.2 x 10^3 cell L^{-1}, of which 30% were cysts. Cysts were very similar to those found at Sepetiba bay, south of the bloom area [6].

The toxin profile was dominated by Neo-STX, followed by GTX2 (Table 1).

The toxin profile is similar to that observed for *A. minutum* isolated from New Zealand [7], in which Neo-STX also dominated, and distint from European isolates in which GTX 4 was the major toxin [8].

This is the first report of vegetative cells of *A. minutum* from the Western South Atlantic. Among species of *Alexandrium*, *A. minutum* apparently occupies a broad niche, and has a large geographical distribution [9-11]. Its present regional and global distribution has being hypothesized to result from bio-invasion, as a result of ballast water translocation [11]. In fact, during a cyst survey for the Globallast project in Sepetiba bight, cysts of *A.* cf. *minutum*, among other, were reported in the region. Sepetiba is an important port subjected to large ballast water discharges, located about 80 km south of the bloom region. Considering a bloom as a manifestation of species opportunism, the fact that *A. minutum* vegetative cells were never before recorded in the region, and the fact that it was first reported as a cyst in a nearby port area, the idea of ballast water introduction appears to be quite attractive to explain its presence in Rio de Janeiro waters.

Together with *Gymnodium catenatum* [12] and *Alexandrium tamarense* [13], this is third marine PSP producing species found in Brazilian coast. Although during the bloom no other harmful effect hater than the aesthetic was observed, this species can affect the flourishing mussel aquaculture in the country.

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PLANKTON*NET: A new and improving tool for studying and monitoring Harmful Algal Blooms

Introduction

PLANKTON*NET is an online family of websites with information about all types of plankton. It is a source of authoritative information about plankton taxa, featuring images, schematics, taxonomic descriptions and glossaries, and soon also online taxonomic keys for different taxon groups. It is also a repository for historic and current distribution data of plankton organisms. Most of the PLANKTON*NET records deal with marine phytoplankton, including harmful algae, but we are also increasingly archiving information on additional taxa, particularly zooplankton and also on freshwater organisms e.g. cyanobacteria.

PLANKTON*NET currently consists of three sites (at the Alfred Wegener Institute for Polar and Marine Research (planktonnet.awi.de), Station Biologique de Roscoff (planktonnet.sb-roscoff.fr/index.php) and University of Lisbon (plankton-net.fc.ul.pt), with two more sites being planned for autumn 2007).

In each of these sites data are entered by the core PLANKTON*NET group, also including the Natural History Museum in London and IPIMAR in Lisbon as important data contributing members, but also by a growing number of external contributors from locations all over Europe and beyond. After a very simple registration process, these external partners can enter their own images as individual images or, more commonly, as part of an image set. They can add and modify records and customize the web interface according to their own needs and requirements.

New features

In all areas of plankton taxonomy and ecology but particularly in the field of HAB research, access is required to a very large number of different online resources. The great diversity of information sources available to an online user can make it very difficult to extract the most up to date or the most relevant information for one's needs. PLANKTON*NET remedies this situation by offering both the opportunity to learn about the taxonomy and ecology of harmful algae as well as a facility for entering observational data from historic data sets as well as current research projects such as cruises etc. As PLANKTON*NET is not only populated by the PLANKTON*NET partners but also by a number of external contributors, this gives us the unique opportunity to produce an easily and openly accessible online inventory

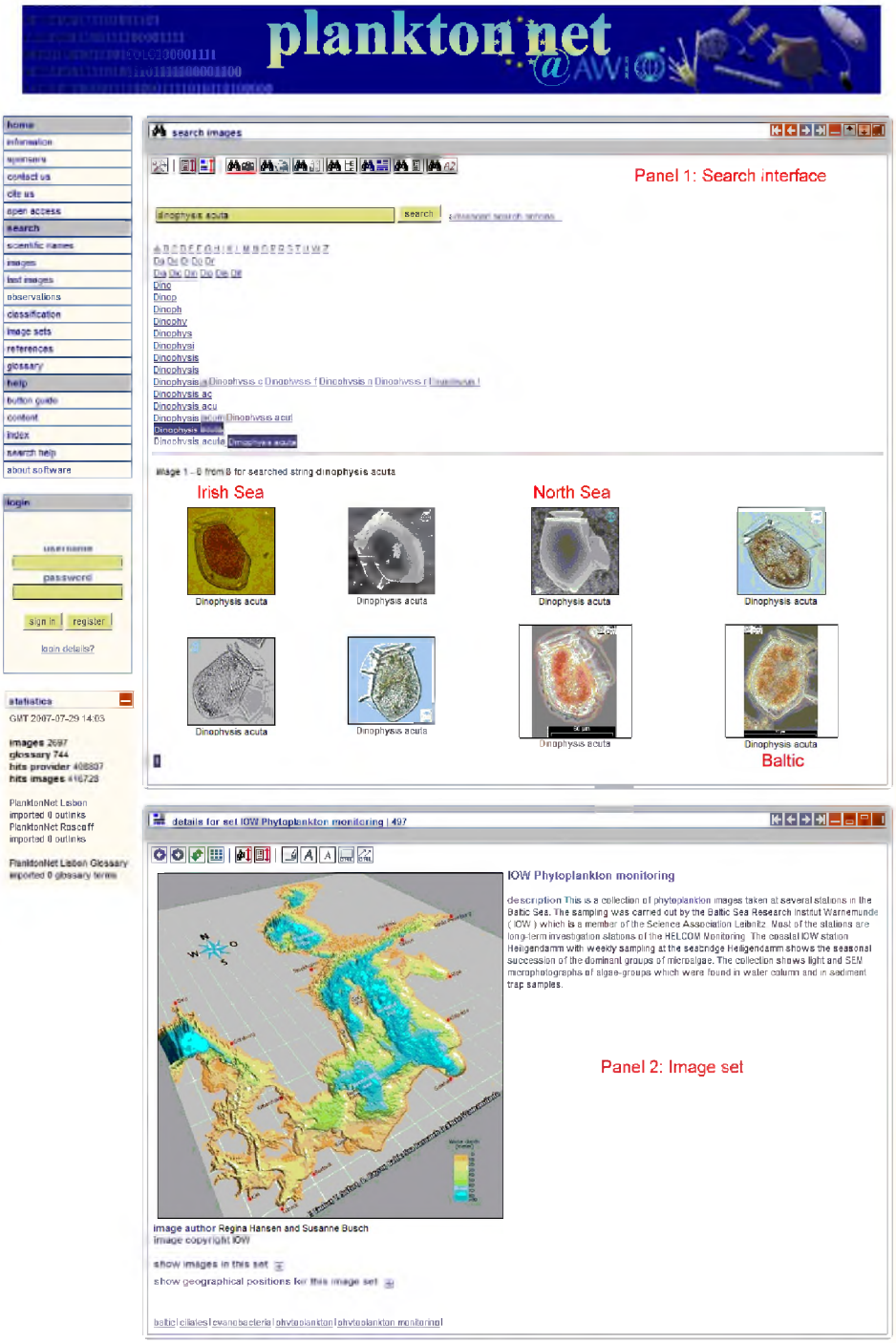

*Fig. 1. Example of PLANKTON*NET layout showing two panels: one for searching images (showing a search result with images from three different locations), and one to browse through and open image sets. This layout is customizable. Panels can be closed and minimized/maximized. They can be moved up and down the screen e.g. to place data input forms and observation search forms adjacent to each other.*

of harmful algae with comprehensive, accurate and up to date information, not only about the taxonomy of organisms but also about their distributions, which are of particular importance in HAB research. An exciting new development in this context is also the design of a central portal through which searches of information can be made in all PLANKTON*NET sites simultaneously. To further enrich the portal, links to a number of additional online databases including library resources and databases containing environmental information are also being created, so that a search e.g. on a given species will retrieve all relevant records from PLANKTON*NET sites, literature references and available environmental information.

To organize the diverse and complex content now at the core of PLANKTON*NET, individual PLANKTON*NET sites have also been redesigned to enable the user to draw the greatest benefit from the PLANKTON*NET resource:

1. A new interface: A panel structure has been established with all panels in one page (e.g. search and data input panels). This system is extremely flexible (each panel can be minimized, closed or be moved up and down the page to customize it). In this way one can for instance enter new terms in the glossary while also carrying out searches on glossary terms (Fig. 1).

2. Tagging: Users can now add their own tags (diatoms, open ocean, cultures, etc.) to facilitate custom-made searches.

3. Custom image sets: One image can now be part of several image sets. In this way a user can produce custom made sets of images, e.g. restricted to harmful algae, particular taxon groups or records from a particular geographic area that can be used in teaching or for other presentations.

4. New search engines The site includes powerful browse and search functions allowing the user e.g. to search for distribution records of a given species, or look for all records in a given geographic location. These search facilities can be used to quickly find information on, for example, scientific names, images or image sets and to browse through the classification tree. Images and distribution data can also be browsed alphabetically.

5. New taxon detail pages: These now include, in addition to images, links to classification trees and short taxa descriptions a graphical representation of all locations for which PLANKTON*NET records of the taxon in question exist (Fig. 2).

Future Plans

At present, while we already have the means for collating detailed distribution records for any given harmful species, this is restricted in terms of its graphical representation, to a display of locations at which a species occurred at some point in time. What we would like in the future is a facility that also allows us to follow the time course of development of a given species, and therefore potentially to track the direction in which a developing bloom is moving. To accomplish this PLANKTON*NET will essentially

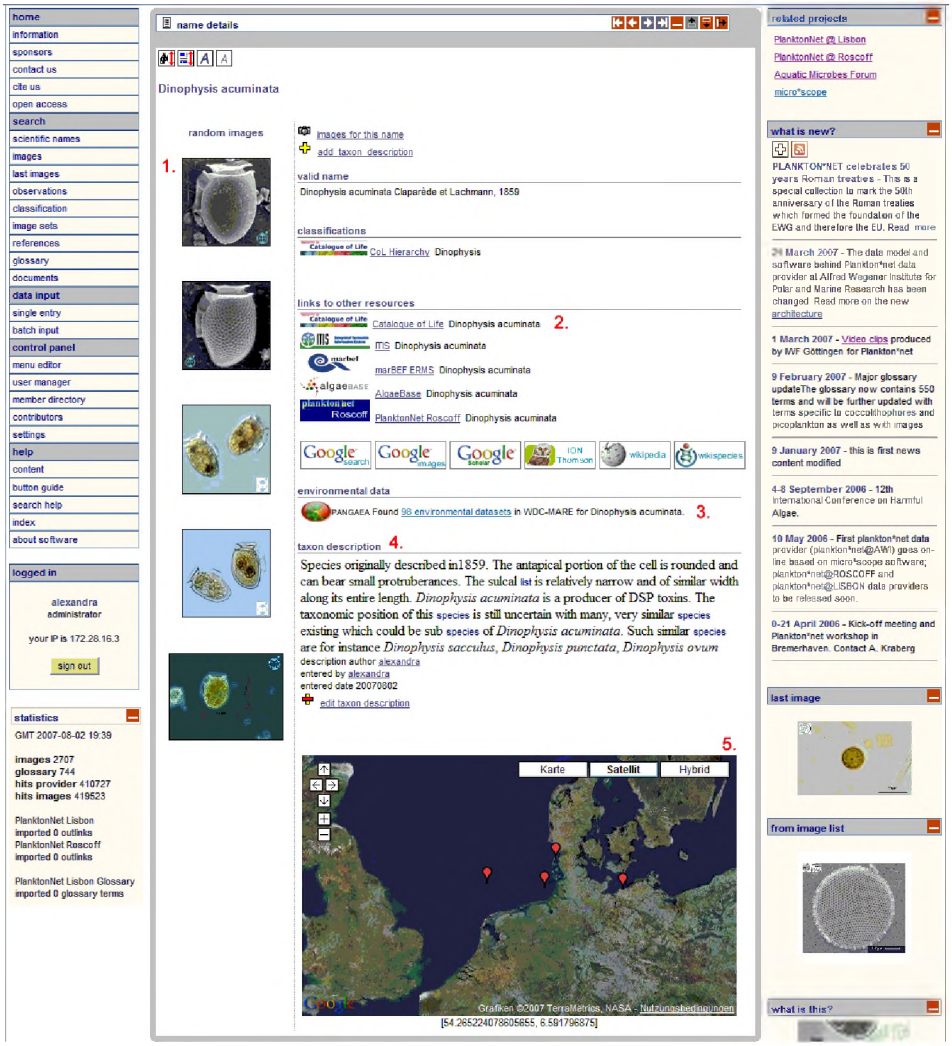

*Fig. 2. Example of a taxon details page showing the different components: 1. Enlargeable images, 2. Links to external resources, 3. Link to the environmental database Pangaea, 4. Brief taxon description, 5. Map showing the locations for which records of the species appear in PLANKTON*NET.*

follow a two pronged approach: 1. The development of the necessary IT tools for mapping species data, and to further increase the data input facilities (e.g. to include toxin data) and 2. Collation of the data to populate the resource so as to form a high resolution observatory that can then also give warnings about the occurrence of a given species. This obviously requires a large joint effort and we therefore seek and encourage collaboration with additional external partners.

If you have any questions regarding PLANKTON*NET, would like to contribute data, or have any suggestions for improvements, please contact the PLANKTON*NET co-ordinator: Alex Kraberg (Alexandra.Kraberg@awi.de) or the local site co-ordinators.

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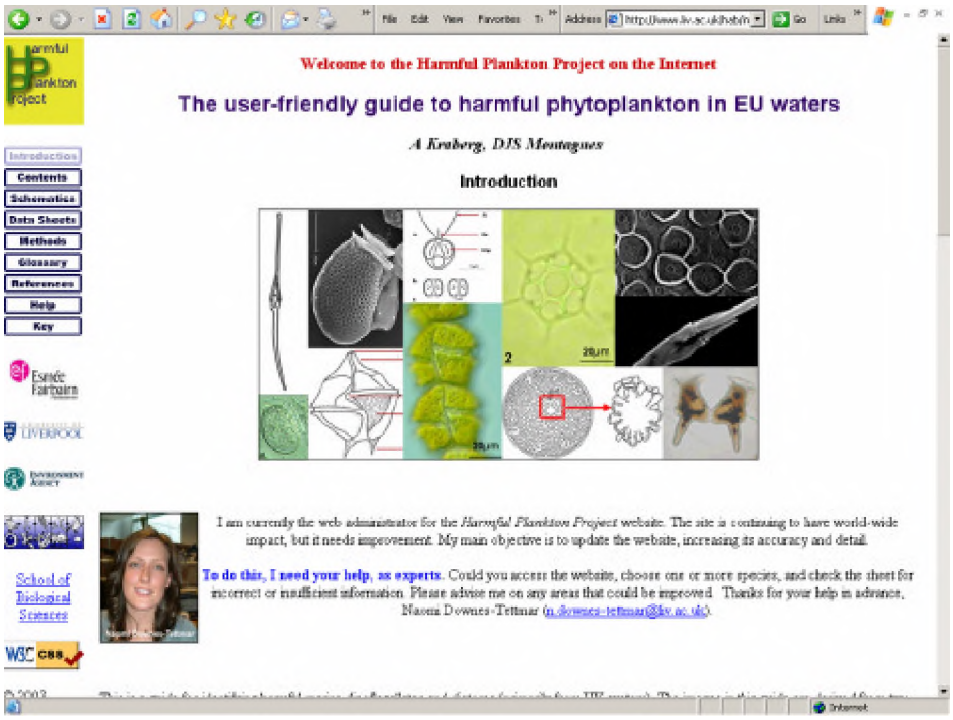
Updates on the User-Friendly Guide to Harmful phytoplankton (in EU waters)

The Identification of harmful algal species (HABs) is often impeded by the large number of sources of information, on the disparate taxonomic groups. Although books and guides exist that aid in their identification, resources still need to be developed to provide relevant, easily accessible, and current information on HABs; electronic resources are a logical direction. Over the last four years, we have been developing an online guide to harmful plankton at *The University of Liverpool* that is globally accessed by 1000's of users (www.liv.ac.uk/hab/intro.htm). For a detailed description of the HAB site and its sister site on planktonic ciliates, see Kraberg *et al.* [1], Kraberg & Montagnes [2], and Strueder-Kypke & Montagnes [3]. In general, these sites have as their centre, a series of "data-sheets" (one for each taxon), but they also includes sampling and fixation methods, references, and a glossary. Furthermore, the entire sites can be downloaded or saved and printed as pdf files. Thus they provide an easily updated, "user-friendly" repository of information for scientific, non-scientific, technicians, and students.

After a brief hiatus, where we lacked funds, the *Liverpool* HAB site is now being developed, thanks to 10 months of support by the Esmée Fairbairn Foundation. One of the main goals of our present work involves



adding and updating datasheets for species, making the resource more comprehensive. An element of this work includes mapping current and historical harmful algal blooms showing their distribution and toxicity levels in an easily accessible format. This is done using NASA World wind software to produce global images akin to Google earth.

Further information is also needed to improve this internet repository. Specifically our goal is to help researchers identify live and Lugol's-fixed samples. To this end we need to incorporate a greater selection of images into the datasheets and further details on key features and similar species. We also need to ensure that our identification criteria are accurate. These goals will only be feasibly reached through collaboration from the HAB community: we encourage researchers holding taxonomic information and expertise about harmful phytoplankton to visit our site and offer assistance. We invite collaboration, comments, and criticisms regarding the website. Interested parties should contact Naomi Downes-Tettmar, Email: n.downes-tettmar@liv.ac.uk.

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• Argentina

First detection of domoic acid produced by *Pseudo-nitzschia* species, Chubut coastal waters, Patagonia, Argentina

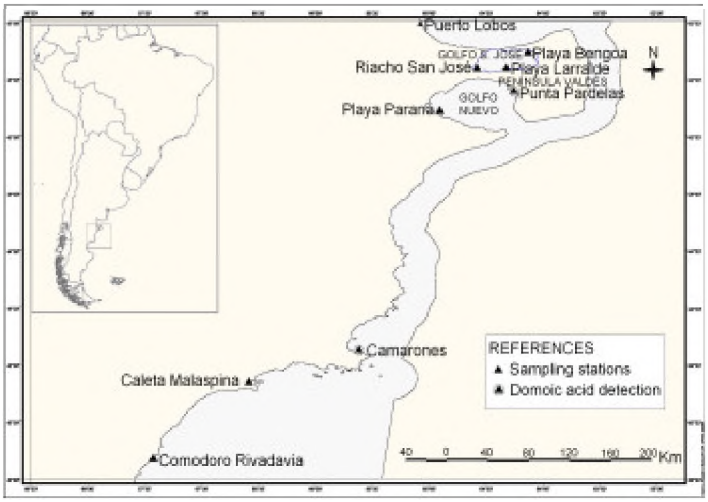

Fig. 1. Study area with sampling stations.

A Harmful Algal Blooms Regional Monitoring Programme has been carried out in Chubut coastal waters (Patagonia, Argentina) since the year 2000. This programme ranges over an extended shoreline (approximately 1,000 km) with bays and gulfs where shellfish are extracted from natural banks, and where there are also mariculture activities. The programme includes monitoring of harmful algal blooms and marine environmental conditions, shellfish toxicity control, educational and training activities and dissemination of information, detection of human consumers' intoxication cases in public health facilities, and closures to ban shellfish extraction and sales. The study area is located approximately between 42º00'-46º00' S and 64º00'-67º30' W.

Phytoplankton samples for qualitative and quantitative analysis and environmental parameters are collected from 14 stations. Analysis for Domoic Acid (DA) toxins was added from October 2005. In the period August 2005 - August 2006, DA in net phytoplankton samples from Punta Pardelas and Bahía Camarones monitoring stations (Fig. 1) was detected in October 2005. Analytical procedures were based on Quilliam *et al*. [1] method, Solid-Phase Extraction (SPE) being added. DA concentration was 0.66 µg/100 ml in a sample from Punta Pardelas (Fig. 2), and at lower levels at Camarones (Fig. 3).

DA is a potent neurotoxic aminoacid produced by some species of *Pseudo-nitzschia* genus which causes Amnesic Shellfish Poisoning. This can be fatal to human beings, sea mammals and birds after consumption of contaminated shellfishes or/and fishes. DA was

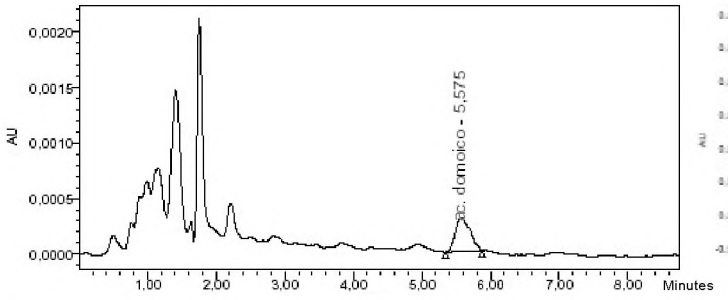

Fig. 2. Domoic acid chromatogram from Punta Pardelas sample.

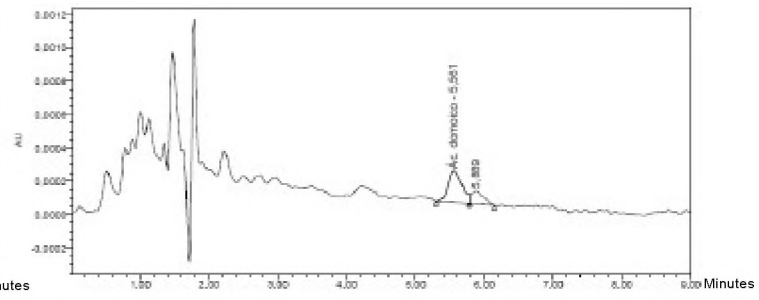

Fig. 3. Domoic acid chromatogram from Bahía Camarones sample.

previously detected in continental shelf waters of the Argentine Sea (Southwestern Atlantic Ocean) in July 2000 associated with *Pseudo-nitzschia australis* in Mar del Plata, province of Buenos Aires. This neurotoxin was present in natural samples of phytoplankton, mussels (*Mytilus edulis*), and muscle tissue and gastrointestinal contents of anchovies (*Engraulis anchoita*) [2, 3].

Net samples were analyzed with a Wild M20 light microscope (LM) and a Jeol JSMT 100 scanning electron microscope (SEM), following Ferrario *et al.* [4]: *Pseudo-nitzschia fraudulenta* and *P. pungens* were identified. *P. pungens* showed strongly silicified and linear fusiform valves with distinctive pointed ends; interstriae are approximately equal in number to fibulae (11-12 in 10 µm). The raphe is not interrupted by an interspace (Fig. 4a-c). *P. fraudulenta* exhibited weakly silicified and convex-sided lanceolate valves with rounded ends; interstriae (18-24 in 10 µm) are slightly denser than fibulae (16-24 in 10 µm). The raphe is interrupted by a larger central interspace (Figs. 5 & 6). This species is morphologically close to *P. subfraudulenta*, but *P. fraudulenta* can be discriminated by evaluating some morphometric data, such as numbers of fibulae and interstriae (approximately equal) and non-parallel lanceolate valve margins [5]. In the study area, *P. fraudulenta* was the most abundant species with a peak of 24,200 cells L^{-1} in Punta Pardelas; *P. pungens* was detected at both stations in low concentrations in qualitative samples.

Temperature, salinity, nutrients (nitrate + nitrite ($NO_3^+ + NO_2^-$), ammonium (NH_4^+), reactive phosphate (RP) and reactive silicate (RSi), chlorophyll a- and Secchi disk depths were determined at Punta Pardelas and Bahía Camarones stations during the August 2005 - August 2006 period. At the Punta Pardelas site, temperature ranged from 10 to 16°C, mean salinity was 33.99 ± 0.14 psu (n=11). $NO_3^- + NO_2^-$ were not detected from spring to autumn while the highest concentration (4 µM) was in winter. NH_4^+ ranged from 0.2 to 2.7 µM. Concentrations of RSi were lower than 1.1 µM from spring to autumn, with a peak of 4.6 µM in winter. Mean RP was 1.0 ± 0.2 µM (n=10). Chlorophyll a (records available only between November 2005 - April 2006) did not rise higher than 0.8 mg m^{-3}. Mean Secchi disk depth was 8 ± 1 m (n=7). At the time of DA detection in October, water temperature was 13°C (3°C higher than September) and salinity 34.04 psu. $NO_3^- + NO_2^-$ and RSi were not detectable. These nutrient concentrations decreased 96.5% and 100% respectively between September and October 2005; NH_4^+ decreased 30%, and Secchi disk depth (11 m) increased 3 m.

At the Bahía Camarones site, (August 2005 - August 2006) temperature ranged between 8 and 15°C. Mean salinity was 33.55 ± 0.11 psu (n= 11). Maximum of $NO_3^- + NO_2^-$ (7.3 µM) was observed in winter;

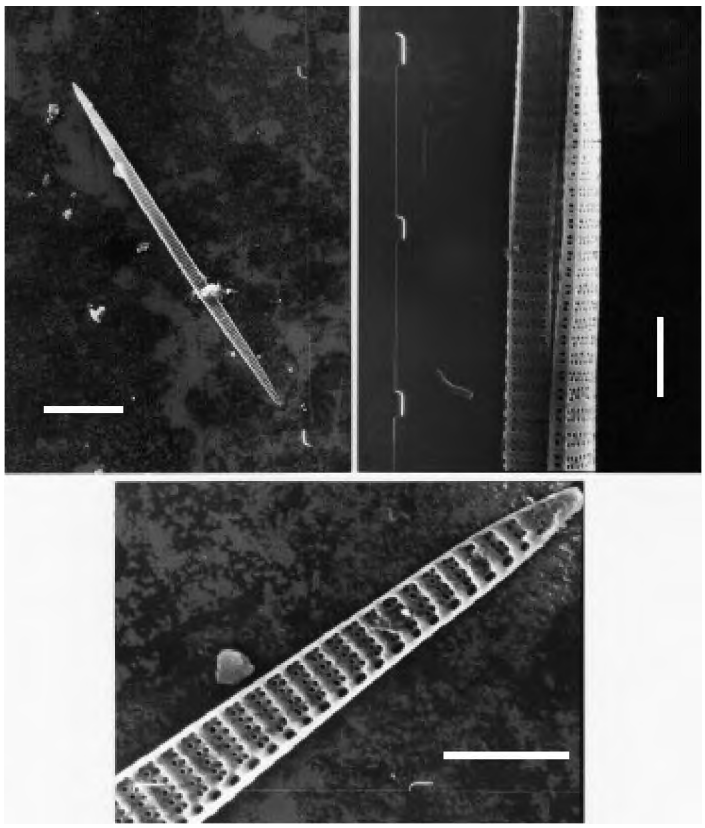

Fig. 4. P. pungens *(a-c). a, internal view of whole valve showing pointed poles. Scale = 20 µm; b, Part of valve in internal and external view showing mantle. Scale = 5 µm; c, valve apex, note the biseriate striae with round poroids. Scale = 5 µm.*

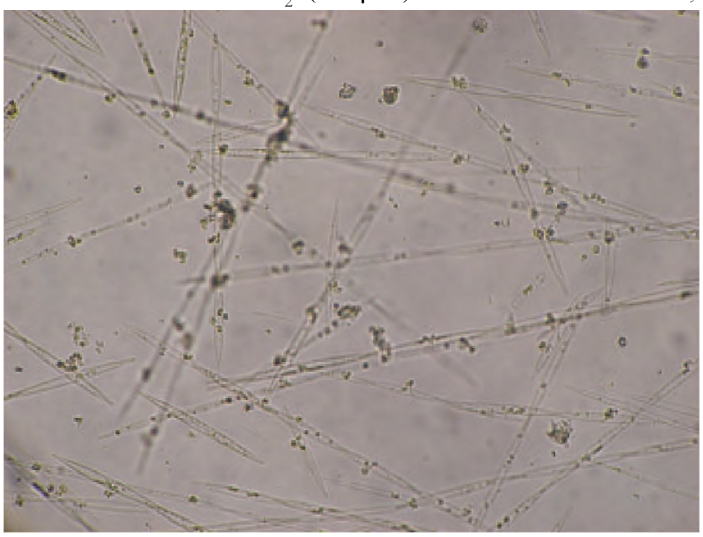

Fig. 5. Light microphotograph from the October bloom of P. fraudulenta *in Punta Pardelas. Scale = 40 µm.*

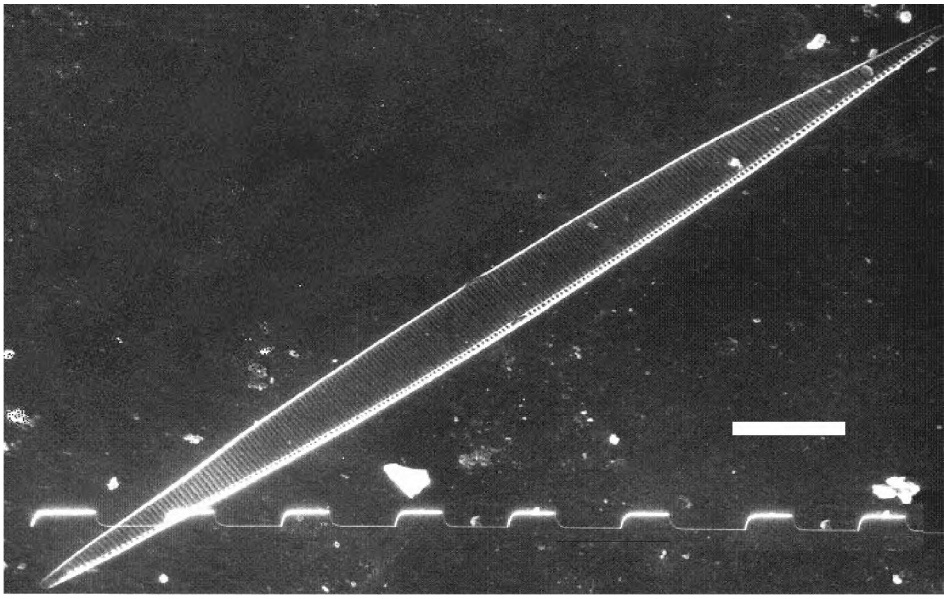

Fig. 6. P. fraudulenta. *Internal view of whole valve showing fibulae and central interspace. Scale = 10 µm.*

most of the period concentrations did not rise higher than 3.5 µM and were not detectable in November and December 2005. NH_4^+ ranged from 0.3 to 2.5 µM and RSi from 1 to 5 µM. Mean RP was 1.1 ± 0.2 µM (n=11). Mean chlorophyll *a* concentration was 2.0 ± 0.3 mg m^{-3} (n=7) and mean Secchi depth was 6 ± 2 m (n=7). At the time of DA detection water temperature was 9°C and salinity 33.65 psu. All nutrients decreased in comparison with September (RSi: 64%; NH_4^+: 45%; NO_3^- + NO_2^-: 34%; RP: 26%); Secchi disk depth (8.3 m) increased 2 m.

P. pungens and *P. fraudulenta* are known to be toxic. Both species co-occurred during the spring bloom at the Punta Pardelas and Bahía Camarones stations where DA was detected. Blooms coincided with nutrient decreases and Secchi disk depth increases in both places. This is the first report of DA production in Chubut coastal waters (Patagonia). The high density of *P. fraudulenta* in the sampling area may indicate that it was the DA producer. In spite of the fact that during this event there were no harmful consequences, we believe that the monitoring programme in Chubut shellfish harvest areas must be continued.

This work was supported by the PNUD ARG 02/018 DON 28385 AR Project.

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Professor Enrique Balech

On August 26th 2007, a few days after celebrating his 95th birthday, Prof. Enrique Balech died in Necochea, Argentina, where he spent the last 50 years of his life.

Enrique Balech Capdeville was born in Telén, La Pampa, Argentina on August 17th, 1912 in a family of French origin. He studied Natural Sciences at the Instituto Superior del Profesorado "J.V. González" in Buenos Aires, and specialized in marine plankton. In 1937 he became chief of the Laboratory of Protistology of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". He later moved to Necochea as chief of the Estación Hidrobiológica Puerto Quequén, until he was dismissed, in 1947, for political reasons. He then worked as a high school teacher to earn a salary, and continued his research while working from home. In 1951 he received a fellowship from the French Government and spent two months at the Station Biologique de Roscoff; as a result he published a pioneering paper on sand-dwelling dinoflagellates (Balech, 1956). He was later granted a John Simon Guggenheim Fellowship to work at the Scripps Institution of Oceanography, La Jolla, California, USA from 1957-1958 (Balech 1962). He published a paper on two new dinoflagellate genera, *Scrippsiella* and *Fragilidium* (Balech, 1959). In 1961 he was employed as a



Enrique Balech at Sherkin Island Marine Station, Ireland in 1989. As a result of this meeting his monograph on Alexandrium *was published in English by this institution.*

Professor at Universidad de La Plata (Argentina), and from 1962 to 1981 was named Researcher of the National Council for Science and Technology (CONICET). Professor Balech was also a member of the National Academy. Between 1964 and 1965 he spent almost a year as a Visiting Researcher at the Department of Oceanography of Texas A&M University, working with phytoplankton of the Gulf of Mexico, the Caribbean Sea, and Antarctica (Balech & El-Sayed, 1965; Balech, 1967). After all these travels, and despite the opportunity of a research position abroad, Prof. Balech decided to return to Necochea, where he was head of the Estación Hidrobiológica Puerto Quequén until he officially retired in 1982. He used to say that he became a full-time researcher after retiring, since it was then that he finally had time for his taxonomic work. In the 1960s, based on studies of dinoflagellates biogeography in the Southwestern Atlantic, he delivered a rather accurate description of the surface currents in this area (Balech 1965), which was confirmed by satellite imagery 20 years later. Enrique Balech authored a large number of publications. In his 'opera magna' (as he used to call it) on the dinoflagellates of the Southwestern Atlantic (Balech, 1988) he illustrated over 330 species, with information on their ecology and distribution. He wrote an illustrated identification manual on Antarctic dinoflagellates (Balech 1976) with descriptions of the species based on the many investigations of Southern Ocean plankton. During his long scientific carrer, he dealt with almost all dinoflagellate genera, describing new species and genera and providing detailed revisions. Just to mention a few: the work on the genera *Protoperidinium* (Balech 1973, 1974, 1994) and *Alexandrium* (Balech 1989, 1995). His monograph on *Alexandrium* (Balech 1995) includes descriptions and illustrations of all the species, a fundamental contribution to the taxonomy of this genus.

Prof. Balech developed his scientific career from the modest facilities of the Estación Hidrobiológica Puerto Quequén, and throughout all these years maintained the museum at the Estación and the historical wooden house, sometimes even using his own money.

He used to visit the Museo Argentino de Ciencias Naturales in Buenos Aires on a monthly basis, bringing fresh news from Necochea, sharing the excitement of researchers on the latest scientific achievements and guiding the students in their thesis work. He was in contact with many scientists all over the world, always prone to scientific discussions and guidance and sharing his immense knowledge with many of us. His lessons went beyond plankton. He taught us that perseverance, tolerance, high academic performance, and solid scientific knowledge are all tools necessary to build a better world.

In addition to his successful career as a scientist he had many other interests. His love for humanity and understanding of people led him to promote the universal language Esperanto; he was a teacher of Esperanto and president of the Argentine League of Esperanto. He published several works in this language and even, in 1951, an Esperanto-Spanish dictionary. He was also a pioneer among environmentalists, and published a book in 1978 "Geocidio. La destrucción del planeta" where he proposed a new combination for *Homo sapiens*: *Homo stultus*, (from Latin, brainless, fatuous) due to the role of human beings in destroying our planet.



Enrique Balech at the third International Conference on Toxic Dinoflagellates, St. Andrews, Canada, 1985 where he was awarded for his outstanding work.

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Friends and colleagues from all over the world.

Future events

NOVEMBER 2007

Course: Taxonomy of Recent Dinophyceae

Wattenmeerstation Sylt of the Alfred Wegener Institut für Polar- und Meeresforschung, List/Sylt, Germany, 18-25 Nov 07.

Organized by Malte Elbrächter.

Registration deadline: 15 Oct 07 (melbraechter@awi-bremerhaven.de).

Course restricted to 10 participants, aimed at people working or intending to work with dinoflagellates (students, technical assitants, scientists) with basic knowledge of dinoflagellates.

Further information: www.awi-bremerhaven.de/Benthic/CoastalEco/index.html.

JANUARY 2008

Regional Meeting on Future Focus and Cooperation in HAB Research. 2nd Asian GEOHAB Meeting

Nha Trang Vietnam, 28 Jan-1 Feb 08.

The meeting will provide an opportunity to develop further ideas and outlines of regional and national research relevant to GEOHAB, establish partnerships and identify research components that may merge into the GEOHAB Core Research Projects.

Abstracts submission: 1 May 07 - 1 Jan 08.

Registration deadline: 21 Jan 08.

Further info: www.geohab.info.

MARCH 2008

SPECIAL SESSIONS ON HABs AT THE 2008 OCEAN SCIENCES MEETING

Harmful Algal Blooms: Interactive Influence of Nutrient Competition, Differential Grazing, and Other Causative Factors

Orlando, Florida, USA 2-7 Mar 08.

Organizers: C.J. Gobler, W.G. Sunda and E. Granéli.

Presentations examining the effects of resource supply, algal growth, mortality processes, and nutrient cycling on HAB dynamics are welcomed, particularly those which explore feedback interactions among these processes. Talks on how such processes and feedbacks interact with other regulating factors such as benthic/pelagic nutrient coupling, physical forcing, climate change, anthropogenic alterations of ecosystems, and life cycles of HAB species are also encouraged.

Abstract deadline: 2 Oct 07. Online submission is highly preferred at www.aslo.org/orlando2008.

Regional and Comparative Studies of the GEOHAB and ECOHAB Programs, 2008 Ocean Sciences Meeting

Orlando, Florida, USA, 2-7 Mar 08.

Organizers: P. Glibert, D.L. Meitiv.

Comparative studies of HABs that occur in similar ecosystems around the world can further understanding of HAB dynamics by establishing the existence of recurrent patterns and revealing fundamental processes governing population development and toxin production. The session will focus on synthesis papers that report on large scale regional HAB projects, or which apply comparative approaches for HABS in upwelling, eutrophic, coastal lagoon or highly stratified systems.

Abstract deadline: 2 Oct 07. Online submission is highly preferred at www.aslo.org/orlando2008.

APRIL 2008

9th Advanced Phytoplankton Course - Taxonomy and Systematics

Stazione Zoologica 'A. Dohrn', Naples, Italy, 5-26 Apr 08.

Applications deadline: 31 Oct. 07.

The Course aims at training and upgrading qualified students in the identification of marine phytoplankton species. It is planned for 20 participants and will consist of theoretical and practical sessions, examining a broad collection of fixed, live and slide material provided by faculty and participants.

Honorary director: G.R. Hasle. Scientist responsible: A. Zingone. Contact: A. Zingone (zingone@szn.it). Secretary: I. Canettieri (i.canet@szn.it).

Further info: www.szn.it/apc9.

OCTOBER 2008

Ciguatera and related biotoxins

Nouméa, New Caledonia, 21-31 Oct 08.

Workshop on the latest developments in ciguatera research, covering environmental influences, causative organisms and socio-economic and medical aspects. It will be held at the IRD Noumea Centre and the SPC symposium facilities, Noumea, New Caledonia.

Further info: www.ird.nc/ciguatera.

NOVEMBER 2008

13th International Conference on Harmful Algae 08

Hong Kong-China, 3-7 Nov 08.

ISSHA is pleased to announce the 13th Int. Conference on Harmful Algae. Supporting organizations include School of Science & Technology, the Open University of Hong Kong and the Association of Harmful Algal Bloom of South China Sea (AoHABSCS).

Further info: www.hab2008.hk.

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HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

http://ioc.unesco.org/hab/news.htm No. 35

• HANA

First IOC/HANA Workshop on Harmful Algal Blooms in North Africa

It was in Casablanca, Morocco, that twenty four experts from Egypt, Tunisia, Algeria and Morocco met from 18 to 20 October 2007, together with their eight guests, to discuss HAB problems in the HANA region. "HANA", or Harmful Algae in North Africa, is the network initiated by twelve young scientists from North Africa while attending an IOC training course in Salammbô, Tunisia in 2003. HANA was endorsed by IOC in 2005 as one of its regional networks for HABs.

The workshop programme was conceived with more than one objective in mind. It consisted of reports and scientific presentations, invited lectures and round tables.

The scientific presentations reflected the considerable work presently carried out by young and more advanced scientists on potentially harmful microalgae in the region. They also provide a picture of the state of the marine environment regarding these issues. This is particularly obvious where economically important bivalve resources are under threat, namely in Morocco and Tunisia, the only two countries of the region where an institutionalized monitoring programme is in place.

In Morocco, seven sites are monitored on both the Mediterranean and Atlantic coasts of the country. Of the diverse potentially harmful species recorded, some are of more concern than others: *Alexandrium minutum* (Nador Lagoon, Mediterranean) causing PSP contamination, *Lingulodinium polyedrum* (Abda Doukkala, Atlantic) contaminating shellfish with DSP, and three *Pseudo-nitzschia* spp. (coastal Atlantic waters). Work has begun on dinoflagellate cysts and their possible implication in outbreaks of toxic species in Walidia Lagoon.

In Tunisia, toxic blooms lead to heavy fish mortality in the Gulf of Gabes in 1995, inciting the authorities to launch

(Cont'd on p. 2)

Modified ichthyoviscometer shows high viscosity in *Chattonella* culture

Introduction

Striking amounts of mucus accompany some blooms of *Chattonella* spp. As *Hornellia marina, Chattonella marina* (Subramanyan) Hara et Chihara was first described in 1954 [1] from viscous, slimy blooms causing huge kills of wild fish. As *Hemieutreptia antiqua, Chattonella antiqua* (Hada) Ono was first described in 1974 [2] from blooms in which an enormous number of young cultured yellowtail died from "disturbance of respiration by mucous films" or "respiratory injury by mucus". A well-developed glycocalyx *invests C. antiqua* cells (Fig. 1), with fine filaments apparently continuous through the plasma membrane. This glycocalyx is microfibrillar, and rich in a neutral carbohydrate-protein complex and acidic complex carbohydrates [3], while *C. marina* glycocalyx shows immunologically mediated adhesion to fish gills [4]. More than any other phytoplankton, *Chattonella* cells also produce reactive oxygen species (ROS) [5], associated with the cell surface, the glycocalyx or both [6-7]. This ROS, as well as its accompanying fatty acids are toxic to bacteria and, separately or in concert, may modulate allelopathic action against ambient bacteria [8]. Nevertheless, since 2000, the ROS

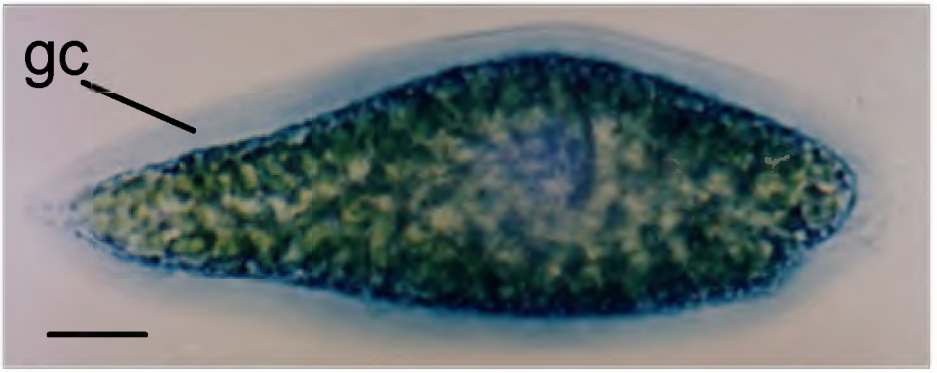

Fig. 1. Light micrograph of Chattonella antiqua *cell illustrating glycocalyx on the cell surface. Scale bar = 5 µm; gc: glycocalyx.*

(Cont'd on p.3)

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

(Cont'd from p. 1)

a monitoring programme in shellfish areas. Most of the toxicity episodes in the last six years appear to have been due to *Karenia selliformis*, though *Alexandrium minutum, Coolia monotis, Karlodinium veneficum* and *Prorocentrum minimum* also occur. Investigation of epiphytic microalgae on *Posidonia oceanica* leaves is just beginning. Of practical importance are the attempts to assess the rate of detoxification in clams.

Algeria did not report any toxicity episodes although fifteen potentially harmful species occur. High biomass blooms of the dinoflagellate *Lepidodinium chlorophorum*, the diatom *Cyclotella meneghiniana* and the coccolithophore *Holococcolithophora sphaeroides* developed in Algiers harbour in 2003, and *Scrippsiella trochoidea* along the coast in 2006.

Phytoplankton investigations in Egypt began with the observations of recurrent red tides in the Eastern Harbour of Alexandria since 1960. The red tides were caused by the then newly described *Alexandrium minutum*. Although no formal monitoring programme is in place, the E. Harbour and neighbouring coastal embayments were continuously monitored for red tides and heavy blooms, although irregularly. Heavy fish kills accompanied the last bloom of *A. minutum* in 1994. Since then, *A. minutum* has become insignificant, replaced by other potentially harmful species such as *Chattonella* spp., *Prorocentrum triestinum, P. minimum,* and *Pseudo-nitzschia* spp. In a new development, a survey of epiphytic microalgae along the coast revealed benthic blooms of *Ostreopsis* spp. and *Oscillatoria* spp.

A variety of issues were dealt with by the six invited lecturers: Phytoplankton Strategies (Tim Wyatt), the IOC-ICES-PICES HAEDAT data base (Monica Lion), Monitoring and Management of Phytoplankton and Phytotoxins (Catherine Belin), Biology, Ecology and Oceanography of *Dinophysis* spp. (Beatriz Reguera), Occurrence of Harmful Freshwater Cyanobacteria Blooms in North Africa (B.Oudra), and the proposed project for cooperation between HANA and DANIDA (Jacob Larsen).

Three round tables were held respectively at the end of each day. They were devoted to free discussions of research priorities (chair B. Reguera), monitoring and management priorities (chair C. Belin) and to HANA business (chair Y. Halim). The round tables agreed on a number of recommendations.

Recommendations of the workshop:

1. All countries of the region should launch monitoring and management programmes of phytoplankton, phytotoxins and the quality of the environment.
2. A training course on taxonomy of harmful algae and on monitoring should be held soon in the region.
3. Selected experts from the HANA region should be trained for toxin analysis at IFREMER, France.
4. Biotoxin experts from the HANA region should be represented in the IPHAB Task Team on Biotoxin regulation.
5. A link to HAEDAT should be added to the HANA web site and HANA data be introduced to HAEDAT.
6. The HANA web site (http://ioc.unesco.org/hab/HANA/hana.htm) should be updated and completed regarding the directory, the list of publications and the list of species.
7. As no representatives from Mauritania and Libya were present, the organizers are urged to insure the participation of all countries of the region at the next meetings.
8. Presentations in French language must be acompanied by slides in English.
9. The second IOC/HANA workshop must be convened in 2009. The invitation of Egyptian participants to host the next workshop at the Department of Oceanography, Faculty of Science in Alexandria, Egypt, was accepted.
10. Extra budgetary funding should be sought to allow more participants from the region to attend the next workshop.

During the third round table, the participants elected the bureau of HANA for the next two years as follows:

HANA chair: Youssef Halim.

HANA Vice chair: Hamid Taleb.

National coordinators: Amany Ismael for Egypt, Hassina Illoul for Algeria, Asma Hamza for Tunisia and Chafik Abdelghany for Morocco.

At the end of the meeting the HANA community agreed unanimously that the workshop had been both successful and enjoyable.

Y. Halim, HANA Chairman. Univ. of Alexandria, Alexandria, Egypt.
Email: youssefhalim@hotmail.com.



Participants in the first HANA workshop.

(Cont'd from p. 1)

produced by *C. marina*, relatively large in amount though it is, has been shown [10-12] insufficient to kill fish. Since 2004, respiratory disturbance by mucus is again considered [11-12], as originally thought by Hada [2] for *C. antiqua*, to be a major factor in fish mortality, perhaps accompanied by a factor producing osmotic distress [9].

Perhaps confusingly, fish mucus [13] and bivalve-mollusc mucus [9] increase the secretion of ROS by *Chattonella*, so ROS production seems likely to be increased by passage through fish and bivalve gills. Whether this gill passage, with attendant exposure to fish and bivalve mucus, likewise cause *Chattonella* to secrete more of its own mucus appears not to have been reported.

In this preliminary study we measured rheological properties, during flow through fish gills, of cultures of five phytoplankton taxa, compared to that of culture medium. Of the taxa tested, several are known to produce mucus. *Heterosigma akashiwo* surrounds itself with a glycocalyx closely comparable with that of *C. antiqua* [20-21] while *Chaetoceros* sp. can provoke damage to and clogging of fish gills associated with production of mucus by the fish [22], and *Skeletonema costatum* uses mucus to regulate cell-cell aggregation [23]. Since in this study only cultures of *C. antiqua* showed increased viscosity, our discussion has centred on this species and its congener *C. marina* which shows comparable harmful effects.

Methods

Cultures of phytoplankton (Table 1) were grown in the ACRO laboratory at 23°C, in modified SWM-3 medium [24] under a light:dark regime of 12h:12h with "daylight" fluorescent illumination at 300 µmol photons·m^{-2}·s^{-1}.

Table 1. Cultures used

Species	IVS model	Date	Growth phase	Concentration during trials (cells.mL^{-1})	Yield stress (Pa) (1)	Max notional cell volume (inluding gc) (µm^3) (2)	Max notional volume fraction (3)
Diatoms							
Skeletonema costatum	Mark 3	13/02/07	Log	403000	ns	3.4 x 10^4	1.4 x 10^{-2}
	Mark 3	16/02/07	Log	652000	ns		2.2 x 10^{-2}
Chaetoceros sp.	Mark 3	13/02/07	Log	220000	ns	1.0 x 10^3	2.2 x 10^{-4}
	Mark 3	16/02/07	Stationary	242000	ns		2.4 x 10^{-4}
Thalassiosira minima	Mark 3	12/02/07	Log	233000	ns	5.5 x 10^2	1.3 x 10^{-4}
	Mark 3	16/02/07	Stationary	606000	ns		3.3 x 10^{-4}
Rhaphidophytes							
Heterosigma akashiwo	Mark 3	10/02/07	Log	43950	ns	1.8 x 10^3	8.2 x 10^{-5}
	Mark 3	11/02/07	Log	107500	ns		2.0 x 10^{-4}
	Mark 3	15/02/07	Stationary	311000	ns		5.8 x 10^{-4}
Chattonella antiqua	Mark 3	11/02/07	Log	1600	ns	7.0 x 10^5	1.1 x 10^{-3}
	Mark 3	14/02/07	Log	6050	ns		4.2 x 10^{-3}
	Mark 3	16/02/07	Log	5300	ns		3.7 x 10^{-3}
	Mark 3	17/02/07	Stationary	19500	9*		1.4 x 10^{-2}
	Mark 2	19/01/07	Old culture	nd	17**		nd

(1) 1 Pa ~0.01 cm water. (2) Cell volumes calculated from largest cell dimensions found in literature, inlcuding glycocalyx when present. (3) Not including volume partly enclosed by seatae of Chaetoceros *sp. * $P<0.1$, $n = 4$; ** $P<0.05$, $n = 3$.*

Rheological (mechanical) properties of seawater and cultures were measured at the scales of flow through the gills of freshly killed juvenile (14 to 15 cm) Faria brown trout (*Salmo trutta*). The fish were kept starved in lake water for several days, and killed by cardiac puncture immediately before use. The genus *Salmo* is euryhaline.

Measurements used the Mark 2 ichthyoviscometer (IVM) [25], or the purpose-developed Mark 3 IVM (Fig. 2). The latter uses much smaller volumes of test medium than the Mark 2 (20 ml per measurement, for a hydrodynamic pressure P sweep of 300 to 0 Pa). It consists of a shortened measuring cylinder (MC) (internal and external diameters 2.9 cm and 3.2 cm respectively) standing in an aquarium (internal dimensions 42.9 x 18.6 cm) containing 6-7 cm of water. The pressure probe and recording components in Mark 3 were the same as in the Mark 2. As there was sometimes drift in the pressure sensor set-up over several minutes, the time scale of a single measurement, we added another new feature, a yield-stress tube (YST). This is a flexible tube with a tap, joining the contents of the cylinder with the contents of the aquarium. During measurements this tap was kept closed, then after completion of each measurement the tube was opened. Because of scale dependence in yield stress Y of the EPS (see Box 2), even when Y was positive in the gills of the fish, it was negligible in the YST (confirmed by visual observation of the water level in the cylinder relative to that in the aquarium). The YST was used to check for any effect of instrument drift on recorded Y.

Each IVM has a characteristic value of J, the volume flow through the fish for every 1 Pa change in hydrostatic pressure. For the Mark 2, J was 3.14 x 10^{-6} m^3 Pa^{-1} [25], but J in the Mark 3 was only about one fiftieth of this, 6.66 x 10^{-8} m^3 Pa^{-1}. Less test material flows through the fish gills in the Mark 3 IVM, so any tendency the test material might have to clog or concentrate during flow should be less marked in the Mark 3 set-up than in the Mark 2. This tendency may have been slightly offset by the smaller fish we used, ~15 g compared with ~25 g used previously [25].

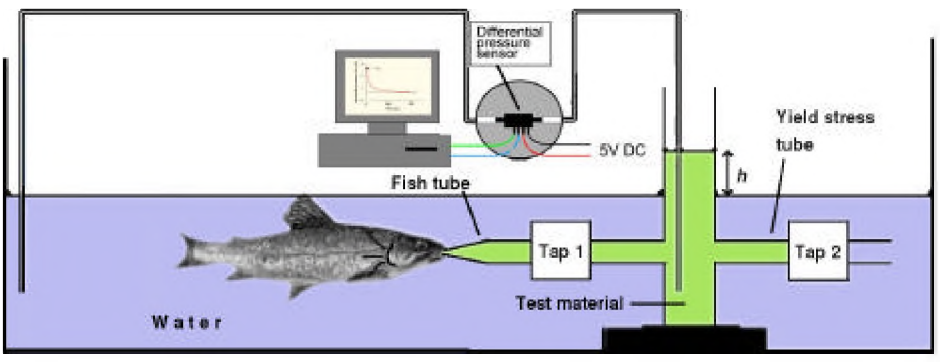

Fig. 2. Sketch of the Mark 3 ichthyoviscometer (not to scale).

Box 1

We use the term *ichthyoviscometry* to mean the measurement of the viscosity of ventilating water as it passes over and between fish gills. Dissolved, colloidal or suspended material may increase the viscosity of this water to the extent that a fish may have to consume more oxygen to fuel the energy needed to pump the water over its gills than it can extract from the water pumped, and it would then suffocate through oxygen lack [14]. Such polymeric material may give the water a viscosity that depends on the deformation type and rate D [18]. One possible manifestation of D-dependent viscosity is a yield stress Y (a resisting pressure which must be overcome before material yields and flows) higher than the head pressure a fish can develop across its gills. In this case ventilation will be impossible and the fish will likewise suffocate [25]. *Ichthyoviscometry* can be carried out using an *ichthyoviscometer* (Fig. 2).

Results

Table 1 shows the results of trials with cultures of three diatom and two raphidophyte species. Even concentrated cultures, whether in logarithmic or stationary phases, of *Skeletonema costatum*, *Chaetoceros* sp., *Thalassiosira minima* and the often ichthyotoxic *Heterosigma akashiwo* showed no statistically discernible yield stress Y. For *Chattonella antiqua*, however, although log-phase cultures of 1600 to 5300 cells mL^{-1} showed Y not significantly different from water, a stationary-phase culture of 19,500 cells$\cdot L^{-1}$ and an old culture in decline-phase showed values of Y of 9 and 17 Pa respectively (0.9 and 1.7 mm water).

Box 2

It has been suggested that viscosity in blooms of the dinoflagellate *Karenia mikimotoi* could kill fish by increasing the energy needed to ventilate their gills, to the extent that the oxygen extracted from the seawater would then be insufficient to fuel this energy [14]. Results obtained using an ichthyoviscometer supported this hypothesis [15] in a study showing that polymeric thickening alone could have killed the fish, but that haemolytic ROS were also produced, which may have significantly contributed.

Since phytoplankton-derived organic matter to some extent produces flocculates [16] and also heterogeneous distribution of viscosity at scales $\leq$~1cm [17], its mechanical properties are likely a function of the flow regimes (shearing, elongational, etc.) [18] and scales of the system constraining the flow [19]. The most relevant flow regimes and scales, however, are hard to identify and characterise in each case, but the aim of an ichthyoviscometer, using the flow-ways between fish gills as the measuring system, is to get the flow regimes and the scales right for measuring rheological thickening during flow in fish gills.

Discussion

A culture of *Chattonella antiqua* with a cell concentration of 20,000 cells mL^{-1} produced a yield stress Y of 9 Pa in the gills of trout, while an old culture in decline produced 17 Pa. Such values of Y are about an order of magnitude higher than those theoretically needed to prevent ventilation and thus suffocate young fish, which produce a maximum head pressure of only 0.6 to 1.5 Pa [26]. If blooms of *Chattonella antiqua* in the field produce similar values of Y, these blooms would easily kill captive fish by preventing gill ventilation. Volume fractions of cells with glycocalyxes (estimated conservatively high) in these cultures did not exceed ~2%, while it requires over 10% of suspended spheroids to significantly increase viscosity [19]. Therefore it is likely that diffuse colloidal EPS, rather then the volume of cells and glycocalyxes, was at least a major contributor in stopping flow of *Chattonella* culture through the fish gills. In *Chattonella*-associated fish kills, toxins, ROS, oxygen levels, biopolymeric structures [27], the role of heterogeneous viscosity (Box 2), flocculation in the high-shear flow between the gills, adhesion or slipping at the gill surface, and fish behaviour might all modify the roles of EPS-modulated yield stress and viscosity increase. These subjects should thus be investigated together in *Chattonella* blooms to promote better understanding of fish mortality with a view to mitigation.

Acknowledgements

We thank G. Claireaux (CNRS - Station méditerranéenne de l'Environnement littoral, Sète, France) for kind help and loan of recording equipment, G. Guillou and Y. Descatoire (both of CNRS-CRELA, L'Houmeau, France), for technical advice and help with drawing Fig. 2, respectively. JM Faurs' fish farm, 19120 Altillac, France provided the trout.

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• France

Identification of *Pseudo-nitzschia multistriata* and *P. subpacifica* from French waters. Were they part of the cryptic flora?



Fig. 1. Map of France showing localities where P. multistriata *(▼) and* P. subpacifica *(●) have been detected.*

Identification of *Pseudo-nitzschia multistriata* (Takano) Takano and *P. subpacifica* (Hasle) Hasle have recently been confirmed with help of scanning electron microscopy (SEM). However, they were previously detected in French waters; the former appeared in 2002 and the latter in 2004, but their identification required further examination for confirmation.

P. multistriata was mostly observed in the autumnal phytoplankton of the Channel and Atlantic Ocean while *P. subpacifica* was mainly detected in the Atlantic during summer (Table 1, Fig. 1).

***P. multistriata*:** First observed with light microscopy (LM), *P. multistriata* was characterized by short chains and sigmoid valve ends in girdle view (Fig. 2A) [1] . The apical axis of cells was 50-75 µm long and the transapical axis 2.9-3.7 µm wide (n=24). Cell overlap in chains was 9-13% of the length. When observed with SEM, valves did not possess a central nodule and fibulae, and interstriae numbered 23-26 and 37-42 in 10 µm, respectively (Fig. 3). The interstriae were parallel or oblique (Fig. 3B) to the transapical axis and the striae contained two rows of poroids. The number of poroids per 1 µm (10-11) was close to the range described for strains from Italy (11-13) [2] and Morocco (11-12) [3]. However, it was the single parameter which differed from the original description where only 5 to 6 poroids in 1 µm were mentioned for this species [4].

***P. subpacifica*:** First observed with LM, its asymmetrical valves drew attention (Fig. 2B). The apical axis of cells was 47-79 µm long and the transapical axis 4-6.3 µm wide (n=32). Chains had a cell overlap of 12-20 % of the length. Occasional surface net samples were collected for SEM examination. A large central interspace was clearly visible (Fig. 4B) and the number of fibulae and interstriae was 14-20 and 28-30 in 10 µm, respectively. The biseriate striae contained 8 to 10 poroids in 1 µm, which is in agreement with other descriptions of this species [3, 5-7]. Molecular analysis of the internal transcription spacer (ITS) sequences was made on cultured material, and results compared with known sequences of *Pseudo-nitzschia* species available in databases. In the absence of data equivalent to *P. subpacifica*, they were identical (100% identity) with three records of *P.* cf. *subpacifica* of which two sequences were from Spanish strains and the third from a Portuguese strain [8].

Owing to the distinctive shape of *P. multistriata* in girdle view, it would have been difficult to overlook this species during more than 20 years of

Table 1. Monthly observations of P. multistriata *and* P. subpacifica.

	J	F	M	A	M	J	J	A	S	O	N	D
P. multistriata								X	X	X	X	X
P. subpacifica						X	X	X	X	X	X	

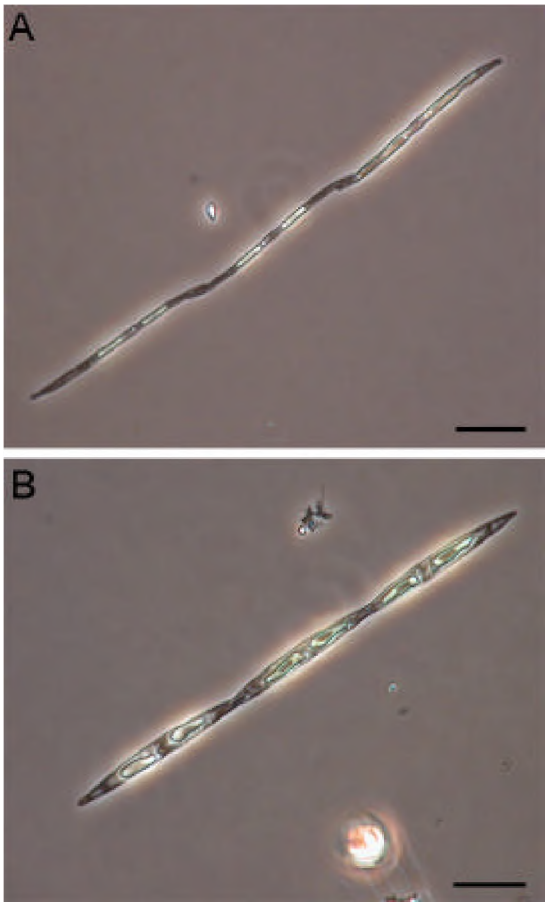

Fig. 2. Light micrographs of Pseudo-nitzschia multistriata *and* P. subpacifica. *(A) Chain of* P. multistriata *in girdle view. (B) Chain of* P. subpacifica *in valve view. Scale bars = 20 µm.*

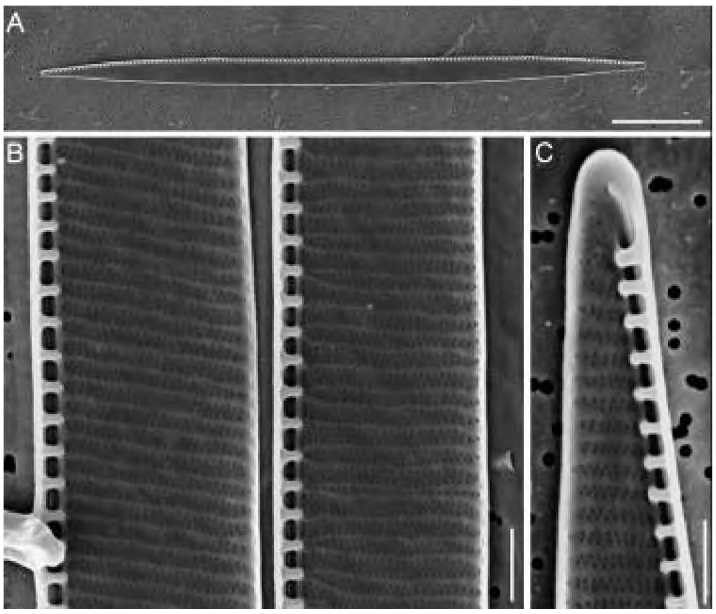

Fig. 3. Scanning electron micrographs of Pseudo-nitzschia multistriata *(A) Valve outline. (B) Part of valves showing two different patterns of interstriae. (C) Tip of valve. Scale bars = 10 µm (A) and 1 µm (B, C).*

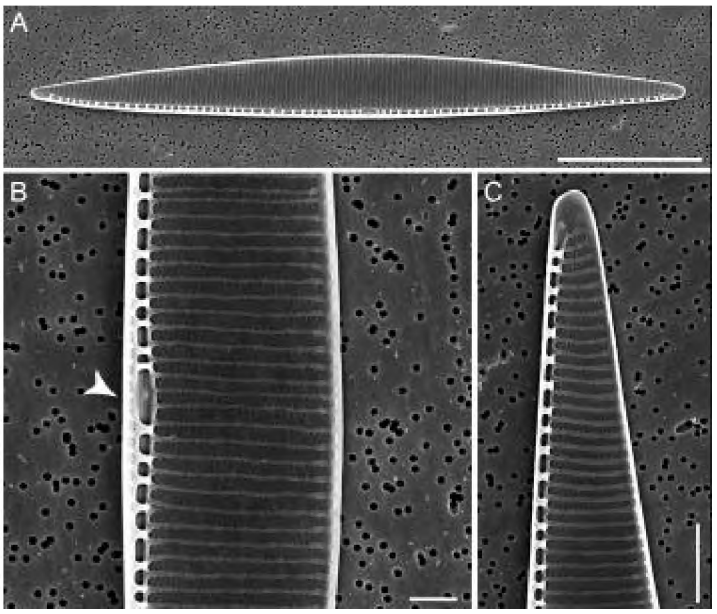

Fig. 4. Scanning electron micrographs of Pseudo-nitzschia subpacifica. *(A) Whole valve. (B) Central part of valve showing central nodule (arrow). (C) Tip of valve. Scale bars = 10 µm (A) and 1 µm (B, C).*

phytoplankton monitoring. Thus, we can reasonably consider that it was not present earlier. In contrast, in spite of its asymmetrical shape in valve view, *P. subpacifica* could have been confused in light microscopy with *P. fraudulenta* with which it co-occurs.

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• Greece

The coincidence of an *Arthrospira - Anabaenopsis* bloom and the mass mortality of birds in Lake Koronia

An extremely dense bloom of the cyanobacteria *Arthrospira fusiformis* (Voronichin) Komárek et Lund and *Anabaenopsis arnoldii* Aptekarj (Fig. 1) occurred on 17 September 2007 in the shallow Lake Koronia. This is the first report of an *Arthrospira* bloom in Greek freshwaters. Cyanobacterial blooms however are common in eutrophic freshwaters in Greece [1-2]. Another dense bloom of the haptophyte *Prymnesium parvum* N. Carter coincided with a mass kill of birds and fish in Lake Koronia three years ago (September 2004) [3].

Lake Koronia (40° 40' 58" N, 23° 09' 33" E, altitude: 75 m a.s.l.) is located in N. Greece and over the past 20 years has undergone a dramatic decrease in water volume, surface area and depth due to anthropogenic activities. During this period the lake water became brackish and in the summer of 2002 the lake dried up completely. After increased rainfall in 2003, water reaccumulated in the lake and in 2004 the water level had a maximum depth of 1 m. In 2007 the prolonged dry and warm period resulted in a maximum depth of just 0.5 m.

Arthrospira fusiformis population density was extremely high with 23 x 10^6 filaments L^{-1} (2.99 x 10^9 cells L^{-1}, or 487.37 mg L^{-1} w/w) close to the highest values reported from African lakes [4-5]. At the same time, *Anabaenopsis arnoldii* exhibited high population density as well (0.12 x 10^9 cells L^{-1}). Euglenophytes were also present in the lake's phytoplankton. Zooplankton consisted only of individuals of the *Brachionus plicatilis* group complex that were present in low numbers.

A mass mortality of water birds was observed to coincide with the *Arthrospira-Anabaenopsis* bloom preceeded in June by a heavy *Microcystis aeruginosa* (Kützing) Kützing bloom (5.6 x 10^9 cells L^{-1}) with its epiphyte *Pseudanabaena mucicola* (Naumann et Huber-Pestalozzi) Schwabe (12.4 x 10^9 cells L^{-1}). The number of dead birds reported by the Hellenic Ornithological Society at the meeting organised by the Prefecture of Thessaloniki was estimated to be two hundred, including mostly flamingos *Phoenicopterus ruber* Linnaeus.

From the literature, it is known that mass mortalities of Lesser flamingos *Phoeniconaias minor* (Geoffroy) can be caused by the toxic *Arthrospira fusiformis* and *Anabaenopsis* species [4-6]. Ballot *et al.* [4] found that *A. fusiformis* strains produce

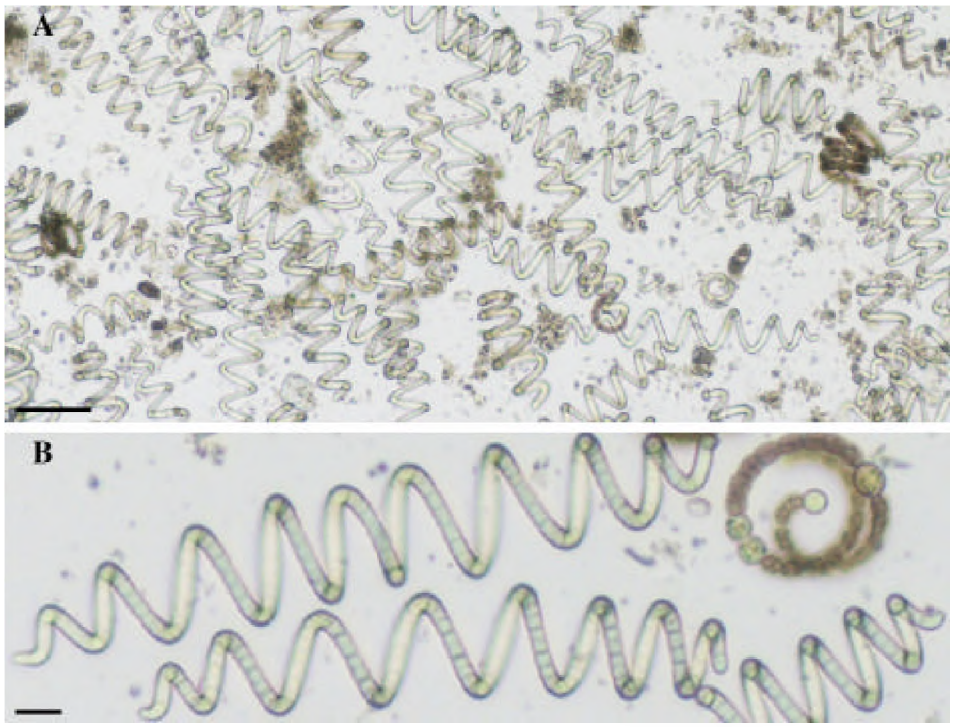

Fig. 1. Light micrograph of phytoplankton collected from Lake Koronia on 17 September, 2007. (A) Phytoplankton dominated by Arthrospira fusiformis. *Scale Bar: 50 µm. (B) Filaments of* A. fusiformis *and* Anabaenopsis arnoldii. *Scale Bar: 20 µm.*

microcystin-YR and anatoxin-a. Lanaras and Cook [7] first raised the possibility that *Anabaenopsis* species produce microcystins, reporting microcystin in a cyanobacterial bloom dominated by *Anabaenopsis milleri* Woronichin in a Greek water body. In 2001, the mass mortality of flamingos *Phoenicopterus ruber* and other water birds in southwest Spain was attributed to microcystins produced by toxic cyanobacteria (*Microcystis*, *Anabaena*) [8].

The bird mortality in Lake Koronia during September 2007 was most likely caused by the heavy bloom of toxic cyanobacteria in the lake. The mass development of *Arthrospira*, *Anabaenopsis*, *Anabaena* and *Microcystis* species in the lake the last three years presents a health risk for wildlife and especially for water birds, and underlines the need for cyanobacteria and cyanotoxins risk assessment and management. This is of great importance considering that Lake Koronia is covered by the Directives 79/409/EEC [9] and 92/43/EEC [10], the RAMSAR Convention (http://www.ramsar.org) and is part of a National Wetland Park [11].

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• Tunisia

First bloom of dinoflagellate *Alexandrium catenella* in Bizerte Lagoon (northern Tunisia)

Bizerte Lagoon is located in northern Tunisia (37º 8'-37º14' N, 9º46'-9º56' E). The surface area is about 128 km^2, maximum width 11 km, and maximum length 13 km; the mean depth is 7 m. The lagoon is connected to the sea via a canal 6 km long, as well as to Ichkeul Lake to the east via the 5 km long oued (= river) of Tinja (Fig. 1). In Tunisia, mussel and oyster farming are developed only in this lagoon.

In the context of the national surveillance network of bivalve production in northern Tunisia, two zones of clam production and five oyster and mussel farms are considered by the phytoplankton and phycotoxin surveillance programme for various products from the lagoon.

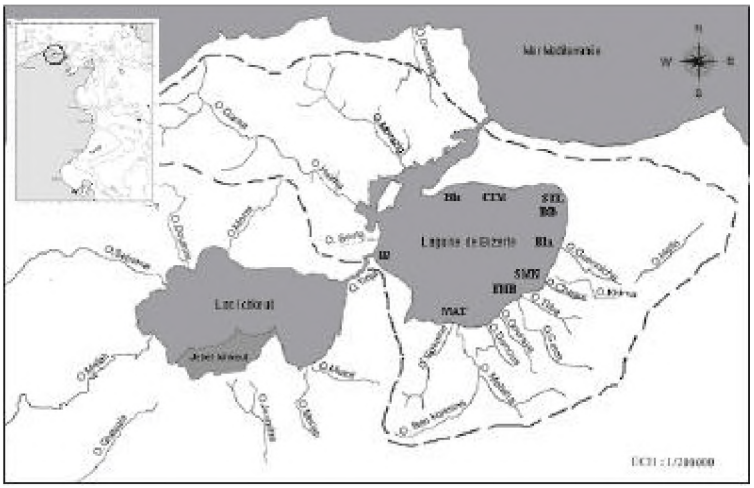

Fig. 1. Location of the sampling stations at the Bizerte lagoon.

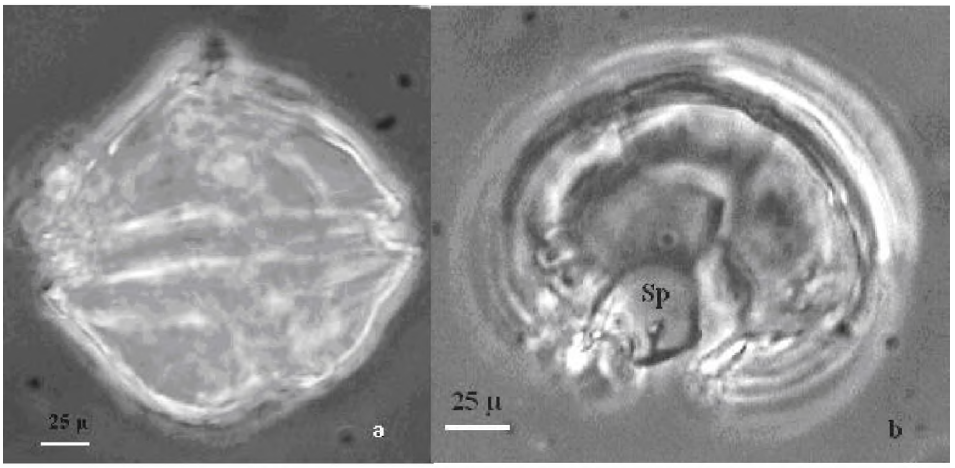

Fig. 2. Microphotograps of A. catenella from *Bizerte Lagoon,Nov 2007. a.General morphology; b. Hypotheca (sp: posterior sulcal plate).*

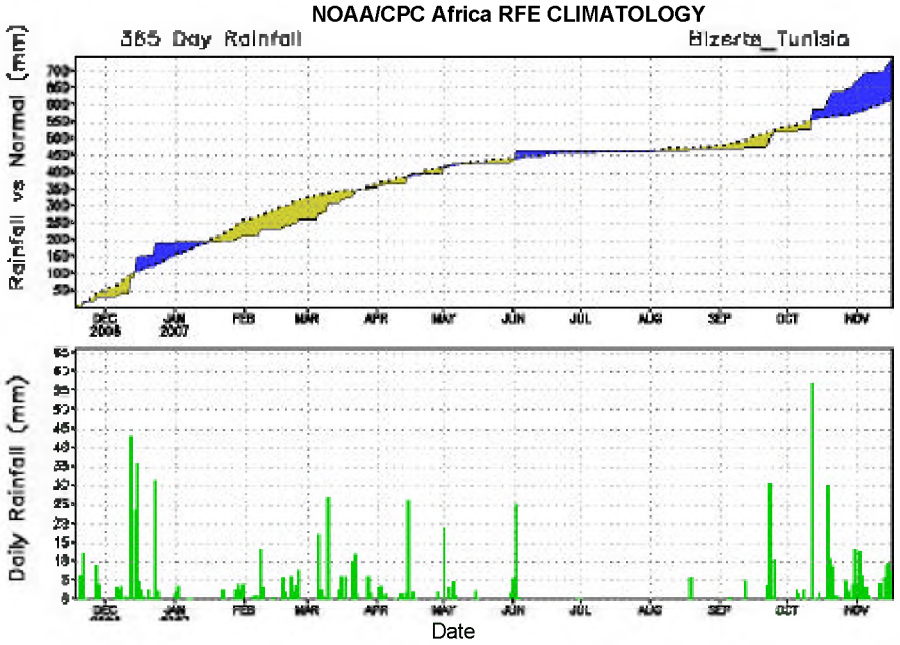

Fig. 3. Rainfall recorded in the Bizerte region during 2007.

For the first time, in November 2007, a bloom of *Alexandrium catenella* was recorded in Bizerte Lagoon (Fig. 2). These proliferations affected especially the sampling sites located on the southern shore of the lagoon (MAT, FMB and SMN). This shore receives several fresh water inputs from the oueds. Moreover, precipitation recorded in the region of Bizerte was more important from October to November 2007. Quantities of daily recorded rain reached approximately 55 mm (Fig. 3).

In the lagoon, *A. catenella* appeared for the first time at the end of the year 2006, between 27 November and 18 December. The densities recorded varied from 100 to 450 cells L^{-1}. During the same period in 2007, much higher concentrations have been observed (20 x 10^4 cells L^{-1}) (Fig. 4). During the 10 years of phytoplankton and phycotoxin surveillance in the area of bivalve molluscs production in Tunisia, mussel contamination by paralytic toxins was recorded for the first time, with concentrations higher than the threshold (80 µg/100 g of flesh) accepted by public health authorities. Values recorded by the Pasteur Institute of Tunis using the mouse bioassay [1] were between 140 and 330 µg/100 g of mussel flesh.

The national surveillance network for bivalve production detected *Alexandrium catenella*, for the first time, in the canal of Tunis in August 1997 [2]. During November 2007, we also recorded high concentrations of *A. catenella*, about 15.3 x 10^4 cells L^{-1}, in the navigation channel of Tunis.

In the Mediterranean Sea, *A. catenella* first appeared in 1983 along the Catalan coast (Spain).[3]. Its distribution area spread along the northwest Mediterranean coast with recurrent blooms in the 1996-1999 period in Barcelona harbour. These blooms were associated with paralytic toxin production [4]. In the Italian waters, *A. catenella* was also observed for the first time in 1999 in the Tyrrhenian Sea (Sardinia).

Toxic events due to the presence of gonyautoxins and saxitoxins have been observed in Thau Lagoon (southern France). Their origin is likely to be *A. tamarense* with suspicions from *A. catenella* [5-7]. Thau Lagoon shares some hydrobiological features with that of Bizerte. The periods when *A. catenella* appeared were the same in both ecosystems. In Thau, a new *Alexandrium catenella/tamarense*

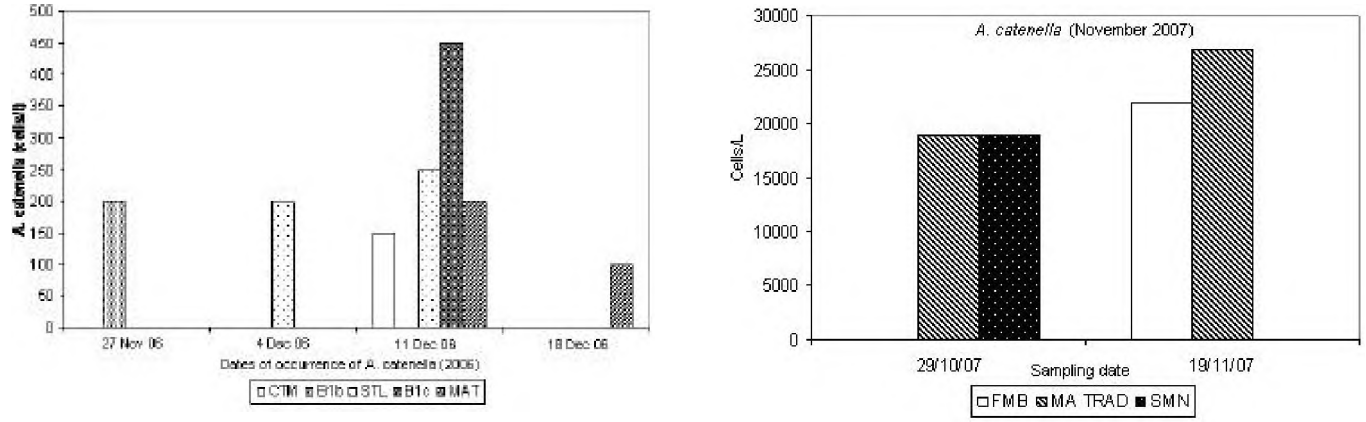

Fig. 4. A. catenella numbers in Bizarte Lagoon, 2006-2007.

event was observed during the year 2001; this one was more important than those in 1999 and 2000. From its consequences, it is comparable to the 1998 crisis. All the shellfish produced in Thau Lagoon (mussels, clams and oysters) reached for the first time the statutory toxicity threshold (80 µg eq. STX /100 g of flesh); toxicity was higher in mussels and clams (close to 500 µg eq. STX /100 g of flesh) than in oysters (about 160 µg eq. STX /100 g of flesh). In Italian waters, *A. catenella* was also reported for the first time in 1999 in Sardinia [8].

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• Tunisia

First detection of *Kryptoperidinium foliaceum* (Stein 1883) in Tunisian waters

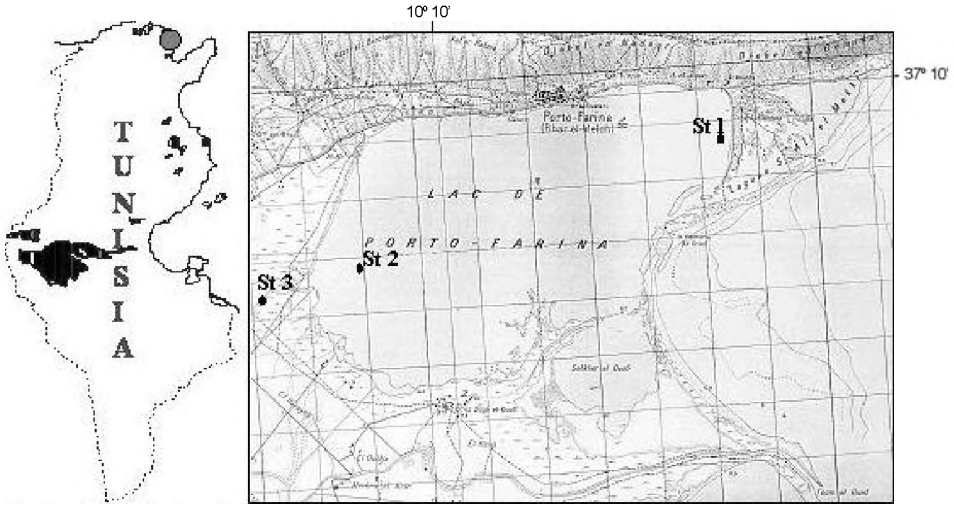


Fig 1. Chart of Ghar el Melh Lagoon showing sampling stations

Fish mortalities were observed during the first fortnight of June in the lagoon of Ghar El Melh «Lac de Porto Farina» in northern Tunisia (Fig. 1). The lagoon has an area of about 3000 ha, and is considered eurohaline on account of the diversity of environments which range from limnetic to hypersaline [1]. Climatic factors play a primary role in control of the physico-chemical parameters of this lagoon due to its shallowness, with a maximum depth of 1.5 m. During June, air temperature ranges from 16.4°C to 27.7°C, and mean rainfall is estimated at 10 mm in the region of Bizerte.

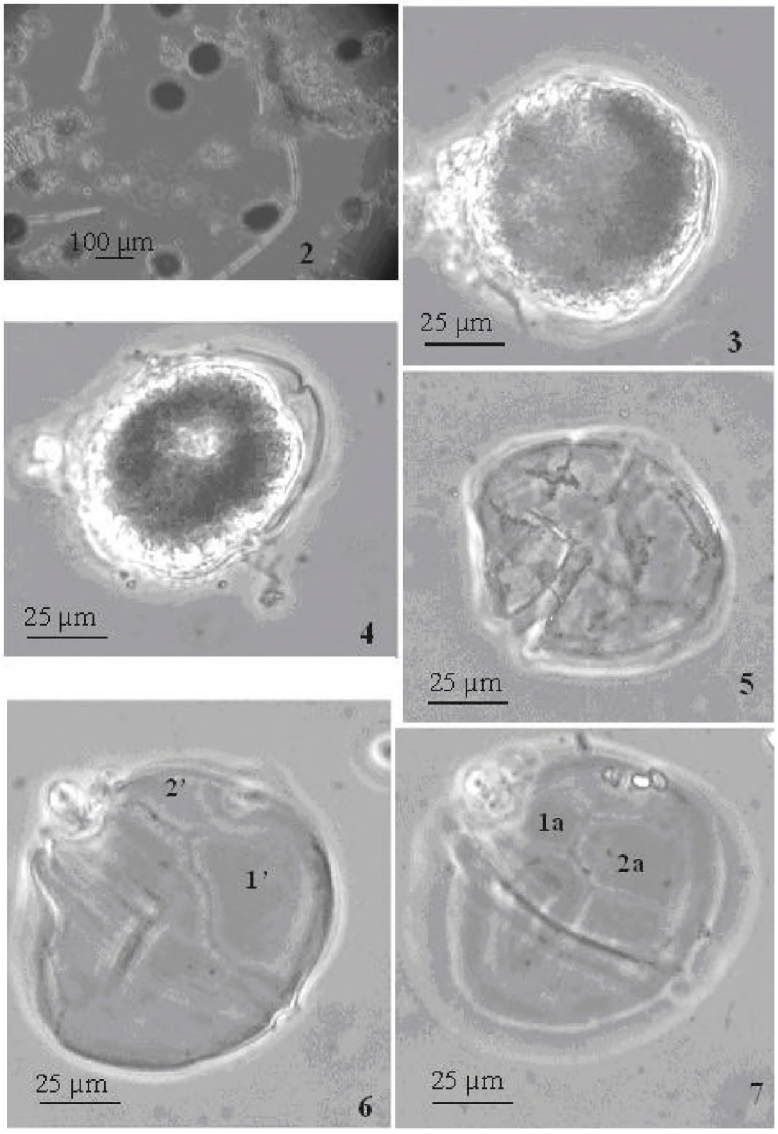
The diversity of the benthic vegetation in this lagoon has varied over time; the dominant plants were *Cymodocea nodosa* and *Zostera* in the 1970s, *Caulerpa prolifera* and *Zostera* in the 1980s, and these are now being replaced by *Ruppia cirrhosa* and *Cladophora* [2]. Among the potentially toxic microalgae recorded in Ghar El Melh Lagoon are *Alexandrium minutum* with densities of the order of $2x10^5$ cells L^{-1} in June 1966 [3], and *Prorocentrum lima* with concentrations higher than $1.5x10^4$ cells L^{-1} in December 1966 [4].

Sampling carried out on 14 June 2007 at 3 stations led to the first detection, in Tunisian coastal waters, of the dinoflagellate *Kryptoperidinium foliaceum* (Figs. 2-7), in concentrations higher than 10^5 cells L^{-1}. This species was previously reported in Mediterranean waters [5]. Samples collected at the rainfall-drainage channel (St 3) had a light brown colour due to high concentrations of *K. foliaceum* ($1.8x10^5$ cells L^{-1}) and of the cyanobacteria *Anabaena* ($1.2x10^5$ cells L^{-1}). The latter is frequently found in freshwaters but can withstand salinities up to 40 psu (Table 1). Concentrations of *K. foliaceum* showed a sharp decline from the peripheral stations towards the central parts of the lagoon. Therefore, stations 1 and 2 are the main bloom sites.

Kryptoperidinium foliaceum cells are spherical with a lateral compression (Figs. 2-4). Their size ranges from 29.3 to 52.0 µm. The thecae are very fragile, which renders observation of plates difficult (Figs 5-7). *K. foliaceum* is mainly found in brackish waters (down to 10 psu). Brownish waters of this species have been observed in different

Table 1. Physico-chemical parameters and phytoplankton concentrations in Ghar El Melh Lagoon (14 June 2007)

Parameter	St 1	St 2	St 3
Water temperature (°C)	27.3	27	29.5
Salinity (‰)	38.9	44	41
pH	8.53	8.71	8.22
Dissolved oxygen (mg/l)	5.14	3.8	5.47
Phytoplankton, cells/l			
Kryptoperidinium foliaceum	$9.8x10^3$	1800	$1.83x10^5$
Scrippsiella subsalsa	0	3400	0
Prorocentrum micans	700	1300	3100
Anabaena sp	$1.1x10^4$	0	$1.22x10^5$



Figs. 2-7. Microphotographs of K. foliaceum *from Ghar El Melh Lagoon.* ***2:*** *cells of* K. foliaceum *and* Anabaena *sp;* ***3:*** K. foliaceum *cell;* ***4:*** *longitudinally flattened cell of* K. foliaceum*;* ***5:*** *theca (ventral view);* ***6:*** *apical plates 1' and 2';* ***7:*** *intermediary apical plates1a and 2a.*

parts of the Atlantic coast in summer between 1986 and 1988, and in spring 1991 following heavy rainfall [7]. It is not a toxic species, but mass mortalities due to anoxia have been reported with high concentrations ($>10^7$ cells L^{-1}). Dense blooms of *K. foliaceum* ($3.5 \cdot 10^8$ cells L^{-1}) were also reported in spring 1998 in South Carolina estuaries (USA) [6]. *K. foliaceum* is included in the list of bloom-forming species, very restricted in space and time, that occur every year in Northern Catalonia (Western Mediterranean, Spain) [8].

Different potentially toxic algal blooms occur in Ghar El Melh Lagoon (Tunisia), a region that should be subject to regular monitoring of HABs and their toxins. The lagoon, an important tourist resort due to its medicinal waters (balnearios), is very enclosed, but may contribute to the propagation of these HAB species into neighbouring coastal waters.

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• Italy

First record of *Prorocentrum lima* on Abruzzo Region coast, W Adriatic

Prorocentrum lima is a neritic and estuarine dinoflagellate (Dinophyceae) epiphytic on macroalgae and epibenthic on sediments or detritus. When found in the water column, it is generally in low abundance. The distribution ranges from tropical to temperate coastal waters. *P. lima* is a small to medium sized obovate cell with a central pyrenoid and a posterior nucleus. Each valve contains 50-80 regular marginal pores and about 60-100 evenly spaced pores on the valve surface.

Cells measure 36.61 µm in length and 25.19 µm in width for a length to width ratio of 1.45. There are 51 ± 1 regular marginal pores and 72 ±1 small spaced pores per valve (average values) (Figs. 1a & 1b). The right valve has a shallow V-shape similar to a triangular excavation with a protruding flange (Figs. 1c & 1d). Finally, in figure 2a and 2b, the central pyrenoid can be seen. Cells were fixed with Lugol's solution and 0.4% formalin respectively [1].

P. lima first appeared on Italian Adriatic coasts was in 2006, in the Emilia Romagna Region (NW Adriatic) (Pompei pers.comm.) and Ancona [2]; it reached the western part of the Abruzzo Region in summer 2007. This species produces DSP toxins (Diarrhetic Shellfish Poisoning) that can contaminate filter-feeding shellfish and cause gastrointestinal illness in humans [3]. The complex of DSP toxins produced by *P. lima* includes okadaic acid (OA), dinophysistoxins (DTX-1,

Table 1. P. lima *cell concentrations inside harbours during June, July and August 2007. Pump samples collected at 0.5 m below surface.*

	P. lima cell concentration (cells · L^{-1})	
date	Pescara Harbour	Ortona Harbor
13 June 2007	-	472,230
09 July 2007	-	255,054
02 August 2007	-	50,650

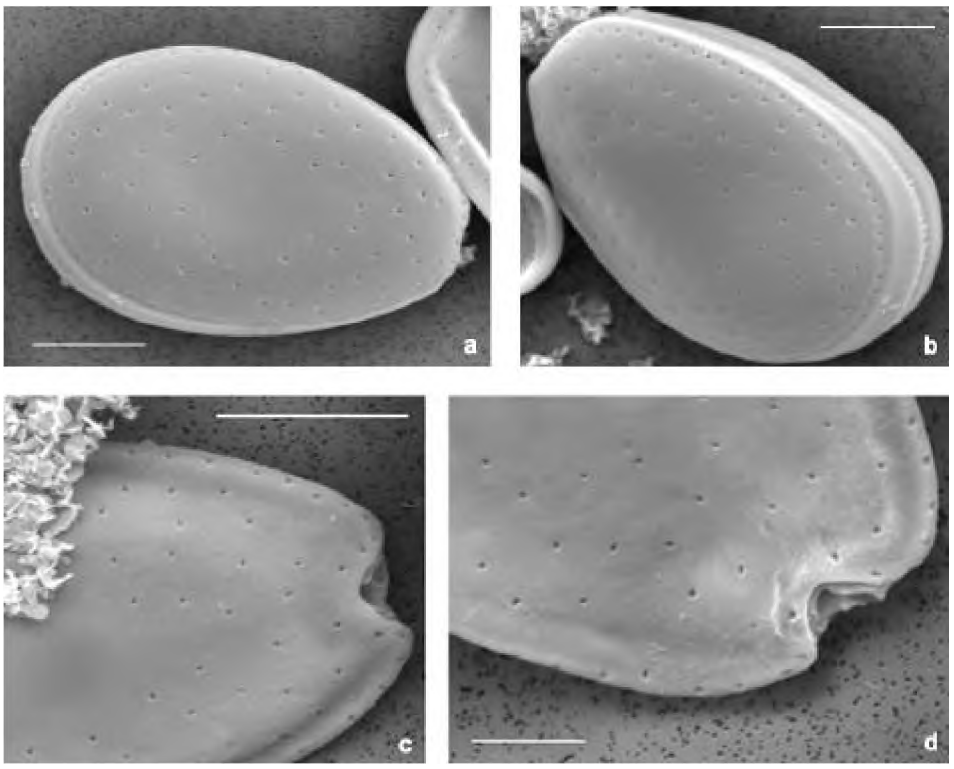


Fig. 1. Scanning electron micrographs of P. lima strain isolated from Ortona harbour. (a) and (b) entire cell showing shape of theca, marginal pores and spaced pores, scale bar = 10 µm; (c) periflagellar area in anterior part of cell. Scale bar = 10 µm; (d) triangular excavation similar to a shallow V-shape with protruding flange. Scale bar = 5 µm.

DTX-2), prorocentrolide and fast acting toxin [4].

This is the first record of *P. lima* together with other toxic phytoplanktonic species collected these coasts. Sampling was carried out at six stations located 500 m and 3000 m offshore from Pescara, Ortona and Francavilla during June, July and August 2007. The stations were located using a GPS (Global Position System) Garmin GPS45. In addition, monthly samples were taken inside Pescara and Ortona harbours (Fig. 3). The harbours are potentially habitats for growth of toxic epibenthic species like *P. lima*, due to the absence of water movements and stagnant environments.

Together with *P. lima*, we also observed other toxic and potentially toxic phytoplankton, including *Alexandrium minutum*, several toxic species of *Dinophysis*, potentially toxic *Pseudo-nitzschia*, *Gonyaulax spinifera*, *Lingulodinium polyedrum*, *Prorocentrum minimum*, and *Protoceratium reticulatum.*

At each station, one water sample was taken at 0.5 m by means of a pump, and net samples collected with a 10 µm mesh phytoplankton net. In the harbour areas, pump samples only were taken, at 0.5 m.

Cell observations were made with a light microscope (ausJENA Telaval 3) at 400x and 1000x magnification, by the Utermöhl method using a volume of 50 ml, while net samples were counted reading the entire well using a volume of 2 ml.

P. lima first appeared on June 13th inside Ortona harbour, both over masses of *Ulva lactuca* and over the reef around the harbour perimeter. Its abundance in June was 4.7 x 10^5 cells L^{-1} at 0.5 m from the surface. This bloom was detected only inside the harbour area (table 1). The chemical-physical conditions were: pH 8.19, water temperature 24.1°C, salinity 36.28 psu, dissolved oxygen 7.7 mg L^{-1}.

Along the coast, *P. lima* was absent, but other toxic and potentially toxic species were present in the monthly samples. *Dinophysis caudata* was the most abundant at the six stations (2.0 x 10^3 cells L^{-1} in net sample; 40 cells L^{-1} in sample collected at 0.5 m), followed by *Lingulodinium polyedrum* (1.5 x 10^3 cells L^{-1} in net sample; 40 cells L^{-1} at 0.5 m), both at Ortona station 500 m from shore (called Ortona 500).

On July 9th and August 2nd *P. lima* concentrations inside Ortona harbour were 2.5 x 10^5 and 5.0 x 10^4 cells L^{-1} respectively (0.5 m sample). These values were lower than the previous month; this species has not been observed in Pescara harbour.

Chemical-physical parameters measured in July and August in Ortona harbour were respectively: pH 8.19 and 8.22 pH; temperature 25 and 22. 1°C, salinity 33.28 e 34.81 psu, dissolved oxygen 7.9 and 7.6 mg L^{-1}.

A periodical decrease in the *P. lima* cellular abundances inside Ortona harbour corresponded a monthly increase outside the harbor itself and specifically at 500 m from the coast of this station during July and August exactly. Its cellular concentrations were 1.0 x 10^3 and 5.0 x 10^2 cells L^{-1} in net sample along the water column respectively; 40 cells L^{-1} on July and 20 cells L^{-1} on August in samples collected at 0.5 m from the surface. These values are not considered an HAB phenomenon.

Chemical-physical parameters

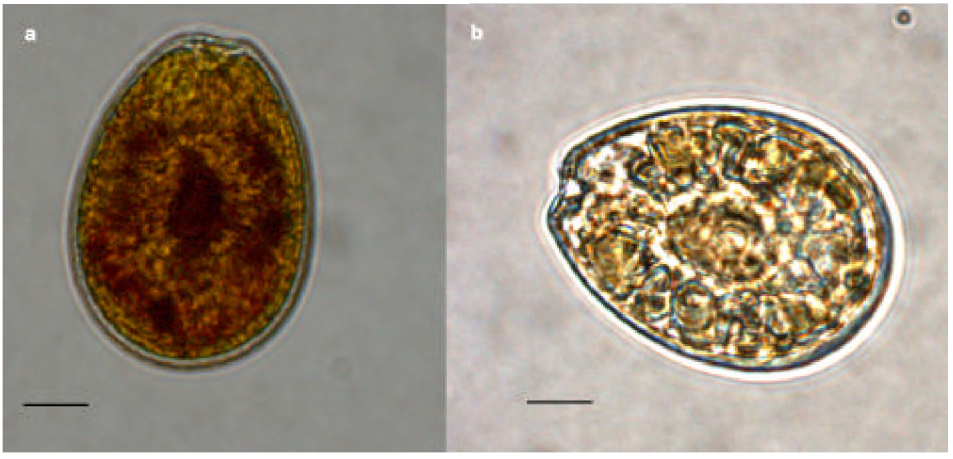
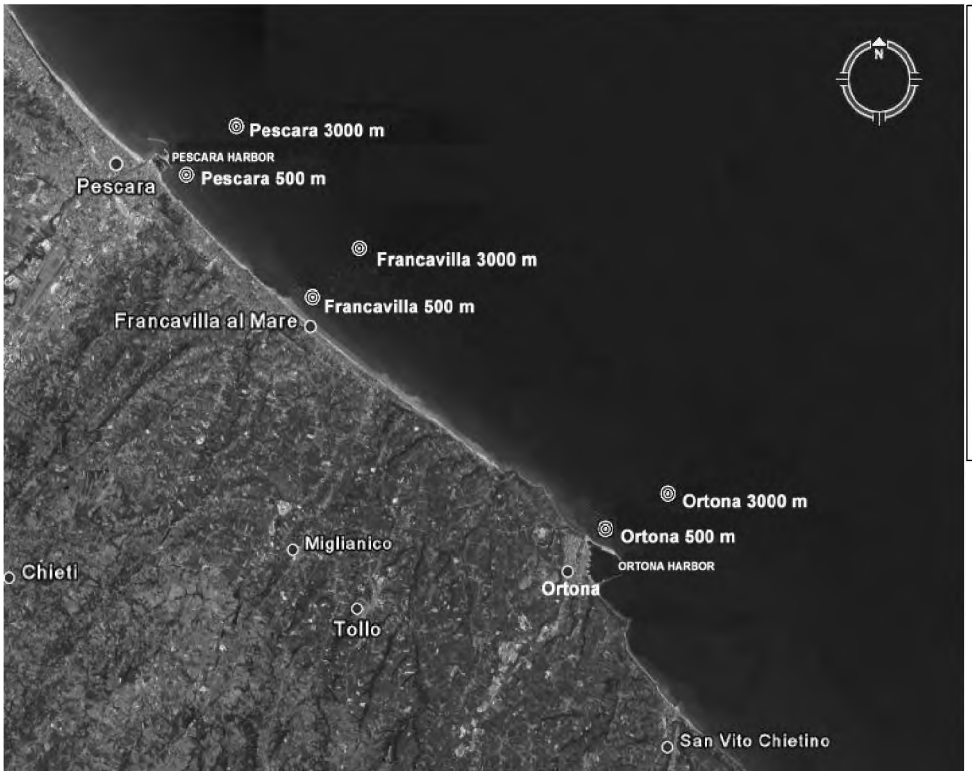


Fig. 2. Light microscopy photographs of P. lima at 1000x magnification to show central pyrenoid. (a) cell fixed with Lugol's solution, scale bar = 10 µm. (b) cell fixed with 0.4% formalin, scale bar = 10 µm.



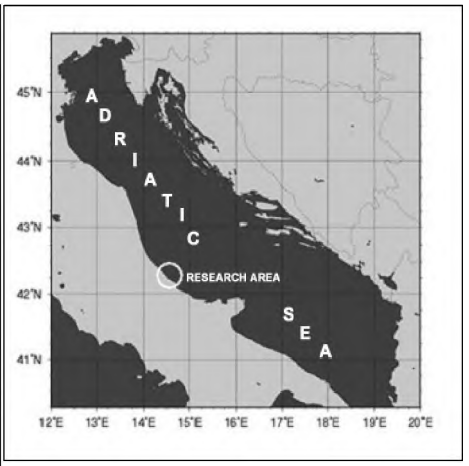


Fig. 3. sampling area.

measured in this sampling point (Ortona 500) during these two last months were: 8.19 and 8.22 pH; 25 e 22‘ 1°C temperature; 33.28 e 34.81 psu salinity; 7.9 e 7.6 mg > L^{-1} dissolved oxygen.

Maximum concentrations of other species these two months were *Prorocentrum minimum* (7.5 x 10^3 cells L^{-1} in net sample, 60 cells L^{-1} in 0.5 m sample) in July at Pescara station at 500 m from shore; *Pseudo-nitzschia* (1.1 x 10^6 cells L^{-1} in net sample, 3.0 x 10^5 cells L^{-1} in 0.5 m sample) in July at Francavilla station at 500 m from the shore; *Lingulodinium polyedrum* (1.4 x 10^4 cells L^{-1} in net sample, 1.4 x 10^2 cells L^{-1} in 0.5 m sample), and *Dinophysis caudata* (4.5 x 10^3 cells L^{-1} in net sample, 60 cells L^{-1} in 0.5 m sample) in August at Pescara station at 3000 m from shore.

Additional studies are needed to observe the periodical presence of *Prorocentrum lima* and other toxic and potentially toxic species along the coasts and inside harbors of Abruzzo region.

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PICES HAB Activities

The North Pacific Marine Science Organization (PICES) Working Group 15 (WG 15) on *Ecology of Harmful Algal Blooms (HABs) in the North Pacific*, with Tatiana Orlova (Russia) and F.J.R (Max) Taylor (Canada) as co-chairs, began in October 1999 as the initial effort in HAB collaboration among PICES member countries (Canada, Japan, P.R. China, Republic of Korea, Russia and the United States). The terms of reference for this 3-year WG 15 were to:

1. Identify the various species involved, and the timing, frequency, and duration of harmful algal bloom events;
2. Develop a regional database of relevant observational parameters associated with bloom events, including maps, bibliography, data sets and techniques, existing programs and expanded list of researchers in the area;
3. Investigate links between bloom events and environmental factors, trophic interactions and possible anthropogenic stress (e.g., eutrophication);
4. Assess the economic, health-related, and environmental impacts arising from harmful algal bloom events in order to improve the ability to predict the occurrence of these events and thus minimize their overall impacts;
5. Suggest areas where critical data are missing and where future research is necessary;
6. Facilitate regional collaborative research efforts at various levels to address these problems.



C. Tomas preparing materials for the practical lab demonstration on harmful raphidophytes.

At the 2003 PICES Annual Meeting (PICES XII) in Seoul, Korea, it was proposed and accepted that the WG 15 activities be expanded, and that an on-going HAB efforts evolve into a HAB Section. The terms of reference of the HAB Section, co-chaired by Hak-Gyoon Kim (Korea) and Vera Trainer (U.S.A.) are to:

1. Develop and implement annual bloom reporting procedures that can be consistent with ICES procedures and therefore incorporated into the broader HAB dataset (HAEDAT). This step will be important in assessing impacts of HAB events and as a research tool to look at patterns that will facilitate efforts to develop predictive capabilities;
2. Create and document national reports of HAB incidents and research in order to inform Section members of new toxins, new developments, and new approaches. These reports target both toxin producing and non-toxic (but harmful) algal species will be included;
3. Raise awareness of current specific needs for scientific advice among PICES member countries by identifying topics of interest, and providing syntheses of the available scientific information on those selected topics.

Membership for the HAB Section can be found on the PICES website at www.pices.int/members/sections/HAB.aspx

The PICES HAB data efforts began during the Seoul meeting with a workshop on "*Harmonization of HAB data: Developing a North Pacific HAB data resource I*". The following year at PICES XIII in Honolulu, U.S.A., the data workshop was titled, "*Harmonization of HAB data: Developing a North Pacific HAB data resource-II*". At PICES XIV in Vladivostok, Russia, PICES collaborators Henrik Enevoldsen and Monica Lion from the IOC, gave a database demonstration, "*Toward the development of a joint IOC-ICES-PICES database*". During that year, year 2000 HAB data for each PICES member country were entered into the Harmful Algal Event Database (HAEDAT), in a joint effort towards developing a global HAB database. The central tasks of the joint PICES-ICES HAEDAT were to:
1. Ascertain the data base process in PICES member countries;
2. Identify potential difficulties in delivery (e.g. confidentiality of data);
3. Assess the needs for the web-based data entry platform and;
4. Make any further modifications needed to represent the unique issues facing PICES member nations.



Selected HAB Section members and guest speakers, PICES XVI, Victoria, BC, Canada, Oct 07.

Another highlight of the PICES HAB Section is the ongoing annual sponsored workshops on selected harmful algae that began with the "*Review of selected harmful algae in the PICES region: Pseudo-nitzschia and Alexandrium*" (2005) then continued with *Dinophysis* and *Cochlodinium* (2006), *Heterosigma akashiwo* and other harmful raphidophytes (2007)", with a plan for a workshop on *Karenia* and *Prorocentrum* at PICES XVII in Dalian, China. These workshops have featured summary presentations by invited experts and practical laboratory demonstrations in phytoplankton identification, toxin analysis, and other methods pertaining to the species of focus for that particular year.

The HAB Section within its parent committee on Marine Environmental Quality (MEQ) also has sponsored a number of special topic sessions on subjects of particular interest and pertinence to HAB researchers in the country hosting the Annual PICES Meetings. These topic sessions include: "*Natural and anthropogenic introduction of marine species*", "*Harmful algal blooms in the PICES region: New trends and potential links with anthropogenic influences*", "*The relative contribution of offshore/inshore HAB development and persistence in the PICES region*". In Dalian, China at PICES XVII, the special topic session will be "*Environmental and anthropogenic influences of species succession towards HAB species dominance*".

Finally, a 3-year PICES HAB International project, sponsored by a grant from the Government of Japan, will help establish and implement monitoring programs in developing non-PICES nations. The sharing of such monitoring information will ensure the security of imported fisheries products, and will protect the coastal environment and ecosystems of Japan and other importing nations. HAB International will focus on assisting in the development of capabilities in Vietnam, Hong Kong, Malaysia, Philippines, Thailand, Mexico, Peru, Chile, but the project is not exclusive of other developing nations that are interested in participating. The primary aims for the pilot training workshops are to establish effective protocols, determine proper equipment, develop appropriate databases, and to design ideal teaching strategies. We seek the support of key national and local regional officials in developing nations, and an understanding of the regional operational structure and laboratory facilities in the countries suitable for hosting the workshops. Preliminary site visits in Year 1 of the project by the workshop organizers will be used to work with country representatives to design the framework for the workshops. We will begin the training workshops in Vietnam during Year 2 using established contacts at regional and local levels that members of the organizing committee have established.

PICES XVII will be held October 23-November 2, in Dalian, China.

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• India

A 'Red tide' caused by the diatom *Coscinodiscus* on the southwest coast of India

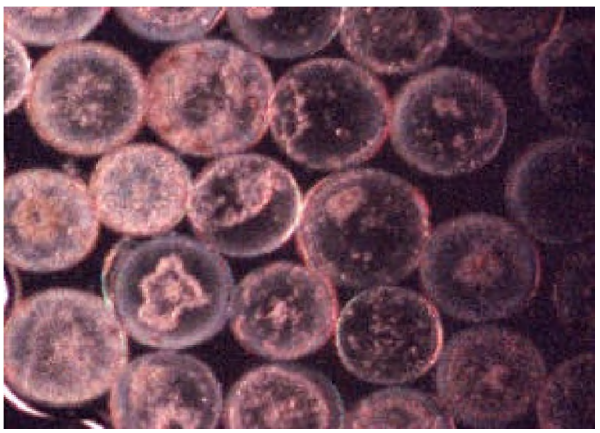

Fig. 1. Coscinodiscus asteromphalus *var.* centralis *Cells x 40.*

The International Council for Exploration of Seas defined phytoplankton blooms as "those, which are noticeable, particularly to the general public, directly or indirectly through their effects such as visible discoloration of the water, foam production, fish or invertebrate mortality or toxicity to humans". These are natural phenomena that occur in the sea under special hydrographical conditions, when the combined effects of grazing are less than biomass production due to growth of phytoplankton, and via advective processes. The accumulation of phytoplankton may be so dense that the water appears coloured red, green, brown, orange, etc. Numerous incidences of these blooms have been reported from many parts of the world ocean including Indian seas. The frequency of algal blooms in the recent past has been related to anthropogenic activities. Along the southwest coast of India, there are several reports of blooms caused by dinoflagellates, and by the diatoms *Fragilaria* and *Hemidiscus*.

During the regular monitoring of Harmful Algal Blooms (HAB) in the Indian EEZ and estuaries, water discoloration due to a bloom of *Coscinodiscus asteromphalus* var. *centralis* was observed off Kodikkal-Calicut (11° 28.43'N, 75° 36.10'E) during the second week of August 2006. Though dinoflagellate blooms have been reported every year during this period, this is the first time a monospecific bloom of this diatom causing red tide has been seen: the bloom spread into the estuary during high tide. In Mahe Estuary, the cell density was so high that the water colour was brownish red.

Surface water samples were collected in a clean plastic bucket about 1.5 km offshore. Temperature, salinity, pH, dissolved oxygen (DO) and nutrients (nitrate, phosphate and silicate) were estimated employing standard techniques. Water transparency was measured with a Secchi disc. Primary productivity, chlorophyll *a*, phytoplankton and zooplankton composition were also estimated. Phytoplankton samples were collected by filtering around 50 litres of surface water using 60 µm mesh bolting silk. The samples were examined microscopically and cell concentrations determined. The cell count of *Coscinodiscus* was $7x10^6$ L^{-1}.

Along the southwest coast of India, red water is reported to occur between August and November, which marks the end of the upwelling period along this coast. Earlier workers have also observed that dinoflagellate blooms are preceded by rains and diatom maxima. Observations reviewed by Brongersma-Sanders [1] show that red tides occur in upwelling areas towards the end of upwelling period and that diatom blooms precede dinoflagellate blooms in these regions. The period when red tides are generally reported coincides with the end of the upwelling season, when the weather is warmer with longer hours of sunshine than during the monsoon months. The hydrographic conditions measured during the bloom period were relatively low surface temperature (28°C) and relatively high salinity (34 ppt). NO_3-N was low (3.185 µmol L^{-1}) while the PO_4-P was high (2.185 µmol L^{-1}) resulting in a rather significant N: P ratio. The reactive silicate recorded (42.27 µmol L^{-1}), was very high for this period, which could be one of the reasons for the diatom bloom. Reduction in nitrate and increase in phosphate content could be a feature associated with the diatom bloom itself; similar increases in phosphate and decreases in nitrate have been reported during blooms by earlier workers. Increased photosynthetic activity resulted in high dissolved oxygen concentration (6.9 mg L^{-1}) in the surface waters. Primary production was 5.6 gC m^{-3} day^{-1} and chlorophyll *a* (206.5 mg m^{-3}) was several times higher than during normal productive periods. Zooplankton biomass was low (11.24 ml $1000m^{-3}$) and was comprised of a few species of copepods, copepodites and nauplii.

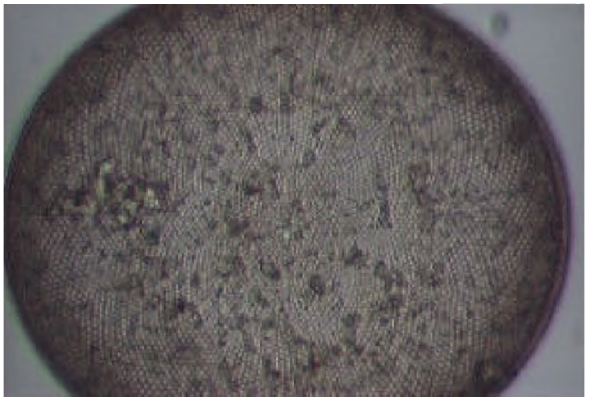

Fig. 2. C. asteromphalus *var.* centralis *Single cell x 200.*

Acknowledgements

The authors are grateful to the Centre for Marine Living Resources and Ecology, Ministry of Earth Sciences, Govt. of India for financial assistance during this study.

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ISSHA President's Corner

Happy New Year.

ISSHA
International Society for the Study of Harmful Algae

2008 promises to be an exciting and busy year for ISSHA

The General Assembly will convene during the 13th International Conference on Harmful Algae the first week in November 2008 in Hong Kong. Prior to the meeting members will have an opportunity to nominate their colleagues for awards as well as nominate candidates to serve as Officers (President, Vice-President, Secretary and Treasurer) and as Council members for the coming 3 year term.

The important dates to note are listed below but, first let me remind you that membership dues expire on 31 December. So check your membership status and renew your membership (via Nina Lundholm nlundholm@bi.ku.dk). You may not serve as a nominator for a candidate unless both you and the candidate are current ISSHA members. ISSHA dues are still only $20 per year and $10 per year for students. With the decreased value of the US dollar, this is quite a bargain.

On or by **3 July 2008 Nominations for Officers and Council Members**.

Article 23. Elections.

l. Any member of the Society may nominate candidates, who shall be members of the Society, for the election as President, vice Presidents, Secretary, Treasurer or Members of Council.

ll. All such nominations, with the name of a seconder and with written consent of the nominee to act if elected, shall be forwarded to the Secretary no later than four months before the General Assembly.

On or by **3 August 2008 Ballots Circulated to Membership**

Article 23. Elections.

lll. Voting shall be by written ballot. Fir this purpose, the Secretary shall circulate ballot forms to all members of the Society three months before the General Assembly. At the meeting, the ballot forms shall be opened and the count made by scrutineers appointed by Council, and the results of the ballot shall be declared.

On or before **19 September 2008 Submit Agenda Items for General Assembly.**

Article 17 General Assembly.

ll. At the General Assembly, members of the Society present shall consider any business brought before them by the Council, or by any member (of which notice in writing has been given to the Secretary at least two weeks before distribution of the agenda).

On or by **3 October 2008 General Assembly Agenda.**

Article 17 General Assembly.

l. Notice of the General Assembly shall be sent to each member by the Secretary at the earliest possible date (normally at least one year in advance), and the agenda of the meeting shall be sent out at least one month before the meeting and posted on the ISSHA website.

The agenda for the 2008 General Assembly will have suggested alterations to the Statues to clarify ISSHA's business year and other minor changes.

ISSHA encourages its diverse membership and would like to have that diversity mirrored in the Committees as well. If you would like to serve on any of the following committees, please let one of the Officers or the Committee Chairs know of your interests.

I look forward to seeing you at the 13th Conference on Harmful Algae in Hong Kong and urge you to visit the ISSHA website at http://www.issha.org for updates on the program and activities. Again, I wish each of you a prosperous and safe New Year.

Pat Tester, President,
Email: pat.tester@noaa.gov

Committee on Elections: Chair: K. Steidinger. Member: S. Bates.

Committee on Membership: Chair: P. Tester. Members: H.-G. Kim, B. Reguera, G. Hallegraeff & T. Smayda.

Committee on Finances: Chair: N. Lundholm. Members: D. Anderson, K. Steidinger, E. Granéli & Y. Fukuyo.

Committee on Conference Program: Chair: Ø. Moestrup. Members: D. Anderson, A. Cembella, B. Dale, G. Doucette & J. Lewis.

Committee on Achievement Awards: Chair: M. Montresor. Members: A. Cembella, H. Enevoldsen, B. Reguera & B. Dale.

Committee on Travel Awards: Chair: D. Anderson. Members: A. Cembella, H. Enevoldsen, B. Reguera, K. Steidinger, Y. Fukuyo & E. Granéli.

Committee on Publications: Chair: J. Lewis. Members: S. Bates, A. Cembella, B. Dale, G. Doucette, H. Enevoldsen, K. Steidinger, P. Tester & A. Zingone.

***Ad hoc* Committee on Special Projects:** Co-chairs: H. Enevoldsen, P. Tester, A. Zingone.

Executive (2004-2007): Please note that all current Officers have agreed to serve until the 2008 election.

President: Pat Tester, Email: pat.tester@noaa.gov

Vice President: Gustaaf Hallegraeff, Email: hallegraeff@utas.edu.au

Vice President: Beatriz Reguera, Email: beatriz.reguera@vi.ieo.es

Secretary: Tracy Villareal, Email: tracy@utmsi.utexas.edu

Treasurer: Nina Lundholm, Email: nlundholm@bi.ku.dk

Past President: Karen Steidinger, Email: Karen.Steidinger@MyFWC.com

Council (2004-2007): D. Anderson (USA), B. Dale (Norway), G. Doucette (USA), Y. Fukuyo (Japan), E. Granéli (Sweden), H.-G. Kim (S. Korea), J. Lewis (UK), Ø. Moestrup (Denmark), M. Montresor (Italy), T. Smayda (USA) and A. Zingone (Italy).

Future events

FEBRUARY 2008

The ICES-IOC-SCOR Working Group on Implementation of GEOHAB in the Baltic

Gothenburg, Sweden, 27-29 February 2008.

The working group will meet to: review progress in implementation of the GEOHAB-BALTIC cooperative research plan, with reports from the projects in progress and reports from projects at planning stage; define processes and plan experiments that are needed for better parameterisation of dynamical HAB models; review the progress of incorporating the Baltic HAB list into the IOC Taxonomic Reference List of Toxic Plankton Algae; and discuss and report on the need of a Baltic HAB researcher network, in the light of the WGHABD comments on WGGIB performance.

OCTOBER 2008

Ciguatera workshop

Noumea, New Caledonia, 27-31 October 2008

The Organizing Committee is in the process of organizing the "Ciguatera and related biotoxins" Workshop. Dates were set in line with those of the 13th International Conference on Harmful Algae to be held in Hong Kong on 3-7 November 2008, as a satellite workshop and to allow participants to make the appropriate travel connections from Noumea to Hong Kong in good time.

The workshop will take place in the IRD and the Secretariat of the Pacific Community (SPC) symposium facilities, both based in Noumea and appropriately located next door to each other.

We would also like to kindly draw your attention to a special roundtable session on toxic marine cyanobacteria,



which will be organised on Tuesday evening, 28 October. The interest has come about as a result of our recent studies in New Caledonia and French Polynesia. This will enable valuable discussions with international scientists involved in this area and should provide the basis for new collaborative research and better use and allocation of funding from donors in the future. All those interested in this area of research are welcomed, and therefore please feel free to extend and share this announcement to your colleagues in this field of research.

For further information, please visit: http://www.ird.nc/ciguatera or ciguatera@noumea.ird.nc.

NOVEMBER 2008

13th International Conference on Harmful Algae 08

Hong Kong-China, 3-7 Nov 08.

ISSHA is pleased to announce the 13th Int. Conference on Harmful Algae. Supporting organizations include School of Science & Technology, the Open University of Hong Kong and the Association of Harmful Algal Bloom of South China Sea (AoHABSCS).

Further info: www.hab2008.hk.

Harmful Algae News

Previous issues of HAN and newsletters of the IOC HAB Programme can be downloaded at http://ioc.unesco.org/hab/news.htm

Requests for subscription

Subscription to HAN is made by sending a request with a complete address to Ms V. Bonnet: v.bonnet@unesco.org.

PHYCOTOXINS

You can also get news on HAB research and events at the Phycotoxins-list at the internet.

The address of this list is: phycotoxins@www.agr.ca

To join the list, send "subscribe phycotoxins" to: listserv@www.agr.ca

Archives are located at www.agr.ca/archives/phycotoxins.html

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Write the Editor NOW with news on your work, HAB events in your country or region, or any other matter you wish to share with HAB scientists and managers worldwide. Harmful Algae News has more than 2000 subscribers.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

http://ioc.unesco.org/hab/news.htm No. 36

• Turkey

Mucilage event associated with diatoms and dinoflagellates in Sea of Marmara, Turkey

The massive presence of mucilaginous organic matter, resulting from planktonic and benthic algal blooms, has become more frequent in many coastal waters around Europe, especially in the Adriatic. The appearance of mucilage in the Adriatic Sea has been reported periodically since 1800, with major mucus blooms during the 1990s [1]. The mucilage phenomenon of the Adriatic Sea had usually been related to extracellular organic matter of phytoplanktonic origin.

An extensive mucilage event consisting of white gelatinous material initially suspended at the surface and in the water column was noticed along the Turkish coast of the Marmara Sea (especially Izmit Bay). Marmara Sea has a rather complex hydrological system, in a zone of transition between dense (salinity 37- 38.5 ‰) and warmer waters originating in the Mediterranean Sea, and cold, lower-salinity water (20-22 ‰) coming from the Black Sea. The pycnocline lies at 10 to 30 m depth and varies seasonally [2].

The first mucilage was observed in mid-autumn 2007 along the north-eastern part of Marmara Sea with temperatures 18.4±1.0ºC. It extended from Izmit Bay to the Dardanelles during the calm weather period; it was denser and of longer duration in Izmit Bay, which is affected by intense industrial activity, and which has a weaker circulation compared to Marmara Sea. To identify phytoplankton species responsible for the mucilage, water samples were collected from surface and deep water at target sites (Fig 1)

(Cont'd on p.2)

• Mexico

A bloom of *Ceratium balechii* in Michoacan, Pacific Mexico

From 5 to 12 June, 2007, the National Autonomous University of Mexico (UNAM) conducted the Talud XI cruise on the Pacific coast of Mexico. While at stations 15 and 16 off Michoacan State, a marked red discoloration was observed. Microscopical analyses revealed the presence of *Ceratium balechii* Meave, Okolodkov et Zamudio [1]. This species, a very frequent bloom forming organism [2](Fig.1A), has been commonly confused mainly with *Ceratium dens* Ostenfeld et Schmidt [3: Fig. 11, see the drawing of the anomalous chain of two individuals e)] and recently, with *Ceratium divaricatum* Kofoid [4]. Nevertheless, the cell morphology is quite distinct. The body is pentagonal, asymmetric, and very different from the above mentioned species. Also, the antapical horns have different size and direction. Meave *et al* [1] propose that according to the roughness and size of the antapical horns there may be two forms, hypothesizing that they result from an adaptation to different water densities. At the moment, we are not able to speculate on this last point, since our observations in a single place and population showed a wide size variability (Fig.1B) as has been shown in cultures [5]. We have observed minute specimens (Fig.1C) as well as some showing teratogenic changes (Fig.1D). This species, unlike *C. dens* and *C. divaricatum*, is

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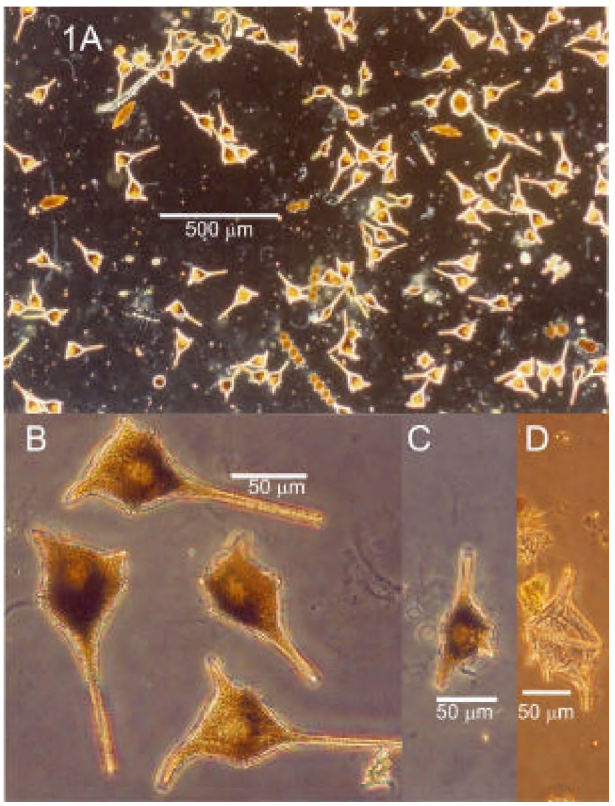

Fig. 1. A) Ceratium balechii, *dark field at 40x, 1 ml sample. B) Four cells of different size. C) Small cell. D) Anomalous specimen with three antapical horns. B-D) Phase contrast, 200x.*

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

(Cont'd from p. 1)

from Marmara Sea (nine sampling stations from Istanbul Strait on October and December 2007, February and March 2008; from Prince Islands on February 2008; and from Izmit Bay on October 2007) during the bloom period (September 2007-March 2008). Temperature, salinity, pH, conductivity and dissolved oxygen profiles were obtained with a SEABIRD CTD probe during the cruise in Istanbul Strait and Prince Island. Some nutrients and chlorophyll *a* analyses were performed according to standard methods. Phytoplankton samples were collected for qualitative and quantitative analyses by Nansen bottle and plankton nets (with mesh size 10µ and 30µ) respectively. Phytoplankton analysis was carried out by the Utermöhl method. During the mucilage event, aggregates were sampled in the water column by SCUBA divers using syringes, and also collected from sediments and mussels. Some environmental parameters during the different sampling periods are summarized in Table 1.

Historically, diatoms have always been considered as the algal group mainly involved in this kind of event due to their abundance in mucilaginous aggregates [3] and for their known extracellular release of polysaccharides [1, 4]. In our observations, high diatom densities were recorded during this period (Fig. 2). During the first days of this bloom, the phytoplankton community was characterized by

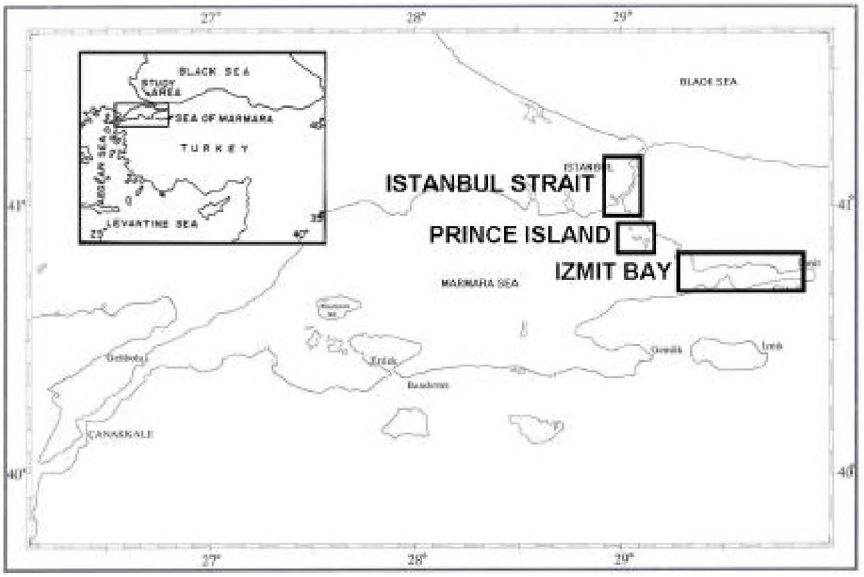

Fig 1. Sampling sites (Istanbul Strait, Prince Island, and Izmit Bay) from Marmara Sea during the mucilage events.

abundant diatom species (*Proboscia alata, Rhizosolenia* sp., *Pseudosolenia calcar-avis*). Total diatom density was more than 10^7 cells L^{-1}. This phenomenon successively increased and spread on a large scale in the Marmara Sea. However, shifts were observed in dominant species during the rest of the bloom period (*Thalassiosira* sp., *Ditylum brightwellii*, *Coscinodiscus* spp., *Leptocylindrus minimus, Skeletonema costatum, Chaetoceros* spp., *Cerataulina pelagica, Cylindrotheca closterium, Pseudo-nitzschia* cf. *seriata*). In February 2008 simultaneously with the diatom bloom (max value 3.9×10^6 cells L^{-1}), the dinoflagellate *Gonyaulax fragilis* became abundant in the mucilage. The presence, in fairly high numbers, of *Gonyaulax fragilis* is reported in several papers [4, 5, 6] concerning the mucilage events in the Adriatic Sea. In our observations, *Gonyaulax fragilis* contributed to the bloom as biomass and possibly to mucilage production, but its density did not reach high numbers; its maximum concentration (36×10^3 cells L^{-1}) was recorded in the pycnocline (15 m) off Prince Island. The dominant species of phytoplankton during the mucilage event were similar at all investigated sites from Marmara Sea. Furthermore, a large scale increase of coccolithophores (especially *Emiliana huxleyi*) was also observed during the mucilage event off Istanbul.

Mucilage can affect marine ecosystems by massive development of

Table 1. Comparison of minimum and maximum values of some parameters at the surface during the mucilage events in 2007-2008, and different periods of study in Marmara Sea [2,7].

Autumn-Winter period of different years	Izmit Bay 1999-2000 (from Aktan et al. 2005)	Izmit Bay 2007-2008 Mucilage Period	Istanbul Strait 1997-1998 (from Aktan et al. 1999)	Istanbul Strait 2007-2008 Mucilage Period	Prince Island 2007-2008 Mucilage Period
Water temperature (°C)	21.2-23.0	17.5	4.8-15.3	6.7-16	7.4-9.6
D. oxygen (mg L^{-1})	8.3-15.6	9.4	7.4-11.0	10.1-10.7	7.9-10.1
NO_2+NO_3-N (µg N L^{-1})	7.7-15.7		1.4-2.8	6.2-8.4	3.8-10.6
PO_4-P (µg P L^{-1})	2.3-14.5		0.0-0.2	0.1	0.0-0.1
T.Phosphorus (µg P L^{-1})	13.0-28.1		0.2-0.5	0.1	
SiO_2-Si (µg Si L^{-1})	57-257		3.0-7.1	0.01- 0.04	0.035-0.043
Conductivity (µS cm^{-1})			29	19.1-19.2	22.5-28.9
pH		8.5	8.32-8.48	9.4-9.6	7.5-9.6
Surface salinity (psu)	20-22	20		17.1-18.1	21.1-25.3
Secchi disc (m)	2.5-5.5	<1	6.0-7.0	5-5.5	3.7-5.5
Chlorophyll a (mg m^{-3})	3.6-7.0	-	0.8-0.9	0.13-1.86	1.3 1-9
Dominant algae in density	dinoflagellates	Single and colonial diatoms	Dinoflagellates coccolithophores (*E. huxleyi*)	Single and colonial diatoms	Single and colonial diatoms
Dominant algae in frequency	dinoflagellates	Single and colonial diatoms	Dinoflagellates coccolithophores (*E. huxleyi*)	Diatoms and dinoflagellates (*G. fragilis*)	Diatoms and dinoflagellates (*G. fragilis*)

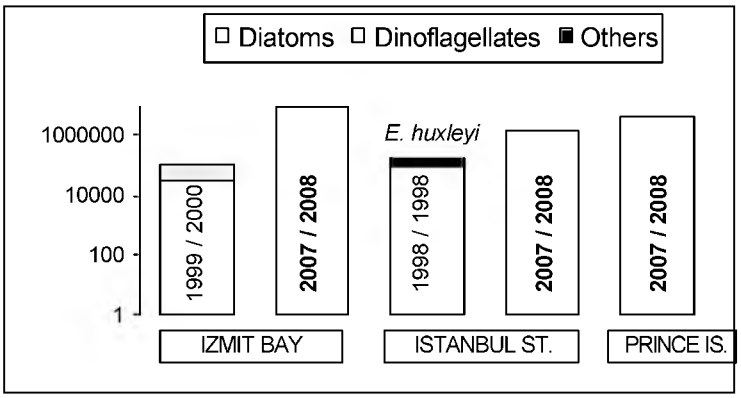

Fig. 2. Comparison of mean densities (cells L^{-1}, logarithmic) of phytoplankton groups during the mucilage events in 2007-2008 and the different periods of study at target sites (Izmit Bay, Istanbul Strait, Prince Islands) of Marmara Sea.

aggregates that cover large areas, and it can also seriously affect fisheries and tourism [1]. During these observations, neither hypoxia nor anoxia nor fish kills were recorded, but the large quantity of mucilage aggregates affected fishing and sport diving activities (personal communications from fishermen and divers). Extensive benthic mucilage aggregates were also observed on the sediments and mussels; the main bloom forming species are planktonic and benthic colonial and single cells of diatoms within the mucilage aggregates on the sediments. During our dive we noticed that dense sedimentation of these aggregates (on sediment, mussels and crabs, etc.) has a negative effect on the benthic ecosystem. Following this preliminary study, we have started a long term monitoring project of these mucilaginous phenomena to explore their distribution and seasonal dynamics, potential causes (nutrients, climatic, hydrographical and oceanographic conditions), consequences (benthic and pelagic ecosystem and fisheries) and the possible relationship with grazers.

Acknowledgements

We thank to Prof. Dr. Bayram Öztürk for providing opportunities for the research cruise, to personnel of R/V Yunus-S for assistance in the field studies and to Mine Çardak, Aysu Gümüsoglu and Esra Balcioglu for assistance in the laboratory.

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associated with upwelling. Even though *C. divaricatum* is very variable [6: Fig. 31], the antapical horns are never directed downwards.

Cortés and Nuñez pointed out two peaks of abundance in spring (March-April) and autumn (October) in the Gulf of California [2]. Our observations in early summer, far outside and south of the Gulf of California, provide a new record in a different climatological and hydrographical region (Fig 2). Surface samples (10 ml sedimented for 24 hours) from 30 stations were observed (400x) and quantified [7], but 1ml was used for stations 15 and 16 due to the higher abundance. The concentrations (Fig 2) are the highest recorded for this species in the Pacific: 1,806 cells·ml^{-1} at station 16 (688 cells·ml^{-1} at station 15). Concurrently, at station 16 were recorded the lowest temperature and salinity (24.52ºC and 34.24 PSU) during the cruise, in agreement with the idea of a relationship of the species with upwelling. Even though *C. balechii* is a non toxic organism, it has been related to fish mortality (together with *C. furca* and *C. falcatum*), mainly due to oxygen depletion.

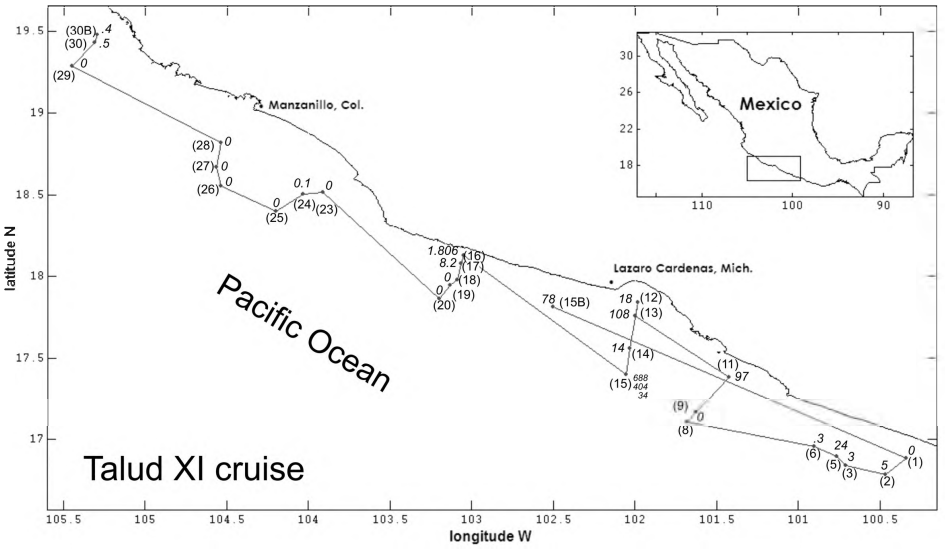

Fig. 2. Map showing the track of the Talud XI cruise. Station numbers in parenthesis and plain numbers are counts of C. balechii *(cells ml^{-1}).*

Acknowledgements

To A. Nuñez-Pasten for his invaluable help in sampling.

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• Italy

What is *Chrysophaeum taylorii* Lewis & Bryan doing in Sardinia (Tyrrhenian Sea, Mediterranean)?

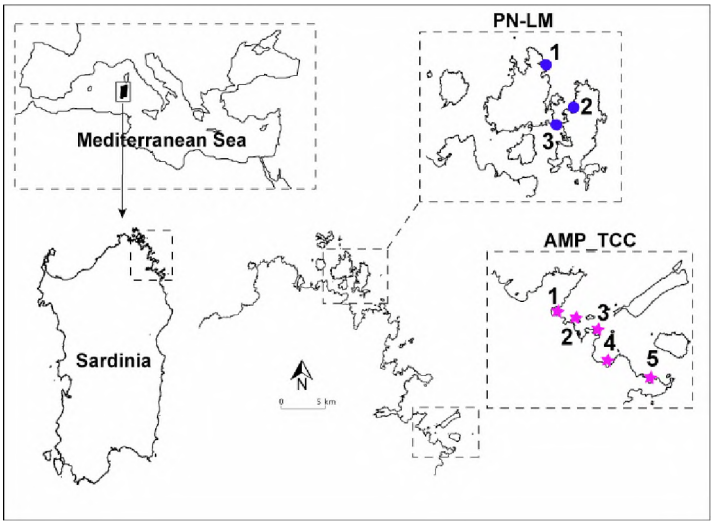

Fig. 1. Geographical localization of the sampled areas interested by the C. taylorii proliferation.

We report the first event of an intense growth of *Chrysophaeum taylorii* Lewis & Bryan (Pelagophyceae) [1] on a broad section of the coast (Fig. 1) of eastern Sardinia (Tyrrhenian Sea, Western Mediterranean) during summer 2007. This species was observed once before in a sample collected off Elba (Tuscany Archipelago) in summer 2005, when its growth was very low (personal communication Sartori & Boddi) and without any kind of *"public nuisance"*. In contrast, this aspect was important in Sardinia where *C. taylorii* covered primarily hard benthic substrate (mainly granitic), and sandy and plant substrates (predominantly *Posidonia oceanica*) in marine areas of high naturalistic and tourist interests.

C. taylorii has never been reported as a possible agent of benthic Mediterranean mucilage. The hyperproduction of benthic mucilaginous material has been documented since the early 1990s along both Italian [2] and French coasts [3]. In the former, the phenomenon has been very intense, especially in the area between Sicily and the Tuscan Archipelago and in the latter along eastern Corsica. In these cases the main producer species were *Chrysonephos lewisii* (Taylor) Taylor and *Nematochrysopsis marina* (Feldman) Billard (Chrysophyceae) and *Acinetospora crinita* (Carmichael) Kornmann (Phaeophyceae) [4-5].

Our studies were performed at the request of the Marine National Park of La Maddalena (NP-LM; www.lamaddalenapark.it) and Marine Protected Area of Tavolara-Capo Codacavallo (MPA-TCC; www.amptavolara.it), whose watch-monitoring had highlighted the anomalous presence of the benthic mucillage in their areas of interest since the beginning of the summer, but with the maximum extension in August. Furthermore, this exceptional and "strange" situation had generated alarm among local people and tourists, whose reports were numerous.

Our activities were also carried out as a member of the BENTOX-NET (www.bentoxnet.it), the network of

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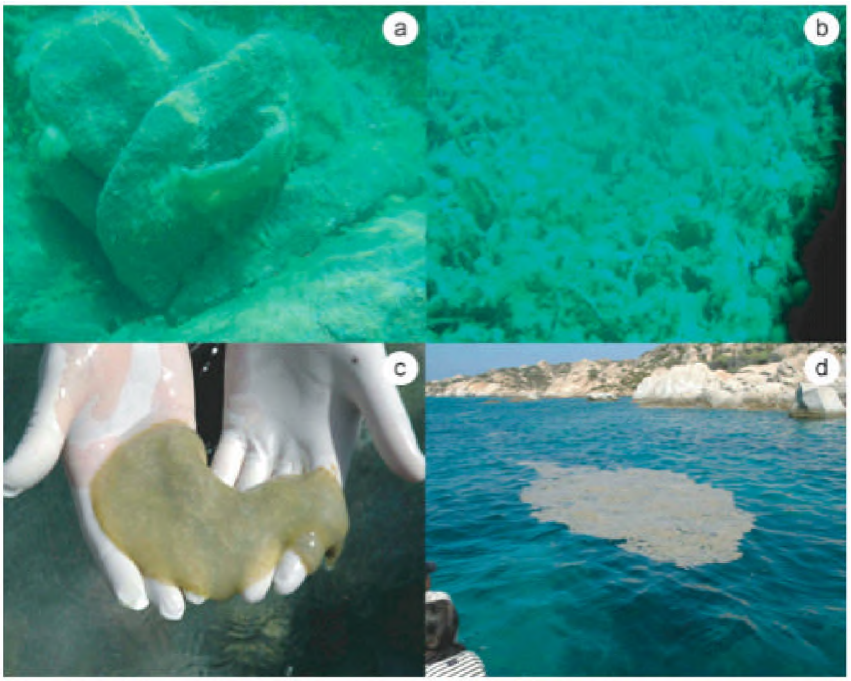

Photo 1. NP-LM. Mucilaginous cover associated to C. taylorii growth on different substrates: on granitic rock (a) and on P. oceanica (b); c) piece of mucilage and presence of gas bubbles; d) mucilaginous patch on the surface.

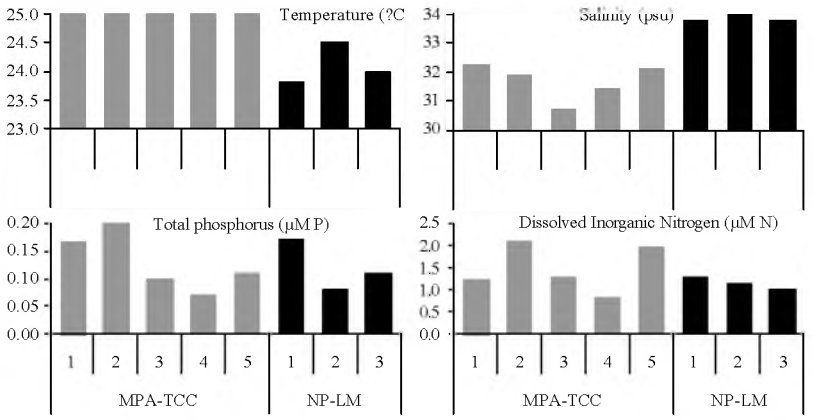

Fig. 2. Environmental data of the two areas sampled.

voluntary Italian researchers for investigation of Harmful Benthic Algae Blooms.

Samplings were carried out at 5 stations in the PMA-TCC on the 8th of August and at 3 stations in NP- LM on the 22nd of August (Fig. 1). Water samples were collected for routine analyses (pH, salinity and chlorophyll *a* [6]; nutrients [7] and for phytoplankton and benthic microalgae studies. Floating and substrate samples of mucilage were also collected and fixed with both modified Lugol's solution and neutralized formaline (4%).

The field observations revealed a vast covering of yellow-green-brown mucilage (Photo 1a-b). The mucilaginous layer was 5-8 cm thick. Large portions came off the substrates due to hydrodynamic stress and/or gas bubbles (evident macroscopically; Photo 1c). Currents in the water column transported the smallest portions; the largest accumulated, forming patches on the surface, also transported by wind and currents (Photo 1d). Temperatures were rather uniform (Fig. 2), but higher in PMA-TCC (up to 25.0 °C) than in NP-LM (between 23.8 °C and 24.5 °C). Salinity ranged from 30.72 to 32.28 psu in PMA-TCC and from 33.78 to 34.01 psu in NP-LM. Nutrient concentrations were fairly low (maxima of 0.20 µM P for total phosphorus and 2.09 µM N for DIN in NP-LM and of 0.17 µM P and 1.26 µM N in PMA-TCC); chlorophyll *a* values were up to 0.96 mg m^3 in PMA-TCC and 1.11 mg m^{-3} in NP-LM (Fig. 2).

Microscopic analyses of unfixed samples of mucilage showed a layered aspect, as a whole of small layers or tubes (Photo 2a). Within the mucilage there were many microscopic organisms but the most abundant was *C. taylorii* (Photo 2b). The cells of this species are peculiar with a really "unusual" aspect, as already pointed out [8-9]. The identification was not easy because this species is typical of coral reefs and its presence in the Mediterranean Sea was not expected. In fact, its distribution is reported in the tropical and subtropical Atlantic, Western Pacific, Micronesia and Australian [9-11]. In these areas it is often considered a nuisance species due to the copious production of mucilage. Sparrow and Heimann [12] indicated *C. taylorii* as species in "identity crisis", because of the increase, in the last 10 years, of strong growth in Australian waters.

The event in Tyrrhenian coastal areas of Sardinia during summer 2007 seems to be an example of this kind of exceptional event, but in very distinct biogeographical and environmental conditions, which raises a long list of questions.

Will this exceptional event turn out to unique for the Mediterranean, or will *C. taylorii* blooms occur in future years? Will this species enlarge its presence? Will it be a nuisance again? Will it affect the benthic habitat and impact local biodiversity? Is *C. taylorii* an allochthonous species introduced into the Mediterranean by human activities? If yes, what was the vector? When did it happen? From which geographical area does it come from?...and many others!

Aknowledgements

To the Directors and personnel of MPA-TCC and NP-LM for their interests, photos and logistic support. To Prof. Sartoni and Dr. Boddi for their kind collaboration. To Maria Grazia Giacobbe and Carmelo Tomas for their friendly help.

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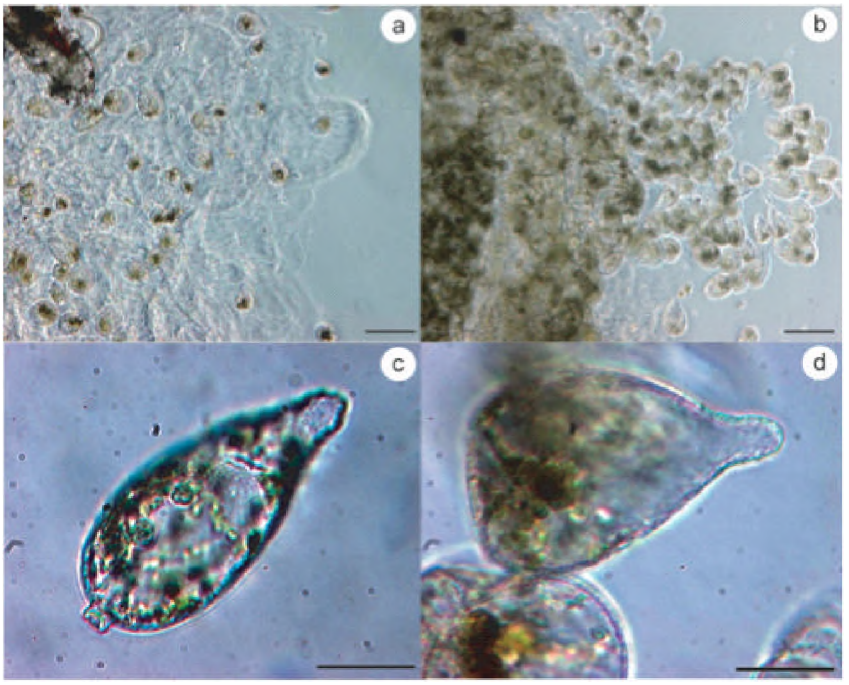

Photo 2. Unfixed samples collected at NP-LM: layered aspect of the mucilagine with intertwined cohered stalks having the cells at their end (a and b; bars=100 µm); characteristic shape of cells with their peculiar tubular invagination (c and d; bars=20 µm).

• Mediterranean and Eastern Atlantic

Is *Gambierdiscus* expanding to new areas?

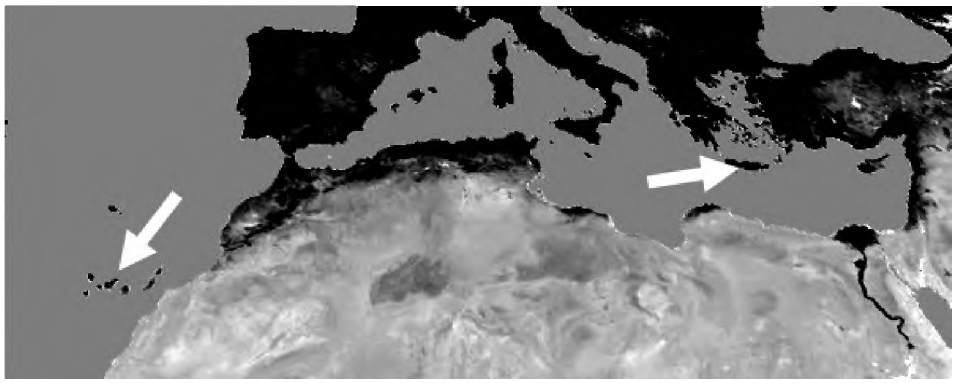

Fig. 1. Map indicating (arrows) the locations of Gambierdiscus *sp. records (Canary Islands, Spain and Crete, Greece).*

Recent findings of *Gambierdiscus* sp. in the Canary Islands, Spain and in Crete, Greece may indicate spreading of this genus into new areas.

The biogeographical distribution of *Gambierdiscus* species was until recently restricted to tropical and sub-tropical areas, specifically in discrete regions of the Pacific and West Indian Oceans and the Caribbean Sea [1]. The dinoflagellate *Gambierdiscus toxicus* is considered the primary causative agent of ciguatera fish poisoning [2], which is estimated to affect more than 25,000 persons per year [3].

Recently, Pérez-Arellano *et al.* [4] reported ciguatera incidents in the Canary Islands (Fig. 1), while Fraga *et al.* [5] documented the presence of *Gambierdiscus* sp. (Fig. 2) in the same area, expanding the distribution of this "tropical" genus. In a search for toxic benthic dinoflagellates in Tenerife waters on March 2004, *Gambierdiscus* sp. was found epiphytically in tide pools. In a later survey, it was also found on drifting seaweeds in La Gomera waters. Although the relation between the ciguatera case and the presence of *Gambierdiscus* sp. is not proved, it is striking that both events happened the same year, and were reported by two unrelated teams, a medical group and a marine biology group.

The detection of *Gambierdiscus* sp. cells on the west coasts of Crete (Fig. 1) in September and October 2007 is the first record of the causative agent of ciguatera in the Mediterranean Sea. *Gambierdiscus* sp. cells were found epiphytically on *Padina pavonica*, *Cystoseira* sp., *Corallina* sp. and *Jania* sp. in abundance levels up to 9.01 cells/gr fresh weight. The water temperature during that time ranged between 23.8 and 26.1 °C, while salinity was about 38.5 psu.

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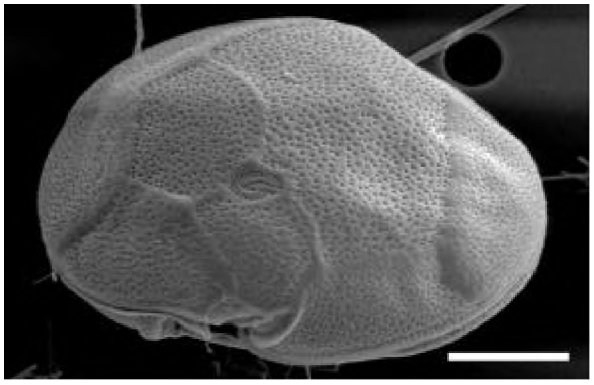

Fig. 2. SEM picture of Gambierdiscus *sp. from the Canary Islands (scale bar: 20 µm).*

Gambierdiscus sp. cells are round to ellipsoidal (Fig. 3), anterior-posteriorly compressed (Fig. 4), with the dorsoventral diameter (DV) and transdiameter (width, W) ranging from 57.12 to 76.16 µm and 52.36 to 78.54 µm, respectively; the DV/W ratio is about 1.02 (±0.07). The apical pore plate (Po) is situated near the center of the epitheca (Fig. 5), but in some cells seems to be displaced towards the ventral area (Fig. 6). Since the initial description of *G. toxicus* [6], five more *Gambierdiscus* species have been described: *G. belizeanus* [7], *G. yasumotoi* [8], *G. pacificus*, *G. australes* and *G. polynesiensis* [9].

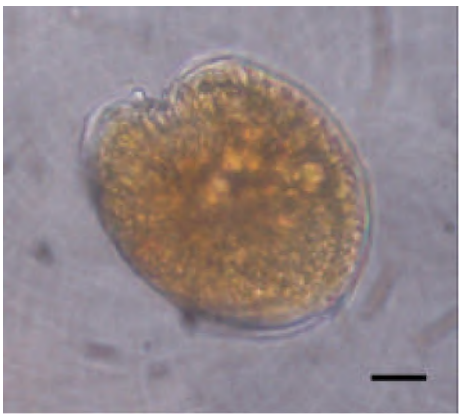

Fig. 3. Live Gambierdiscus *sp. cell from Crete in apical view (scale bar: 15 µm).*

However, Tester *et al* [10] suggested the existence of more species, and asked "if the type *G. toxicus* description included multiple species"; this situation makes the systematics of *Gambierdiscus* fluid. The taxonomy of *Gambierdiscus* is far from clear as new results of molecular and morphological studies contradict in part those of Chinain *et al* [9, 11].

In both cases, Canary Islands and Crete, *Gambierdiscus* co-occurred with other epiphytic dinoflagellates of the genera *Ostreopsis*, *Prorocentrum*, *Coolia* and *Amphidinium*, as is usual in ciguatera related assemblages.

The occurrence of representatives of what was

once considered a tropical or subtropical genus, like *Gambierdiscus*, accords with suggestions of several researchers regarding climate change impact on the geographical expansion of tropical microalgae [12], and the "tropicalization" of the Mediterranean Sea in recent years [13]. This fact, as well as the *Ostreopsis* species range expansion in the Mediterranean during the last 5-7 years, and their toxicity [14-18], constitute a serious threat to human health by ciguatera and palytoxin intoxications.

These two new records of *Gambierdiscus* sp. raise some questions. 1) Are they really spreading to new areas, or were they found because they were searched for? To the best of our knowledge there are no published studies of benthic dinoflagellate communities on either the Canaries or Crete. *Gambierdiscus* sp. was reported in the 1950's in the Cabo Verde Islands by Silva [19] under the name of *Goniodoma* sp.. Her description is so detailed that there are no doubts that it is *Gambierdiscus*. As the Canaries are not so far form the Cabo Verde Islands they could belong to the same population. 2) Are the Greek and Canarian *Gambierdiscus* related? 3) Could *Gambierdiscus* in the East Mediterranean be the result of an invasion via the Suez Canal (a lessepsian immigrant), or through the Strait of Gibraltar?



Fig. 4. Live Gambierdiscus *sp. cell from Crete showing anterior-posterior compression (scale bar: 10 μm).*

If the presence of *Gambierdiscus* in the Mediterranean is the result of a recent introduction, it could be a consequence of global warming, expressed in the Mediterranean as an increase in sea surface temperatures, especially in the Levantine Basin. The possible introduction of *Gambierdiscus* to the East Mediterranean as a likely consequence of climate change was discussed a few months before its finding in Crete, at the 4 th European Phycological Congress [12].

To provide answers to these questions, morphological, genetic sequences and toxicological analyses of cultured strains of these *Gambierdiscus* species are being carried out.

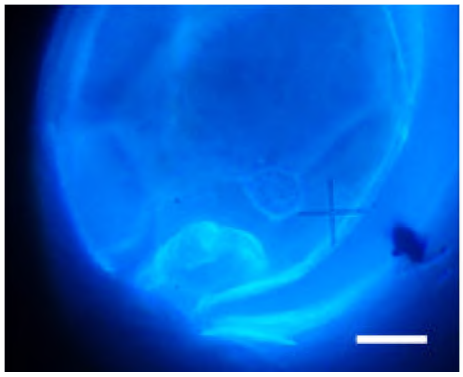

Fig. 6. Calcofluor-stained Gambierdiscus *sp. cell from Crete showing Po and 1' plate (scale bar: 10 μm).*

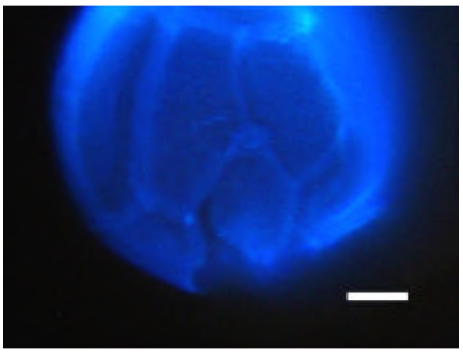

Fig. 5. Calcofluor-stained Gambierdiscus *sp. cell from Crete showing Po and 1' plate (scale bar: 10 μm).*

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The Poseidon Adventure Redux – NORCOHAB Cruise in North Sea

Many will remember the original *Poseidon Adventure*, a 1972 disaster film (literally and critically!) about a capsized passenger ship on her way to the scrap yard, which was pushed to her limits by the new owners to save on dismantling fees. The more recent "Poseidon adventure" reported here as the NORCOHAB P-352 cruise aboard the *R.V. Poseidon* (Kiel) (Fig.1) highlights a much more successful (and better directed) effort with significant scientific discoveries to her credit. This cruise, endorsed by GEOHAB and under the auspices of the Core Research Project on *HABS in Fjords and Coastal Embayments*, was undertaken to study the coastal oceanographic processes and mechanisms underlying the dynamics of key toxic bloom species and the biogeographical distribution of their toxins in the water column. Following the prescribed format of GEOHAB research the studies were *international*, *multidisciplinary* and *comparative* with the ultimate aim of *modeling* dynamics and behaviour. A critical sub-set of target taxa, primarily *Alexandrium* and other toxic gonyaulacoid dinoflagellates, but also *Pseudo-nitzschia* and *Dinophysis* species, were selected for special attention on the basis of their important role in bloom ecology and human health, their likely occurrence in high abundance during the cruise programme, the availability of molecular probes and approaches for their detection and identification and their production of known toxins for on board chemical analysis. Of course we also kept our eyes and minds open to new information, such as an opportunistic search for the source of azaspiracid toxins, of which more later.

The cruise transects were from Bremerhaven, Germany across Dogger Bank with detailed sampling initiated along the Scottish east coast from the Firth of Forth to the Shetland Islands (Fig. 2). For comparison, sampling was also conducted within the Norwegian Current on the south coast of Norway and then along the north coast of Denmark. In addition to primary and secondary transects, drift stations were sampled over several days on the Scottish and Danish coasts to obtain time series data within a given water mass. Standard physical oceanographic parameters (temperature: ° C, salinity: psu, s_t) plus current velocity were supplemented with biooptical measurements with multiple profiling fluorosensors and various passive optical profilers (for turbidity and diffuse attenuation), including a hyperspectral radiometer. The use of multiple fluorosensors of differing wavelengths enabled the identification of sub-surface patches attributable to cyanobacteria. Although concentrations of the key toxic microalgae were below detection by passive optical sensors, the optical data provided a more accurate mapping of the higher turbidity and particle loads (including phytoplankton) along the Scottish coast than on the Norwegian coast, presumably due to higher riverine run-off and tidal flux.

The timing of the cruise was carefully planned to coincide with the historical bloom period of these species (especially *Alexandrium*) based on information kindly provided by the IMR, Flødivigen, Norway (Einar Dahl) and the FRS Marine Laboratory, Aberdeen, Scotland (Eileen Bresnan). Alas cruel Nature saw fit to initiate and then terminate any such blooms prior to our arrival on the scene. An unusually warm winter-spring transition in 2007 appears to have accelerated the typical bloom succession and we were left with mainly grazers and detritivores accompanied by relatively few pigmented dinoflagellates and some rather unhealthy diatom chains at most stations around the North Sea perimeter from early June to early July.



Fig. 1. The NORCOHAB P-352 research cruise vessel at port-of-call in Arendal, Norway.

Our working hypothesis was that bloom dynamics of key toxic species in the North Sea are regulated at least as much by "top down" factors such as grazing by copepods and protists as by "bottom up" factors, including light, temperature and nutrients. Since such "top down" field studies are grossly under-represented in modelling simulations of toxic bloom dynamics, we conducted on board grazing experiments to quantify grazing losses of *Alexandrium tamarense*, caused by

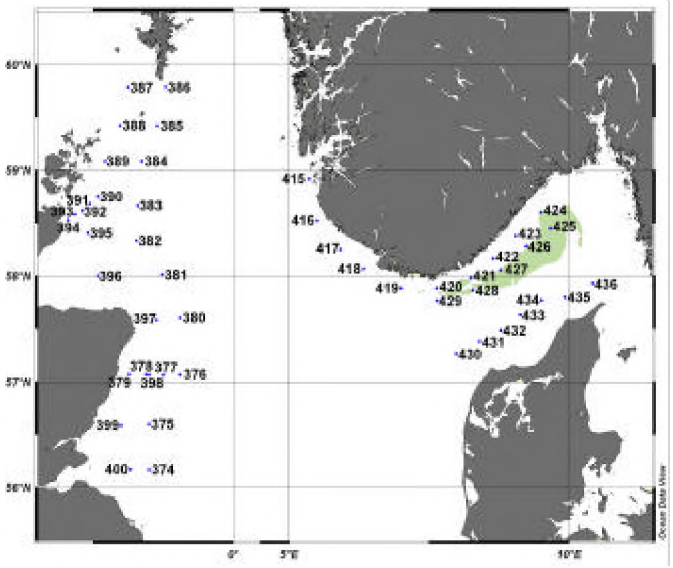

Fig. 2. Map of the primary and secondary transect stations sampled for HAB organisms and their toxins in association with physical and bioptical parameters in the coastal North Sea.

dominant metazoan and protistan grazers. In the absence of natural *Alexandrium* blooms at high cell concentrations, we added fluorescently labelled live *A. tamarense* cultured cells of known toxin content to different size fractions of natural grazer populations. A strong reduction in abundance of labelled cells at stations with high micrograzer abundance showed that *A. tamarense*-specific grazing coefficients may be as high as 2 d^{-1} with almost 80% of total grazing caused by micrograzers in the <200 µm fraction (Fig. 3). The hypothesis about the importance of micrograzers appears to be sustained.

Other on board grazing experiments were conducted to test reciprocal effects of grazing on *Alexandrium tamarense* when exposed to three different copepod species selected from natural communities. Specifically, we examined the changes in cellular PSP toxin content and transcriptomic response of *A. tamarense* via microarrays. An apparent toxin defense response from *A. tamarense* against copepod grazing varied among different copepod species, and was highest for the largest suspension feeder *Calanus helgolandicus*. However, *C. helgolandicus* also seemed best adapted for feeding on *A. tamarense* cells and showed the best fitness among the copepods at the end of the incubation period.

One of the major objectives was to apply liquid chromatography coupled to a highly sensitive triple-quadrupole mass spectrometer (LC-MS/MS) to identify and quantify phycotoxins at trace levels throughout the cruise. With this equipment on board we were able to respond directly to relevant findings on the composition and concentration of toxin is various size-fractions and plankton assemblages from various depths in near-real time (i.e. between stations). The analytical instrumentation provided a gold-mine of information, allowing us to characterize many toxin derivatives (of spirolides, yessotoxins, saxitoxin/gonyautoxins, domoic acid, okadaic acid/dinophysistoxins, azaspiracids) from particulate fractions from the water column at sub-picomolar concentrations. Amazingly, in spite of the rather low concentrations of putative causative organisms, phycotoxins were found at most stations around the perimeter of the North Sea from plankton samples. Such sensitive analytical methods proved crucial to the dynamics determination of the occurrence and fate of toxins in various components of the planktonic food web.



Fig. 3. The predatory tintinnid Favella ehrenbergii *with an ingested fluorescently labelled* Alexandrium tamarense *cell.*

One highlight of the *Poseidon* cruise was the on board determination of azaspiracids in plankton samples, and the subsequent isolation and culture of the causative organism of azaspiracid poisoning (AZP) (no it is *not Protoperidinium*!). Of course we were lucky, but following Pasteur's dictum that: "in the field of observation, chance favors only the prepared mind", this was still a worthy accomplishment. The proximal source of azaspiracid (AZA) is a small photosynthetic dinoflagellate of approximately 12 -15 µm long-axis diameter, which was found at stations with high AZA-1 concentrations. The AZA-producing dinoflagellate has now been described morphologically and with reference to molecular phylogenetic analysis for several genes from single cell isolates collected off the Scottish east coast. The major azaspiracid component (AZA-1) was almost ubiquitous in the North Sea, but its abundance varied significantly (Fig. 4). In the western North Sea, AZA-1 was

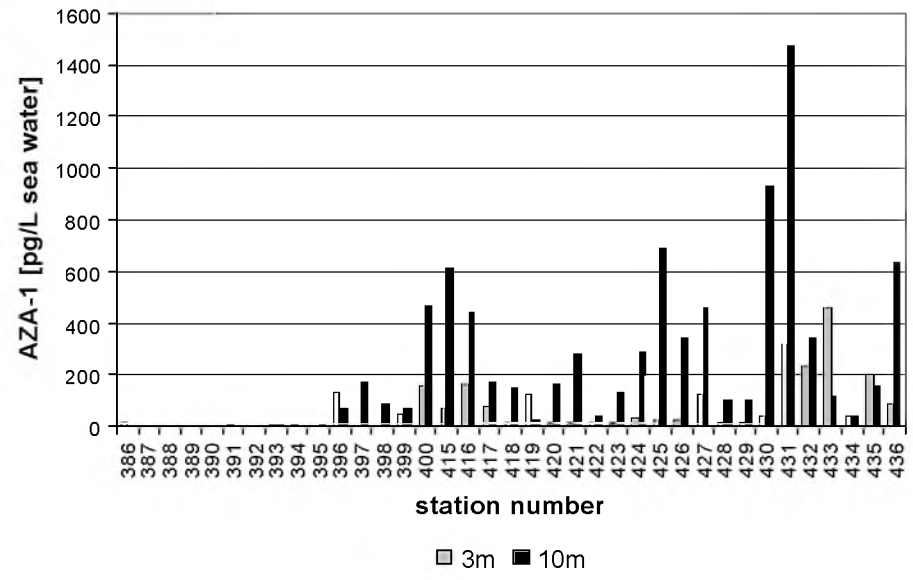

Fig. 4. The concentration of azaspiracid-1 (AZA-1) in the particulate size-fraction 3 – 20 µm collected from two water column depths (3 and 10 m) from stations in the coastal North Sea.

• USA

First toxic *Dinophysis* bloom observed in the Gulf of Mexico, USA

Dinophysis acuminata (Dinophyceae, Dinophysiales) is a thecate dinoflagellate that produces okadaic acid, the toxin responsible for diarrhetic shellfish poisoning (DSP). Although toxic blooms of *D. acuminata* have been a serious problem in Europe [1], relatively few reports of human illness have been linked to DSP in the US [2]. Blooms of *D. acuminata* have been reported on the US east coast [3, 4], but cell concentrations were not high enough and/or populations did not persist long enough to result in significant shellfish intoxication. Consequently, no routine monitoring of *Dinophysis* or other DSP-related HAB species is conducted in the US [2].

Now, for the first time, both elevated concentrations of *D. acuminata* and DSP intoxicated shellfish have been detected in US waters, in central Texas in the western Gulf of Mexico (Fig. 1), requiring shellfish beds to be closed to harvesting.

Early warning of this first toxic *Dinophysis* bloom was provided by a new automated particle imaging system [5]. Imaging FlowCytobot combines video and flow cytometric technology to capture images for plankton identification and to measure chlorophyll fluorescence associated with each image. From the high resolution images, organisms ranging from 10 to ~100 µm can be identified, often to genus or even to species.

The instrument was installed in the pier laboratory at the University of Texas-Marine Sciences Institute (Port Aransas, TX; Fig. 2) in September 2007, as part of a NOAA/CICEET project to monitor *Karenia brevis*, another toxic dinoflagellate that blooms periodically in the Gulf and that can cause neurotoxic shellfish poisoning. No *K. brevis* blooms were observed in the region this year, but the imaging approach's flexibility allowed this unexpected plankton species to be studied equally well.



Fig 1. Port Aransas,Texas, on Mustang Island, a barrier island along the coast of central Texas, Gulfof Mexico, USA.

In early February 2008, *Dinophysis* cells (1-5 per mL) were detected through manual inspection of cell images. By late February estimates were >100 per mL (Fig. 3). Because these levels exceed the typical background level for the genus in this region, manual sampling was conducted by the Texas Department of State Health Services (DSHS) to confirm the species, toxicity and abundance in coastal waters from Port Aransas harbor and adjacent bays. The bloom consisted primarily of *D. acuminata* (Fig. 3), although *D. fortii* and *D. caudata* were also present at much lower abundance. Okadaic acid was confirmed in plankton samples collected 25 February by liquid chromatography/ mass spectroscopy. Four days later, collection and analysis of shellfish samples showed significant accumulation of DSP toxins. On 7 March, the Aransas, Corpus Christi, and

most abundant along the southern Scottish coast, but further north around the Orkney and Shetland Islands, AZA-1 was only detected at trace levels. In general, AZA-1 concentrations were higher in the eastern North Sea with highest levels found in the southern Skagerrak.

Further NORCOHAB expeditions in the North Sea are planned under the comparative GEOHAB framework. Following the proof of concept for integrated operation of chemical analytical instrumentation and biooptical profilers for detection of HABs and their toxins in near-real time, we will continue work on the biogeographical distribution of toxigenic organisms and their toxins in relation to food web transfer and chemical ecology of key species in coastal waters.

*Reported by A. Cembella with contributions from B. Krock, U. Tillmann and U. John, AWI students and technicians. Thanks to B. Cembella (*Optimare *AG) and O. Zelinski (Bremehaven Hochschule) and co-workers.*

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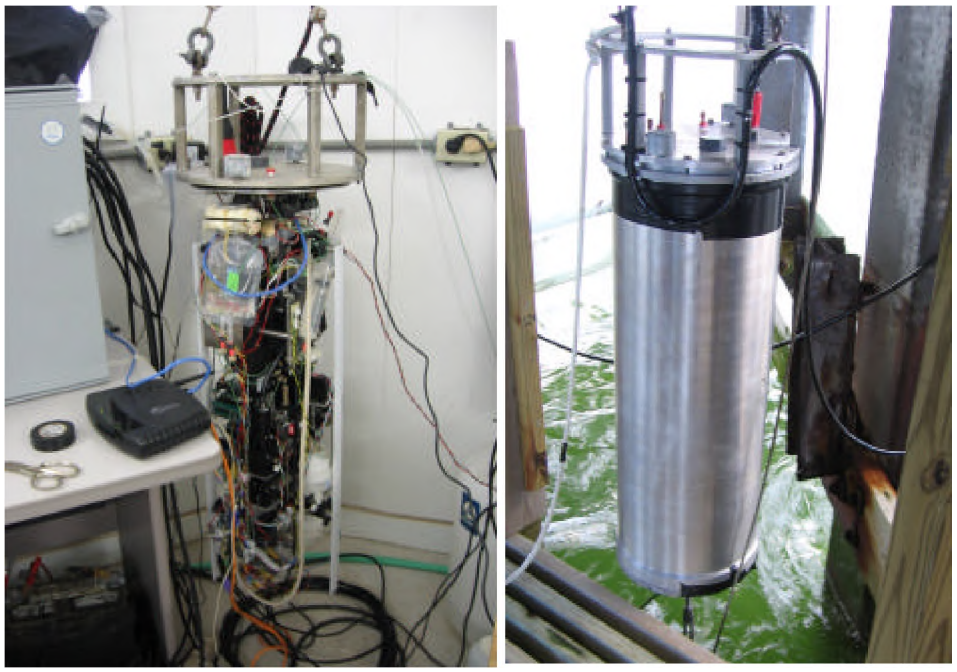

Fig. 2 Imaging FlowCytobot (version 3) as constructed for deployment from the Port Aransas Pier. Internal view (left) while being configured and aligned in the laboratory, and after enclosure in the pressure housing (right) designed for submersible applications.

Copano Bays were closed to shellfish harvesting, and shellstock harvested on or after 1 March were recalled [6]. Timing of this closure and recall of local oyster harvests was fortunate as it allowed organizers of a scheduled Oyster Festival – an annual community event that typically attracts 30,000 people—to purchase oysters from elsewhere.

Monitoring by Texas DSHS continued throughout March in the Port Aransas region. During this time, observed *Dinophysis* concentrations remained >5 cells mL^{-1} in Aransas and Copano Bays, but the bloom did not appear to impact other regions of the Texas coast. At the Port Aransas pier, *Dinophysis* counts from Imaging FlowCytobot exceeded 20 cell mL^{-1} during most of March, with the exception of a few days mid-month when counts decreased to <1 cell mL^{-1} (Fig. 3). Imaging FlowCytobot sampling shows that the bloom is continuing in April and the 3 bays remain closed to shellfish harvesting.

Because Imaging FlowCytbot has collected over 31 million images at the Port Aransas pier since 1 January 2008, automated image analysis and classification is essential for application to taxon-specific bloom detection. A preliminary set of images selected from the time series have been inspected, manually identified, and then used to train a machine learning algorithm for automated classification, as described in Sosik and Olson [7]. This analysis approach not only provides taxon-specific abundance estimates at times of specific interest, but also permits detailed bloom dynamics to be investigated over extended periods of time (Fig. 3). From this information it will be possible to study aspects of bloom ecology such as initiation and patchiness.

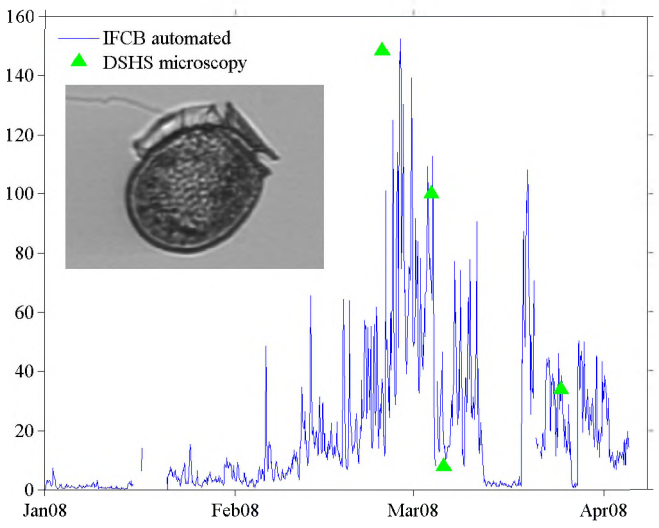

Fig. 3. Preliminary time series of Dinophysis *abundance at the Port Aransas pier from automated analysis and classification of images collected by Imaging FlowCytobot. DSHS counts from conventional microscopic analysis are shown for comparison. Inset:*

This successful event response has demonstrated that continuous and automated methods for monitoring coastal waters can provide real-time detection and early warning of harmful algal bloom (HAB) events.

Further development of automated image classification, involving extraction of image features and supervised machine learning algorithms, will enhance our ability to monitor the abundance of individual phytoplankton taxa at relevant temporal scales for HAB prediction.

Acknowledgements

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• India

"Green tide" of *Noctiluca miliaris* in the Northern Arabian Sea

On the west coast of India, discoloured water events caused by a variety of organisms and sometimes associated with mass mortality of local marine fauna, have frequently been reported [1-7].

During an oceanographic cruise onboard *FORV Sagar Sampada* in the second week of March 2007, an intense green colouration accompanied by soup like consistency was observed in the upper layers of Northern Arabian Sea. The phenomenon in its full intensity with intermittent patches were found to be spread over an area of around 30 km^2. The physical and chemical characteristics of two stations were sampled along with a non-blooming region. The location of the blooming area was in Lat. 21° 50' 32 N, Long. 66° 09' 38 E and Lat. 21°50' 63 N, Long. 67° 03' 55 E, and that of the non-bloom region was in Lat. 21° 00' 33 N, Long. 65° 01' 21 E. The bloom organism was *Noctiluca miliaris* Suriray, a nonphotosynthetic, heterotrophic and phagotrophic dinoflagellate. Innumerable motile prasinophyte endosymbionts (*Pedinomonas noctilucae* (Subr.) Sweeney) were found within the *Noctiluca* cells giving the deep green hue to the surface waters.

Water samples were analysed for pH, dissolved oxygen (DO), chlorophyll *a* and nutrients according to Parson *et al* (Table 1). The CTD profiler recorded temperature and salinity. Phytoplankton samples preserved in 1-3% neutralised formalin were used for qualitative and quantitative analysis.

Cells have a size range from 200-600 µm in diameter. *Noctiluca* density was 4x 10^6 cells m^{-3} in bloom stations. *Noctiluca miliaris* was found along with 19 other species of phytoplankton, which included nine species of diatoms (*Chaetoceros brevis, C. didymus, Navicula digito-radiata, N. elegans, N. lyra, Rhizosolenia alata, R. hebetata, R. imbricata, Thalassionema nitzschioides*) and ten species of dinoflagellates (*Ceratium furca, C. trichoceros, C. vultur, Diplopeltopsis minor, Diplopsalis lenticula, Prorocentrum micans, Protoperidinium obovatum, P. oceanicum, P. pyriforme, P. steneii*). Among the diatoms, *Rhizosolenia* formed the major constituent. Co-occurrence of *Noctiluca miliaris* and *Rhizosolenia* spp. has been reported previously [8].

Dissolved oxygen values ranged between 4.96 and 5.09 ml L^{-1}. Inorganic phosphate was below the detection limit, presumably due to consumption by the bloom species. Nitrate values were higher and might be due to the presence of *Noctiluca*. Prasad and Jayaraman [9] reported a similar trend in nitrate concentration during *Noctiluca* swarming. The relatively high chlorophyll *a* value at the bloom stations (21.9 and 21.3 mg m^{-3}) compared to the non-bloom station (0.97 mg m^{-3}) is due to the flagellates associated with *Noctiluca*. Relatively stable low temperature, high salinity, calm sea and muggy weather are known to favour proliferation and blooming of *Noctiluca*.

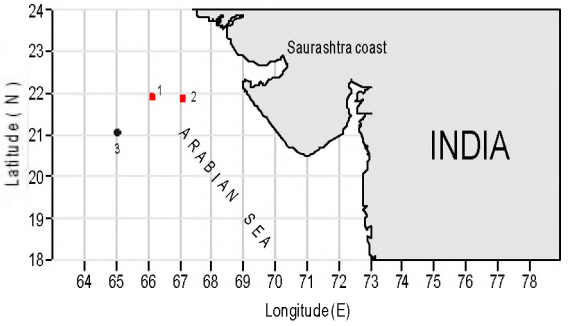

Fig. 1. Map of study area.

Zooplankton biomass was very high at the bloom stations (6090 ml 1000 m^{-3} and 7790 ml 1000 m^{-3}) compared with the non-bloom station (985 ml 1000 m^{-3}). Copepoda, Chaetognatha, Cladocera, fish eggs, Amphipoda, Heteropoda, *Lucifer*, *Oikopleura*, salps, doliolids, and siphonophores were abundant.

Surveys in this area during the last seven years have shown that *Noctiluca* blooms in the Northern Arabian Sea every year during this period. The regular occurrence of the bloom in the same area, the role of wind in nutrient enrichment, and the role of the symbiont *Pedinomonas noctilucae* in *Noctiluca* blooms need detailed investigations.

Table 1. nd - not detectable*

Parameter	*Noctiluca* bloom stations		Non bloom region Station 3
	Station 1	Station 2	
Temperature (°C)	25.71	25.56	26.01
Salinity (ppt)	35.96	35.98	36
pH	8.33	8.31	8.36
Dissolved oxygen (ml l^{-1})	4.96	5.09	5.38
Nitrate (µmol l^{-1})	4.735	5.038	1.678
Phosphate (µmol l^{-1})	nd*	nd	nd
Silicate (µmol l^{-1})	0.201	0.015	1.602
Chlorophyll *a* (mg m^{-3})	21.9	21.3	1.24
Cell count (Cells m^{-3})	4x 10^6		*Noctiluca miliaris* absent

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Book review

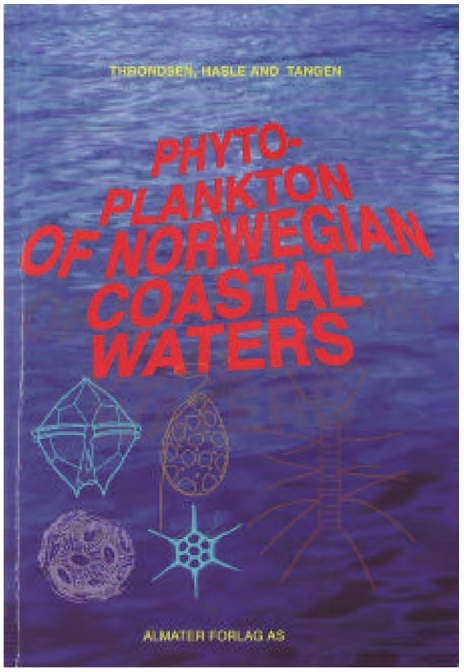
Throndsen J, Hasle GR and Tangen K 2007. *Phytoplankton of Norwegian Coastal Waters* (Almater Forlag AS, Olso) 343 pages, b&w illustrations, photos, color plates, soft cover.
Price £ 96 at www.nhbs.com/title.php?tefno=157807. Also available from Balogh International (www.balogh.com/~balogh) for $ 205. ISBN (13) 978 82-7858-086-8.

A few years ago, on a visit to Oslo, Jahn Throndsen showed me a book to which he contributed: "Norsk Kystplankton Flora". I marveled at the wonderful drawings of phytoplankton it contained. A year later, on a visit to Roscoff, Jahn pulled out a copy and offered it to me, autographed. I was thrilled, but at the same time frustrated because Norwegian does not make easy reading, at least for me. I was wondering whether this book would someday be translated into English. This is now done and the result is outstanding.

This book is limited to the phytoplankton of coastal waters around Norway but still should be extremely useful for European readers since Norwegian coasts are influenced by quite different water masses including Arctic, Atlantic, and Baltic ones. Still, as Jahn Throndsen cautions in his introduction, "users abroad (should) be especially careful when identifying the species and seek comparison and advice from local floras and other taxonomical publications".

Those familiar with the book "Identifying Marine Phytoplankton" (IMP) coordinated by Carmelo Tomas, probably currently one of the most used taxonomical reference for phytoplankton, will feel at home, since the three authors of the present book were also major contributors to IMP. The book is organized along division and classes with major sections dealing with dinoflagellates, diatoms and coccolithophorids. However, while in IMP, flagellates are grouped together at the end of the book, here they are grouped according to divisions. For example, Chrysophyceae are treated next to diatoms in division Hetorokontophyta and Pavlophyceae next to coccolithophorids in division Haptophyta. This is much more coherent but make things a little more difficult for novices. The taxonomy is really up to date as recently established groups such as Pinguiophyceae are mentioned, even though they do not contain any genera reported for Norwegian waters. Even species that have been described after the publication of the Norwegian edition (e.g. the Dictyochophyceae *Verrucophora farcimen*) have been included in the English version.

For each major algal class, a compact introduction is provided summarizing the systematics of the



class, the cell morphology and the specialized nomenclature (for example plate patterns for dinoflagellates); A general key follows for the class. Each species is then described and illustrated by at least one drawing and sometimes by a microscopy picture. Information on the species habitat in Norwegian coastal waters is also provided. For many genera, detailed keys are also provided. This organization (i.e. description and illustration grouped together) is in fact more handy that the one used in IMP where illustrations are organized in plates resulting in physical separation between the species descriptions and drawings, forcing the reader to move back and forth between the pages of the book.

As with all productions, there are of course a few things that could be improved. A short presentation of the current view of protist phylogeny could have been useful to illustrate the fact that phytoplankton is highly diverse and spread over many protist groups. Also it would seem more logical to place upfront methodological considerations concerning plankton sampling and plankton preparation for microscopy, instead of sending them to the end of the book. Concerning the material covered by the book, the inclusion of some heterotrophic groups such as choanoflagellates or ciliates could be questioned. Obviously, they are often present in phytoplankton samples and the book will help people to identify them. However since the coverage of these groups is much less exhaustive than that of the photosynthetic groups, it may induce some readers to downplay their actual diversity or to wrongly identify some species. Finally there are a few new minor errors or omissions. For example p.12, the Prochlorophyta (cyanobacteria containing chlorophyll *b*) have been known for quite a while to be polyphyletic, all genera are in fact *bona fide* cyanobacteria, and Prochlorophyta are best forgotten. Also *Telonema* (p. 279) is no longer *incertae sedis* but belongs to a new phylum called Telonemia.

I think all plankton taxonomists should be very grateful to Grete Hassle, Karl Tangen and Jahn Thondsen for having offered to the scientific community and beyond, this compilation resulting from the many years they spent looking under the microscope, taking pictures and making these wonderful drawings. If phytoplankton taxonomists

PHYCOTOXINS

You can also get news on HAB research and events at the Phycotoxins-list at the internet.

The address of this list is: phycotoxins@www.agr.ca

To join the list, send "subscribe phycotoxins" to: listserv@www.agr.ca

Archives are located at www.agr.ca/archives/phycotoxins.html

from other countries (and I am thinking here of my own country, France) had left similar legacies, our field would be much richer. One would only wish that all this information soon becomes available on the Internet either through a dedicated website or even better through collaborative initiatives such as Plankton*Net (e.g. planktonnet.sb-roscoff.fr) or the Encyclopedia of Life (www.eol.org). Such dissemination would probably not hurt book sales since people who can afford it would want to buy a hard copy, but would help many field taxonomists throughout the world who may have little access to books.

In conclusion, although I have not yet used this book myself to practice phytoplankton identification, I am deeply convinced that this book is soon going to be sitting next to numerous microscopes (and probably often stolen…) along with IMP, and I can only highly recommend purchasing it.

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FAO-SIDA-UNESCO-MIRCEN International Workshop on Shellfish Safety from Harmful Algae and Biotoxins

Harmful algal blooms and shellfish toxicity are a global problem affecting shellfish safety and international trade. It is estimated that about 2000 cases of shellfish toxicity occur annually with 15% mortality. The economic impact of this problem has been estimated to be US$ 82 million in United States alone. In south and south-east Asia, there have been several reports of fish kills and shellfish poisoning. India had two recorded outbreaks of paralytic shellfish poisoning (PSP) and toxin involved in diarrhetic shellfish poisoning (DSP) has been detected in shellfish in India. Malaysia, Indonesia and China have experienced outbreaks of PSP. However, the dinoflagellate species involved in toxic episodes vary in the region. Bivalve production by aquaculture has been growing and in 2005, 84% of bivalve production came from aquaculture. China is emerging as a major producer of bivalves; China presently accounts for 80% of world bivalve production by aquaculture. To ensure the safety of shellfish, it is important to have monitoring programmes for harmful algae and biotoxins. However, a major concern has been the expertise required to identify the small proportion of toxic species in water samples. Technology for detection of biotoxins has been undergoing rapid developments with improvements in analytical capability. Sophisticated analytical procedures such as Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) and immunoassays are being applied for toxin detection.



Considering the global importance of the problem and the need for capacity building in this area, an International workshop on "Safety of Shellfish from Harmful Algae and Biotoxins" was held at the UNESCO Microbial Resources Center (MIRCEN), College of Fisheries, Mangalore, India from January 21 to 25, 2008. Dr. Indrani Karunasagar was the Workshop Coordinator. The workshop was jointly sponsored by FAO, Swedish International Development Agency (SIDA) and UNESCO MIRCEN. The workshop included lectures and practical sessions on identification of harmful algae and their cysts; biotoxins and their detection by bioassay, immunoassay, HPLC and liquid chromatography mass spectrometer (LC-MS) techniques, and monitoring as a component of integrated shellfish safety management. The workshop included 23 participants from Vietnam, Malaysia, Indonesia, Thailand, China, Morocco, Yemen, Sweden and India. The faculty for the workshop included Dr. Y. Fukuyo from University of Tokyo, Japan, Dr. J. Larsen, IOC Center on Harmful Algae, Copenhagen, Denmark, Dr. Dave Clarke from Marine Institute, Galway, Ireland, Dr. Iddya Karunasagar from FAO, Rome, Dr. Ann-Sofi Rehnstam-Holm and Dr. Anna Godhe from Sweden, Dr. Indrani Karunasagar and Dr. B.B. Nayak from India. The sessions were highly interactive and the Workshop material included Manuals and CDs. In addition to lectures and practical sessions, the programme included presentation of the status of work on harmful algae and biotoxins in the countries represented in the workshop. Experiences from 10 countries were presented.

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Towards measuring mucus events

Are aquatic "mucus", "slime" and "froth" events, like harmful algal blooms [1], increasing worldwide in both extent and intensity? The last six numbers of *Harmful Algae News* (HAN) Nos. 31-36 (2006-2008), report the following mucus events:

• "Cream or beige coloured foam" produced by *Alexandrium monilatum* in Costa Rica [2];

• "Abundant foam forming stripes parallel to the coast" in a bloom of *Chattonella marina* and *C.* cf. *ovata* in the Gulf of California, Mexico [3];

• Three reports of benthic, pelagic and neustonic occurrences of polymer matrices apparently produced by *Ostreopsis ova* as well as *O.* cf. *siamensis* in the Mediterranean. Cogestri *et al.* [4], showed mucilage flocs and cells detaching from the substrate and floating in the water, while Totti et al [5] found *O. ova* to produce a brown velvet-like mat, shown by scanning electron microscopy to consist of a matrix of fibrils, covering rocks, seaweed and mollusc shells. Barone [6], furthermore, demonstrated that *O. ova* cells use this network, to entangle and trap prey, rather like spiders use their webs except that, unlike spiders, they do it collectively. These webs may also entrap potential predators, thus protecting their creators;

• Along a 65-km stretch of the coast of NE Spain large patches of floating green-brownish mucus were reported [7]. Dominant organisms in the mucus included *Gonyaulax fragilis* and *Pseudo-nitzschia,* but since *G. fragilis* was shown to secrete mucoid material from its apical pore, it seems likely that this species may be the origin;

• In a report on *Microcystis aeruginosa* in a city reservoir in Colombo, Sri Lanka, it is pointed out the species may form thick layers or mats at the water surface [8], underlining the inevitability that surface algal and cyanobacterial blooms to some extent control air-water fluxes. There are three examples in the present issue:

• "Soup-like consistency" in green *Noctiluca* blooms covering around 30 km², in the Arabian Sea, off NW India [9];

• The pelagophycean *Chrysophaeum taylorii* was found in 2007 investing substrates of rock, sand and seagrass on the NE coast of Sardinia, with a yellow-green-brown mucilaginous cover up to 5-8 cm thick. Bubbles formed in this cover and caused it to float up to the surface, producing visible patches. It was sufficiently abundant to alarm tourists and local people. [10];

• In the Sea of Marmara, Turkey, from December 2007 to March 2008, white mucilaginous aggregates were noticed near the surface. SCUBA divers then found that similar mats also occurred on the bottom, covering benthos [11]. This phenomenon, newly reported for the area, was compared to that of "mare sporco" that has occurred sporadically for centuries in the Northern Adriatic [12].

These reports of mucous events linked to algal blooms are of course only a tiny proportion of the literature. Much more exists on this subject, and still more on the properties of small mucous particles, including transparent exopolymeric particles (TEP) and marine and lake snow [13] as well as models of their flocculation and break-up [14-16].

Progress has been better in understanding and measuring the mechanical properties of the air-water surface through 2-dimensional (2D) shearing [17], and more particularly during 2D compression-dilation cycles, accompanied by electrochemical measurements showing how the molecules restructure and exchange with the bulk phase [18, 19].

Yet it is in the bulk phase of the medium, where changes in the mechanical properties of the water are likely to impact plankton ecology most. Progress in this field is slow both at scales of mm to cm [20-23], and, using at atomic force microscopy at those of polymer molecules [24], and little understood by many HAB research teams.

Like marine algal slime, blood too is thicker than water, and huge advances were already being made nearly 30 years ago in the rheology of blood [25]. Qualitative descriptions of mucus are extremely valuable, but were sufficient training in marine rheology methods available to teams working on HABs, we might see comparable advances in understanding HAB dynamics. To this end, I propose that a Working Group on marine mucus properties be set up. This will be particularly focussed on algal blooms, as it is where algae are most concentrated that they have most potential to thicken the water. Anyone interested is welcome to contact the author directly, Esther at the XV th International Conference on Rheology, Monterey, USA, or the 13th International Conference on Harmful Algae, Hong Kong, November 3-7 2008.

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Reflections from the GEOHAB Scientific Steering Committee:

Ocean Colour Remote Sensing and Harmful Algal Blooms: a Rush of Blood?

Development and application of optical sensors and remote sensing is central to many marine research programmes, agencies and activities. GEOHAB addresses development of improved remote observation systems for harmful algae, and activities have included a science workshop on real time observation systems for harmful algae and publication (early 2008) of an UNESCO monograph on oceanographic methodology entitled 'Real-time Coastal Observing Systems for Marine Ecosystem Dynamics and Harmful Algal Blooms: Theory, Instrumentation and Modelling' edited by Marcel Babin, Collin Roesler and John Cullen. A critical role of these activities is to identify gaps in knowledge, and to stimulate development and improved application of relevant techniques.

Ocean colour remote sensing offers considerable potential for the observation of harmful algal blooms (HABs); such potential remains largely unfulfilled due to the sizeable uncertainties associated with applications in the coastal zone. Recent studies suggest that space-based ocean colour sensors allow the routine coastal detection of high biomass phytoplankton blooms, or the identification of phytoplankton functional types. However, there are still fundamental problems with the application of rigorous ocean colour techniques in optically complex coastal waters. The ocean colour and harmful algal bloom scientific communities would gain a great deal by identifying and addressing these problems, rather than prematurely adopting under-developed techniques.

Ocean colour radiometry has several fundamental steps: Radiance is measured at the top of the atmosphere; to get the oceanic signal the atmospheric signal has to be removed (typically accounting for more than 80% of the total signal). The derivation of geophysical parameters such as chlorophyll *a* concentrations from this corrected signal, uses various empirical or analytical algorithms. Further ecologically meaningful products, such as anomalies, can then be calculated. In the HAB-prone coastal zone, all of these steps suffer from considerable uncertainty. Atmospheric correction schemes considered suitable for the open ocean often perform poorly in turbid, atmospherically complex coastal waters where a lack of suitable validation data hampers our ability to analyse and improve these vital procedures. In addition, an incomplete understanding of the underlying causes of ocean colour variability means that the various techniques typically employed to derive HAB products are at best ambiguous.

There are five broad techniques employed with regard to HAB or phytoplankton assemblage type detection:

1. Bright water is historically often used to discriminate non-harmful coccolithophore blooms and can be used for operational, i.e. non-quantifiable, detection of abnormally high turbidity waters that can circumstantially be associated with given groups such as harmful cyanobacteria (e.g. *Nodularia spumigena* in the Baltic Sea).
2. Discrimination based on spectral signature of targeted phytoplankton species or groups is successful for a small number of cases e.g. cyanobacterial blooms containing the spectrally distinctive phycocyanin pigment (see Figure 1).
3. Several approaches based on chlorophyll anomalies relative to climatologies have been proposed as a means to raise warning of possible high biomass HAB events. While operationally promising to some extent, those approaches are subject to ambiguities caused by the anomalies being partially or wholly due to variability

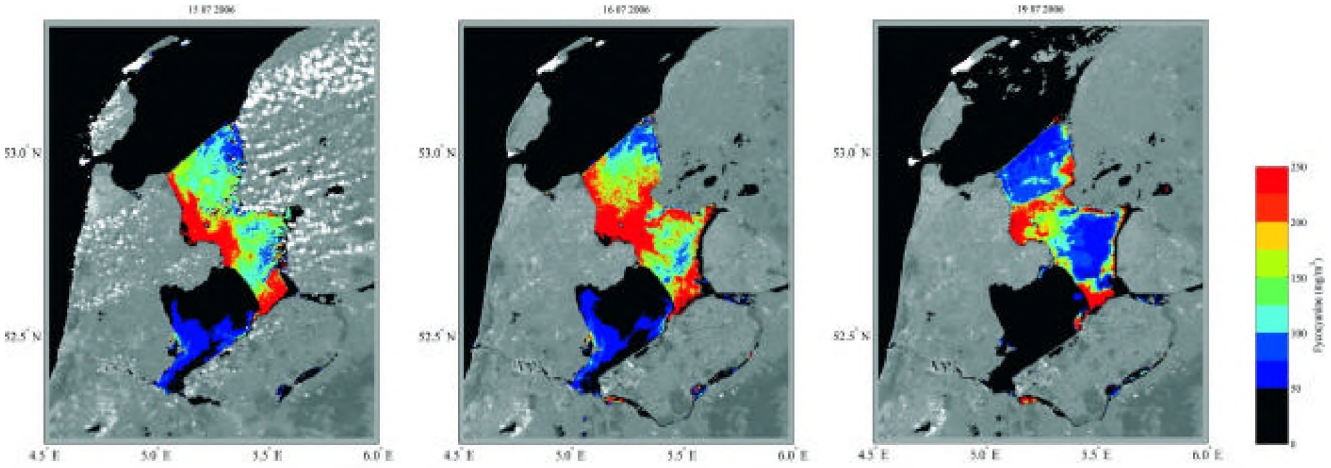

Fig. 1. Spatial distribution of the phycocyanin pigment in the Netherlands eutrophic Lake IJsselmeer (courtesy of Stefan Simis). Phycocyanin is associated with the presence of cyanobacteria such as Aphanizomenon flos-aqua *and* Microcystis *sp. Those results were obained using the algorithm from Simis et al. (2007) applied to imagery from the European sensor MERIS (Full resolution), collected over three days during the July 2006 heatwave. This short time series illustrates the potential of ocean colour remote sensing for monitoring with great spatial detail the temporal evolution of highly dynamic phytoplankton blooms.* Reference: S.G.H. Simis, et al., Remote Sensing of Environment 2007, 106: 414-427.

in atmospheric properties, dissolved organic colour, or non-chlorophyllous particles. As illustrated in Figure 2, the use of ocean colour to detect phytoplankton is often, in coastal waters, limited by the fact that other seawater constituents (e.g. coloured dissolved organic matter) are present in such high quantities that they simply hide any other constituents. Other approaches alternative to the use of the blue and green part of the reflectance spectrum may allow circumventing this problem.

4. Analytical algorithms, based upon inverse optical modelling of water column constituents, are promising as alternatives to standard geophysical algorithms but also suffer from poor atmospheric correction, and an incomplete understanding and parameterisation of the variable optical properties of phytoplankton and other water constituents.

5. The use of anomalies in the reflectance spectrum has recently been proposed to distinguish broad phytoplankton groups, based primarily on statistical relationships with chemotaxonomic data derived from High Performance Liquid Chromatography (HPLC) pigment measurements. However, recent studies suggest that the variability in the reflectance targeted by such algorithms is primarily associated with the varying concentration of non-algal constituents and that such methods are not robust.

Aside from atmospherically-related problems with absolute radiometric measurements, many of the problems lie in the fundamental question of identifying phytoplankton groups or species, and whether such groups can be distinguished using ocean colour. For example, HPLC pigment methods are potentially a very precise means of measuring phytoplankton pigment concentrations, but variations in accessory pigmentation are often only a minor source of ocean colour variability, and the chemotaxonomic interpretation of HPLC data can often be ambiguous. Allometric or size based ocean colour techniques also show promise, but whilst phytoplankton size variability across marine systems is characterised to some degree, our ability to translate this into useful information is limited by a lack of routine particle size observations. There is therefore a need for the ocean colour community to engage constructively with the phycological community, to adopt less ambiguous routine means of identifying phytoplankton community structure, e.g. traditional cell counts, flow cytometry, genetic techniques, or cell sizing techniques if it is to pursue HAB or phytoplankton functional type research in an ultimately meaningful way.

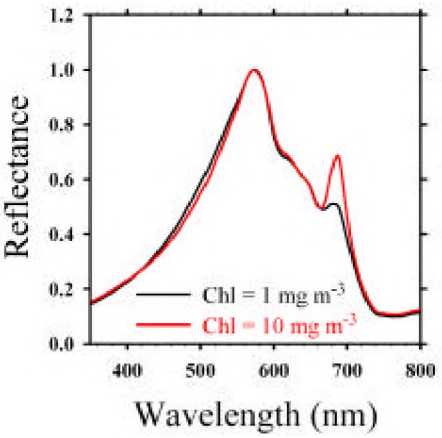

Fig. 2. Spectra of the water-leaving reflectance calculated using a radiative transfer code (Hydrolight 4.2) and optical properties for coloured dissolved organic matter and suspended non-algal particles typical of the Baltic Sea. Two different concentrations of chlorophyll a were used to set the optical properties of phytoplankton. Note that the two spectra were normalized to their respective area under the curve. This figure illustrates the fact that increasing the amount of phytoplankton by an order of magnitude in such coastal water has only subtle effects on the shape of the reflectance spectrum in the blue and green domains. It has, however, a very significant impact on reflectance in the red part of the spectrum where chlorophyll a fluorescence stimulated by sunlight can be readily observed (peak centred around 683 nm).

The way forward

The way forward lies in a suite of measures: true validation rather than rationalisation of results as plausible; sensitivity studies exploring the causal influences and assemblage detection limits across a wide variety of water types; a drive to put confidence limits on ocean colour products from a regional perspective. Consequently there is a requirement for a skill assessment strategy for ocean colour products which requires both the quantification of product uncertainty and the definition of minimum acceptable performance levels. Ocean colour techniques that do not outline plausible causal mechanisms may be of regional operational value, but are unlikely to be useful across a variety marine ecosystems, and are therefore of limited value in advancing our understanding and global observation of HABs as ecologically prominent phenomena. The greater involvement of multi-disciplinary scientific communities is extremely important – most notably HAB phycologists and oceanographers, in addition to ecophysiological modelers, who have specific and theoretically demanding requirements with regard to error analyses. The challenge is to give these distinct scientific communities some degree of ownership of ocean colour products as experimentalists. Considerable gains can be made from better understanding mechanisms underlying variability in the red portion of the ocean colour spectrum, most sensitive to phytoplankton backscattering at high biomass as demonstrated by research in hyper-trophic freshwater systems. Red wavelengths may also yield valuable information on physiology and phytoplankton functional type through sun-induced fluorescence, currently measured by several ocean colour sensors but remaining very much an underexploited phenomenon. However, the starting point almost certainly lies not only in the ocean, but in the ocean-atmosphere system: validation and improvement of the atmospheric correction across a variety of coastal regions is critical.

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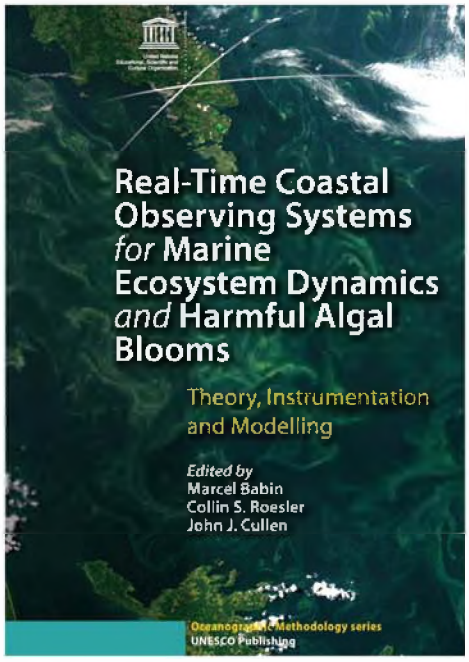
REAL-TIME COASTAL OBSERVING SYSTEMS FOR MARINE ECOSYSTEM DYNAMICS AND HARMFUL ALGAL BLOOMS:

THEORY, INSTRUMENTATION AND MODELLING

The proliferation of harmful phytoplankton in marine ecosystems can cause massive fish kills, contaminate seafood with toxins, impact local and regional economies and dramatically affect ecological balance. Real-time observations are essential for effective short-term operational forecasting, but observation and modelling systems are still being developed. This volume offers guidance for developing real-time and near real-time sensing systems for observing and predicting plankton dynamics, including harmful algal blooms, in coastal waters. It explains the underlying theory and discusses current directions in research and monitoring.

Topics treated include: › coastal ecosystems and dynamics of harmful algal blooms; › theory and practical applications of in situ and remotely sensed optical detection of microalgal distributions and composition; › theory and practical applications of in situ biological and chemical sensors for targeted species and toxin detection; › integrated observing systems and platforms for detection; › diagnostic and predictive modelling of ecosystems and harmful algal blooms, including data assimilation techniques; › observational needs for the public and government; and › future directions for research and operations.

This anthology should inform the work of researchers and environmental monitors as well as teachers and trainers concerned with understanding the causes, predicting the occurrences and mitigating the effects of harmful algal blooms in marine ecosystems.



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GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

GEOHAB developments

Five new people were welcomed onto the Scientific Steering Committee (SSC) of GEOHAB by chairman Robin Raine (Ireland) at the recent SSC meeting in Annapolis, USA between 9-11 April 2008. Elisa Berdalet (Spain), Suzanne Roy (Canada) and Icarus Allen (UK) now act as liaison between the SSC and the GEOHAB Core Research Projects on Stratified Systems, Coastal Bays and Fjords, and Eutrophic Systems respectively. Stewart Bernard (South Africa, remote observations) and Liam Fernand (UK, physical oceanography) will participate on a Task Team on Observations through which formal links between GEOHAB and GOOS can be established. This team, led by Bengt Karlson (Sweden), will initially update the IOC-IPHAB-GEOHAB recommendations for real time HAB observation systems, and make recommendations regarding HAB-observations in the ChloroGIN (http://chlorogin.org/contact.php) pilot areas among other activities.

Discussion on progress in regional research focused on the success of the 2nd GEOHAB Asia meeting, organised by Ken Furuya (Japan) and hosted at the Vietnamese Institute of Oceanography in Nha Trang by Nguyen Ngoc Lam. At this meeting, and subsequently, a document was developed on the likely effects of large scale nitrogen additions in the sea, following a proposal to dump large amounts of urea in the Sulu Sea (Glibert et al. 2008 Marine Pollution Bulletin, doi 10.1016/j.marpolbul.2008.03.010. The SSC were delighted to learn that the government of the Phillipines had refused permission for this activity. GEOHAB has issued a statement on this activity which is posted on the website (www.geohab.info).

Robert Magnien and Quay Dortsch (NOAA) were among agency representatives who were invited to the SSC meeting for a briefing on US HAB programs. An evening outreach session was also organised by Pat Glibert (Horn Point, MD, USA) at the Chesapeake Bay Program Office in Annapolis Maryland where four presentations on GEOHAB were made followed by a small reception.

Further points under discussion at included arrangements for a workshop on modelling the biophysical interactions relevant to the promotion of HAB events is being organised by the GEOHAB SSC and will be held in summer 2009. The formal announcement for this will be in June 2008.

Robin Raine, Chair of the GEOHAB Scientific Steering Committee.
Email: robin.raine@nuigalway.ie



The GEOHAB SSC in front of the Annapolis Synthesis Center of the University of Maryland, Center for Environmental Science, Annapolis, USA.

ISSHA
International Society for the Study of Harmful Algae

ISSHA President's Corner

Exciting things are happening. We are less than 6 months away from the 13th International Conference on Harmful Algae 3-7 November 2008 in Hong Kong and there are a number of very busy local and international organizing committee members making preparations.

ISSHA Auction

Plans for the ISSHA Auction are underway. We are soliciting items for the ISSHA Auction. If you can recommend a vendor you would like ISSHA to contact for a contribution, please send me contact information. Also, be thinking about you might bring to the Auction. I know that Allan Cembella will have the now famous Florida Red Tide - Mote Sunglasses Case back to see if he can recover his investment. Books, reprints and prints are always popular items and they travel well. See you at the ISSHA Auction in November!

Awards

Just a reminder that by 1 June 2008 Lifetime Achievement Award and Young Scientist Award Nominations should be sent by e-mail to Marina Montresor (mmontr@szn.it), Chair of the Committee on Achievement Awards.

Officer and Council Nominations

These nomination are due on 3 July 2008. Dr. Karen Steidinger has agreed to help vet the nominations. Please submit candidates to her at Karen.Steidinger@MyFWC.com. You may also send nomination to the ISSHA Secretary, Dr. Tracy Villareal at T.Villareal@mail.utexas.edu.

Pat Tester, ISSHA President,
Email: pat.tester@noaa.gov

Future events

Events in Food Safety, Toxins and Related Areas, AOAC Toxins Task Force 2008:

1) Dallas, Texas Sept 21-24, AOAC Int. Annual Meeting:

The 2008 AOAC International Annual Meeting brings together prominent senior-level analytical chemists and microbiologists to present research and share ideas with common scientific interests in the areas of biodefense, agricultural, food, drug, and environmental sciences.

There will be an open session of the Marine and Freshwater Toxins Task Force Meeting.

More info at: www.aoac.org/meetings1/122nd_annual_mtg

2) Seattle and Tacoma, Washington, June 15-20.

Rapid Test Kit Workshop, Seattle, WA Shoreline Laboratory and Hotel Nexus, June 15-17.

ELISA and Dipstick Tests for Natural Toxins, Vet. Residues & Allergens - Including lectures and extensive hands-on experience, kits from several vendors, this session in association with Pacific NW AOAC Annual Meeting. Spots are available on limited basis, so if you are interested in the workshop please contact: James_Hungerford@hotmail.com

3) Pacific NW AOAC Annual Meeting, Tacoma, WA,June 18 - 19.

There will be a specific seminar on Marine and Freshwater Toxins.

More info at: www.aoacpacnw.com





OCTOBER 2008

Ciguatera and related biotoxins

Nouméa, New Caledonia, 21-31 October 2008.

A workshop on the latest developments in ciguatera research, covering environmental influences, causative organisms and socio-economic and medical aspects.

Deadlines:

· Submission of abstract: 1st June.
· Early registration: 1st June.
· Late registration: 15th September.
· Submission of full papers: 31st December.

For additional information: nwww.ird.nc/ciguatera

NOVEMBER 2008

The 13th International Conference on Harmful Algae 2008

Hong Kong - China, 3-7 November 2008.

The International Society for the Study of Harmful Algae (ISSHA) is pleased to announce that the 13th International Conference on Harmful Algae will take place in Hong Kong on 3-7 November, 2008. Supporting organizations include School of Science & Technology, the Open University of Hong Kong and the Association of Harmful Algal Bloom of South China Sea (AoHABSCS).

The conference will address all issues related to the causes and effects of marine and freshwater harmful microalgae and, to serve as a forum for the exchange of new research results and relevant ideas among researchers, industry, government and all other intereseted groups.

Important dates:

· Abstract submission deadline: 1st June.
· Early registration: before 1st June.
· Late registration: 1st June - 15th Sept.
· Deadline for Submission of Full Papers: 31st December.

More info at: www.hab2008.hk

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HARMFUL ALGAE NEWS

Compiled and edited by Tim Wyatt, Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello 6, 36208 Vigo, Spain; Tel.: +34 986 23 19 30/23 19 73; Fax: +34 986 29 27 62; E-mail: twyatt@iim.csic.es and Mónica Lion, Centro Científico y de Comunicación sobre Algas Nocivas COI-IEO, Apdo. 1552, 36200 Vigo, Spain; Tel.: +34 986 49 21 11; Fax: +34 986 49 20 03; E-mail: monica.lion@vi.ieo.es

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

http://ioc.unesco.org/hab/news.htm No. 37

• France

Co-occurrence of two *Alexandrium species* in Thau Lagoon

The occurrence of *Alexandrium* species involved in paralytic shellfish poisoning (PSP) in Thau lagoon (French Mediterranean) (Fig. 1) is known since 1998 [1-2]. Blooms usually occur in spring and autumn, and cause economic damage to shellfish farming that represents 10% of national production. Maximal vegetative cell densities reached 4.5 × 10^6 cell L^{-1} and 16 × 10^6 cell L^{-1} in October 2003 and November 2004 respectively. Such cell concentrations resulted in red tides during these periods (Fig. 2). Regarding species identification, thecal plate observations and the detection of complex toxin profiles in natural populations sampled in 1998 originally suggested the co-existence of *Alexandrium tamarense* and *Alexandrium catenella* [2]. These suspicions were confirmed by recent observations that some cells were characterized by unusual size and morphology (Fig. 3).

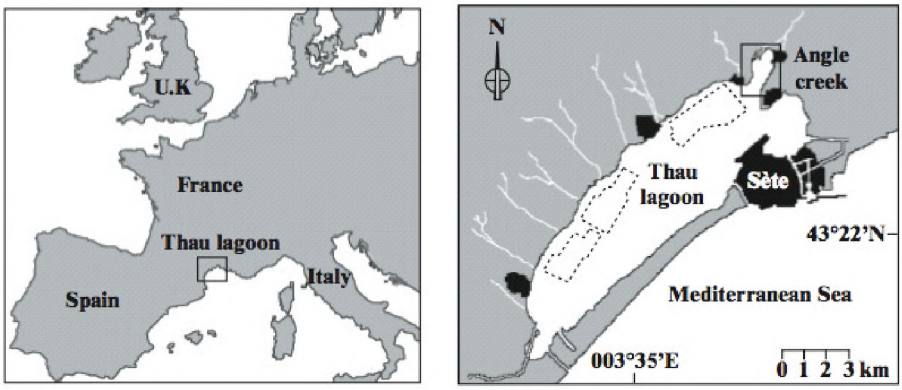

Fig. 1. Thau lagoon is located 30 km southwest of Montpellier (Hérault, South France). Shellfish farming sectors (dashed lines) represent 1/5 of total surface of the lagoon.

For a clarification, during blooms in 2007, more than 400 monoclonal strains were established by single cell isolation from seawater samples collected in the creek of l'Angle (NE part of lagoon, Fig. 2). Following genomic DNA extraction, the ribotype of ≈200 *Alexandrium* sp. Strains, originating from both spring and autumn 2007 blooms, was resolved by

(Cont'd on p.2)

• Brazil

Ostreopsis ovata blooms on Rio de Janeiro coast

There are sporadic reports of the occurrence of the epiphytic-benthic dinoflagellate *Ostreopsis ovata* on the Brazilian coast. *Ostreopsis* sp. strains were isolated from São Sebastião channel, São Paulo State (23°46'S, 45°21'W) in 2001 [1]. In summer 1998/1999 and 2001/2002, *O. ovata* blooms were reported in Arraial do Cabo (22°59' S, 42°00' W), in Rio de Janeiro State. In 1998, a biofilm of *O. ovata* cells was seen covering epilithic macroalgae [2–3]. *O. ovata* blooms were associated with sea urchins (*Echinometra lucunter*) deaths, and palytoxin analogues were identified in extracts from the *O. ovata* natural population [2]. In November 1998 two human deaths occurred as a consequence of seafood poisoning in Cabo Frio City, near Arraial do Cabo, but it was not clear if they were linked to the *O. ovata* bloom [2].

In Santa Catarina, on the southern Brazilian coast (27°16'S, 48°19'W), high *O. ovata* abundance (10^5 cells L^{-1}) was associated in January 2004 with a *Trichodesmium erythraeum* (Cyanobacteria) bloom and attached to the benthic diatom *Licmophora* sp. [4]. In March 2006, *Ostreopsis ovata* and *Prorocentrum lima* were the dominant species associated with macroalgae in Pernambuco, in the Brazilian northeast (8°32'S, 35°00'W) [5].

A project aiming to identify and quantify epibenthic dinoflagellates associated with macroalgae on the eastern Rio de Janeiro coast and to investigate community dynamics was established in June 2006. The study area is characterized by rocky shores covered by granite boulders, ending in a sand bottom with no influence from rivers (Fig. 1). A survey in a broader area was performed at 10 stations along the eastern Rio de Janeiro coast in summer 2007 and two sampling stations were monitored from June 2006 and throughout 2007 (Nascimento *et al.*, *in*

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The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

(Cont'd from p. 1)

sequencing the (ITS1-5.8SrDNA-ITS2) nuclear ribosomal DNA region.

Results revealed the presence of two ribotypes in Thau lagoon seawater: the formerly known "Temperate Asia" *A. catenella* and the "Western European" *A. tamarense*, in proportions of 44/56 in spring and 70/30 in autumn. Such co-occurrence in natural populations has been reported from Scottish coastal waters, i.e. *A. tamarense* vs. *Alexandrium ostenfeldii* [3]. However, in mixed phytoplankton assemblages *Alexandrium* species are difficult to distinguish. To settle this matter, a rapid and selective method was developed to identify the two species isolated from field samples; it consists of a semi-multiplex allele specific PCR using ribotype-specific primers. These newly designed primers lead to the specific amplification of a PCR product that has a different size in each species, thus enabling rapid identification based on amplicon length.

Several questions remain unanswered and require further investigations. When did *A. tamarense* colonize Thau lagoon? How can we explain the co-occurrence of two *Alexandrium* species also observed in Temperate Asia and Western Europe respectively? Are the dates and vectors of introduction similar? For investigating the origin of *A. catenella*, population genetic investigation is in process using microsatellite markers. Microsatellite markers are also developed to investigate *A. tamarense* origin and, maybe, its mechanism of introduction. The analysis of strains derived from cyst-germination from stored sediments collected since 2002 might supply useful information about the successive colonizations.

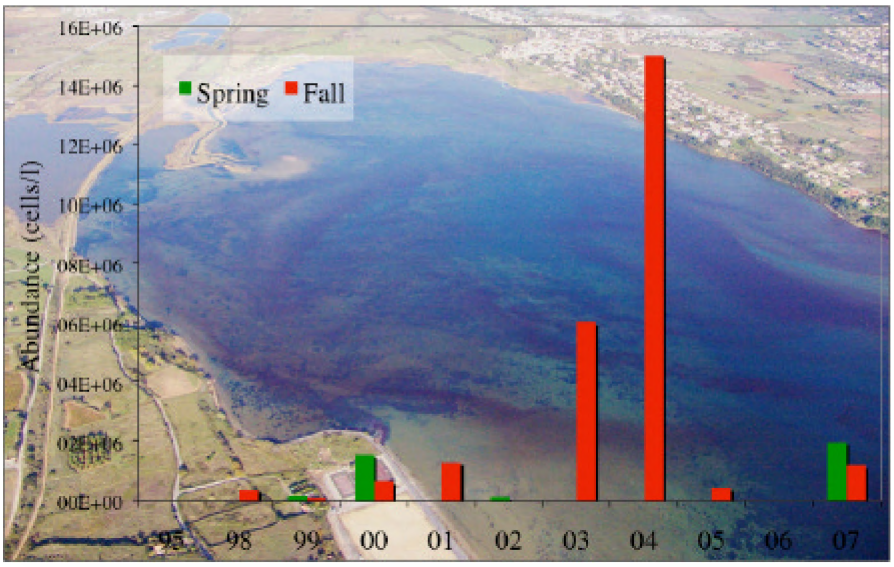

Fig. 2. Graph shows changes in cell density of Alexandrium *sp. blooms in Angle Creek, Thau lagoon with time. Background photography shows red tide during bloom of* Alexandrium *sp.*

References:

1. Lilly EL *et al* 2002. *J Plankton Res* 24: 443–452
2. Masselin P *et al* 2001. *In Harmful Algal Blooms 2000,* Hallegraeff *et al* (eds), IOC of UNESCO.
3. John U *et al* 2003. *Eur J Phycol* 38: 25–40.

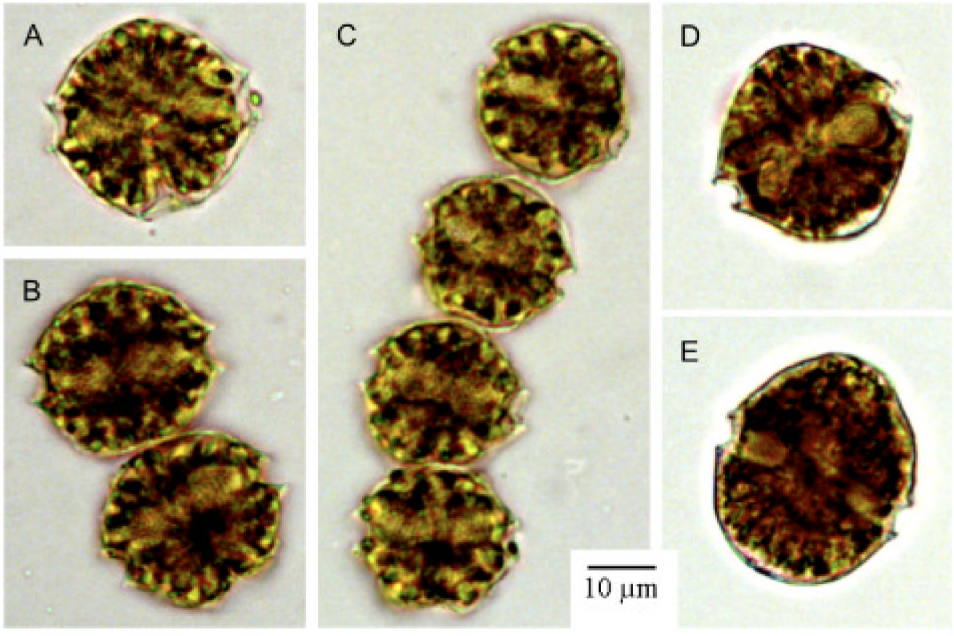

Fig. 3. Vegetative cells of Alexandrium *sp. from 2007 blooms cultivated in laboratory: single-cell (A), 2 cell- (B) and 4 cell-chains (C) observed in* Alexandrium catenella *strains; single-cell (D) and 2 cell-chain (E) observed in* Alexandrium tamarense *strains.*

B. Genovesi[1], E. Masseret[1], M-S. Shin-Grzebyk[1], D. Grzebyk[1], P. Berrebi[2], P-A. Gagnaire[2], M. Laabir[1], Y. Collos[1], A. Pastoureaud[3] & A. Vaquer[1]

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[2]Université Montpellier 2, UMR 5554 Institut des Sciences de l'Evolution, équipe Génétique et Environnement-Métapopulations, Conservation et Co-évolution, Montpellier, France.

[3]IFREMER Laboratoire Environnement Ressources/LR, BP 171, 34203 Sète, France.

Harmful Algae News

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prep.). A number of potentially toxic dinoflagellates were identified on macroalgae surfaces, such as *O. ovata*, *P. lima*, *Prorocentrum emarginatum*, *Prorocentrum rhathymum*, *Coolia* cf. *monotis* and *Amphidinium* cf. *operculatum*. The identification and quantification of dinoflagellate cells in macroalgae samples was performed in a counting chamber on an inverted microscope and taxonomic confirmation was performed under epifluorescence using calcofluor dye.

In the first 6 months of the study, two *O. ovata* (Fig. 2) blooms were recorded in the region. The first event occurred in June 2006 at Búzios, when *O. ovata* and *P. lima* were found associated with the macroalgae *Laurencia* sp., *Amphiroa fragillissima* (Rhodophyta), *Sargassum vulgare* (Phaeophyta) and *Codium intertextum* (Chlorophyta). *O. ovata* densities reached 1.5×10^5 cells g^{-1} fresh weight (FW) (1.2×10^6 cells g^{-1} dry weight) of *Laurencia sp.* (Table 1). Mean *O. ovata* densities in *S. vulgare* (n=3) samples was 7.2×10^4 cells g^{-1} FW of macroalgae (5.1×10^5 cells g^{-1} dry weight). In *C. intertextum* lower densities were registered, of 1.7×10^3 cells g^{-1} FW (1.6×10^4 cells g^{-1} dry weight). *P. lima* was also observed on macroalgae samples, in densities that varied between 100 and 1302 cells g^{-1} FW (Table 1).

At the Forno embayment in Arraial do Cabo, a yellowish-brown biofilm was seen covering the epilithic algae community on 5 December 2006 (Fig. 3a). At this time, *O. ovata* densities reached 2.1×10^4 cells g^{-1} FW in a mixed sample of *A. fragilissima* and *Jania capillacea* (Rhodophyta). Rocky shores at the Forno station are dominated by coralline red algae such as *A. fragilissima*, *Amphiroa brasiliana*, *Amphiroa beauvoisii* and *J. capillacea*. *P. lima* was not observed in samples during the *O. ovata* bloom. Although samples from other places along the coast were not analyzed at that time, it is likely that the *O. ovata* bloom was widespread through a larger area, as the biofilm was also observed covering macroalgae in a nearby embayment on 27 November 2006 (Fig. 3b).

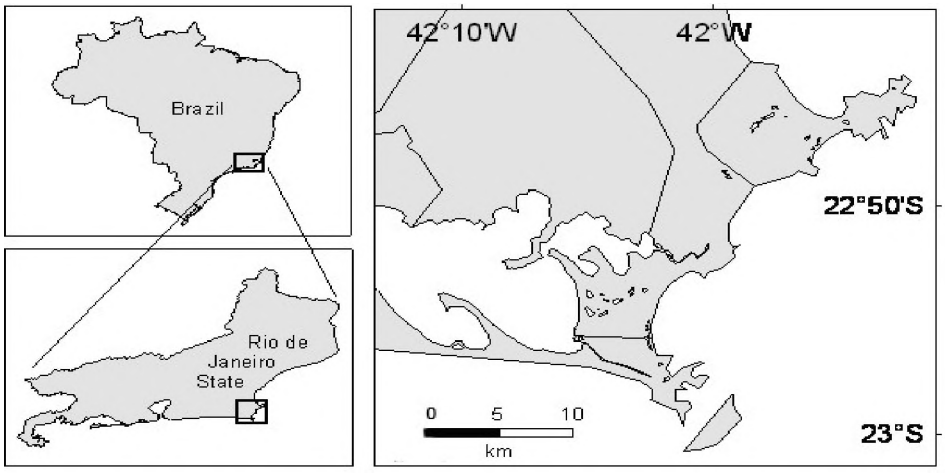

Fig. 1. Eastern Rio de Janeiro coast (Brazil) and location of sampling sites.

O. ovata densities at the Forno embayment two weeks before the bloom (21 November 2006) ranged between 24 cells g^{-1} FW on *J. capillacea* and 392 cells g^{-1} FW on *A. fragilissima*. Warm (average air temperature 24.1 ºC) and calm (average wind speed 4.5 m s^{-1}, 8.6 mm of rain) weather conditions that prevailed between 21 November and 5 December 2006 provided optimal conditions for bloom formation.

Early in 2007, intense rainfall (105.4 mm) and strong winds (mean of 7.6 m s^{-1} with gusts of 19.4 m s^{-1}) that occurred between 1 and 5 January 2007 caused a marked decrease in *O. ovata* densities. Moreover, the Arraial do Cabo region is characterized by a coastal upwelling [6] and such an event was recorded in 9 January 2007, contributing to surface cooling. On 23 January 2007, *O. ovata* maximum density at Forno embayment was 1,337 cells g^{-1} FW on *A. fragilissima*; on this occasion, *P. lima* was observed at densities between 30 and 230 cells g^{-1} FW on *A. fragilissima* and *P. emarginatum* and *A.* cf. *operculatum* were also recorded in lower densities.

O. ovata densities on the eastern Rio de Janeiro coast are among the highest reported in the literature. Vila *et al.* [7] recorded peak densities of *Ostreopsis* sp. to be 5.9×10^5 cells g^{-1} FW in *Halopteris scoparina* during July 1997 on the Catalan coast (Spain), and Aligizaki & Nikolaidis [8] reported maximum *Ostreopsis* densities of 4.0×10^5 cells g^{-1} FW on the North Aegean coast (Greece). The highest density of *O. lenticularis* was estimated to be 2.3×10^5 cells g^{-1} FW in *Dictyota* at Laurel Reef, Puerto Rico (Ballantine *et al.*, 1985) while in Hawaii the highest *Ostreopsis* sp. density was 1.8×10^4 cells g^{-1} FW in *Pterocladiella capillaceae* in November 2001 [9].

O. ovata cells were isolated and cultures are currently being kept in the laboratory for further work. *Ostreopsis* species are known to produce putative

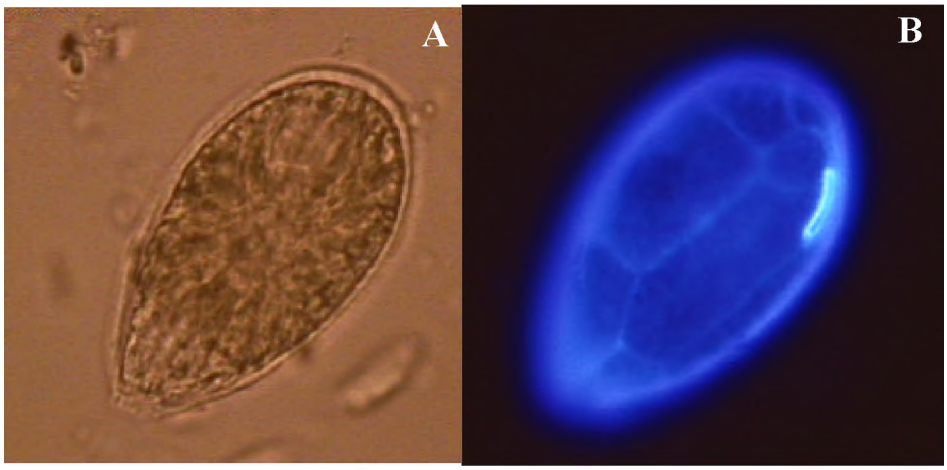

Fig. 2. Ostreopsis ovata *cells from Búzios. (A) Light microscopy, (B) Epifluorescence with calcofluor. Scale bars = 10 µm.*

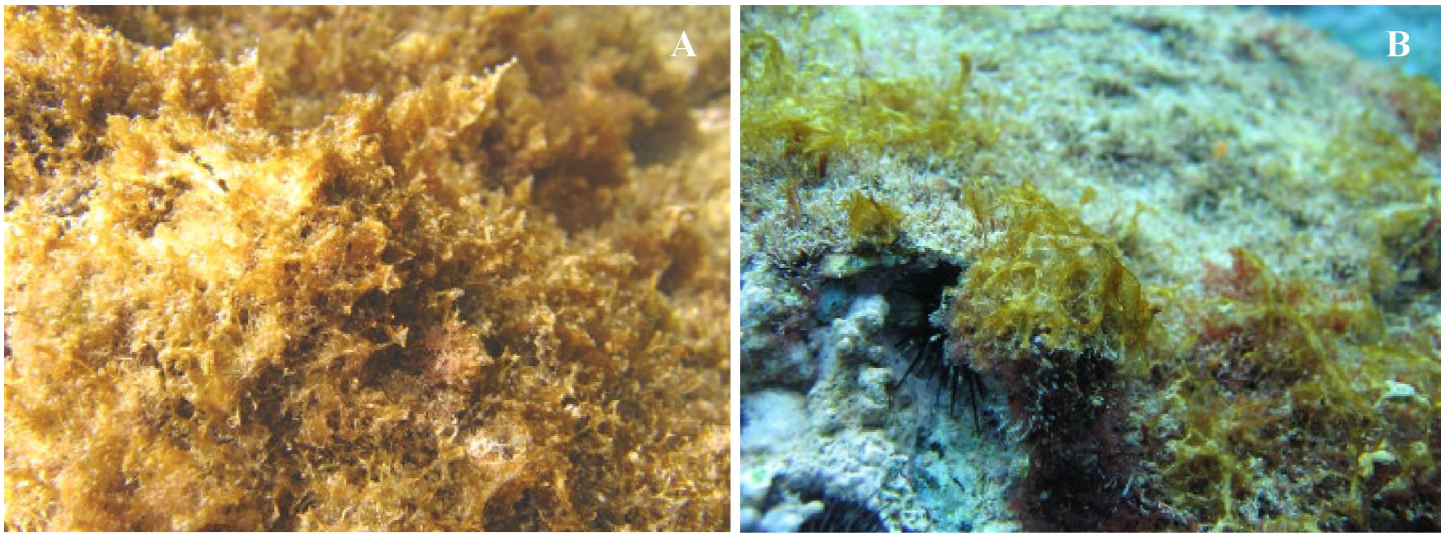

Fig. 3. Biofilm of Ostreopsis ovata *covering the red algae* Amphiroa fragilissima *(A) at Forno embayment on 5 December 2006 and (B) at Cabo Frio Island on 27 November 2006.*

palytoxin. Toxin analyses of a strain of *O. ovata* isolated from the Rio de Janeiro coast have shown the presence of palytoxin analogues [10]. Two *O. ovata* strains isolated during the current study showed haemolytic activity that was inhibited by ouabain (unpublished data). Palytoxin was confirmed as the causative agent in human seafood poisoning through the consumption of crabs and fish [11–12]. Moreover, over the last few years *Ostreopsis* species, mainly *O. ovata*, were involved in the intoxication of people who were exposed to marine aerosols along the Italian coast. Symptoms reported included rhinorrhoea, broncho-constriction, coughing, fever and dermatitis [13].

On the eastern Rio de Janeiro coast, aquaculture activities (particularly the mussel *Perna perna*), have been expanding in recent years, and fisheries and tourism are important for the local economy. These activities are vulnerable to the impacts of harmful algae blooms in the area, and considering the potential of this phenomenon, a project has begun to study the seasonal dynamics of epibenthic dinoflagellates associated with macroalgae in the region.

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Table 1. Ostreopsis ovata *and* Prorocentrum lima *absolute (n = 1) or average (n = 2 or 3) densities (cells g^{-1} FW) in macroalgae samples from Búzios and Arraial do Cabo, Rio de Janeiro, Brazil.*

Búzios, 10/06/2006		
	Ostreopsis ovata	*Prorocentrum lima*
Laurencia sp. (n=1)	151,420	1,302
Sargassum vulgare (n=3)	71,630	936
Amphiroa fragilissima (n=1)	33,184	735
Codium intertextum (n=2)	1,689	100
Arraial do Cabo, 5/12/2006		
	Ostreopsis ovata	*Prorocentrum lima*
Amphiroa fragilissima + *Jania capillacea* (n=2)	13,114	0

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• Mexico

First record of *Cochlodinium fulvescens* in Mexican Pacific

At the beginning of March 2008, a *Cochlodinium* bloom occurred in Bahía de Mazatlán, Sinaloa, México (23°11'11.8" N, 106°26'46.2" W, Fig. 1). To study this event, surface samples were collected and fixed with Lugol's solution. Some natural aliquots were reserved for taxonomical identification and culture establishment. Fixed samples were used to determine phytoplankton composition and *Cochlodinium* abundance.

At first we supposed that the bloom was caused by *C. polykrikoides*, but with the isolated material, we observed that coloration, shape and morphology of the cells and chains, and specially the intermediate position of the sulcus between the semicircular cingulum at the dorsal side (Fig. 2b), match the recently described *Cochlodinium fulvescens* [1]. In culture, paired cells or chains of 4 and 8 cells developed (Fig. 2). In dorsal view, the sulcus and a reddish orange pigmented body were easily observable (Fig. 2c). Cell shape was sub-spherical to ellipsoidal, the epitheca was hemispherical, and the hypotheca with the posterior end was slightly excavated by the sulcal groove. Mean length and width were 41.3 µm and 37.2 µm (n = 30), respectively. In field samples four cell chains were observed, but the addition of the Lugol's solution promotes breaking of the chain into pairs.

Average *C. fulvescens* population density ranged from 3.8×10^5 to 4.9×10^5 cells L^{-1}, and appeared with a bloom of *Pseudo-nitzschia* spp. (1.7×10^6 to 4×10^6 cells L^{-1}). The bloom was also included other dinoflagellates such as *Ceratium furca* (1.3×10^5 cells L^{-1}), and *C. balechii* (1.1×10^4 cells L^{-1}). No adverse effects were observed during the bloom.

Until now, only 7 species of *Cochlodinium* had been recorded in the Mexican Pacific: *C. catenatum, C. citron, C. faurei, C.* cf. *miniatum, C. pirum,* and *C. polykrikoides* [2]. *Cochlodinium catenatum* and *C. polykrikoides* are the most important because of the harmful blooms recorded in Bahía Banderas [3] and Bahía de La Paz [4, 5]. The isolation and culture procedures led to the first detection in the Mexican Pacific of the chain forming *Cochlodinium fulvescens*. Bahía de Mazatlán is one of the most important areas with frequent red tides (both harmful and innocuous), and where the historic record is the most extended and accurate [6]. No previous evidence of *Cochlodinium* species morphologically similar to *C. fulvescens* was found.

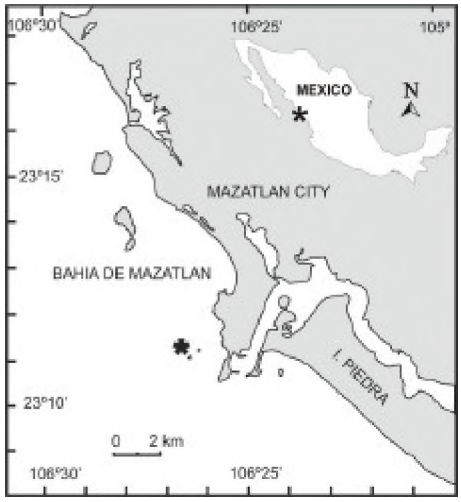

Fig. 1. Location of Cochlodinium fulvescens *bloom in Bahía de Mazatlán, Sinaloa (Mexico).*

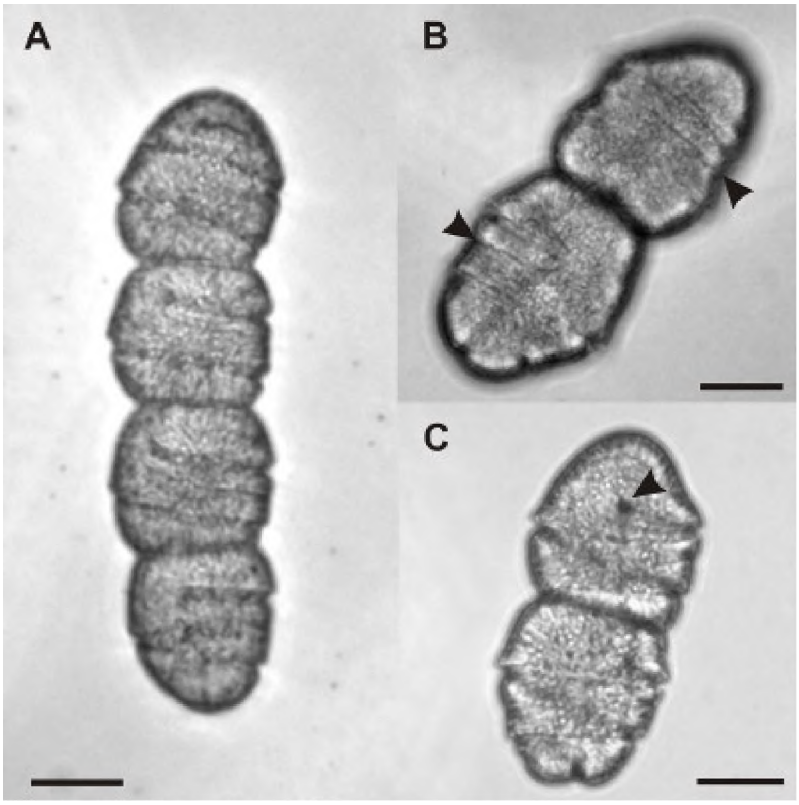

Fig. 2. Light micrographs of Cochlodinium fulvescens. *(A) chain of four cells, (B) chain of two cells; arrowheads indicate sulcus position, (C) dorsal view showing the reddish orange pigmented body. Scale=20 µm.*

Cochlodinium fulvescens occurs in the Seto Inland Sea (Japan), Hurun Bay (Indonesia), British Columbia (Canada), and California (USA) [1, 7, 8]. The phylogenetic tree inferred from partial (D1-D6 regions) LSU rDNA sequences clearly differentiated between *C. polykrikoides* and *C. fulvescens*; however, these species are closely related as a sister group [8]. In addition the morphology of the chain forming dinoflagellates of this genus is very similar [7]; it is therefore possible that *C. fulvescens* on the Mexican Pacific coast had been identified previously as *C. polykrikoides* or *C. catenatum*.

If we succeed with culture establishment, considering that *C. fulvescens* in Canada has been reported as a red tide forming dinoflagellate related to substantial mortalities of farmed salmon [9], we will corroborate the ichthyotoxic properties and life cycle.

• Mexico

Myrionecta, Gyrodinium and *Katodinium* bloom in Gulf of California

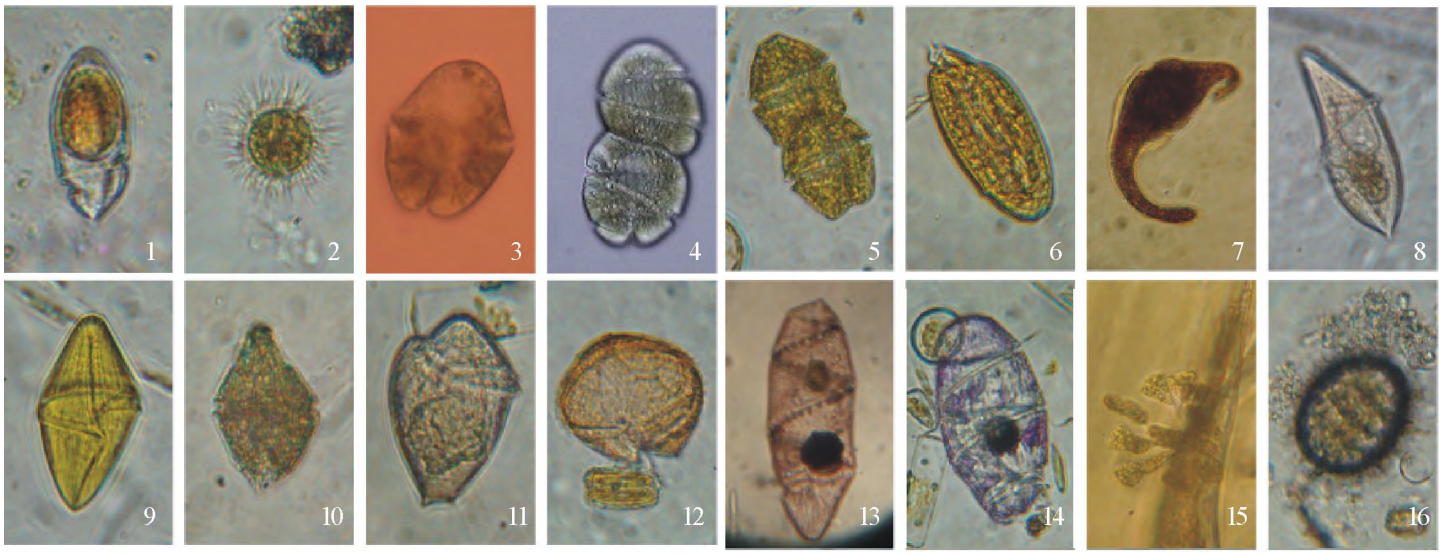

Plate 1. (1) Katodinium glaucum, *(2)* Myrionecta rubra, *(3)* Gyrodinium instriatum, *(4)* Cochlodinium polykrikoides, *(5)* Gymnodinium catenatum, *(6)* Torodinium robustum, *(7)* Gyrodinium falcatum, *(8)* Gyrodinium spirale, *(9)* Gyrodinium dominans, *(10)* Gonyaulax polygramma, *(11)* Gyrodinium *sp., (12)* Protoperidinium *sp. feeding on the diatom* Thalassiosira *sp., (13)* Warnowia polyphemus, *(14)* Warnowia violescens, *(15)* Licmophora *on a copepod tail, (16) cyst of* Polykrikos *sp.*

Microalgae blooms are frequent and periodic throughout the year in Bahía de La Paz, part of the southwestern Gulf of California. This bay has experienced frequent blooms, especially since 1984; more than 40 blooms and 25 bloom-forming taxa have been identified here [1, 2]. Bloom-forming species belong to different phytoplankton groups: dinoflagellates, diatoms, raphidophytes, planktonic cyanobacteria, and the ciliate *Myrionecta rubra*. This study describes a two-day multi-species bloom that occurred near the sandbar 'El Mogote' that separates the shallow lagoon from the main bay. On 17–18 June 2008, red patches appeared near El Mogote in Bahía de La Paz; (Sample A; 24°10.7'N, 110°22.09'W; Sample B; 24°11.1'N, 110°21.90'W). Samples were taken with a plastic bucket and preserved with Lugol's solution. Another sub-sample was taken for live phytoplankton analysis. Sea surface temperature was recorded. Ciliate and phytoplankton abundance were estimated and species identified in sedimentation chambers under an inverted microscope. Live samples of the red tides were used for identification of naked dinoflagellates.

A total of 45 microalgae species and one ciliate were identified. Dinoflagellates were by far the most abundant group in abundance and species richness (30 taxa). They were followed by diatoms (12), silicoflagellates (2) and 1ebridian (Table 1 lists the more abundant taxa). The total density (phytoplankton and the ciliate) at the sampling stations was similar on both days, varying between 4.97×10^6 cells L^{-1} on the first and 4.25×10^6 cells L^{-1} on the second day. The ciliate *Myrionecta rubra* and the two naked dinoflagellates *Gyrodinium instriatum* and *Katodinium glaucum* were the most abundant bloom species (Table 1, Plate 1). Highest numbers the first day were *M. rubra* (3.42×10^6 cells L^{-1}), *G. instriatum* (98×10^3 cells L^{-1}) and *K. glaucum* (41×10^3 cells L^{-1}). A shift in the dominant species occurred during the second day with highest numbers of *G. instriatum* (3.24×10^6 cells L^{-1}), *K. glaucum* (620×10^3 cells L^{-1}) and *Myrionecta rubra* (160×10^3 cells L^{-1}). Previous blooms of *G. instriatum* have occurred in this bay at the end of summer and linked to breakdown of the thermocline. *M. rubra* red tides are common during the upwelling season (2). *K. glaucum* is normally solitary, however two cell chains were found; this is an easily recognizable species in live and lugol fixed samples. No previous blooms of *K. glaucum* have been reported in the Gulf of California. Other

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naked dinoflagellates such as *Gymnodinium catenatum*, *Cochlodinium polykrikoides*, *C. fulvescens*, *Gyrodinium spirale* and *Torodinium robustum* were also collected. *K. glaucum*, *Warnowia violescens*, *C. fulvescens* and *T. robustum* are reported for the first time in Bahía de La Paz. *Amphidinium acutissimum, C. fulvescens*, *Ebria tripartita*, *Gyrodinium dominans*, *G. pepo*, *W. polyphemus* and unidentified *Gyrodinium* species are the first reports from the Gulf of California. Identification of live samples was an opportunity to observe several mixotrophs (such as *Protoperidinium* spp.) feeding on *Thalassiosira* sp. (Plate 1, Fig. 12). One of the most common dinoflagellate predators along the coasts of Baja California Peninsula is *Noctiluca scintillans* [3], recently observed feeding on *G. catenatum* (chains of 4–16 cells per *Noctiluca* cell) in Bahía de Los Angeles, Bahía Concepción, and Bahía de La Paz which are large bays in the Gulf of California. Large (>20 µm) naked and thecate heterotrophic dinoflagellates may form large biomasses in response to spring blooms of diatoms [4] and dinoflagellate blooms (this study). Other genera of naked heterotrophic dinoflagellates such as *Gyrodinium*, *Polykrikos*, *Pheopolykrikos* and *Warnowia* were observed in the examination of live phytoplankton samples. The importance of the heterotrophic component of marine dinoflagellates has not previously been reported for any area of the Gulf of California. An important finding was the epizoic symbiosis between the diatom *Licmophora* sp. on the copepod carapace.

The bloom occurred under upwelling conditions characteristic of this time period, accompanied by low water temperature (20 to 21°C) and winds of 3.0 to 4.5 m s^{-1}. Upwellings are common events during the late autumn to early spring [5, 6]. However, a short period of upwelling has been reported at the end of spring that is related to the SE winds [7, 8]. Variations in the wind regime from changes in the position and strength of high and low pressure systems cause short-term variability in upwelling-downwelling cycles within each season [9]. Temperatures are lower than typical (26–28 °C) for this month [6]; however, similar low water temperature (18–21 °C) have been recorded in this month in this area under similar meteorological conditions [7, 8]. In essence, blooms in Bahía de La Paz at this season are regulated by upwelling currents that bring nutrient-enriched water and probably re-suspended dinoflagellate cysts to the surface. Germination of cysts and blooms of phytoplankton are promoted by higher levels of nitrates and phosphates. In our samples, some unidentified dinoflagellate cysts and *Polykrikos* sp. cysts were found. Some species such as *C. polykrikoides*, *G. instriatum*, *G. catenatum*, *G. polygramma*, *L. polyedra*, *P. hartmannii*, *P. kofoidii*, and *P. schwartzii* are cyst-forming dinoflagellates [10]. The presence of cysts and cyst-forming dinoflagellates are related to the upwelling that occurred in the week prior to and during the bloom.

Table 1. Abundance (10^3 cells L^{-1}) of microalgae and the ciliate M. rubra found in Bahía de La Paz during a two-day bloom in June 2008. Note: The original table has been shortened by omission of less common species. Readers interested in the full list should contact the authors.

	Sample A	Sample B	Sample A	Sample B
CILIATES	17/06/2008	17/06/2008	18/06/2008	18/06/2008
Myrionecta rubra	3420	2850	160	89
DINOFLAGELLATES				
Cochlodinium polykrikoides	12	14	16	22
Gonyaulax polygramma	4	32	36	12
Gymnodinium catenatum	16	12	42	79
Gyrodinium instriatum	980	833	3100	3420
Gyrodinium spirale	1	4	27	2
Karenia c.f. mikitori	10	12	17	13
Katodinium glaucum	414	750	620	560
Pheopolykrikos hartmannii	1	6	35	21
Polykrikos schwartzii	11	9	31	14
Protoperidinium claudicans	4	1	4	60
Torodinium robustum	0	2	6	40
DIATOMS				
Asteromphalus heptactis	8	4	18	20
Hemiaulus sinensis	2	0	10	8
Leptocilyndrus danicus	8	4	0	12
Licmophora sp. (on copepod caparace)	0	0	28	0
Pseudo-Nizschia spp.	6	5	11	14
Rhizosolenia hyalina	20	17	24	17
Thalassiosira sp.	3	12	1	2
TOTAL ABUNDANCE	4976	4615	4216	4454

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• Mexico

New HAB records in the Gulf of California

In a previous contribution, we reported on HAB's on the surface waters of Mazatlan Bay, Sinaloa, Mexico over the 23 years from 1979 to 2002 [1]. At the time, we mentioned *Scrippsiella trochoidea* and *Prorocentrum dentatum* among the species responsible; we now know these were wrongly identified using light microscopy. Our recent observations of the collections held at the Plankton Laboratory (ICMyL-UNAM, Mazatlan Unit) allow us to examine both dinoflagellates using SEM equipment (JEOL/EO JSM 6360) on previously washed and alcohol dehydrated samples dried at CO_2 critical point (Samdri-795 equipment) and, finally gold covered using a Fine Coat Ion Sputter LFC-1100. The images obtained were appropriate for review the ultrasctructure and correctly identify the specimens as *Pentapharsodinium trachodium* Indelicato *et* Loeblich 1985 [2] and *Prorocentrum donghaiense* Lu 2001 [3], respectively.

P. trachodium have a scrippsielloid shape, 20–36 µm long and 15–28 µm wide (N=64). The plate disposition is orthoperidinioid, with the formula: po, x, 4', 3ª, 7'', 5c (4c+T) 4s, 5''', 2'''' (Fig.1A–D). The Sp plate is short and never reached the cingulum, showing the dorsal apithecal tabulation bipesioid. All plate surfaces with the exception of plate x are covered by small pimples. Also, trichocysts surrounded by circles of five pimples are widely distributed on the plates surfaces (Fig.1D). A gelatinous apical stalk emerges from the apical pore complex (not shown). Blooms always occurred as bright reddish patches.

P. donghaiense is a small elongated cell measuring 16.5–20.8 µm long and 7.5–10.8 µm wide (N=65). The valves are covered by small knobs (density 9-10 per μm^{-2}), sometimes interconnected by a ridge (Fig. 2 A–F). We observed two kind of pores: trichocysts and mucocysts, which bear different ornamentation on the inner and outer sides of the valves. The former present a triangular space on the outer side and are more prominent on the inner valve (Fig. 2B-D). The periflagellar region is very similar to that reported by Pertola *et al.*, 2003 for *P. minimum* [4], but in *P. donghaiense*, the flagellar and auxilliary pores are oval shaped (Figs. 2E). The features reported here, and others that were not reported in the original description of *P.*

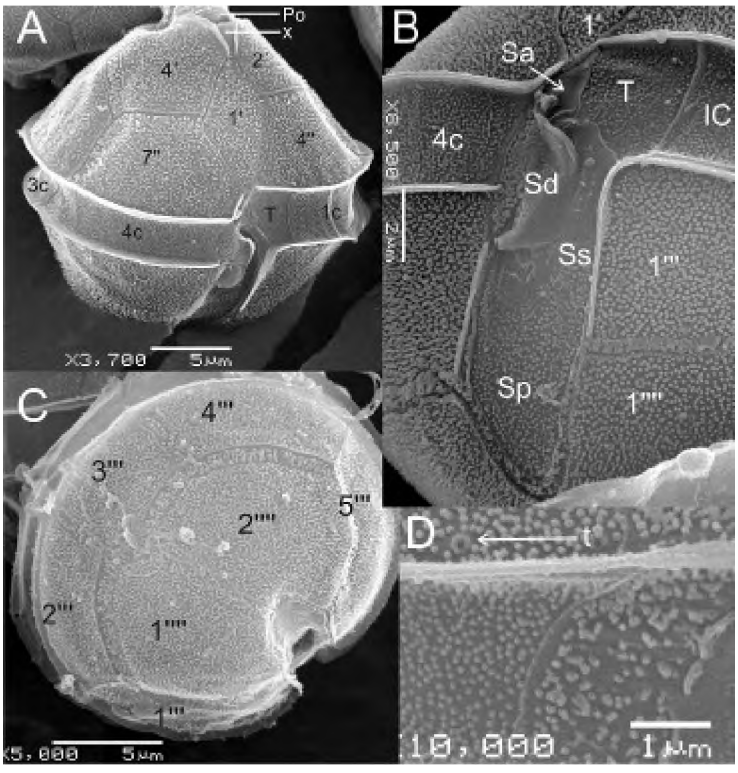

Fig. 1. Pentapharsodinium trachodium. *A) epitheca showing plate 1 'ortho. B) sulcal plates. C) hypotheca. D) detail of ornamentation of thecal surface, showing difference between the widespread and unorganized pimples and the neatly organized circles surrounding trichocyst pores.*

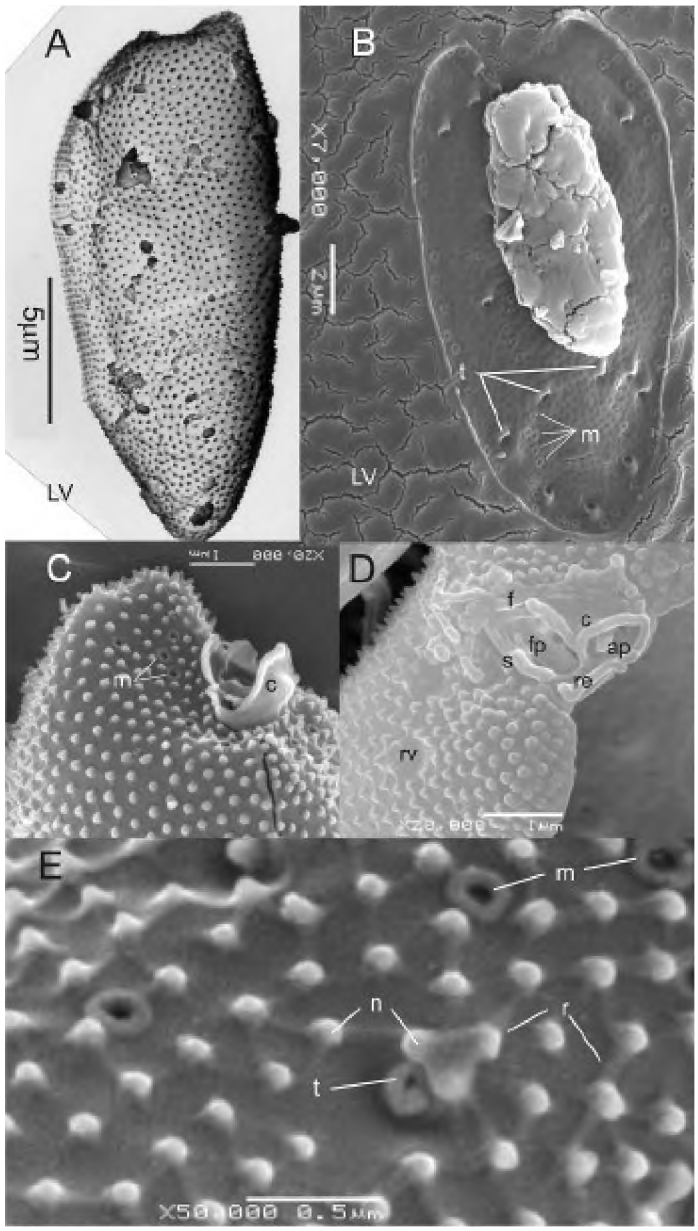

Fig. 2. Prorocentrum donghaiense. *A-B) left valve showing dorsal elongation. C-D) outer and inner side of valves. Mote trichocysts pores (t), rows of 4 or 5 mucopores (m) and, knobs (k). E) apical region showing two pores, flagellar (fp) and auxiliary (ap). F) sagittal view of periflagellar region showing the "ear shaped" collar sheats © with a periflagellar structure similar to that typified by Pertola* et al., *(2003), single tooth (s), forked tooth (f) and, ridged edge (re).*

• India

Karenia mikimotoi bloom in Arabian Sea

A harmful algal bloom (HAB) occurred from July to September 2004 on the Kerala coast of the Arabian Sea. *Karenia mikimotoi*, a dinoflagellate, accounted for nearly 98% of the standing crop of >20 µm fraction of phytoplankton. Mass mortality of fish coincided with the bloom event. Algal cell density peaked at 9.0×10^4 cells L^{-1} during the second week of September. Levels of PO_4^{3-} (10.5 µmol L^{-1}) and of Fe (2 mg L^{-1}) were high compared to corresponding seasonal values of 0.5 µmol L^{-1} and 0.2 µmol L^{-1} respectively. Dissolved oxygen levels had dropped from 5.6 to 0.2 mg L^{-1} by the end of the bloom. Covering of the gill lamellae by mucous released by the bloom and hypoxia following collapse of the bloom may have caused fish mortality. A noxious odour and respiratory problems among children on the coast were also noticed.

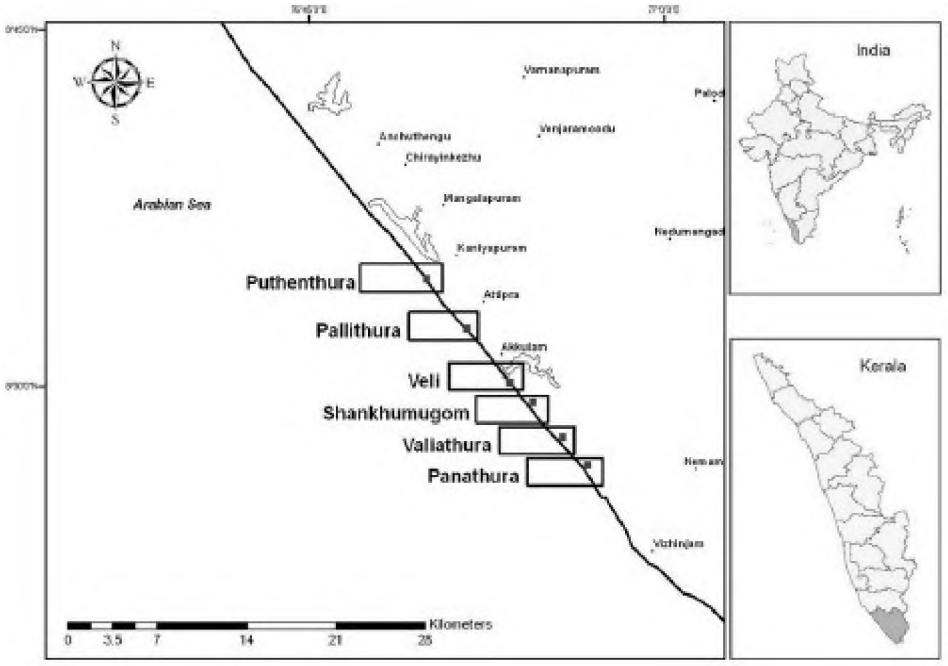

Fig. 1. Location of algal bloom and sampling sites.

Bloom conditions were recorded from mid-July to mid-September 2004 off the coast of Thiruvananthapuram, west India, between latitudes 8º 22' N - 8º 34' N and longitudes 76º 48' E - 76º 59' E. Six transects were established at 4 km intervals along the coast, at Puthenthura, Pallithura, Veli, Shangumugam, Muttathura, and Panattura, respectively from north to south (Fig. 1). On each transect, two stations were occupied, one near shore and one 25 km offshore, 25 km, as well as a few intermediate stations between these. Water samples were collected at all stations at 1 metre depth. Phytoplankton was sampled with a 20mm net and fixed with Lugol solution. Shellfish samples for toxin analysis were collected from the area, and stored frozen in the laboratory. Dead finfish strewn along the coast were also examined.

Standard physico-chemical procedures were followed [1]; 0.45µ filtered water samples were used for NO_2, NO^{3-}, $PO4^{3-}$ determinations following Brewer and Riley [2] and Grasshoff [3]. Iron was analyzed by the methylene blue method [4]. Chlorophyll a concentrations were determined by JGOFS protocol [1]. Species composition was determined in 1 ml sub-samples: identifications were based on keys in UNESCO [5], Santhanam *et al* [6] and Hallegraeff *et al* [7].

The temperature of the water, when the bloom was first noticed on July 15 varied from 28.22°C near shore to 28.52°C offshore, and salinity from 33.9‰ near shore to 35.7‰ offshore. Dissolved oxygen levels varied from 0.2 to 5.6 mg L^{-1}. pH varied from 7.8 near shore to 8.2 offshore. Concentrations of PO_4^{3-} were abnormally high, 10.5 µmol L^{-1} near shore and 5.0 µmol L^{-1}offshore. Concentrations of NH_4^+ varied from 5.5 µmol L^{-1} near shore to 4.2 µmol L^{-1} offshore, nitrite from 0.2 µmol L^{-1} near shore to 0.3 µmol L^{-1} offshore, and NO_3^- concentrations were in the range of 4.8 µmol L^{-1} near shore to 3.2 µmol L^{-1} offshore (Table 1).

Concentrations of iron were rather high, in the range of 2.7 near shore compared to 1.1 mg L^{-1} offshore.

donghaiene, has been submitted for publication [5].

P. trachodium has been observed 10 times in Mazatlán Bay, blooming in April 1980, November 1985, April 1986, July 1989, April 1990, March 1994, from March to August 1996, June 2000 and March 2003. *P. donghaiense* occurred only twice as the dominant organism, in April 1985 and April 1994; during March, April and May 1990 and 2000, it followed the dominant species *P. balticum.* For further biogeographical information, see reference [1].

Acknowledgements

To Arturo Núñez-Pasten for assistance during field sampling.

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Table 1. Variations in Physiochemical conditions during July to September 2004.

	July 15		Sep 9		Sep 23	
	Near shore	Offshore	Near shore	Off shore	Near shore	Off shore
Temp (°C)	28.22	28.52	29.63	29.41	30.33	30.18
pH	7.8	8.2	8.1	8.3	8.4	8.3
Salinity (‰)	33.9	35.7	34.6	34.2	34.0	34.1
DO (mg L^{-1})	5.6	5.4	4.8	5.0	0.2	2.8
Phosphate (?mol L^{-1})	10.5	5.0	5.4	3.8	4.0	1.6
Ammonia (?mol L^{-1})	5.5	4.2	1.8	1.0	0.5	0.5
Nitrite (?mol L^{-1})	0.2	0.3	0.1	0.3	0.2	0.2
Nitrate (? mol L^{-1})	3.4	3.2	2.3	1.8	2.2	2.6

Predominant winds during the bloom period were onshore, southwesterly and westerly. Winds from the SW accounted for 32% and SSW for 22% of the total time, with wind speed and frequencies as follows: 1-5 kmph (0.8%), 6-10 kmph (1.2%), 11-15 kmph (27%), 16-20 kmph (65.5%), and 21-25 kmph (5%).

A total of 18 algal taxa consisting of 14 diatoms and 4 dinoflagellates were identified in the bloom. Diatoms included *Chaetoceros lorenzianus*, *Thalassionema nitzschioides*, *Thalassiosira eccentrica*, and *Skeletonema costatum*. Of the dinoflagellates present, *Karenia mikimotoi*, *Ceratium macroceros*, *Protoperidinium depressum* and *Prorocentrum micans*, *K. mikimotoi* accounted for >90% of the phytoplankton.

There was a conformity in phytoplankton abundance between near shore and offshore. For example at station Veli, the cell density was around $3.0x10^3$ L^{-1} near shore, and 2.0×10^2 L^{-1} offshore on July 15. By Sept 9, it had risen to 9.4×10^4 L^{-1} near shore and 4.4×10^4 L^{-1} offshore. By Sept 23, it had decreased to 2.0×10^4 L^{-1} near shore and $5.0x10^3$ L^{-1} offshore. Chlorophyll a increased from 8.8 mg m^{-3} in July 15 to 721 mg m^{-3} by Sept 9th, followed by a decrease; maximum chlorophyll values compare favorably with those reported in high density blooms elsewhere. The bloom-affected area was covered with slimy mucus, partly washed ashore.

The onshore and poleward winds do not lead to upwelling. In our study there was no increase in salinity, or decrease in temperature, which suggests absence of upwelling. The high concentrations of nutrients (5.5 µmol L^{-1} NH_3, 10.5 µmol L^{-1}, PO_4^{3-} 2.7 mg L^{-1} Fe) can be attributed to heavy runoff. This was corroborated by unusually high precipitation (60 mm in two days) during this period. Thus the explosive algal growth can be attributed to a sudden increase in nutrients from the land; the role of nutrients in the evolution of HAB has been documented [10]. Many HAB species display a preference for ammonia over other nitrogenous nutrients, as reflected in the present case, where NH_4^+ concentrations were high, whereas NO_2^- and NO_3^- were low [11].

As the bloom declined, a massive fish mortality of *Rastrelliger kanagurta*, *Sardinella longiceps*, *Arius arius*, *Seganus javus*, *Psettodes erumei*, *Monacanthus hispidus*, *Diodon hystrix*, and *Anguilla* sp. occurred. Examination of the dead fish revealed heavy mucous coatings on their gills. Fish kills are often marked with an odour resembling rotten eggs, characteristic of hydrogen sulphide (H_2S), and perceptible even at concentrations of 0.002 mg L^{-1}. It is highly toxic to most organisms. In the present studies, H_2S concentration reached 1.6 mg L^{-1}.

The presence of high concentrations of ammonia may have stressed the fish, affecting their metabolism and immunity responses. The situation was further aggravated by the anaerobic conditions with release of H_2S, as the bloom ended. Further, mucus released by algae during the bloom covered the gill filaments of the fish, leading to asphyxiation and death, consistent with the other reports.

The prevailing winds carried the putrid smell from the decline of the bloom and decaying fish towards the coast, along with H_2S released under anaerobic conditions. All these airborne contaminants resulted in irritation of eyes and nasal membranes, coughing, sneezing and in some cases even, asthmatic attack among the coastal population.

Acknowledgements

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• India

Microcystis aeruginosa bloom on Southwest coast of India

Photosynthetic prokaryotes like Cyanobacteria (blue green algae) are cardinal elements of aquatic ecosystems. Blue green algae proliferate quickly in stagnant lakes with high nutrient levels, during less turbulent warm seasons, resulting in 'blooms' that turn the water green, often with floating layers of green scum. Toxic blooms of cyanobacteria have been observed elsewhere in the aquatic ecosystems [1]. Several species of cyanobacteria produce both neurotoxins and hepatotoxins often causing health problems for humans and affecting standing crop of the finfishes. *Microcystis* is a prominent genus of toxic cyanobacteria. Morphologically a few species belonging to the genus form dense aggregations of cells floating on the surface of the water forming thick layer of green scum. Such growth can produce bad smell and affect the aesthetics of freshwater bodies and can be often lethal to livestock and humans [2].

The Chalakudy river is considered to be the lifeline of central Kerala. It originates from the Anamalai region, which is a collection of hills and the river transverse through Parambiculam, Kuriyarkutty, Sholayar, Karapara and Anakayam joining Periyar near Puthenvelikara, in the Ernakulam district emptying into the Arabian Sea at Azheekode. Chalakudy River performs a pivotal role in shaping the economic prospects of Kerala. It helps in power generation, domestic water supply, irrigation, tourism, Industrial production, collection of various inorganic resources and fisheries.

During the first week of March 2008, water discolouration of the river Chalakudy near the Kanakkankadavu regulator cum bridge (Fig. 1) was reported in the press. The surface water was green with floating layers of green scum (Fig. 2), spreading in an area of around 5 km^2. This regulator cum bridge, which prevents the entry of saline water from Cochin estuary into the river during the summer months, tend the locals to depend on this water for agricultural and domestic uses.

Detailed analysis of the samples revealed that the discolouration was due to *Microcystis aeruginosa* a Cyanophycean member (Fig. 3). *Microcystis* is one of the common algal species found in the river. This alga is capable of producing a hepatotoxin 'microcystin' which in high concentrations could be fatal to humans, livestock, and pets. However, only itching and irritation of the skin among the locals was reported. The physico chemical characteristics of the bloom area are shown in Table 1. *Microcystis* bloom samples were taken and preserved for the toxin analysis and molecular taxonomic studies.

Influence of nutrient contents in surface water receiving high radiation influences the abundance and distribution of phytoplankton. Lower reaches of Chalakudy river receive large quantities of effluents from domestic source and effluents from bone meal factory. The shutters and regulator dams raised during the premonsoon season prevents easy mixing of seawater from the highly saline Cochin estuary during the warm months to the Chalakkudy river. This is sure to increase the phosphorous content of the surface water where this nutrient will otherwise act as a limiting factor. This could be the reason for a bloom of the Cyanobacteria *Microcystis aeruginosa* during this period. It may be assumed that during the bloom the phytoplankton



Fig. 1 Map showing the Microcystis *bloom area.*

is quantitatively abundant and qualitatively poor resulting in low diversity. This situation according to Margalef (1978) represents the climax phytoplankton community structure the time frame of which is controlled by nutrient enrichment and change in N:P ratios.

The stagnant condition resulting from lack of flow of water might have resulted in a 'dystrophic' condition supporting large biomass of less number of species among which *Microcystis aeruginosa* occupied around 98% of the biomass.

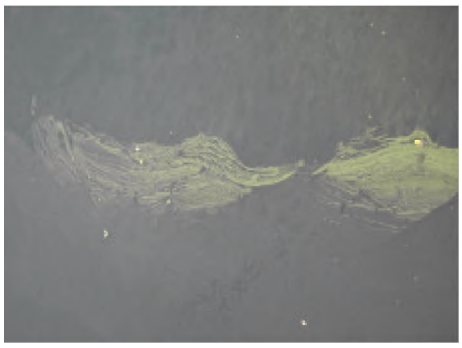

Fig. 2. Microcystis *bloom.*

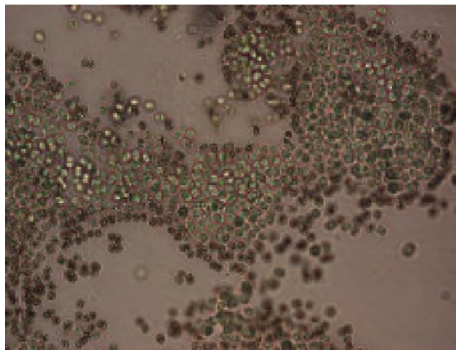

Fig. 3. Microcystis *cells x 100.*

Table 1. Physicochemical parameters of Microcystis *bloom area.*

Parameter	Bloom area
Temperature (°C)	31
Salinity (ppt)	0
pH	8.4
Dissolved Oxygen (mg l^{-1})	6.28
Nitrate (µmol l^{-1})	4.11
Phosphate (µmol l^{-1})	8.147
Silicate (µmol l^{-1})	25.64
Chlorophyll *a* (mg m^{-3})	23.4
Cell count (Cells m^{-3})	4 x 10^8

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Write the Editor NOW with news on your work, HAB events in your country or region, or any other matter you wish to share with HAB scientists and managers worldwide. Harmful Algae News has more than 2000 subscribers.

GEOHAB Modeling Workshop

The Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) program is planning a workshop to help integrate modeling activities into GEOHAB Core Research Projects (CRPs) and regional/national projects. The workshop will be open to any interested students and scientists, but the number of participants will be limited by the size of the meeting space. The workshop will be held at the Martin Ryan Institute, National University of Ireland, Galway, Ireland on 15-19 June 2009. Inexpensive accomodations will be available on campus for students. The organizing committee for the workshop is chaired by Dennis McGillicuddy (USA); other members include Wolfgang Fennel (Germany), Marcel Babin (France), Marina Levy (France), Peter Franks (USA), Icarus Allen (UK), and Ken Furuya (Japan). CRP chairs Patrick Gentien, Grant Pitcher, Pat Glibert, and Allan Cembella have also participated in the planning. Information about the meeting is available at www.geohab-models.org.

Objectives

The objectives for the meeting will be to

- stimulate modeling activity in GEOHAB Core Research Projects (CRPs);
- entrain researchers at all levels (students, postdocs, faculty, etc.) into HAB modeling;
- facilitate dialog between model developers and HAB researchers involved in process studies through joint training sessions;
- improve understanding of HAB processes through linkage of models, *in situ* observations, and remote sensing;
- foster linkage between HAB modeling and the broader community of biogeochemical, ecosystem, and population dynamics modeling;
- highlight species-specific aspects intrinsic to HAB modeling: autecology, behavior, species interactions, toxin production, etc.;
- improve capabilities for prediction of HABs and quantitative assessment of their skill;
- encourage the use of advanced data assimilation techniques in HAB modeling;
- encourage the use of observing system simulation experiments (OSSEs) in array design;
- improve forecast products and their dissemination to maximize their benefit to the user community; and
- develop a written glossary for terminology.

Structure

The workshop will consist of four connected elements:

1. Plenary talks comprised of (a) invited reviews on HAB modeling and other relevant approaches (ecosystem modeling, population dynamics modeling), and (b) contributed talks on models and observations in support of the CRPs.
2. Dialogue seminars given by HAB observationalists and modelers. Specific modeling needs of the CRPs will be identified; implementation plans will be developed, utilizing existing modeling infrastructure where practical, and identifying needs for additional model development where gaps exist.
3. Tutorials and training on model design and application of models (geared toward students involved in CRPs).
4. Student mentoring.

Product

A set of peer-reviewed contributions will published in a special issue of a suitable journal, such as *Journal of Marine Systems* or perhaps an open-access journal. The tentative title of the special issue is *Modeling biophysical interactions in harmful algal blooms: processes and methods.*

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9th IOC-AECID-IEO Course on Harmful Phytoplankton



Picture of participants and teachers in the 2008 Vigo course.

The 9th IOC-AECID-IEO Course on Harmful Phytoplankton was held in the Oceanographic Centre of Vigo, Spain, in June 2008. Like in previous years, this course was jointly sponsored by the Intergovernmental Oceanographic Commission (IOC) of UNESCO, the Spanish Agency of International Cooperation and Development (AECID) and the Spanish Institute of Oceanography (IEO).

The course focused on taxonomy of harmful marine microalgae, and was intended for Latin-American and African experts having experience in microalgae identification, responsible for setting up or strengthening their national or regional harmful marine microalgae monitoring programs.

For the first time in the Vigo Centre, the course had a distant learning part participants should complete before attending the classes held in Vigo. This distant learning was supported by the IOC Science and Communication Centre on Harmful Algae of Copenhagen. Jacob Larsen was the person in charge of its supervision, using the Absalon e-learning platform at the Copenhagen University. This part of the course comprised approximately 45 hours of individual learning and was developed from 5th May – 6th June.

The second part of the course was held in Vigo from 11th to 27th June. It comprised 93 hours of lectures and practical sessions on the identification of harmful algae and cysts, as well as introductory lessons on marine biotoxins, monitoring and management of harmful events, chemical taxonomy and molecular tools, ecology of microalgae, cultures and sampling techniques at sea and in the laboratory. Participants were also able to report on their national HAB events, exchanging impressions on their experience.

11 scientists from Argentina, Brazil, Chile, Ecuador, El Salvador, Peru, Spain and Venezuela attended the course.

This year we had the invaluable contribution of Jacob Larsen from IOC SCCHA, Denmark and Celia Villac from Universidade de Tabauté, Brazil, as guest teachers, as well as our usual collaborators for this kind of courses: Santiago Fraga, Isabel Bravo, Beatriz Reguera, Sonsoles González-Gil, Lourdes Velo, Francisco Rodríguez and Pilar Rial from the IEO, Vigo; José Franco, Beatriz Paz and Pilar Riobó from IIM-CSIC, Vigo; Yolanda Pazos and Fabiola Arévalo from INTECMAR, Vilagarcia de Arousa; María Rodriguez-Velasco from the EU-CRLMB, Vigo; Emilio Marañón from the Universidade de Vigo and Monica Lion from the IOC-IEO SCCHA, Vigo.

Participants contributed actively to the classes and we enjoyed a superb work atmosphere.

If you are interested in this kind of IOC training activities, please check our web site http://ioc.unesco.org/hab/ for complementary information.



Taxonomy practical class with Jacob Larsen.

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Second Asian GEOHAB Meeting

The Second Asian GEOHAB Meeting was held at the Institute of Oceanography in Nha Trang, Vietnam on Jan. 31–Feb. 1, 2008 as a sequel to the Asian GEOHAB Meeting held in Tokyo on March 15–16, 2007. The first meeting reviewed current activities on HAB issues in Asia with 20 presentations on a broad range of topics (fol.fs.a.u-tokyo.ac.jp/1stAsianGEOHAB/abstracts.pdf). The second meeting was more focused to identify key questions and research components for drafting a plan for the development of Asian GEOHAB to launch international cooperative research. The meeting was attended by more than 60 participants from 11 countries. Sixteen presentations were made and regional priority was well addressed. Species highlighted include *Prorocentrum donghaiense*, *Cochlodinium polykrikoides*, *Karenia mikimotoi*, *Noctiluca scintillans*, *Pyrodinium bahamense* var *compressum*, *Alexandrium minutum*, *Dinophysis* spp., and benthic dinoflagellates. Most presentations focused on scientific aspects on ecology and oceanography of HABs; some presentations introduced on-going national and international projects in Asia. In addition, the urea dumping issue in the Sulu Sea, Philippines, was reviewed, and its ecological consequences relevant to HAB issues were discussed. The programme and abstracts of the meeting are available at http://ioc.unesco.org/hab/AsianHAB%20abstractBook_Latest_Lam.pdf.

After the presentation sessions, subgroups convened to identify specific questions that can be addressed in a comparative context, and to recognize what it would take to address these questions including co-operative research, workshops, joint cruises and capacity building. Four subgroups were formed on 1) biogeography and biodiversity of HAB species, 2) nutrients and eutrophication, 3) adaptive strategies, and 4) comparative ecosystems. It was agreed to prepare a report of this meeting as a GEOHAB publication based on materials used in the presentations, and the discussion of the establishment of a GEOHAB regional program, HABs in Asia. An international editorial team was formed from participants to prepare a document on Asian GEOHAB.

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Ocean fertilization with urea

In late 2007 a private corporation based in Australia proposed enrichment of the Sulu Sea, Philippines, a region of high biodiversity, with thousands of tonnes of urea to stimulate algal blooms and sequester carbon.

Scientists in the Philippines immediately reacted when they learned about the project. They wrote letters to government officials and open letters published in local newspapers. In parallel, the international scientific community reacted promptly and from a GEOHAB meeting in Vietnam in January 2008, as well as other networking, emerged a view point paper with 57 authors for the 'Marine Pollution Bulletin' [1]. The paper outlined the scientific reasons why the proposal was flawed. These reasons were conveyed to the Philippine authorities.

The concern over urea enrichment to generate plankton blooms for carbon credits is multifaceted. Urea is preferentially used as a nitrogen source by some cyanobacteria and dinoflagellates, many of which are neutrally or positively buoyant, making them less likely to sink to the deep sea. Biological pumps to the deep sea are classically leaky, and the inefficient burial of new biomass makes the estimation of a net loss of carbon from the atmosphere questionable at best. The potential for growth of toxic dinoflagellates is also high, as many grow well on urea and some even increase their toxicity when grown on urea. Many toxic dinoflagellates form cysts which can settle to the sediment and germinate in subsequent years, forming new blooms even without further fertilization. If large-scale blooms do occur, it is likely that they will contribute to hypoxia in the bottom waters upon decomposition. Lastly, urea production requires fossil fuel usage, further limiting the potential for net carbon sequestration. The environmental and economic impacts are potentially great and need to be rigorously assessed.

This debate finally resulted in the rejection of the project by the Philippine authorities, however the parties behind the initiative are said to be seeking alternative locations.

The GEOHAB Scientific Steering Committee in April 2008 issued an Advisory Bulletin on Urea Fertilization, which can be found at the GEOHAB web site www.geohab.info.

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ISSHA
International Society for the Study of Harmful Algae

ISSHA President's Corner

As I write this on 8-8-08 my thoughts go toward China, the exciting happenings at the opening of the 2008 Summer Olympic Games and the army of athletes that will compete. In just a two short months a small army of phytoplanktologists, toxicologists, ecologists, oceanographers, public health officials, marine resource managers and remote sensing experts will also be arriving in China to enjoy the 13th International Conference on Harmful Algae (ISSHA). It will be held in Hong Kong from 3-5 November 2008. Please visit ISSHA's website to learn more about the exciting things the Co Organizers Prof. K. C. Ho and Prof. M. Zhou and Local Organizing Committee have planned for us.

Auction

The ISSHA Auction was one of the most popular events at the Copenhagen Conference and it will be back by popular demand. The Auction is fast becoming a tradition. It is an opportunity for the Conference participants to bid on donated items that range from signed reprints, books, photos, micrographs or craftsmanship (paintings, drawings, carvings etc.), homemade wine, beer or other specialities. The auction items are donated by ISSHA members and sponsors from around the world and the proceeds are used for the benefit of ISSHA members. This year ISSHA was able to offer partial travel support to more that 25 students to attend the 13th Conference.

Prof. Barrie Dale will serve as the auctioneer and provocateur ensuring the evening will be exciting, fun filled and a bit over the top! Please help us make the 13th Conference auction a success. We need donations for the auction and only your imagination limits what can be donated for this very worth effort. Please contact the secretariat at secretariat@hab2008.hk) to provide a description of the items you plan to donate. There will be a list of items, updated frequently, at the ISSHA website www.issha.org.

Election

ISSHA members will soon be receiving their ballot to elect new Officers and Council Members to lead ISSHA for the next 3 years. We are extremely pleased to offer an exceptional slate of candidates for the coming term and know you'll be inspired by their fine leadership. Take time to return your ballot and influence the future of the Society.

With best regards, I look forward to seeing in Hong Kong in November,

Pat Tester, ISSHA President.
Email: pat.tester@noaa.gov.

Future events

OCTOBER 2008

Ciguatera and Related Biotoxins Workshop

Nouméa, New Caledonia, 21-31 October 2008.



A workshop on the latest developments in ciguatera research, covering environmental influences, causative organisms and socio-economic and medical aspects.

First call–tentative programme covers: toxinogenesis, environmental influences, chemistry of involved ciguatoxins, clinical treatment and folk remedies, toxicology and mechanisms of action, ciguateric fish species, socio-economic impacts, collection and use of clinical and epidemiological data, and risk assessment and management.
Additional info: www.ird.nc/ciguatera

JUNE 2009

7th International Conference on Molluscan Shellfish Safety

Nantes, France, 14-19 June 2009.

Programme will include epidemiology, risk assessment, toxin monitoring, analytical methods in microbiology and biochemistry, health consumer protection, management of farming/harvesting areas, post-harvest treatment, and macro-economic analysis of the repercussions of shellfish-farming crises. This conference will be an exciting forum for scientists, regulators and producers, which will make an important contribution to limiting health risks associated with microbiological, toxinic and chemical contamination of coastal areas. The conference will also address the identification of chemical contaminants and target species that represent significant risks for consumers. Finally, it will aim to improve the development of high performance detection tools to prevent and/or monitor contamination, and will thereby contribute to supporting existing marine environment monitoring networks.

Important dates:

- Abstracts submission: 15 Dec, 08.
- Abstract authors notified of selection: 1 Feb, 09.
- Early registration price: until 15 Mar, 09.
- Normal registration price: 16 Mar–29 Apr.
- Late registration price: from 30 Apr, 09.
- Deadline for online hotel reservation: May 11, 09.
- Deadline for online registration and correspondence: Jun 1, 09.

P. Lassus, ICMSS09 organiser and host. Email: icmss09@ifremer.fr

NANTES 2009
7th International CONFERENCE
Molluscan Shellfish Safety



See you in Hong Kong at the 13th International Conference on Harmful Algae!

3-7 November 2008
www.hab2008.hk

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

http://www.ioc-unesco.org/hab No. 38

Reflections on the 13th International Conference on Harmful Algae

The 13th international conference on harmful algae was convened in Hong Kong from November 3–7, 2008. The meeting was attended by approximately 375 participants from 22 countries. Historically, this represents a decrease in attendance from recent conferences, which have had more than 500 participants and representation from 40–50 countries. The decrease may reflect the financial crisis that was sweeping the world at the time of the meeting, as well as funding shortfalls for HAB research in a number of countries. This was certainly the case with a number of colleagues from the U.S. who usually attend our conferences, but were unable to do so because of a lack of travel funds. There was also a shortage in travel funds for students, postdocs, and for scientists from developing countries.

The venue was unique in that the meeting was held at the Disneyland Resort located outside Hong Kong. The main park with its rides and attractions was a 10-minute walk from the hotels and the conference rooms, but the Disney theme was still evident everywhere, from the names of the conference rooms (Cinderella, Sleeping Beauty), to pictures and statues of Disney characters, to the elevators that called out the floor numbers with the voice of Mickey Mouse. The facilities and rooms were modern and spacious. Hong Kong is an extraordinary city, and those who made trips downtown experienced the spectacular skyline and the other sights and sounds that few will forget. Conference convener K.C. Ho and his assistant Ginnie Kai worked very hard to coordinate a meeting that entertained the participants while also providing opportunities to communicate in formal and informal settings, to interact socially, to form new friendships, and to renew old ones.

As I look back on the conference, I am struck by the maturity of our field. In my summary talk, I went back through the past HAB conferences, pointing out how the complexity of the

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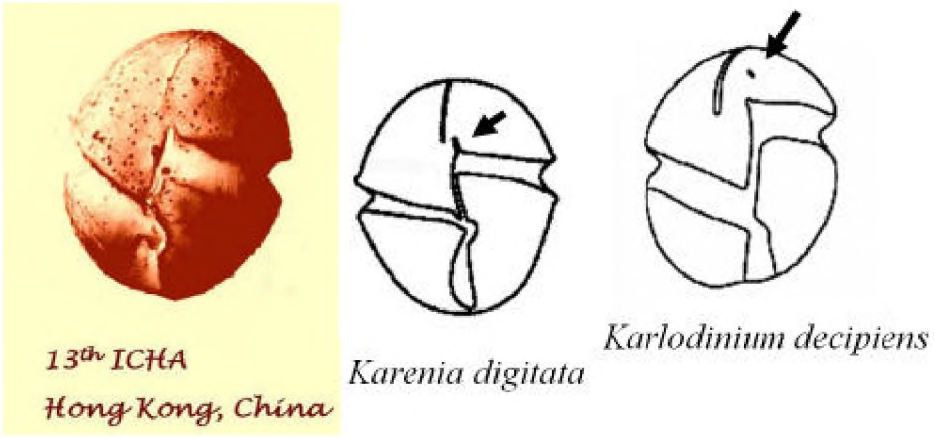
Who is the Hong Kong dinoflagellate on the ICHA13 conference logo?



Karenia digitata Yang, Takayama, Matsuoka & Hodgkiss 2000 as featured on the ICHA13 logo was newly described as the causative organism of the extraordinary red tide and fish kills in Hong Kong waters in 1998 [1]. This event which originated offshore attracted significant media coverage and caused 35B dollars (RMB) loss. This species was allocated to the genus *Karenia* because of the presence of a straight apical groove and the absence of a ventral pore then considered to be diagnostic for the genus *Karlodinium* [2], while the species name refers to the finger-like (*digitata*) protrusion of the sulcus on to the ventral epicone surface. Unfortunately, no live type cultures of *K. digitata* are available and no molecular sequences were obtained, something which is strongly recommended to be included in modern HAB species descriptions (as discussed by Karen Steidinger in Hong Kong). At the HAB13 conference, Miguel de Salas, Ben Mooney, Gustaaf Hallegraeff and co-workers presented a stunning 5 new *Karlodinium* species obtained from a single cruise in the Southern Ocean south of Tasmania [3]. One of those new species, *Karlodinium decipiens* de Salas (*decipiens* means deceiving!),

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now known from Tasmania (Australia) and Spain bears a striking similarity to *Karenia digitata*, except for the presence of an inconspicuous ventral pore and the absence of the finger-like sulcal protrusion. Soon after lodging our LSU rDNA sequences of this taxon in GenBank we were approached by Haifeng Gu of the Third Institute of Oceanography, Xiamen, China, who had established cultures from Hong Kong waters with an identical sequence.

We carefully considered the possibility that *Karlodinium decipiens* could be a junior synonym of *Karenia digitata*. However, the morphological differences between the descriptions of the two species are significant, and further communication with Takayama who prepared the original scanning micrographs of *Karenia digitata* confirmed that the material from Hong Kong and Japan never contains a ventral pore. A further *Karlodinium* species described by de Salas and co-workers, *K. ballantinum* de Salas, now known from Tasmania and Italy (Zingone, unpublished), while genetically undoubtedly being related to *Karlodinium*, does not possess a ventral pore however. As many of you may remember, the significance or not of the presence or absence of a ventral pore for the taxonomy of the PSP dinoflagellate genus *Alexandrium* was hotly debated for nearly two decades. Perhaps *Karenia digitata* still belongs in *Karlodinium* (it is not flattened like most *Karenia*) but potentially is distinct from *Karlodinium decipiens* now also known from Hong Kong. The fish-killing mechanism by *Karlodinium* blooms, whether due to karlotoxins (only known thus far from *K. veneficum* and *K. conicum)* [4], reactive oxygen species, lipophycotoxins [5] or other still uncharacterized chemical fractions, also remains to be fully elucidated.

References

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2. Daugbjerg N *et al* 2000. *Phycologia* 39: 302–17
3. De Salas M *et al* 2008. *J Phycol* 44: 241–257
4. Mooney BD *et al* 2009. *J Phycol* 45 (1) (in press)
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field had grown through time, with new species or toxins or poisoning syndromes or impacts being discovered every few years. In recent meetings, the nature of that discovery has changed, as has the pace. We still learn of an occasional new toxin, but for the most part, they are derivatives or metabolites of known compounds. Another sign of the maturity of our science was the sophistication of the analytical instrumentation and approaches being applied, as well as the complexity of the molecular techniques and numerical models used. These are state of the art in every way.

A conference summary is truly a challenging undertaking given the structure of the meeting. For the first time, our HAB conference had three concurrent sessions, making it difficult to obtain a broad perspective on the science presented. Accordingly, I sought the assistance of a number of colleagues and obtained their insights and opinions, some of which are reflected here.

When I asked my colleagues what struck them about this meeting, a frivolous but common answer was "the food". It clearly is part of Chinese culture to treat guests to a fine meal, to feed them until they can eat no more with food still on the table, and this conference certainly achieved that goal. Beyond that however, there were a number of scientific highlights that I offer here with a concurrent apology to all those who might not be recognized, despite equally important scientific contributions. These are simply my own observations, interspersed with those of some colleagues, and clearly represent a limited view of the large and diverse conference.

One of the major findings reported at the meeting was the identification by **Tillmann** of the causative organism for azaspiracid shellfish poisoning (AZP) — a new dinoflagellate species which will be published in the next issue of Eur. J. Phycol. This was an important discovery for several reasons. It reversed the long-held view that AZP toxins are produced by *Protoperidinium crassipes*; it also demonstrated how well a team of scientists can work together. I was very impressed by the way an LC-MS instrument was taken on board a research vessel and used to screen water samples for minute quantities of HAB toxins. Once azaspiracids were detected in a sample, the team was able to size fractionate the plankton and analyze those fractions to see where the toxin was, and then to isolate many of the organisms in the appropriate size range, testing them all for toxicity. This led to concurrent taxonomic, genetic, and toxicological characterizations of this important organism. It was a beautiful piece of teamwork.

The second highlight for me was new information on *Dinophysis*. At HAB 12, **Park** and his colleagues opened a major door into the study of the DSP syndrome by demonstrating they could culture *Dinophysis acuminata* by feeding it the ciliate *Myrionecta*, after the latter had consumed the cryptophyte *Teleaulax*. As expected, two years later, the Hong Kong conference had a number of papers that followed up on this breakthrough. Some explored, but did not resolve, whether *Dinophysis* chloroplasts are permanent plastids (strongly indicated by ultrastructure of chloroplast membranes; **Garcia, Moestrup**) or kleptoplastids stolen from *Teleaulax* via *Myrionecta* (suggested by chloroplast gene sequence data and by flow cytometric separation and real-time PCR of dividing chloroplasts during the cell cycle of *D. norvegica*; **Minnhagen**). This talk entertained the audience with its clever artwork, shown in the figure below. **Park** used long-term starvation studies to address this question, and concluded that the plastids in *Dinophysis* were permanent. Stay tuned for a resolution of the issue, perhaps in Crete at HAB14. Other important contributions were by **Hansen** on *Dinophysis* grazing and nutrition, and

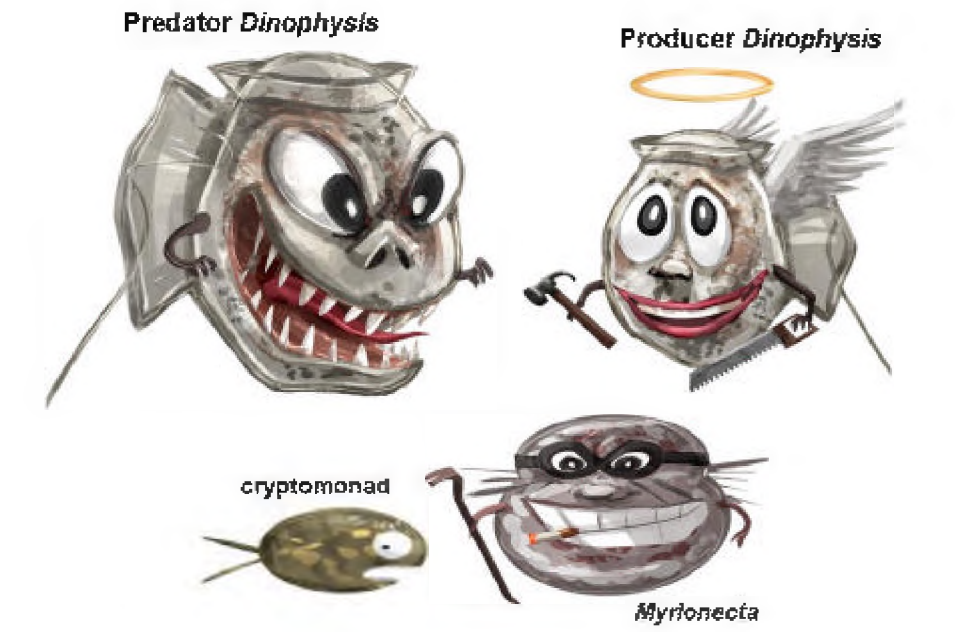

Illustration by Mats and Susanna Minnhagen.

by **Reguera** who elucidated further aspects of the ecology and complex life cycle of *Dinophysis*, including indications that these cells may have a novel approach to sexuality whereby one cell engulfs another. This mechanism is distinct from the manner in which *Dinophysis* species feed on *Myrionecta* using a peduncle, and is also different from the more normal "fusion" of gametes in many dinoflagellates. The conference also provided a valuable opportunity for those working on *Dinophysis* species to meet together and discuss their findings in an informal, after-session meeting, looking for common pathways to progress.

Another highlight was a talk by **Scholin** who described a major development in our field – not a new one, but one that has to be viewed as a major step towards a technology that I believe will ultimately revolutionize the way we manage and study HABs. Chris described the latest developments of the Environmental Sample Processor (ESP), a robotic device that can be moored remotely and programmed to collect water samples, filter them, and perform all of the complex chemistries (e.g., sandwich hybridization and qPCR) needed to not only identify and count HAB cells, but their toxins as well, the latter using antibody-based methods developed by **Doucette**. This instrument has been under development for some time, but recent advances are still impressive. The ESP and instruments like it are needed to transition our genetics, toxicity and physiology into management. The ESP takes technologies developed in laboratory settings and brings them into the field in a way that has direct practical applications. This is especially important these days given the major investments in Ocean Observing Systems (OOSs) worldwide. Of the many societal benefits attributed to these OOSs, "red tide or HAB detection and forecasting" is often listed, yet the vast majority of these observing networks have no capabilities to detect or measure HAB cells or toxins. The ESP can provide them.

A molecular approach to HAB population analysis based on microsatellites was used by **Nagai** and co-workers to study the relationship between HAB populations in different areas of the world, from *Alexandrium* species in the Mediterranean (**Masseret**), western Europe (**Alpermann**) or Asia (**Genovesi**); there was a particularly interesting study of *Cochlodinium* in Japanese and Korean waters in which **Nagai** and colleagues identified distinct populations and biogeographic boundaries, but also showed that the flow between these populations could be related to human activities such as species dispersal facilitated by the pearl oyster industry.

Another area of great progress also involved molecular biology, this time following up on another breakthrough first announced at HAB 12. There, **Kallmann** identified the saxitoxin gene cluster for the first time –the Holy Grail for those working on toxin genetics. He identified and characterized the gene cluster in cyanobacteria, and I fully expected that in Hong Kong we would see a burst of papers built on that discovery. That was true in part, as there were several talks about cyanobacterial toxin genes — their evolution (**Murray**), where they might have been acquired, how common they are, and so forth. Another important study was by **Stucken** who not only confirmed the presence of the gene cluster in other cyanobacterial species, but also found "tailoring" genes with the cluster –genes that take the toxin backbone and make changes to it to form derivatives. This progress with cyanobacteria was noteworthy, but it is also worth pointing out that two years after that major discovery, we heard nothing about saxitoxin genes in dinoflagellates. A number of research teams are working on this, including my own, but none have succeeded as yet. This demonstrates how difficult it is to do this type of genetic work on dinoflagellates, even when a potentially homologous set of genes from another organism is available to guide that search.

Yet another molecular highlight was that of **Lin** on the spliced leader sequence in dinoflagellates; this is a base sequence unique to dinoflagellate mRNAs, and part of the dinoflagellate molecular architecture that facilitates splicing of gene transcripts. This was not a new report, as he and **Van Dolah** have separately reported this finding previously, but this was the first opportunity for many to see how the spliced leader could be used to address questions about dinoflagellate ecology and physiology. This is important because it can be used like a piece of Velcro or a hook to pull dinoflagellate gene transcripts from a sample. In this way, one can take a complex assemblage of organisms and isolate the transcripts from just the dinoflagellates in that sample. It is thus possible to do gene regulation studies in the field, to see how certain cells respond to the environment around them without interference from the other organisms that are present.

Once again, we have a new tool that opens up doors for many different lines of investigation.

Nagasaki gave a detailed study of host and viral bloom dynamics that included reproduction, infection, host range, genetics, and even molecular structure. He showed us that multiple viruses may be present in a *Heterocapsa circularisquama* bloom, each infecting distinct susceptible populations. If similar host/virus relationships are widespread (and it appears they are), then this might be a major evolutionary force driving the high level of cryptic speciation among HABs.

Another general comment relates the complexity of the systems that we study. Mingjiang **Zhou's** plenary talk emphasized that we cannot just study HABs in isolation. We need to identify the linkages with climate change, land use practices, alteration in trophic structure, eutrophication, and many other factors before we can begin to understand bloom dynamics at a level that can lead to effective management. Several papers demonstrated the value of this comprehensive approach.

Talks on toxin chemistry described diverse analytical approaches, such as a method using surface plasmon resonance for PSP toxin detection (**Campbell**), or in vitro cell-based assays as screens for toxicity, and SPATT bags for passive toxin adsorption and detection (**Hermann, Fux**). **Quilliam** demonstrated the power of LC-MS techniques – in effect the "universal method" for biotoxin analysis, or at least for lipophilic toxins. But, his identification of more than 90 metabolites of yessotoxins, or 67 spirolides (and still counting) made it clear that there is a need for functional assays and other methods that can quantify and integrate the potency of these different toxin congeners and metabolites in samples. The chemists are increasingly able to identify compounds of many different types in samples, but the potency of many of those is not known. Managers are bewildered by the plethora of toxins being described, and they can legitimately ask whether they need that much detail for their decisions. In those cases, functional assays like bioassays, immunoassays, and receptor-based assays hold great promise, though of course, they have their own problems.

There were many talks on genomics, with presentations of EST libraries and even entire genomes for a number of HAB organisms (**Cembella, Gobler, Yang, Lin**). but in most cases, the results were underwhelming, given a common problem facing all such studies with HABs – namely that the majority of the genes that can be identified are common, "housekeeping" genes, and the ones that we are most interested in (like toxin genes) are not found in databases, and thus are "unknowns". A clear challenge for those working in this field is to slowly characterize the thousands of HAB genes of unknown function. Microarray studies abounded, but while providing useful indicators of changes in gene expression, the biological and biochemical interpretations present considerable difficulties in HAB organisms. **Place** argued that new approaches are needed that look specifically at gene products that are about to be synthesized (by focusing on polysomes), rather than looking to mRNA pools alone. On a different tack, some talks and posters evidenced a sophisticated understanding of the genetic mechanisms and structure underlying gene expression in several key HAB species. **Van Dolah, Place**, and **John** all described studies of polyketide synthases (PKSs) – compounds linked to toxin production in three different HAB species.

Molecular probe technology is firmly established in HAB science – in many ways, HAB workers have paved the way for this approach to cell counting in other areas of phytoplankton ecology since we were among the first to need species-specific detection methodologies. Demonstrating how far this field has advanced, **Medlin** described a hand-held sensor for molecular probe-based detection of 17 HAB species using electrochemistry, while **Chen** showed how the use of electrochemiluminescence in sandwich hybridization assays could achieve higher sensitivity and more stable assays. **Dyhrman** and others showed how quantitative PCR could be used to provide cell abundance estimates that correlate well with shellfish toxicity measurements in monitoring programs. Another highlight was the application of sniffer dogs on research vessels to spot floating whale feces from which minuscule domoic-acid producing *Pseudo-nitzschia* diatom fragments could be visualized (**Doucette**).

In a manner analogous to the chemistry talks, molecular approaches to species identification have started to unravel the complexities of our plankton communities to a level that can boggle the mind. What was formerly thought to be a population of a single species can now be shown by microsatellites or other approaches to be a community of many different individuals. This information is very interesting and important scientifically, but just as with the many new toxin congeners and metabolites, from a practical perspective at least, it is sometimes more important to pull back to the big picture, to ignore the detailed population structure within a HAB community so that information useful for management decisions can be made.

At many previous HAB meetings, microbial interactions and allelopathy have been considered interesting concepts, but their significance for HAB bloom dynamics and toxin production remained unclear, poorly understood and sometimes met with scepticism. However, HAB13 proved a significant turning point, not so much due to significant advances, but more to the widespread acceptance that allelopathic (algal-algal) and microbial interactions (among algae-bacteria and viruses) *are* important influences on phytoplankton population dynamics. While only 10 oral and 6 poster presentations at the meeting focused specifically on microbial interactions, many presentations acknowledged the potential of microbial interactions to explain their data.

No "Eureka moments" emerged, but evidence of steady progress was abundant. Much of the work presented continued to carefully document the microbial partners in the interactions, and the diversity of stimulatory (**Bolch**), algicidal (**Vargas**) physiological and toxicological effects attributed to microbial interactions with other algae, algal grazers (**Hogfors, Sun** and

Weigand) and other fauna/flora. Chemical "warfare" is clearly widespread in the marine environment.

Significant challenges remain before we can advance our understanding of microbial interactions. The bewildering array of potentially synergistic and antagonistic interactions among the microbial community is now well documented and experimental models presented by **Bolch** and co-authors offers one way to proceed. However, we clearly must combine chemical, biochemical and molecular approaches to unravel the mechanisms and compounds mediating microbial interactions if we are to understand how algal cells respond at the physiological and molecular level. A number of presentations (e.g. **Yin, Zheng, Ma)** have taken tentative steps in this direction, but much more is needed.

Likewise, the role and influence of bacteria in production of toxins (STX, domoic acid, etc) remains unresolved. In this context, one interesting talk that certainly warrants further study was that by **Takata** who showed that the dinoflagellate *Alexandrium* can produce domoic acid after addition of a bacterium isolated from an area where bivalves accumulate this toxin. The conclusion was that domoic acid is produced by multiple species of phytoplankton when certain species of bacteria contact them. But, which partner produces the toxin? Or is it a combined effort? If verified, this reopens the discussion started by **Kodama** decades ago about the role of bacteria in saxitoxin production by dinoflagellates.

There were several dozen talks or posters on cyanobacteria in addition to those related to toxin genetics described earlier. There were presentations on bloom control and prevention using "cyano-cidal" bacteria **(Nybom),** novel chemical treatments, and even aquatic macrophytes to mitigate toxins, nutrients, and blooms **(Nimptsch).** Several talks or posters **(Pinto, Wickramasinghe, Downing, Esterhuizen)** highlighted the increasing concerns over the new cyanotoxin: beta-methylamino-L-alanine (BMAA), a possible cause of Alzheimer's disease. New cyanotoxin detection methods were described,



including Ultra- Performance Liquid Chromatography-Tandem Mass Spectrometry **(Oehrle)** and solid phase adsorption toxin tracking technology (SPATT; **Wood).**

In a pattern that characterizes most of our HAB conferences, there were relatively few talks on bloom control, though several papers addressed impact mitigation techniques. A plenary talk by **H.K. Kim** was a fascinating presentation of the many innovative strategies used by the Koreans to control *Cochlodinium* blooms threatening fish farms. The use of clay was highlighted, including an entertaining analysis of the choreography of the ships that disperse it in different patterns ("merry-go-round","cranes wing", "frontal", and "parallel"). There was also an informative analysis of ecological impacts as well as the surprisingly low costs of these control operations.

Gustaaf Hallegraeff provided the following perspective on taxonomy at the Hong Kong Conference. **Karen Steidinger** reviewed the evolution of the morphospecies concept as it applies to the dinoflagellates *Karenia, Alexandrium* and *Pyrodinium*. Increasingly, DNA sequences are included in new species descriptions, a practice strongly encouraged and which perhaps should be formalized in the codes of nomenclature. **Medlin** outlined the protocol for correcting numerous taxonomic misidentifications associated with widely used GenBank sequences. A lively discussion ensued as to how much genetic distance separates species. **Hallegraeff** and colleagues described 5 new species of *Karlodinium* (including a new karlotoxin producer, **Mooney, Place)** from a single cruise in the Southern Ocean south of Tasmania. While most species were well supported by morphology and genetics, where large genetic distances received limited morphological support, the recommendation was to further scrutinize morphological ultrastructure. Similarly, **Litaker, Tester** and colleagues redefined 10 *Gambierdiscus* species based on combined morphology and rDNA sequences. We should not forget that morphospecies remain the backbone of HAB monitoring, and we echo Max Taylor's early words of wisdom, to "discriminate to the level required to answer the question you are asking". An important breakthrough by **Tillman** and German colleagues has been the identification of the causative dinoflagellate organism of AZP, to be named *Azadinium spinosum* in a phylogenetic position intermediate between the Peridiniales and Gonyaulacales. This can now pave the way for trophic transfer studies.

Grant Pitcher provided an overview of modeling presentations. There is a clear need he feels for the incorporation of models in many aspects of HAB

research, to ensure progress within this field, but an assessment of the presentations at this Conference suggests this need is not being met. Of the approximately 190 oral presentations, only 6 or 7 specifically addressed the use of models while 2 or 3 of the 142 posters included the use of models.

The low number of presentations incorporating models in research activities does not bode well for the HAB scientific community and this shortcoming needs to be addressed. The use of models is specifically required for predictive purposes and although many scientists in this field motivate their research through the promise of prediction, few presentations at this meeting have addressed this using models. One of the reasons for this is a shortage of modeling capacity in many countries, especially for modelers interested in coupling physics to biology. This is clearly an area where support is needed to train modelers for work on HABs, as identified as a priority activity in GEOHAB.

Species-specific models are important in the prediction of HABs, yet of those presented, most do not progress beyond the use of physical models utilized in predicting bloom transport or dispersion. Those that incorporate a biological component typically only attempt to predict biomass (red tides).

On the positive side, a small number of presentations at this meeting did incorporate the use of models in examining bloom dynamics, of *Pseudo-nitzschia* **(Palma, Davidson)**, and of *Alexandrium* **(Fauchot, Anderson, Levasseur)**. The *Alexandrium* models presently demonstrate the most promise as they are "cyst-based" and through this approach are able to successfully initialize models. A noteworthy development was the successful forecast of a major regional bloom of *A. fundyense* by **Anderson** and co-workers for the Gulf of Maine in 2008. Those workers also are predicting a decade or more of frequent and high toxicity blooms for the region, based on analyses of cyst abundance and past patterns of toxicity following major blooms.

Finally, climate change was a theme that a number of speakers pursued. **Dale** demonstrated that it is possible to separate large-scale weather patterns from anthropogenic influences in the fossil record of dinoflagellate cysts. **Balode, Graneli, Tester**, and others discussed possible effects on HAB species of global warming. In my view, this is yet another area where we need to exercise caution, since it is too easy for the public and the press to conclude from these types of studies that certain HAB species will become more abundant and problematic simply because they will grow faster as global temperatures increase. This is where Zhou's emphasis on the complexity of the systems we study comes into play again – that we cannot study these species in isolation. Climate change will affect zooplankton, fish, currents, rainfall, winds, sunlight, and many other factors that are inter-related with HABs. Without detailed analyses of these factors, it is difficult to say whether global warming will decrease or enhance the threat from a given HAB species in a region. Climate change and global warming will continue to be important topics at future HAB conferences, but we need to keep this "big picture" perspective in mind. One forecast that many will agree on is that climate change will drive broad shifts in the distribution of HAB species. This will bring them into areas not historically affected, and thus ill prepared to deal with them. Conversely, this also means that some areas with HAB problems may find those problems disappearing, as species no longer find a region suitable for growth.

HAB13 was a meeting that highlighted the breadth and maturity of our field. I personally am not fond of three concurrent sessions, and could return to two sessions at most for future meetings, with more posters and more poster viewing time. I miss the old days, when we had single sessions, where the chemists sat with the taxonomists and everyone learned about each other's fields. Those days are gone forever, sadly, but perhaps we can keep a semblance of that community feeling when we all meet in Crete in 2010.

I want to express special thanks to the following individuals who provided ideas and in some cases, written text that was useful in both the oral and written versions of this summary: Ian Jenkinson, Per Juel Hansen, Allan Cembella, Uwe John, Pat Glibert, Susanna Minnhagen, Greg Doucette, Gustaaf Hallegraeff, Christopher Bolch, Grant Pitcher, Chris Gobler, Linda Medlin, and Jane Lewis.

This conference was noteworthy for the way it showcased Asian HAB science. Japan has always been a leader in our field, and continues to demonstrate this with a number of papers again at this meeting, including a talk by **Kodama** about the manner in which saxitoxins can be bound by proteins within scallops. Korea also continues to work at the forefront of a number of topics with papers like those of **Park** on *Dinophysis*, by **H.G. Kim** on bloom mitigation, **Jeong** on heterotrophic and mixotrophic dinoflagellates, **C.H. Kim** on *Cochlodinium* cysts, and **Tang** on *Cochlodinium*. But a new highlight was the overall contribution from Chinese HAB scientists. Here I have a personal perspective to offer. In 1992 I made my first trip into China to work with Professor Qi in Guangzhou. At the time, in the entire country, there were only a handful of scientists who worked on algal blooms, and most were taxonomists. Laboratories had almost no equipment or funding, culture collections were non-existent, and the threat from algal toxins was very poorly understood along the vast coastline. In contrast, in Hong Kong we saw a huge range of topics presented by more than 50 Chinese participants. Plenary talks by **Zhou** and **Wu**, and presentations from many others, ranging from large-scale oceanography to genetics, proteomics, toxin chemistry, numerical modeling, taxonomy, toxicology, and physiology. The transformation is truly remarkable, and this meeting made that progress very evident, and drew attention to the many challenges faced by China with its expanding HAB problems.

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GEOHAB issues at 13th ICHA

Several presentations during the 13th ICHA Conference addressed key questions identified within the GEOHAB Core Research Projects. Two papers were presented, from the EU HABIT (Harmful Algal Blooms in Thin Layers) project, one given by **Lourdes Velo-Suárez** on the demonstration of how modelling can explain the disappearance of a *Dinophysis acuminata* bloom through physical dispersion. The other was by **Hazel Farrell** showing the transport of a high density sub-surface thin layer of *Dinophysis acuta* in a coastal jet along the coast of Ireland. On the subject of *Dinophysis*, a presentation by **Beatriz Reguera** showed new findings on *Dinophysis* nutrition. The use of FISH probes to carry out comparative studies on the dynamics of three co-occurring *Alexandrium* species was shown by **Nicolas Touzet**. The importance of comparative studies in ecology was emphasised by **Robin Raine** who compared *Alexandrium minutum* dynamics across Europe, and also demonstrated by **Grant Pitcher** who gave a talk on the effects on HABs of irregularities in the shape of coastlines adjacent to upwelling regions. Upwelling and the prediction of *Pseudo nitzschia* blooms was the topic presented by **Sofia Palma**, who showed a consistency in the time interval between blooms and (weak) upwelling event. Prediction of *Alexandrium* blooms in the estuaries of Brittany was discussed by **Juliette Fauchot** who showed the importance of physical dilution in controlling the timing of blooms there. A colourful and thought-provoking talk was given by Ian Jenkinson who rather graphically gave a lecture on the effects of biological substances such as mucopolysaccharides on the viscous properties on water and hence their effect on small-scale mixing.

Ecology and oceanography were the topics of two plenary lectures, one by **Paul Harrison** who explained how blooms originating on the shelf were maintained by nutrients coming in the Pear River plume. The talk by **Ming Jhang Zhou** focused on eutrophic coastal waters of the East China Sea.

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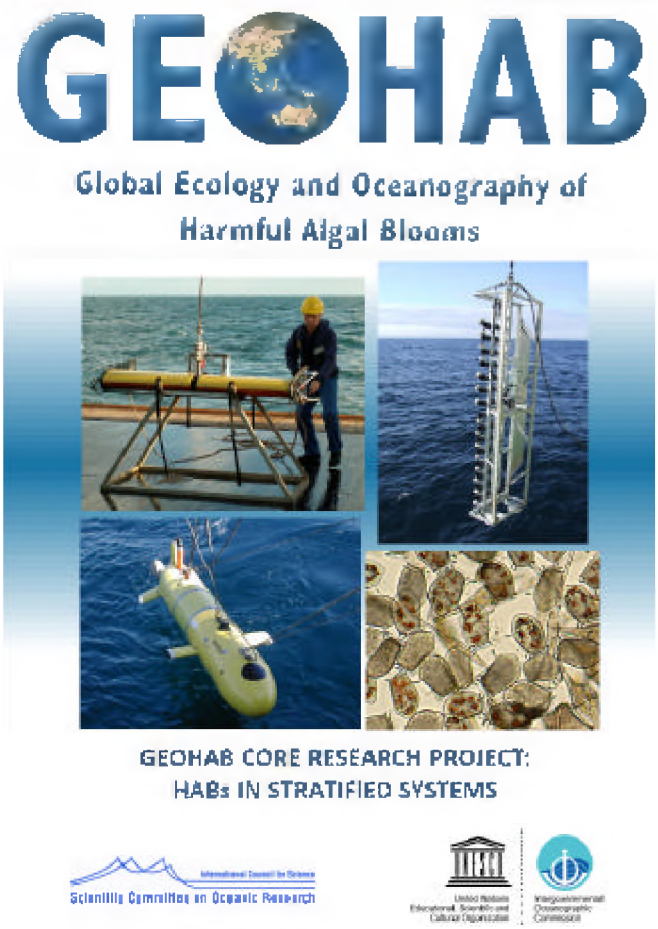
New GEOHAB release

The *GEOHAB Implementation Plan* (GEOHAB, 2003) specifies the formation of Core Research Projects (CRPs) related to four ecosystems types: upwelling systems, fjords and coastal embayments, eutrophic systems and stratified systems. These CRPs are initiated through small, focused open science meetings.An open science meeting on HABs in Stratified Systems was held in Paris, France, 5–8December 2005. The Meeting concentrated on small scale hydrographic features which may be encountered in any of the above mentioned environments. The present report outlines the justification and research priorities for the study of relationships between HABs and stratification, as well as some of the new approaches and advanced instrumentation that may be considered.The following key questions were identified:



What are the relative contributions of biological and physical processes to the initial formation of thin layers in stratified systems?

What are the key processes defining the different strategies that maintain phytoplankton in thin layers?

What are the biological and chemical outcomes of the physical concentration of plankton into high-density thin layers?

What causes a high-density population in a thin layer to collapse?

Download it at www.geohab.info

• Argentina

SEM observations of *Pseudo-nitzschia* from the Beagle Channel: *P. seriata* in the southern hemisphere?

Pseudo-nitzschia seriata (Cleve) H. Peragallo is known as a potentially toxic diatom species [1, 2] and has also been related to domoic acid accumulation in mussels, resulting in the recurrent closure of shellfish harvesting in Prince Edward Island, Canada [3, 4]. Its strong morphological resemblance to *P. australis* Frenguelli has led in the past to common misidentifications and also to great confusion about the worldwide distribution of both species [5]. However, based on an extensive literature survey, it has been noted that *P. seriata* is confined to cold waters of the Northern Hemisphere while *P. australis* is found in the Northern as well as the Southern Hemispheres [6].

Traditionally, the presence of striae composed of 3–5 rows of poroids has been the most distinctive character to discriminate *P. seriata* from *P. australis*, which has only 2 rows of poroids per striae [5, 7]. Nevertheless, this difference is not definite, since it has been demonstrated that strains of *P. seriata* can also show only two rows of poroids in laboratory cultures [2]. As a consequence, the density of poroids per striae seems to be the most reliable character to discriminate between them (4–5 and 6–8 in 1µm in *P. australis* and *P. seriata*, respectively) [8]. Unfortunately, time-consuming examination using electron microscopy is essential to observe this ultrastructural character.

Located in extreme southern South America, the Beagle Channel separates islands of the Tierra del Fuego Archipelago (Fig. 1). The biggest settlement on the channel is Ushuaia in Argentina followed by Puerto Williams in Chile, two of the southernmost settlements of the world [9]. Within the framework of a monitoring program of toxic algae carried out in the eastern sector of the Beagle Channel, we focused on the diversity of *Pseudo-nitzschia* species for the first time in this area. For this study, phytoplankton net samples were examined by phase contrast, DIC and scanning electron microscopy (SEM) following traditional methods. Sampling points included Bay Brown (inside and outside) and Punta Paraná (Fig. 1) where both biological and oceanographic variables have been weakly examined. These areas are usually closed to harvesting each year during the summer, when toxic algal blooms are a serious problem for consumer's health and therefore to the economy of the area [10].

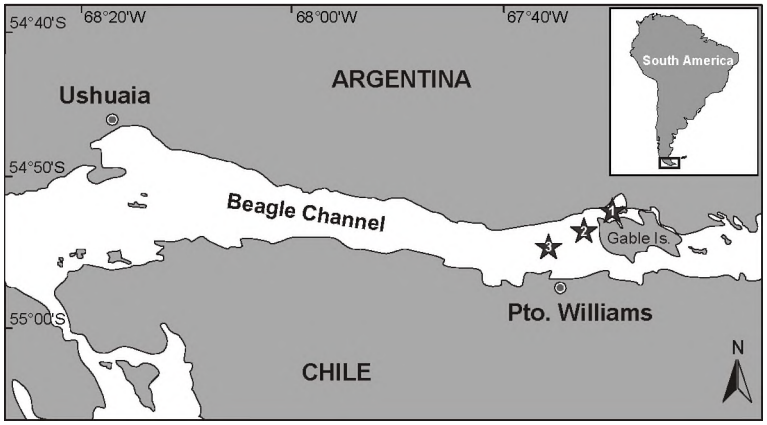


Fig. 1. Map of the study area showing the location of sampling sites in the Beagle Channel, Argentina.

Based on the valve shape and morphometric data (length, width, density of striae and fibulae and the lack of central interspace), light microscopy observations suggested the presence of *P. australis* in the Beagle Channel. This was not too surprising considering that this species is commonly found both in coastal and shelf waters of the Argentine Sea [11–13]. However, subsequent SEM analyses revealed the fine structure of the striae, which disagreed with classical descriptions of *P. australis* [5, 7]. In contrast, it was strikingly similar to unusual specimens of *P. seriata* isolated from Scottish waters [2]. The analyzed specimens showed slightly asymmetrical lanceolate valves with rounded ends (Figs 2a–b), 80.5–103.5 µm long and 7–8.9 µm wide. Interstriae and fibulae were usually distributed in equal number (14–19 in 10 µm) and without a central interspace (Figs 2a–c). The striae were composed of two rows of small poroids (7–8 in 1 µm) (Fig. 2d), but in some cases, a few single poroids (incipient striae?) were visible between them (Fig. 2b).

The observed specimens are in conflict with classical descriptions of both *P. seriata* and *P. australis* (Table 1). The presence of striae mainly perforated by two rows of poroids (typical of *P.*

Table 1. Morphometric summary of specimens observed in the Beagle Channel, Argentina. Data in Fehling et al. (2004) and Hasle & Lundholm (2005) are added for comparative purposes.

Taxa	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Interstriae (in 10 µm)	Rows of poroids	Poroids (in 1µm)
Observed specimens (n = 18)	80.5–103.5	7–8,9	14–19	14–19	2 (+ a few single poroids)	7–8
P. seriata (Hasle & Lundholm 2005)	91–160	4,6–8,0	14–20	14–20	2–more, usually 4	6–8
P. seriata (Fehling et al. 2004)	–	4,6-6	14-20	14-20	2 (+ 1)	6–8
P. australis (Hasle & Lundholm 2005)	75–144	6,5–8,0	12–18	12–18	2	4–5

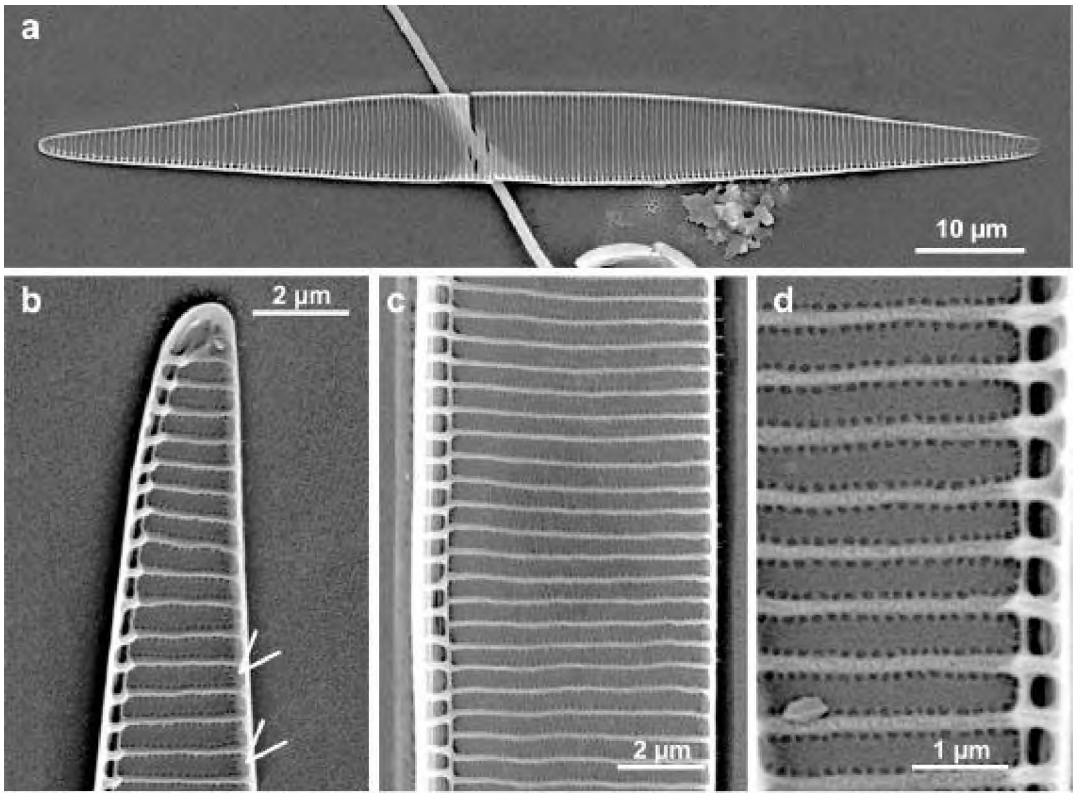


Fig. 2. SEM micrographs of specimens found in the Beagle Channel (Argentina), showing the valve shape, apical and central part details.

australis) but in number of 7–8 in 1 µm (as in *P. seriata*) was rather disconcerting. However, the results are similar to those unusual specimens of *P. seriata* identified in Scottish waters by means of TEM analyses and DNA sequencing [2]. Unlike the traditional description of *P. seriata*, which has 3–5 rows of poroids per striae [5], Scottish strains showed two rows of poroids plus just a few single poroids, or lack a third row visible between the two rows of poroids; whereas a third complete row was only rarely observed [2]. These authors attributed this unusual morphology to the fact that the cells had been kept in culture for approximately six months before the TEM observations. Therefore, our field study could provide additional information supporting the finding of this unusual morphology in natural populations and prompt new questions regarding the morphological differentiation of *P. seriata* and *P. australis*. Moreover, if the identity of specimens collected in the Beagle Channel could be confirmed as *P. seriata* by additional TEM and molecular analyses, the geographical distribution of this species would be greatly extended.

Given the increasing number of semi-cryptic *Pseudo-nitzschia* species being mentioned [e.g. 14–17], we consider that further more detailed morphological investigations in combination with molecular data are required to elucidate the variability and geographical distribution of *P. seriata* and *P. australis*. With this in mind, we are now attempting to establish cultures of these specimens, and have also started to examine other diatom samples from different regions of Argentina for this unusual morphology. Likewise, it would be highly beneficial to work in collaboration with colleagues with previous experience in DNA extraction, amplification and sequencing of species from the genus *Pseudo-nitzschia*.

Acknowledgements

This work is part of a specific agreement between the Government of Tierra del Fuego Province and CADIC, entitled "*Monitoreo de condiciones biológicas y oceanográficas determinantes de floraciones algales tóxicas en la zona de Almanza, Canal Beagle*". It was supported by grants PIP-5603 CONICET and PICT 25509 ANPCyT.

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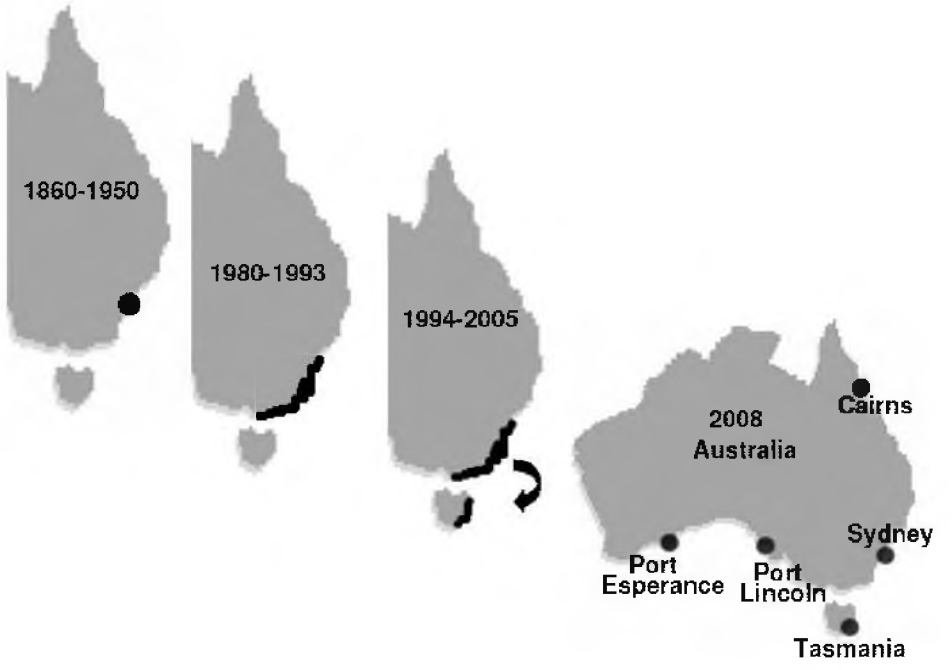
• Australia

Recent range expansion of the red-tide dinoflagellate *Noctiluca scintillans* in Australian coastal waters



Aerial photograph of red Noctiluca *slicks in Parsons Bay, Tasmania (Australia), in March 2002. The circular salmonid fish farm pens accumulated up to 4m thick surface scums of* Noctiluca *acting as an irritant to the fish (photo: J.Marshall, University of Tasmania).*

The large (200–1000 μm diameter) phagotrophic dinoflagellate *Noctiluca scintillans* has long been present in Australian New South Wales coastal waters, with the first report of bioluminescence in Sydney Harbour (34ºS) dating back to 1860 [1]. Dakin and Colefax 1933 [2] observed this species to be a minor component of the phytoplankton of New South Wales coastal waters and Wood 1954 [3] in his extensive Australia-wide net phytoplankton surveys reported this taxon to occur only in Australian east coast estuaries. The first visible *Noctiluca* "red tide" event was not reported until August 1982 in Lake Macquarie, central New South Wales [4]. However, from a rare bloom former in the 1980s, starting in 1993 this organism has developed into one of the most prominent red-tide organisms in Sydney coastal waters, soon after the commissioning of 3 deepwater ocean sewage outfalls off Australia's largest coastal city. El Niño years such as 1997 have exhibited the densest *Noctiluca* blooms [5], which occasionally have forced closures of Sydney public beaches during spring and summer. While *Noctiluca* was virtually absent during a seasonal study of Sydney coastal waters during 1978-79 [6], in a repeat survey in 1997-98 the species was present in 61% of samples [4]. Irregular influxes of *Noctiluca*, most likely carried by the East Australian Current (moving up to 40 km/day), have also been seen in Port Phillip Bay, Melbourne (since 1993) and Tasmania (since March 1994). No *Noctiluca* bloom reports are known from Tasmania (43ºS) prior to this date. A bloom event which apparently started off the East Coast of Tasmania in September 2001 culminated in March 2002 into a significant threat to the salmonid aquaculture industry in Nubeena on the Tasman Peninsula. *Noctiluca* blooms have persisted in Tasmanian waters ever since, notably in the period 2001–2006. While in New South Wales this dinoflagellate proliferates at water temperatures of 19-24ºC, in colder Tasmanian waters it now has established permanent overwintering populations and thrives even in winter months at temperatures of 10-12ºC. We speculate that the gradual warming of East Coast Tasmanian waters (up 1.6ºC in the past 50 years which is 3x the global average warming), associated with a greater influence of the East Australian Current [7], has paved the way for the apparent range extension of this warm-water



Apparent range extension of Noctiluca scintillans *in the Australian region, comparing distribution records in 1860-1950, 1980-1993 (expansion of blooms in the Sydney region), 1994-2005 (range extension into Tasmania), and in 2008 (first reports in Queensland, West Australia and South Australia).*

organism into Tasmanian waters. An example of a similar recent climate-change driven range expansion from New South Wales waters into Tasmanian waters is the spiny sea urchin *Centrostephanus rodgersii* [8]. In the last decade some 36 species of marine fish have shown similar changes in distribution in Tasmania, including range shifts further south and the appearance of new fish species never previously recorded in the area.

Finally, in the year 2008, the first tropical red *Noctiluca* bloom was reported from Cairns Harbour in Queensland in March, followed by bloom reports from Port Esperance, Western Australia, and Port Lincoln, South Australia, in May-June. The latter two events are most likely associated with an extension of the Leeuwin Current. The organism had never previously been reported in Queensland, Western Australia (monitored since 1993 [9]) nor Southern Australia (monitored since 1994 [10]). This "climate-change hotspot" is now increasingly being monitored by sophisticated Continuous Plankton Recorder technology (AusCPR) operating between Brisbane, Tasmania and Antarctica [11].

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In Memoriam

Ralph Lewin of the Scripps Institute of Oceanography, known to some as 'the father of green algae genetics', and maybe best known for his work on prochlorophytes, died last month. As well as his science, he left us a collection of verses. HAN readers may enjoy the two printed here.

Toxic Blooms

Toxic blooms, toxic blooms
Vie with smog and sonic booms,
Bigger, thicker, redder tides
Taint the sea with plankticides.
Toll the tocsins! Tides of doom!
Woe to us the bloody bloom.

Neurotoxicants attack
Herrings in the Skagerrak,
Decimating fishes that
Used to throng the Kattegat,
Dooming them to dismal fates:
Death by dinoflagellates.

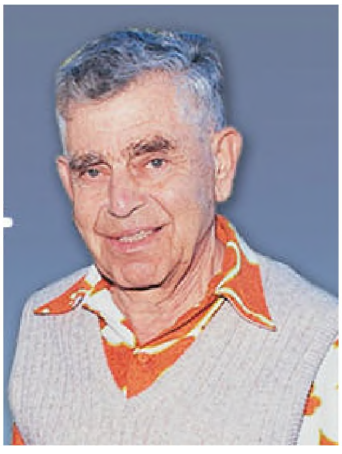

Photo from the Scripps News Web site (http://scrippsnews.ucsd.edu)

Phycology by fiat

The blue-greens are not algae any more … any more
It seems that they're not lost but gone before.
By a stern decree despotic,
Since they're all prokaryotic
The poor blue-greens can't be algae any more.

Euglena's not an alga any more … any more,
Whatever people thought of it before.
Its form is somewhat plastic
And its pellicle's elastic,
So *Euglena*'s not an alga anymore.

Spirogyra's not an alga any more … any more,
Though it passes nuclei from pore to pore.
Since it hasn't swimming gametes
It's reclassified with *Trametes*.
Spirogyra's not an alga anymore.

Red algae are not algae anymore … any more
Though the reddist weeds abound on every shore.
Since their microsporulation
Is devoid of flagellation,
The red algae can't be algae anymore.

You cannot regard an alga as a plant … as a plant,
However much you want to, you just can't.
If it's not too late to switch
You should throw away your Fritsch,
For the algae are not algae anymore.

• Angola

Okadaic acid in the clam *Semele proficua* in Luanda Bay, Angola

Diarrhoetic Shellfish poisoning (DSP) is a gastroenteritis caused by ingestion of bivalve molluscs contaminated with dinoflagellate toxins. Japan was the first country to report this toxin [1], but DSP is now known in many parts of the world. The areas most affected are Japan and Europe. Studies have shown that the DSP syndrome is usually triggered by dinoflagellates of the genus *Dinophysis*. The dominant symptoms, which typically develop within 4 hours and may last up to 3 days, include diarrhoea, nausea, vomiting and abdominal pain.

In Africa, the first DSP episode was recorded on the coast of South African in 1991, and attributed to *Dinophysis acuminata* [2]. In the Austral region, including the coasts of Namibia [3] and Angola [4], various species of *Dinophysis* have been recorded, but shellfish in these two countries have not previously been tested for DSP. Here we report the occurrence of DSP toxins in shellfish in Luanda.

From 6 June to 10 December 2007, a pilot programme to detect biotoxins in bivalve molluscs was conducted in Luanda Bay, where there are large landings of shellfish from natural banks, consumed only locally. Twenty samples of *Semele proficua f. radiata*. (common name *ameijoinha*) were bought from artesanal fishermen and frozen until analysis.

Analyses of whole body extracts of the *Semele* samples were run in the Biotoxin Laboratory of the Instituto Nacional dos Recursos Biológicos (INRB) in Lisbon, Portugal, following the LC-MS method [5]. Okadaic acid (OA) was detected in eight samples (Table 1). Okadaic acid was present mainly in August and September, with a maximum concentration on 15 August (7.4 µg/100g). Other foci were recorded on 4 July and 15 October.

Rangel & Silva [4] noted that phytoplankton density is highest on the Luanda coast when the weather is changing from winter to summer (August to October), coinciding with the highest okadaic acid levels measured. Luanda Bay is affected by strong anthropogenic pressures including eutrophication.

Table 1. Okadaic acid concentrations (µg/100g) in Semele proficua *samples Detection limit: 0.8 ± 0.2 µg/100g; quantification limit: 2.7± 0.8 µg/100g.*

Sampling date	OA (µg/100g)
06.06.07	0
20.06.07	0
04.07.07	4.8
18.07.07	0
02.08.07	0
08.08.07	2.9
15.08.07	7.4
20.08.07	6.9
27.08.07	2.1
10.09.07	2.5
18.09.07	0.0
24.09.07	5.6
01.10.07	0.0
08.10.07	0.0
15.10.07	2.2
22.10.07	0.0
19.11.07	0.0
26.11.07	0.0
03.12.07	0.0
10.12.07	0.0

Dinophysis known on the Angola coast include *D. acuminata, D. caudata, D. fortii, D. norvegica* and *D. tripos* [4]. *Prorocentrum*, which also produces DSP toxins, is present in Luanda waters too. Although OA was detected, we unfortunately did not determine which species were present in the water on these dates, so we cannot assign the presence of this toxin to either of these genera.

Acknowledgements

To the Instituto Nacional de Investigação Pesqueira that supported travel and accomodation in Portugal, to the Biotoxin Laboratory of INRB/ IPIMAR, and to Ms Alcina and Ms Delfina for help with sample preparation.

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Proceedings of the 12th International Conference on Harmful Algae have recently been released. Copies of this publication can be ordered through the IOC site at the following link:

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RANDOM SAMPLES

HAB13









Photos from the 13 ICHA Web site (www.hab2008.hk) and courtesy of L. Escalera, B. Lapointe, I. Baula, L. Velo & D.M. Anderson.

ISSHA President's Corner

The International Society for the Study of Harmful Algae (ISSHA) sponsored the 13th International Conference on Harmful Algae (ICHA), held in Hong Kong (3-7 November 2008), and celebrated there on 6th November its Sixth General Assembly.

Pat Tester (President) opened the meeting. The minutes from the previous assembly were approved and council members summarized the activities and achievements of ISSHA in the previous two years: Nina Lundholm (Treasurer) presented the financial statement, covering the period September 2006 to October 2008, and detailed expenditures and funds raised from member's fees, book sales, and the auction; Don Anderson (Travel Awards Committee) thanked the Conference Organizers, the Society, and UNEP-MAP for providing partial financial support to 26 young scientists (19 predoctoral, 2 master and 5 post docs) from 16 countries, who presented oral and poster communications at the Conference. Øjvind Moestrup (Conference Organization Committee) announced the recent publication of the proceedings from the previous conference (12th ICHA, Copenhagen, September 2006). These include 111 communications accepted from 140 submissions reviewed.



Karen Steidinger, Yasumoto Life Time Achievement Award (bottom left), and Iris Baula, Honorable Mention for Student Oral Presentation (top left) with supervisor and colleagues.

Beatriz Reguera (Achievement Awards Committee) reviewed the procedure followed by the Committee to examine nominations, by ISSHA members, for the *Yasumoto Lifetime Achievement Award* and the *ISSHA Young Scientist Award*. The same committee had the task of securing double evaluations for 56 students who competed for the *Maureen Keller Award* for best oral and poster communications.

2008 was a election year in ISSHA with a major renewal of its Executive and Council. Results of the elections were presented by Karen Steidinger (Elections Committee). Three members of the previous executive— first vicepresident Gustaaf Hallegraeff (Australia), second vicepresident Beatriz Reguera (Spain), now new President, and Treasurer Nina Lundholm (Denmark)—were elected to continue serving the society in the new executive, with the newly elected second vicepresident Jennifer Martin (Canada) and Secretary Karin Rengefors (Sweden), and past president Pat Tester (USA). The whole Council was renewed: new members are: Lorraine Backer (USA), Eileen Bresnan (Scotland), Marta Estrada (Spain), Martha Ferrario (Argentina), Esther Garcés (Spain), Kin Chung Ho (China), Rita Horner (USA), Ichiro Imai (Japan),



Members of the old and new ISSHA Executive and Council pose at the Jumbo Conference Banquet. From left to right, front row: Ichiro Imai, Gustaaf Hallegraeff, Beatriz Reguera, Pat Tester, Karen Steidinger, Rita Horner, K.C.Ho; back row: Lincoln MacKenzie, Greg Doucette, Hak-Gyoon Kim, Henrik Enevoldsen, Don Anderson, Ian Jenkinson, Øjvind Moestrup, Nina Lundholm, Barrie Dale, Jennifer Martin, Jane Lewis and Eileen Bresnan.



The last Conference Proceedings presented by Øjvind Moestrup.

Ian Jenkinson (France), Anke Kremp (Finland), Lincoln McKenzie (New Zealand), Clarisse Odebrecht (Brazil), Jong-Gyr Park (Korea) and Carmelo Tomas (USA).

Henrik Enevoldsen (*Ad hoc* Committee on special projects) related future plans concerning the HAB-MAP project, that will be linked to the Encyclopedia of Life (EoL) initiative under the name HAIS (Harmful Algae Information System). The proposal is to map the biogeography of all species which cause toxic outbreaks—ASP, AZP, CFP, DSP, NSP, PSP, YTX, spirolides, fish kills—with references. The IOC Taxonomy Reference List will be merged into the world register of marine organisms (WoRMS/OBIS).

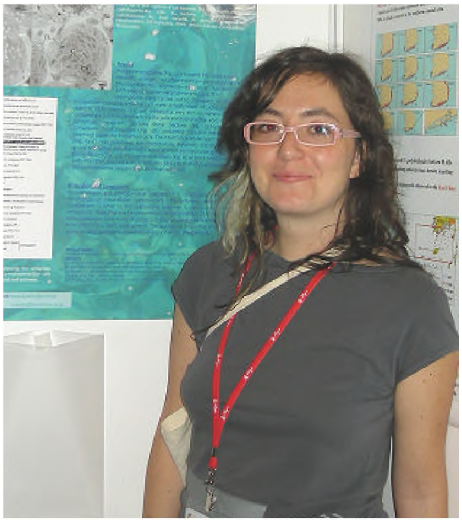
All ISSHA members present welcomed the bid by Hak-Gyoon Kim (Pukyong National University) and colleagues to host the 15th ICHA (October 2012) in Changwon City (Busan, Korea).

Following the success of the first ISSHA auction (Copenhagen 2006) to raise funds for the society, a second one was arranged when the assembly adjourned. Once again, Barrie Dale thrilled the audience with hilarity and his sense of ***mise en scène.***

During the 13th ICHA closing ceremony, details of the 14th ICHA venue (Hersonissos, Crete, Greece, November 2010) were presented by Kalliopi Pagou (Hellenic Centre for Marine Research, Athens).

All achievement awards were announced during the luxurious conference banquet that took place on 7th November at the *Jumbo Kingdom Floating Restaurant* in main Hong Kong Island.

The *Yasumoto Lifetime Achievement Award* was given to Karen Steidinger (Fish and Wildlife Research Institute, Florida, USA), for her contributions to dinoflagellate taxonomy, biology and ecology. Karen is a warm, generous and expansive character and has made major contributions to the HAB community with her articles, books, projects and scientific management, and her frequent participation in international courses on advanced taxonomy. Her work is recognized by taxonomists in the new genus *Karenia*—formerly *Gymnodinium breve*—a nuisance dinoflagellate species in the Gulf of Mexico and one of Karen's main research interests. *Karenia* segregates a group of naked dinoflagellates with distinct characteristics from those of the old *Gymnodinium* complex. The *Young Scientist Award* was given to Rosa Figueroa. Rosa finished her PhD studies (IEO-Vigo, and ICM-Barcelona, Spain; University of Lund, Sweden 2001-2005) three years ago, and has already made a substantial contribution to the description of life histories and survival strategies of a good list of dinoflagellate species.



Laura Escalera, Best Student Poster.

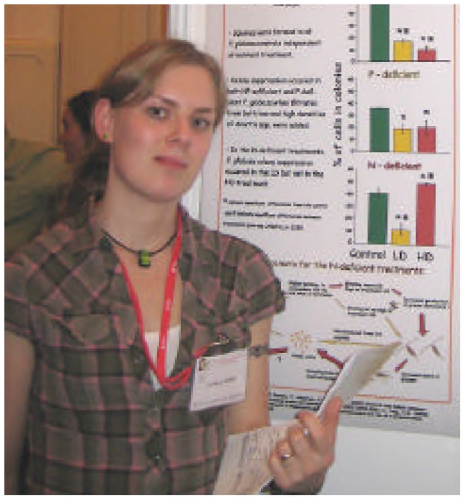


Shi Hong, Best Oral Presentation with John Hodgkiss.



Rosa Figueroa, Young Scientist Award.

Awards for the best oral presentations were given to Shi Hong[1] (University of Xiamen, China) and Iris Baula[2] (University of the Philippines), and for best posters to Laura Escalera[3] (IEO-Vigo, Spain) for work carried out at University of Hiroshima, and to Veronica Lundgren[4] (University of Kalmar, Sweden).



Veronica Lundgren, Honorable Mention Student Poster.

1. H. Shi & J. Zhang. *Clone and identify mazEF: a toxin-antitoxin module in cyanobabteria* Microcystis aeruginosa
2. I.U. Baula, R.V. Azanza & Y. Fukuyo. *Modern Dinoflagellate Cysts From a Mariculture Area in Pangasinan, Northwestern Philippines*
3. L. Escalera, S. Yoshimatsu, K. Takishita, K. Koike & K. Koike. *Cyanobacterial endosymbionts in the benthic Dinophysioid dinoflagellate* Sinophysis canaliculata*?*
4. V. Lundgren & E. Granéli. *Grazer induced colony formation in* Phaeocystis globosa *(Prymnesiophyceae): Influence of different nutrient conditions*

B. Reguera, ISSHA President.
Email: beatriz.reguera@vi.ieo.es

Future events



Harmful Algae News

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APRIL 2009

IPHAB meets in 2009

The IOC Intergovernmental Panel on HABs will meet 22-24 April 2009 at UNESCO Headquarters in Paris. The Panel is the forum to discuss and prioritise international activities and cooperation on HAB.

The 2009 meeting will in particular discuss capacity building; the GEOHAB Research Programme; biotoxin regulation and human health; the Harmful Algae Information System and the International Ocean Data Exchange; HAB observations and their inclusion in GOOS Regional Alliances; harmful algal events, coastal zone management and linkages with coastal eutrophication; and formulation/endorsement of specific objectives for regional activities.

Download invitation letter and more detail at www.ioc-unesco.org/hab

Erratum to 'Padmakumar, K.B. *et al.* 2008. *Harmful Algae News 27: 11-12'.*

In this article, authors' addresses have been incorrectly printed and should read as follows:

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The editor apologizes to authors and readers for the inconvenience caused by this error.

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

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First Report of *Dinophysis acuta* in culture

Several species of the genus *Dinophysis* associated with production of DSP toxins bloom every year during spring-summer in coastal waters of Huelva (Southwestern Andalucía, Spain). *Dinophysis* cf *acuminata* and *D. acuta* are the main species responsible for harvesting closures of natural shellfish beds of *Donax trunculus* and other commercially important species in the region. Cultures of *Dinophysis* may contribute to a better knowledge of their ecophysiology, life cycle and toxin production.

Following the discovery of *Myrionecta rubra* as an optimum prey for mixotrophic species of *Dinophysis* [1], attempts to cultivate our local species started at the *Laboratorio de Control de Calidad de los Recursos Pesqueros de Andalucía*. Cultures of *D.* cf *acuminata* were established in 2007 after overcoming manipulation and culture problems associated with the culture of its prey, *M. rubra*, fed with cryptophytes [2]; both the ciliate and the *Teleaulax*-like cryptophyte were isolated from our local waters. Cultures of *D. fortii* [3], *D. caudata* [4] and *D. infundibulus* [5] were recently reported by other authors. Here we report, for the first time, the establishment of cultures of *Dinophysis acuta* that have been maintained in our laboratory since summer

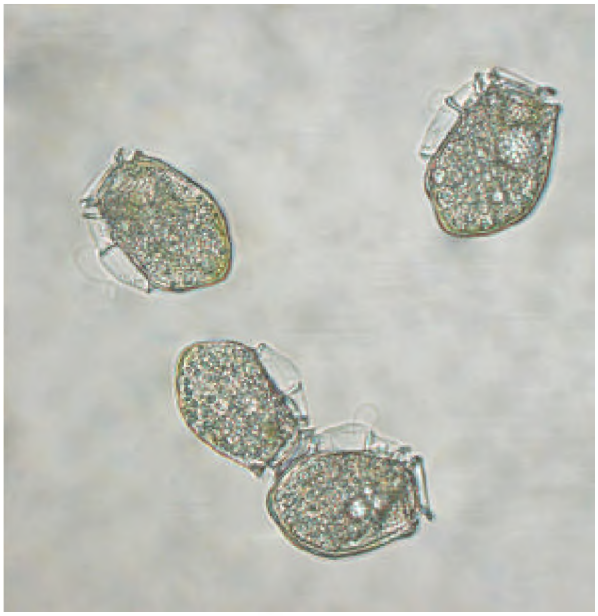

Fig. 1. Laboratory culture of Dinophysis acuta *under prey (*M. rubra*) limitation.*

(Cont'd on p.2)

Long term maintenance of *Dinophysis acuta* in co-culture with *Chaetoceros* sp.

Here we report on a series of experimental settings aiming at culturing *Dinophysis acuta*. Cells of *D. acuta* were isolated in December 2005 from vertical net hauls (20 µm) collected from Lisbon Bay, Portugal (38º 41.34' N; 9º 24.54' W).

In the laboratory, single cells of *D. acuta* were isolated by micropipette under an inverted microscope, and transferred to 4 well 2-ml culture plates (NUNC ref. 176740) with the following media: 1) filtered seawater from the isolation site; 2) aged autoclaved seawater from the isolation site; 3) f/2-Si. In addition, cells of a non-chain forming *Chaetoceros* sp., abundant at the time of isolation, were added to two of the wells in the above experimental settings. Cultures of *Chaetoceros* sp. were also established for further use and were regularly added to the *Dinophysis* cultures.

All cultures were maintained in a culture cabinet at 15ºC±1ºC (Aralab Fitoclima 750E), with overhead illumination of 20 µmol photons · m 2 · s 1, supplied with cool white fluorescent lamps, and a light:dark cycle of 14:10h. Cells of *D. acuta* were transferred fortnightly in groups of 6 cells/well.

Of all conditions tested, only cells of *D. acuta* maintained with autoclaved aged seawater, with addition of *Chaetoceros* sp., lasted until August 2006 (9 months). In cultures with f/2-Si *Chaetoceros* sp. always overgrew *Dinophysis*. The maximum number of *D. acuta* cells/well recorded after 15 days was 16. Immediately after isolation, cells had yellow brown pigmented chloroplasts (Fig. 1a); these faded with time. After 2 months in culture, small *D. dens*-like cells (Fig. 1b) were observed. When *D. dens* cells were transferred to new medium, they were observed to divide but never lasted more than 3 transfers. Only transfers of *D. acuta* cells and of mixed groups of *D. acuta* and *D. dens* allowed higher transfer numbers. When only normal-sized cells were transferred, eventually a mixed culture would develop, and small cells would tend to outnumber *D. acuta*. It is not clear whether the increase in small cells was mainly due to small cell division or to the progressive conversion of *D. acuta* to *D. dens* as suggested by the observed

(Cont'd on p. 2)

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(Cont'd from p. 1)

2008.

Individual cells of *D. acuta* were isolated from samples collected in the course of weekly sampling of the Andalucian Monitoring Programme for the control of sanitary conditions in shellfish production areas in Huelva (Andalucía). *D. acuta* was grown in L1 medium (without silicates), at 33 psu, 18.5°C and a 14:10 L:D cycle. *Myrionecta rubra* was added as prey every 2-3 days. Both the ciliate and the cryptophyte cultures were kept at the same temperature and with the same culture medium as the dinoflagellate.

D. acuta grew well with *Myrionecta* prey, reaching concentrations above 10^5 cell · L^{-1}. Under starvation, division stops, but the cells are able to survive for several months.

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(Cont'd from p. 1)

depauperating divisions described by Reguera et al. [1] (Fig. 1c). No mating pairs were ever observed. Tetrads of cells aligned with their ventral and dorsal margins in parallel to each other and similar to those illustrated from cultures of *Dinophysis fortii* [2] were recorded in July 2006 (Fig. 1d). The nature of these tetrads is still unresolved.

No direct evidence of *Dinophysis* feeding on *Chaetoceros* sp. was observed. The possible ingestion of small bacteria-like particles was observed by immobile cells on the bottoms of the wells that generated trapping currents with the cingular flagellum. However, this was common in all the experimental settings, and does not account for the much longer survival of cells in aged autoclaved sea water with *Chaetoceros* sp. The same experimental setting described above was used for cells of *D. fortii, D. caudata* and *D. acuminata*, but the maximum time in culture was 5 months. All species eventually produced small cells and in contrast to *D. acuta* the engulfment/conjugation process was observed. Tetrads were also observed in *D. fortii.*

Since Park et al. [3] reported the cultivation of *D. acuminata* feeding on *Myrionecta rubra*, other species of the genus have been successfully established in cultures with the same prey. To our knowledge this is the first report of *D. acuta* kept in culture conditions for 9 months and of other *Dinophysis* species for more than 5 months without feeding on *M. rubra*. These results suggest *Chaetoceros* sp. may act as a possible nutritional source for *D. acuta,* but further work is needed to better clarify the nature of this interaction.

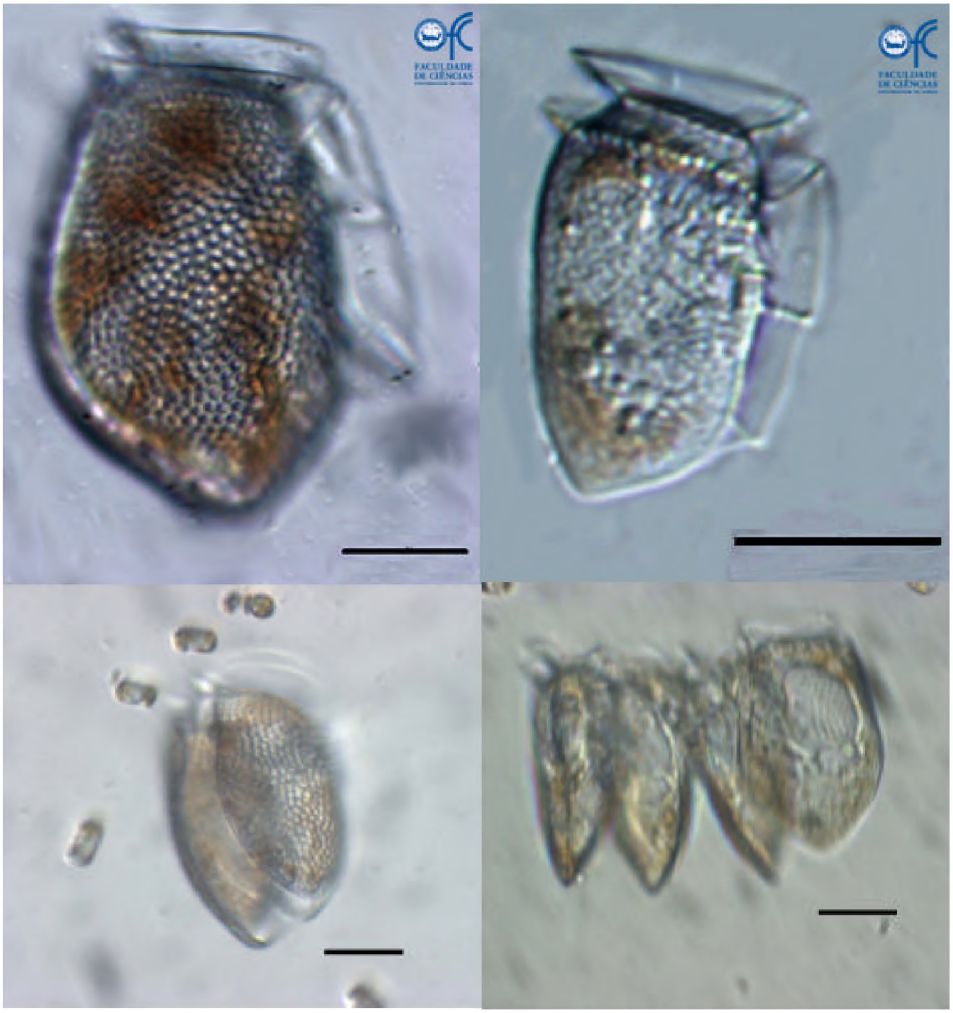

Fig. 1. Different stages of D. acuta*: a) Normal-sized vegetative cell and b) Small* D. dens*-like cell (both in http://planktonnet.awi.de/); c) A dividing pair leading to smaller-sized cells; d) A tetrad of uncertain nature. Scale bar = 20 µm.*

Acknowledgements

To Beatriz Reguera and Teresa Moita for their support and enthusiasm. This work was funded by project FCT-HABCOL—Marine HAB culture COLlection: a critical tool for phytoplankton studies—(PDCT/MAR/60086/2004)

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• France

Chrysochromulina outbreaks, and large species in French Atlantic waters



Fig. 1. Study areas during the cruises (blue = HABIT 2006, grid pattern = RAMVIL).

Outbreaks of *Chrysochromulina* spp. are not unusual in French Atlantic waters from spring to autumn, and densities can exceed 10^7 cells L^{-1} in spring. In 2008, such a bloom was observed south of Brittany during the RAMVIL cruise (31 May - 05 June) (Fig.1). Cells are usually small in size (only a few microns) but since 2006, large ones have been observed. They measured 14 µm to 20 µm long with a rather short haptonema. They were roundish to heart-shaped or with an irregular outline (Fig. 2). Moreover, some of them were sheathed by a meshwork pattern visible in light microscopy (Figs. 2B–C). From a sample of July 2006 collected during the HABIT 2006 cruise (Fig. 1) and containing numerous large cells, a preliminary examination of scales by TEM has been made. The major type of scale (0.8-1.2 x 0.6–0.9 µm) is round to oval in shape and patterned with radiating rectangular perforations arranged in 7-9 concentric circles (Fig. 3A). But much larger scales (3.4-3.7 x 2.7–2.9 µm) were found with a faint monomorphic surface patterning, a distinct rim (0.13 µm), and a cruciform centre (Fig. 3B). The former type resembles that of *Chrysochromulina* sp.7 described by LeRoi & Hallegraeff [1], while the latter, larger scale may belong to the outer meshwork, interpreted as an alternate stage of the complex life cycle [2, 3]. Further observations are needed to identify these large species that are not currently recorded by the monitoring, but are able to dominate the genus in summer or autumn waters with a potential threat of toxicity.

Acknowledgements

The authors acknowledge the scientists and crew of R/V *Thalassa* and *Côtes de la Manche* and especially Drs B. Reguera, R. Raine and P. Gentien.

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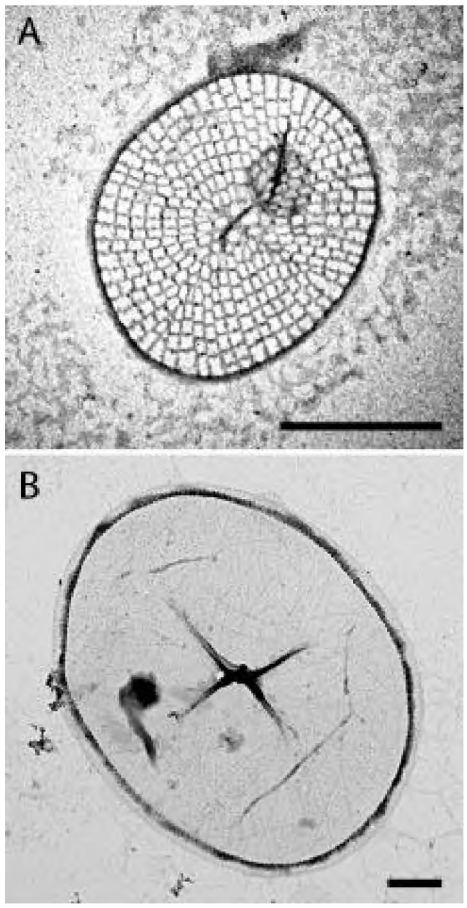

Fig. 3. TEM micrographs of Chrysochromulina *scales. (A) A scale with radiating rectangular perforations arranged in concentric circles. (B) A large scale with a distinct outer rim and a cruciform centre. Scale bars = 0.5 µm.*

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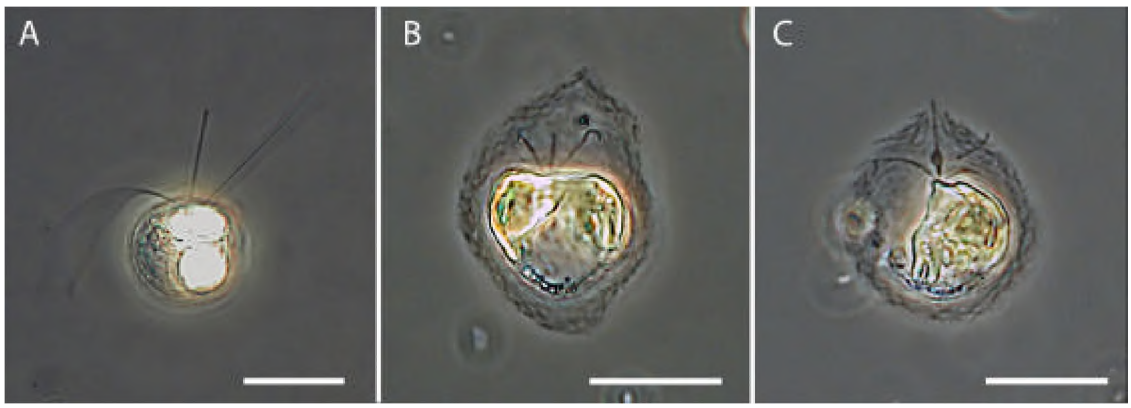

Fig. 2. LM micrographs of living large cells of Chrysochromulina. *(A) A round cell. (B, C) Two other shapes of cells with an outer meshwork. Scale bars = 20 µm.*

• Italy

Ostreopsis cf. *ovata* on Abruzzo coast, W Adriatic

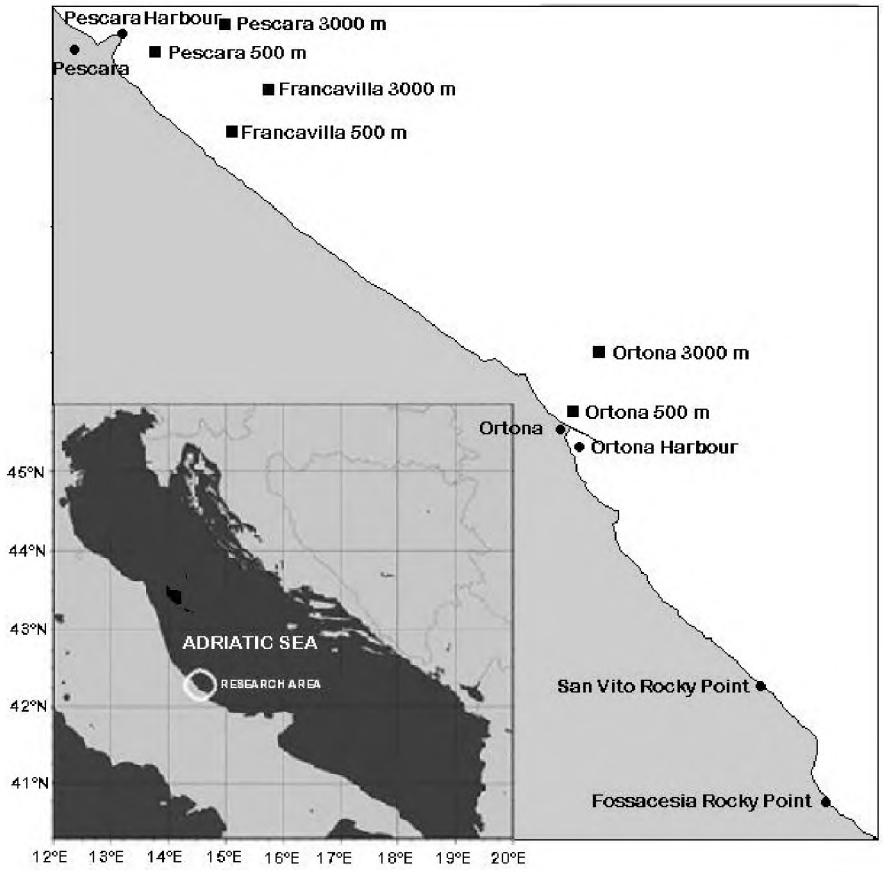

Fig. 1. Research Area.

Ostreopsis belongs to the family Ostreopsidaceae: cells are narrow, ovate in dorso-ventral view with biconvex, drop-shaped theca, scattered pores and eight sulcal plates [1, 2]. The life cycle includes a resting stage, probably a hypnozygote [3]. This species is occasionally planktonic but generally benthic on macroalgae, rocks, sediment or detritus aggregates, and forms a mucilaginous matrix within which the solitary cells can move [4]. *Ostreopsis ovata* produces palytoxin-like compounds [5]. The production of putative palytoxin with other palytoxin-like compounds by *Ostreopsis ovata* from Italy has recently been confirmed [6].

Blooms of *O. ovata* can cause hypoxia, anoxia and benthic invertebrate kills by forming mucilaginous layers hosting thousands of cells covering both biotic and abiotic substrates, as occurred on the Tuscany coast in 1998 [7]. The presence of *O. ovata* in the Mediterranean Sea is traced back to the 1970s on French coasts. It was first detected on the western coast of Italy (Tyrrhenian Sea) in the 1980s [8]. In the last decade, massive blooms of this species have become more frequent, above all in the Tyrrhenian and Ligurian Seas, causing death of benthic organisms and human health problems such as respiratory difficulties and skin irritation [9].

O. ovata was first detected in the southern Adriatic, and later in northern areas, off Ancona and Trieste [5], and on the Abruzzo coast last summer. Here we record the presence of *O.* cf. *ovata* in Abruzzo coastal waters.

Pump samples, 0.5 m below the surface, were taken at six stations located 500 m and 3000 m from the coasts of Pescara, Ortona and Francavilla (Fig. 1). Samples were also collected at these stations with a 10 mm mesh phytoplankton net. Monthly sampling also took place inside Pescara and Ortona harbours and along the rocky banks of Chieti Province (San Vito and Fossacesia), during June, July and August 2008. Stations inside harbour areas and on rocky banks were selected to localize epiphytic and epibenthic species like *O. ovata*. In the harbour areas and on the rocky banks, samples were taken only at 0.5 m below the surface, without a net. But more accuracy was obtained by taking samples in areas rich in macrophytes such as *Ulva lactuca*.

Observations were made by light microscopy (ausJENA Telaval 3) at 200x, 400x and 1000x magnification (Figs. 2 a & b). Epifluorescent microscopy was used for plate tabulation (Zeiss Axiophot microscope at 200x, 400x and 1000x magnification) following the Calcofluor method [10] using fluorescent brightener 28 (Sigma – Aldrich) (Figs. 3 a & b). For SEM

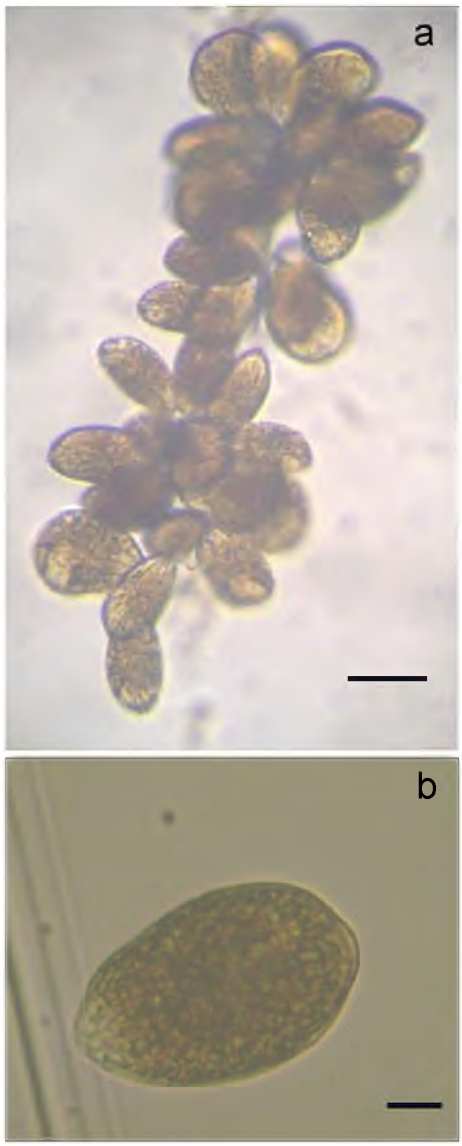

Fig. 2. Light microscopy photographs Ostreopsis *cf.* ovata*: a) cells stuck to Petri plate, 200x, scale bar = 50 µm; b) single cell where is possible to observe the drop-shaped theca, 1000x, scale bar = 10 µm.*

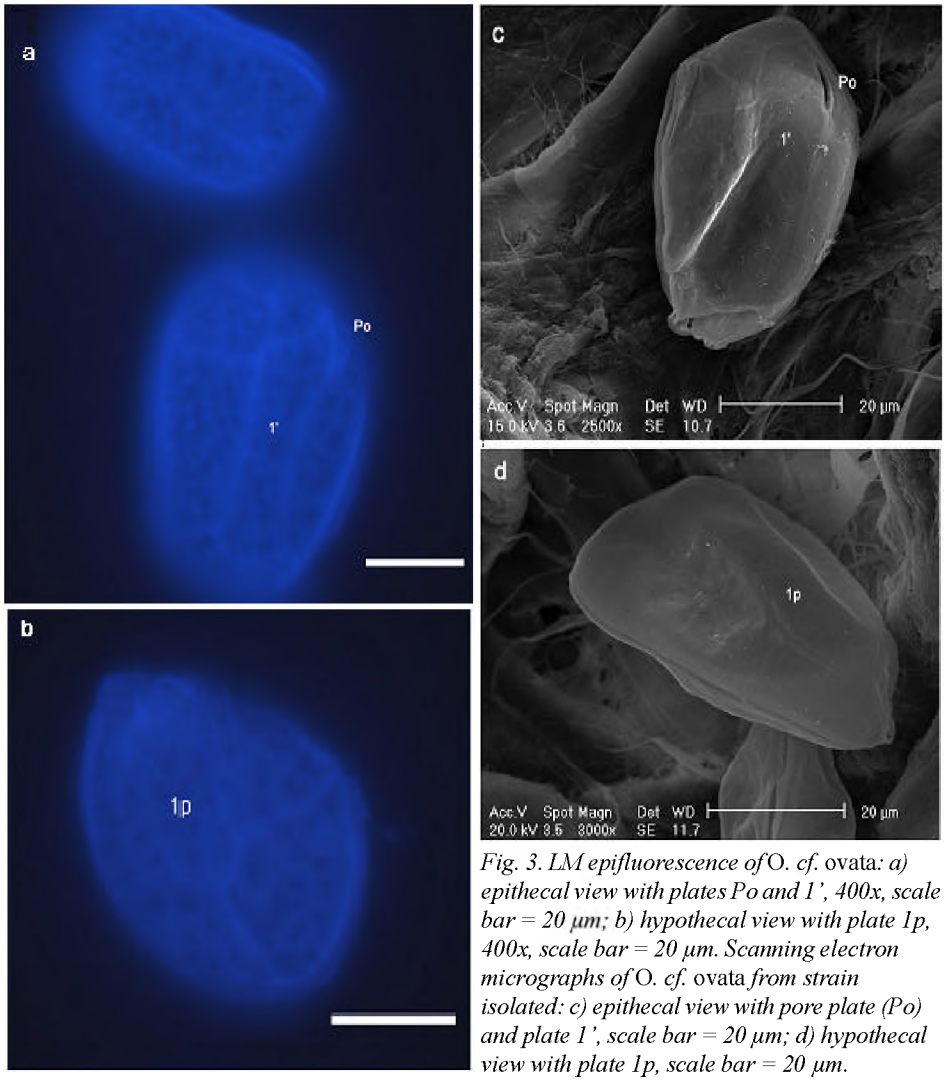

Fig. 3. LM epifluorescence of O. *cf.* ovata*: a) epithecal view with plates Po and 1', 400x, scale bar = 20 µm; b) hypothecal view with plate 1p, 400x, scale bar = 20 µm. Scanning electron micrographs of* O. *cf.* ovata *from strain isolated: c) epithecal view with pore plate (Po) and plate 1', scale bar = 20 µm; d) hypothecal view with plate 1p, scale bar = 20 µm.*

analysis of *O.* cf. *ovata*, in particular of *O.* cf. *ovata* isolated from culture, cells were fixed in 4% formaldehyde. After storage for three days at 4°C, cells were washed 3X in distilled water, dehydrated in alcohol series up to 100%, dried with CO_2 critical point (CPD), sputter coated with Au/Pd and examined on a Philips XL–30–CP electron microscope [12] (Figs. 3c & d).

Cells of *O.* cf. *ovata*, were ovoid to oblong (Figs. 2 a & b) with many golden chloroplasts. Live cells swam in semicircular movements, but mostly remained attached to a substrate (macrophytes or Petri dish). Figure 3 clearly shows the pore plate (Po) and plate 1' in epithecal view and plate 1p on the hypotheca, so, morphologically this species can be assigned to *O.* cf. *ovata*. But as this species has very variable dimensions, only molecular characterization will definitively resolve the taxonomy.

Ostreopsis cf. *ovata* first appeared on August 27th 2008 in Ortona harbour and on the rocky Fossacesia bank, especially on *Ulva lactuca*. Densities in surface samples at these two sites were 40 cells L^{-1} and 3.6 x 10^3 cells L^{-1} respectively. Chemical-physical parameters for the two stations were pH 8.37 and 8.48; water temperature 27 and 28°C; psu 35.80 and 36.30; dissolved oxygen 6.6 and 6.4 mg L^{-1}; the species was absent at other stations, but other toxic and potentially toxic species were present in these monthly samples.

Together with *O.* cf. *ovata*, we also observed other toxic and potentially toxic phytoplankton. In the samples from Pescara and Ortona harbours, and San Vito and Fossacesia banks, only *Prorocentrum lima* and *Fibrocapsa japonica* have been observed. *Prorocentrum lima* was present in June 2008, as in summer 2007 [14] in Ortona harbour and on San Vito bank. Cell densities in surface samples were 1.2 x 10^2 cells · L^{-1} and 20 cells · L^{-1} respectively. *Fibrocapsa japonica* was present only in July 2008 and only inside the harbour areas. Cell concentrations in surface samples were 1.0 x 10^3 cells · L^{-1} and 5.0 x 10^2 cells · L^{-1} respectively. These two species were absent at the 500 m and 3000 m coastal sampling stations. But other toxic and potentially toxic species were observed during June, July and August 2008 (maximum concentrations in 0.5 m net samples): *Prorocentrum minimum* in June 2008 at Francavilla 3000 m from coast (4.8 x 10^2 cells · L^{-1} and 11.0 x 10^3 cells · L^{-1} respectively); *Pseudo-nitzchia* in July 2008 at Ortona 3000 m from coast (8.1 x 10^3 cells · L^{-1} and 44.0 x 10^4 cells · L^{-1} respectively); *Dynophysis sacculus* in August 2008 at Pescara 500 m from coast (20 cells · L^{-1} and 5.0 x 10^2 cells · L^{-1} respectively)

Additional studies are needed to observe the periodical presence of *Ostreospis* cf. *ovata* and other toxic and potentially toxic species along the coasts, inside harbours and on rocky banks of the Abruzzo Region.

Acknowledgements

We thank Dr. S. Teodori and Dr. A. Teodori for help; Dr M. Pompei for comments and discussions.

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• Argentina

SEM observations of *Pseudo-nitzschia* from Beagle Channel: *P. seriata* in the southern hemisphere?



Fig. 1. Map of the study area showing the location of sampling sites in the Beagle Channel (Argentina). Images: Google Earth.

Pseudo-nitzschia seriata (Cleve) H. Peragallo is known as a potentially toxic diatom species [1, 2], and is related to domoic acid accumulation in mussels, resulting in the recurrent closure of shellfish harvesting in Prince Edward Island, Canada [3, 4]. Its strong morphological resemblance to *P. australis* Frenguelli has led in the past to common misidentifications and to confusion about the worldwide distribution of both species [5]. However, based on an extensive literature survey, it has been noted that *P. seriata* is confined to cold waters of the Northern Hemisphere while *P. australis* is found in both Northern and Southern Hemispheres [6].

Traditionally, the presence of striae composed of 3–5 rows of poroids has been the most distinctive character to discriminate *P. seriata* from *P. australis*, which has only 2 rows of poroids per striae [5, 7]. Nevertheless, this difference is not definite, since strains of *P. seriata* can also show only two rows of poroids in laboratory cultures [2]. As a consequence, the density of poroids per striae seems to be the most reliable character to discriminate between them (4–5 and 6–8 in 1 µm in *P. australis* and *P. seriata*, respectively) [8]. Unfortunately, time-consuming examination using electron microscopy is essential to observe this ultrastructural character.

Located in extreme southern South America, the Beagle Channel is a strait separating islands of the Tierra del Fuego Archipelago (Fig. 1). The biggest settlement on the channel is Ushuaia in Argentina followed by Puerto Williams in Chile, two of the southernmost settlements of the world [9]. Within the framework of a monitoring program of toxic algae carried out in the Easter sector of the Beagle Channel, we focused on the diversity of *Pseudo-nitzschia* species for the first time in this area. Phytoplankton net samples were examined by phase contrast, DIC and scanning electron microscopy (SEM) following traditional methods. Sampling points included Bay Brown (inside and outside) and Punta Paraná (Fig. 1) where both biological and oceanographic variables have been examined weekly. These areas are usually closed to harvesting each year during summer, when toxic algal blooms are a serious problem for consumer's health and therefore to the economy of the area [10].

Based on valve shape and morphometric data (length, width, density of striae and fibulae and the lack of central interspace), light microscopy observations suggested the presence of *P. australis* in the Beagle Channel. This was not too surprising considering that this species is commonly found in coastal and shelf waters of the Argentine Sea [11–13]. However, subsequent SEM analyses revealed the fine structure of the striae, which disagreed with classical descriptions of *P. australis* [5, 7]. In contrast, it was strikingly similar to unusual specimens of *P. seriata* isolated from Scottish waters [2]. The specimens showed slightly asymmetrical lanceolate valves with rounded ends (Figs. 2a–b), 80.5–103.5 µm long and 7–8.9 µm wide. Interstriae and fibulae

Table 1. Morphometric summary of Beagle Channel specimens. Data from references [2, 8] added for comparison.

Taxa	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Interstriae (in 10 µm)	Rows of poroids	Poroids (in 1µm)
Observed specimens (n = 18)	80.5–103.5	7–8.9	14–19	14–19	2 (+ a few single poroids)	7–8
P. seriata [8]	91–160	4.6–8.0	14–20	14–20	2–more, usually 4	6–8
P. seriata [2]	–	4.6–6	14–20	14–20	2 (+ 1)	6–8
P. australis [8]	75–144	6.5–8.0	12–18	12–18	2	4–5

were usually distributed in equal number (14–19 in 10 µm) and without delineating a central interspace (Figs. 2a–c). The striae were composed of two rows of small poroids (7–8 in 1 µm) (Fig. 2d), but in some cases, a few single poroids (incipient striae?) were visible between them (Fig. 2b).

The observed specimens are in conflict with classical descriptions of both *P. seriata* and *P. australis* (Table 1). The presence of striae mainly perforated by two rows of poroids (typical of *P. australis*) but in number of 7–8 in 1 µm (as in *P. seriata*) was rather disconcerting. However, the results are similar to the unusual specimens of *P. seriata* identified in Scottish waters by means of TEM analyses and DNA sequencing [2]. Unlike the traditional description of *P. seriata*, which has 3–5 rows of poroids per striae [5], Scottish strains showed two rows of poroids plus a few single poroids, or lack of a third row visible between the two rows of poroids; a third complete row was only rarely observed [2]. These authors attributed this unusual morphology to the fact that the cells had been kept in culture for approximately six months before the TEM observations. Therefore, our field study could provide additional information supporting the finding of this unusual morphology in natural populations and prompt new questions regarding the morphological differentiation of *P. seriata* and *P. australis*. Moreover, if the identity of specimens collected in the Beagle Channel could be confirmed as *P. seriata* performing additional TEM and molecular analyses, the geographical distribution of this species would be greatly extended.

Given the increasing number of semi-cryptic species mentioned for the genus *Pseudo-nitzschia* [e.g. 14–17], we consider that further more detailed morphological investigations in combination with molecular data are required to elucidate the morphological variability and geographical distribution of *P. seriata* and *P. australis*. With this in mind, we are now attempting to establish cultures of these specimens and are examining other diatom samples from different regions of Argentina for this unusual morphology. It would be highly beneficial to work in collaboration with colleagues with previous experience in DNA extraction, amplification and sequencing of species from the genus *Pseudo-nitzschia*.

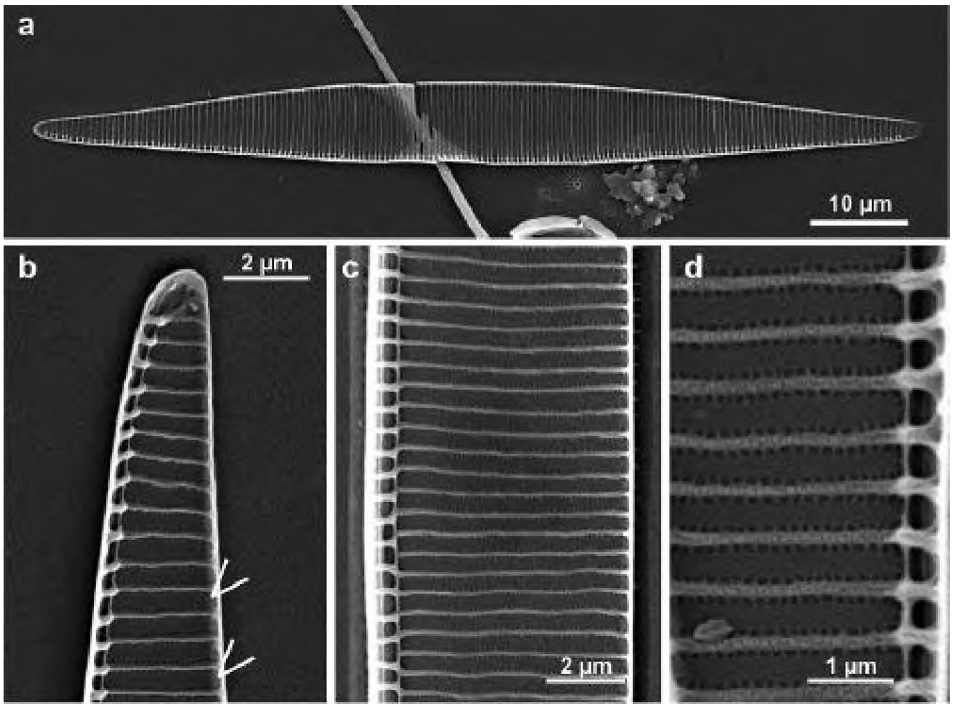

Fig. 2. SEM micrographs of specimens found in the Beagle Channel, Argentina, showing the valve shape, apical and central part details.

Acknowledgements

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Molecular identification of *Ostreopsis* species in Mediterranean coastal waters using fluorescent oligonucleotide probes

Epiphytic and benthic dinoflagellate *Ostreopsis* species are present in coastal areas of the Mediterranean Sea [1–4]. *Ostreopsis* cells colonize a variety of substrates, such as macroalgae, rocks, mussel shells and benthic invertebrates; cell abundance varies between areas, from a few cells to 10^5 cells g^{-1} fw on macroalgae samples. This genus has been detected sporadically in the Mediterranean basin since 1970 (Max Taylor, pers. comm.). However, since the late 1990s, massive blooms of the species *O. ovata* have become harmful to humans and the marine ecosystem along the Italian and Spanish coasts [5]. The impacts of such high biomass proliferation of *Ostreopsis* spp. are severe for both the marine environment, resulting in benthic fauna and fish mortality, and human health, resulting in respiratory and skin problems due to exposure to marine aerosols and seawater [6]. In the Eastern Mediterranean, natural *Ostreopsis* populations occur and cause the contamination of shellfish tissues with putative PLT (palytoxin) compounds [7].

Identification of *Ostreopsis* species is difficult due to variability in the morphological and morphometric features both in field samples and cultured material under optical, epifluorescence and scanning electron microscopes. In the Mediterranean, analyses based on ribosomal phylogenetic and morphological studies grouped *Ostreopsis* spp. isolates into two distinct species, *O. ovata* and *O.* cf. *siamensis*, and within each species all individuals were genetically identical; both species produce palitoxin-like compounds [8]. Furthermore, based on new ribosomal sequence data the toxic genotype *O. ovata* has a wider distribution than the toxic *O.* cf. *siamensis* in coastal areas [9], and is also expanding into colder northern areas, such as the northern Adriatic Sea, with massive recurrent blooms [10].

Genus- and species-specific molecular primers for PCR based assays have previously been used for the detection of the genus *Ostreopsis* and species *O. ovata* and *O.* cf. *siamensis* in several field samples from different Mediterranean coastal sites [11, 12], providing a rapid and species-specific molecular assay. In this study, a fluorescent *in situ* hybridization assay (FISH) for species-specific, quantitative, and rapid detection of the two toxic species of *Ostreopsis* was developed. Species-specific rRNA-targeted fluorescent oligonucleotide probes were designed based on the LSU sequences of more than 80 isolates from the Mediterranean Sea and worldwide, and a FISH-TSA hybridization protocol was performed using cultured material of *O. ovata* and *O* cf. *siamensis* and validated on field populations of *Ostreopsis* spp.

Ostreopsis cells were isolated using a micropipette, and monospecific cultures were maintained in F/4 medium at 21 ± 1 °C with a 14:10 h (light:dark) photoperiod. Illumination was provided by fluorescent tubes with a photon irradiance of 100 mmol photons m^{-2} s^{-1}.

Field samples were collected from three different sites at different periods during blooms or occurrences of *Ostreopsis* spp. (mean abundance of 10^2 to 10^5 × cells g^{-1} fw): at Conero Riviera, NW Adriatic Sea in September 2008, Taormina, Ionian Sea in July 2007 (samples kindly provided by M. G. Giacobbe) and San Felice Circeo, Tyrrhenian Sea, in July 2006 (samples kindly provided by R. Congestri). *Ostreopsis* spp. cells were scraped from mats and macroalgal thalli, fixed in formalin at 4% and then processed for FISH–TSA analysis and PCR-based assay using the species-specific probes and primers for toxic *O. ovata* and *O.* cf. *siamensis*, respectively. The preserved samples of cultured cells and natural populations were successfully processed by the whole cell hybridization assay, as well as in the PCR assay.

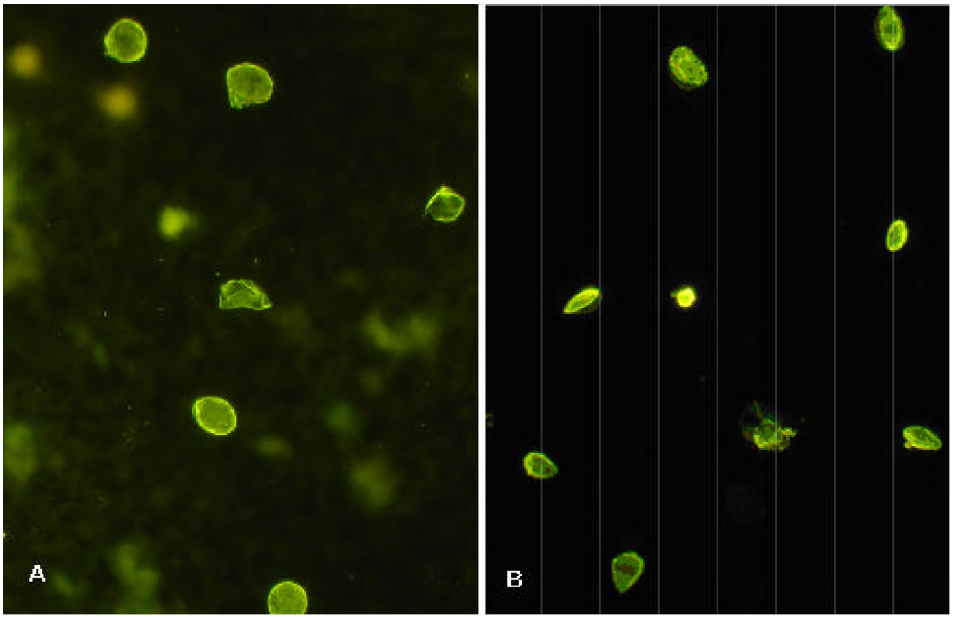

Fig. 1. Epi-fluorescence microscopy: hybridized cells of Ostreopsis siamensis *CNR-T5 (A) and* O. ovata *CBAL (B) with species-specific rRNA probes.*

The whole cell fluorescence assay clearly indicated that rRNA-targeted probes were appropriately hybridized to rRNA of *O. ovata* and *O.* cf. *siamensis* cultured material (Fig. 1). Similarly, the EUK1209R probe used as a positive control yielded positive hybridization, whereas the EUB338R (negative control) probe did not stain any cells of

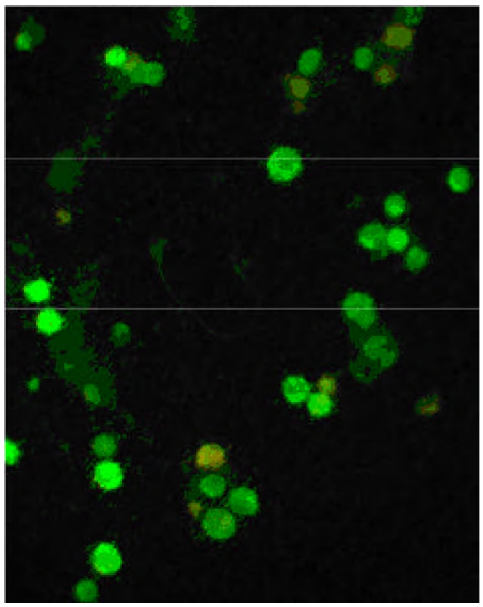

Fig. 2. Epi-fluorescence microscopy: hybridized cells of natural populations of Mediterranean Ostreopsis *spp. with* O. ovata *specific probe.*

Ostreopsis spp. When the FISH-TSA method was applied to field samples from an *Ostreopsis* bloom the rRNA target probe for *O. ovata* properly hybridized to natural population cells (Fig. 2); no fluorescence signal was detected using the *O.* cf. *siamensis*-specific probe. The finding that monospecific blooms were dominated by the genotype *O. ovata* was also confirmed by the species-specific PCR amplification: PCR amplification reactions were positive only for the presence of *O. ovata* in all examined samples. Cell enumeration based on the FISH-TSA assay of cultured and field samples was compared with the traditional light microscopy counting method of formalin-fixed cells and the efficiency of positive detection was comparable for the two methods, whereas uncertainty regarding species-specific identification remained unresolved using traditional light microscopy.

To summarize, we have developed a specific and rapid FISH-TSA method using molecular rRNA-target probes for the identification and enumeration of two toxic species of *Ostreopsis* that are found along the Mediterranean coasts. The data shown are preliminary results of the molecular validation in field samples and it will necessary to test the sensitivity of the whole cell fluorescence assay on a discrete number of natural samples that may contain both species of *Ostreopsis* and different levels of cell abundance. The FISH-TSA method may be used as an alternative to the PCR-based assay for the detection and monitoring *O. ovata* and *O.* cf. *siamensis*. The method does not require a high degree of experience; further, the FISH-TSA method can confirm visually whether or not a fluorescent signal is specific to the targeted species by epifluorescence and light microscopy. Thus, with this whole cell hybridization assay information on species-specific cell identity and counts may be obtained during surveys; this information may be used together with environmental parameters to understand the bloom dynamics of these toxic *Ostreopsis* species.

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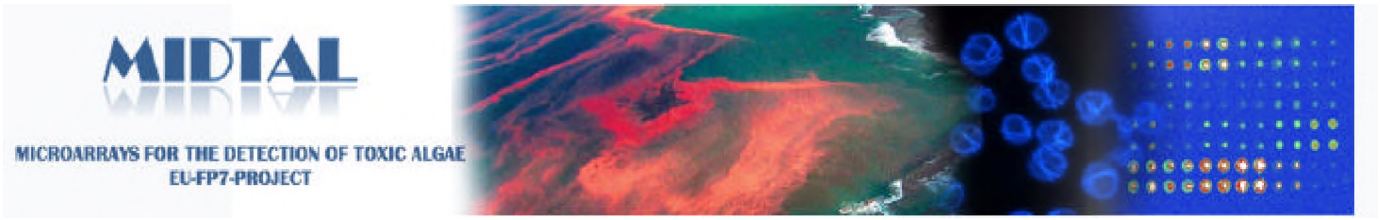

MIDTAL is a new FP7 project entitled Microarrays for the Detection of Toxic Algae. It started on Sep 1st, 2008, is funded by the **Theme 6 Environment (including climate change)** of the European Commission, and will continue for 45 months. Ten partners make up the consortium and include scientists from 7 European countries and the USA. Funding for the USA partner will be applied for through NSF or NOAA. Partners include Marine Biological Association (co-ordinator), Stazione Zoologica Anton Dohrn, University of Kalmar, Instituto Español de Oceanografia, Martin Ryan Institute, National University of Ireland, University of Oslo, University of Westminster, DHI, Instituto Technoloxico para o control do Medio Marino de Galicia, and University of Rhode Island. Some partners are exclusively involved in probe design and testing, whereas others are responsible for making calibration curves from established cultures and others are devoted to taking field samples for two years to validate the microarray.

Microalgae in marine and brackish waters of Europe regularly cause "harmful effects or blooms", considered from the human perspective, in that they threaten public health and cause economic damage to fisheries and tourism. For adequate management of these phenomena, monitoring of microalgae is required. However, the effectiveness of monitoring programmes is limited by the fact that it is time consuming and morphology, as determined by light microscopy, may be insufficient to give definitive species and toxin attribution. In many countries, once cell numbers reach a threshold level, then shellfish are selected for toxin analysis by mouse bioassay. In others, both are mandatory before harvesting. The mouse bioassay is used to ensure toxins do not exceed control levels and is employed more frequently when potentially toxic cells have been identified as present. Molecular and biochemical methods are now available that offer rapid means of both species and toxin detection. MIDTAL will target rapid species identification using rRNA genes. These target rRNA genes include regions that are so variable that they are species or even strain specific. These regions can be targeted for probe design to recognize species or even strains. Antibody reactions to specific toxins produced by these microalgae are also included because harmful algal populations may be patchy and escape detection, and probes against the toxins may allow an early warning in shellfish. Microarrays are state of the art technology in molecular biology for the processing of bulk samples for detection of target RNA/DNA sequences. Existing rRNA probes and immunoassays for toxic algal species/ strains and their toxins will be adapted and optimized for microarray use to strengthen the EU's ability to monitor for toxin-producing microalgae. The purpose of MIDTAL is to support the **common fisheries policy** and to aid national monitoring agencies by providing new rapid tools for identification of toxic algae and their toxins, so that they can comply with EC directive 91/1491/CEE potentially reducing the need for the mouse bioassay.

The methodology MIDTAL plans to develop in order to support monitoring can be summarised as follows:

- Take a water sample, add a known amount of a *Dunaliella* culture as an internal control for extraction and hybridisation efficiency
- Extract total RNA and DNA
- Extract total toxins
- Apply RNA to microarray and determine the fluorescent signal of the hybridised probe to its target RNA. If insufficient RNA is available, then PCR products of the rRNA genes will be used as an alternative
- Apply toxins to microarray and determine the fluorescent signal of the hybridised probe to its target antibody.
- Extrapolate using novel algorithms to cell counts per litre using calibration curves
- Compare manual phytoplankton counts using Light Microscopy or FISH probes, where appropriate, with signals from microarray to validate monitoring effectiveness.

The more accurate counts (e.g. distinction between toxic and non-toxic strains) of species numbers that can be achieved, the less the need for the mouse bioassay to determine if toxic algae or toxins are present. Monitoring for toxic algae using molecular techniques will allow more samples to be analysed in a shorter time period and with greater accuracy, without the need to have personnel trained in taxonomy. It will offer a near real time analysis of the ecosystem, and offer an early warning system for all European countries with aquaculture and toxic algal problems. Mitigation actions can be implemented immediately rather than to wait 3–5 days for results to be returned from the national monitoring agencies using traditional methods. Instead, results can be available in 3–4 hours. Such a development, offering the prospect of handling more detailed field sampling than previously possible will also allow us to advance knowledge of the ecology of 'harmful' species.

We will follow a hierarchical probe approach to the choice of probes for our microarray. Below are listed the probes/ species that we plan to have on our microarray. Some have already been adapted and tested on the microarray during the EU FP6 project FISH AND CHIPS, others are being developed as part of this project. At the end of the project, a workshop is planned to train other potential users in the use of the microarray.

At our start-up meeting (Feb 16-20, Bremerhaven, Germany), all participants received training in probe design using the ARB program,

Objectives include:

1. to test and optimise existing rRNA probes for toxic species, and immunoassays for toxins, for their application to a microarray
2. to design and test the specificity of any new probe needed
3. to construct a universal microarray from the probes tested and optimised by all partners for the detection of harmful algae and their toxins
4. to provide national monitoring agencies with a rapid molecular tool to monitor toxic algae, to validate or replace traditional monitoring methods
5. to integrate European efforts to monitor coastal waters for toxic algae



Fig. 1. Participants at the start-up meeting of MIDTAL. Participants from left to right, first row: Hanne Kaas, Francisco (Fran) Rodríguez, Linda Medlin, Beatriz Reguera, Lucie Maranda, Robin Raine; second row: Jane Lewis, Yolanda Pazos, Jixin Chen, Wiebe Kooistra, Paulo Salomon, Claus Jørgensen, Bente Edvardsen, Gary McCoy, Pasquale de Luca.

microarray hybridisation and analysis with a GenePix microarray reader and phylochip analyser program for the analysis of hierarchical probes on a microarray. We standardised how all the calibration curves would be made, and how the field samples would be collected. For further information on the project, please visit our website: www.midtal.com.

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Taxonomic level	Probe	Specific for	On array
Domain	*Euk 328, 1209*	all eukaryotes	+
Class	*DinoB, DinoE12*	all dinoflagellates	+
	Hetero01	Heterokonta	+
	PRYM 01, 02, 03	Haptophyta, Prymnesiophyceae	+
Clade	*Dinophy GL B-12, Dinophy GL G+6*	phytoautotrophic *Dinophysis*	
	CLADE1	*Chrysochromulina* & *Prymnesium*	
Genus	*Dinophy FL A-5, Dinophy FL G-8*	all *Dinophysis*	
	PSNGENUS	all *Pseudo-nitzschia* ex *delicatissima*	+
	ALEX	all *Alexandrium*	+
	PLIMA/PAQ D	benthic *Prorocentrum*	
	PCH-1, PCH-3	*Pseudochattonella* spp	
Species	*AMIN D+1*	*Alexandrium minutum*	+
	NA1, ATNA02	*Alexandrium tamarense*, North America	+
	AOST01, AOST02	*A. ostenfeldii*	+
	CPOLY01	*Chrysochromulina polylepis*	
	GBREVE	*Karenia breve*	
	GMIKI01	*Karenia mikimotoi* species complex	
	PLIMAE, PLIMAB	*Prorocentrum lima*	
	PMICA02	*P. micans*	
	PMINI H, PMINIC	*P. minimum*	
	PSNAUS A-8	*Pseudo-nitzschia australis*	+
	PSNMUL A+4	*P. multiseries*	+
	PSNPUN A-12	*P. pungens*	+
	Prymparv	*Prymenesium parvum*	
	Hetaka	*Heterosigma akaskiwo*	
	Dacum B	*D. acuminata*	
	Dacut B	*D. acuta*	
	Dnorv B	*D. norvegica*	
	Dinophy GL H-3	*D. acum. / D. norv.* clade	

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Ciguatera and some Caribbean customs

Caribbean fishermen boast of being able to detect when a fish is "ciguato" (contaminated with ciguatera toxins) by using folk methods, such as carrying out their own "bioassay" by feeding a piece of the fish to a cat, checking if the scales fall off a freshly caught fish, or if its eyes are bulky, that they vehemently claim to be appropriate tests. In San Andrés Island (Colombia), fishermen claim they can recognize the barracuda with ciguatoxins by the colour of its blood. They make a longitudinal cut along the spinal cord of freshly caught fish: if the blood is black, the animal is supposed to be toxic and is thrown back to the sea; if the blood has a normal reddish colour, it is safe to eat it. Obviously, none these folk methods are based on scientific evidence.

Recently I heard about a ciguatera episode that occurred in the Cuban world heritage city of Trinidad (21º05'–21º15' N, 79º45'–80º07' W; Fig. 1). A fisherman's family ate a carangid (*Caranx latus* Agassiz, 1831), known in Cuba as "jurel", and one hour later the parents and two children showed clear symptoms of intoxication that were diagnosed and treated as ciguatera. This event was not particularly newsworthy, since Ciguatera Fish Poisoning (CFP) is endemic in the Caribbean, even in Cuba, despite the efficient epidemiological control and fisheries regulations implemented by the Ministry of Fisheries (MIP) to avoid these events. It turned out that the family referred to had carried out their home bioassay, by feeding their cat in advance with a piece of the affected fish. The cat had eaten the fish with apparent relish and no signs of rejection. But I found out it was quite a fat cat, of over 3 kg weight. One and a half hours after ingestion of the fish, the cat started to get fidgety, uneasy, and to exhibit anomalous behaviour, followed by vomiting. It was too late for the family, who had finished their dinner 30 minutes earlier. All they could do was wait, and the reaction soon came. The local health authorities confirmed they suffered from CFP. This story shows that a simple home bioassay, that may have helped in many other cases, did not work on this occasion because the family did not consider the ratio between the amount of fish administered and the size of the cat.

In Cuba, MIP resolution 457/96 forbids the capture or landing of fish included in a list of potentially toxic fish (determined by species and size). The specimen eaten by the family of this story was a large fish, given as a present by a fisherman friend, so beyond the reach of the commercial regulations. In Puerto Rico, large carangids and barracudas have a bad reputation as carriers of CFP toxins, and their sale is forbidden.

Epidemiological records in Cuba show there were in the past two CFP outbreaks in the same region of the country associated with the ingestion of the same kind of fish, then misidentified as *Caranx fallax*. In an outbreak in 1986, 26 consumers got sick after eating "jurel" [1]. Young cats were used for the "bioassays". This practise was abandoned in toxicity studies, but juvenile cats were used in others.

The carangid misidentified as *Caranx fallax* in the epidemiological study of Rojas-Valladares et al. [1] and elswhere [2, 3] is *Caranx latus*, called "jurel común" or "Gallego" ("Galician jurel") (Fig. 2). There are two more described species of the same genus, the "cibi amarillo" (*Caranx bartholomaei*) and the "tiñosa" or "tiñosa prieta" (*Caranx lugubris*), easily recognized by fishermen and also included in the list forbidden for sale. The "jiguagua" (*Caranx hippos*) and the "cibi carbonero" (*Caranx ruber*) are also in the list of potentially toxic species [4, 5]. Despite resolutions and regulations, CFP is still a chronic problem in Cuba. Between 2001 and 2006, there were 570 reported cases of fish intoxication, 72% of which were due to CFP [6]. The picúa (barracuda, *Sphyraena barracuda*) and jurel (*Caranx latus*) were responsible for 43% and 16% of the cases respectively.

Within the Caribbean region, CFP is a major food safety problem with a large socio-economic impact; it also affects tourism. Countries such as Puerto Rico, United States and Cuba, have implemented regulations to forbid the capture and sale of species with a high risk of containing CFP toxins. Unfortunately in many other Caribbean countries, the lack of medical awareness and public education is responsible for a high number of hidden cases of CFP, the symptoms of which are confused with other kinds of seafood intoxication.

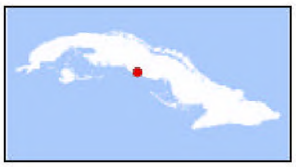

Fig.1. Location of Trinidad in SW Cuba

The IOC Regional Working Group ANCA (**A**lgas **N**ocivas en el **CA**ribe) is seriously concerned by the extent of CFP in their region, and for more than 10 year has been promoting the dissemination of basic knowledge on CFP, the fish species that cause it, the risky areas, the characteristic symptoms of CFP in the Caribbean region, and with a predominance of gastrointestinal disorders, which contrasts with the syndrome in the Pacific Region where neurotoxic symptoms predominate.

There is an urgent need for economic techniques to detect different toxins of the CFP complex (which includes palytoxins and DSP toxins in the same black box) in contaminated fish. Currently available techniques are expensive and difficult to implement. Public health campaigns to educate people are also needed to alleviate the impact of CFP in the Caribbean region.

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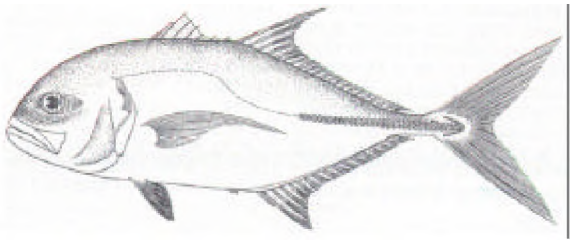

Fig. 2. 'Jurel' or 'gallego' (Caranx latus).

First Coordination meeting of the IAEA–ARCAL Technical Cooperation Project to address impacts of HABs in the Caribbean region

From 27th to 30th of January 2009, the Government of Cuba through the Centro de Estudios Ambientales de Cienfuegos (CEAC) hosted in La Habana (Cuba) the first coordination meeting of the International Atomic Energy Agency (IAEA)–ARCAL Technical Cooperation project on "Designing and Implementing Systems for Early Warning and Evaluation of the Toxicity of Harmful Algal Blooms (HABs) in the Caribbean region. Applying Advanced Nuclear Techniques, Radioecotoxicological Evaluations and Bioassay" in the coastal zones of Chile, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Haití, Honduras, México, Nicaragua, Uruguay and Venezuela.

Reports on the socio-economic impacts of Harmful Algae Blooms in Caribbean countries are increasing in parallel with the increasing exploitation of their coastal areas (tourism, aquaculture). Accumulation of Paralytic Shellfish Poisoning (PSP) toxins in shellfish associated with blooms of *Pyrodinium bahamense* var *compressum* on the Pacific coast, and of *Gymnodinium catenatum* on both Caribbean and Pacific coasts, has caused hundreds of human intoxications and nearly a hundred fatal cases during the last two decades. Ongoing monitoring programmes are often restricted to control of shellfish exports, and do not guarantee the health safety of the vast majority of the population that makes its living from artisanal fisheries.

Ciguatera Fish Poisoning (CFP), caused by consumption of certain tropical fish, has been reported as the main source of human intoxication in the region and represents a serious problem for tourism, the fishing industry and public health in the countries concerned. Ciguatera is endemic in the Caribbean region. Several hundred cases are recorded each year, but many more are unreported due to a lack of medical awareness and public education. The limited knowledge in the region of the toxic algal species involved and the degree of HAB toxicity is aggravating the problem.

The objective of this 5-year project, managed by the IAEA Technical Cooperation Department in Vienna, with technical support of the IAEA Marine Environment Laboratories in Monaco, is the transfer of isotopic techniques for the early detection, quantification of PSP and CFP toxins, and for the reconstruction of HABs occurrence history. The methods concerned are the Receptor Binding Assay and the dating of sediment cores with recent and fossil cysts analysis. The information generated will contribute significantly to the mitigation of the negative socio-economic impacts of PSP and CFP in Latin America.



Coordination meeting with Dr. Guillermo García-Montero, President, IOC Sub-Commission for the Caribbean and Adjacent Regions (IOCARIBE) and Manuel Fernández Rondón, President, Agencia de Energía Nuclear y Tecnologías Avanzadas (AENTA, Cuba)

During the meeting, a workplan was established for the whole duration of the project based on the country reports presented and on the significant contributions of experts from the National Oceanic and Atmospheric Administration (Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC, US), from the Intergovernmental Oceanographic Commission (IOC–IEO Science and Communication Centre on Harmful Algae, Vigo, Spain) and from the Vice-Chair of the IOC–ANCA (Algas Nocivas en el Caribe) regional group. Reference Laboratories will be equipped and selected demonstration sites will serve for implementation criteria, and reference guidepreparation, to increase the knowledge and skills of, the local human people.

The experience gained in a similar IAEA project in Chile coordinated by Prof. Benjamín Suárez-Isla, will be used for this technology transfer to other countries of the Caribbean and Latin America region, making this project a good example of South-South collaboration.

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[4]IAEA,Vienna,Austria.

The Agency is grateful for the support provided to its Marine Environment Laboratories by the Government of the Principality of Monaco.

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• Cuba

New toxic benthic dinoflagellates from south central Cuba

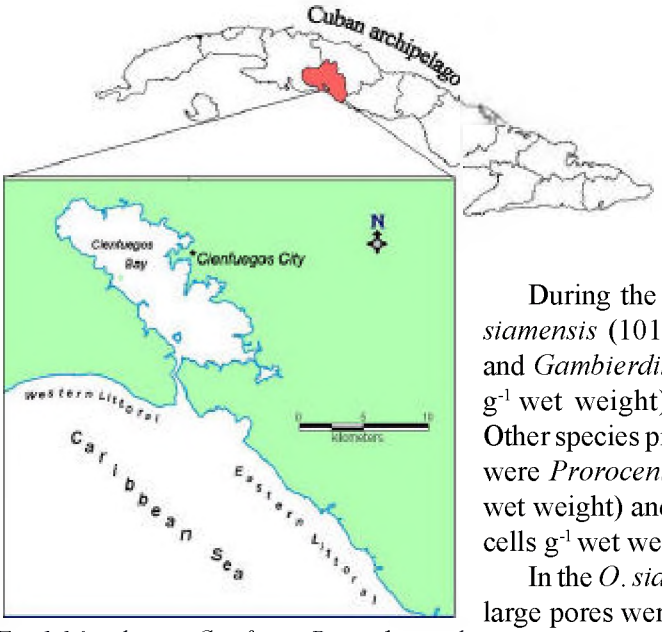

Fig. 1. Map showing Cienfuegos Bay and coastal waters, Cuba.

Coastal Cienfuegos Province in south-central Cuba (Fig. 1) is very diverse, with bays, lagoons, mangroves, coral reefs, and brackish rivers. The dry season lasts from November to April, and the rainy season from May to October. This province, like other regions in Cuba, is affected by ciguatera episodes [1]. Until 2005, there had only been one study of the taxonomy and ecology of potentially toxic dinoflagellates in Cienfuegos Bay, which is estuarine. This study revealed the presence of *Prorocentrum belizeanum* and *P. lima*, the latter being dominant [2, 3]; but the typical agents of ciguatera such as *Gambierdiscus toxicus* had not been recorded.

Following the passage of Hurricane Dennis through the region in July 2005, water quality studies were initiated in the eastern coastal region of the province. *Ostreopsis* sp. (9.3×10^2 cells L^{-1}) and *Gambierdiscus* sp. (2.3×10^2 cells L^{-1}) were detected in the water column in a shallow bay where macroalgae and seagrasses abound, in 2006. The presence of these species led to a preliminary study of benthic dinoflagellates in the bay, and to a seasonal study in 2007, using a mixture of the most abundant macroalgae in the area as substrate; these were the red algae *Ceramium nitens* and *Spyridia clavata*, followed by the green *Bryopsis plumosa* and *Caulerpa sertularioides*, and the brown *Padina* sp.

During the dry season, *Ostreopsis siamensis* (101.3 cells g^{-1} wet weight) and *Gambierdiscus toxicus* (53.4 cells g^{-1} wet weight) were the dominants. Other species present in lower numbers were *Prorocentrum lima* (9.2 cells g^{-1} wet weight) and *Ostreopsis ovata* (4.6 cells g^{-1} wet weight).

In the *O. siamensis* specimens, only large pores were present on the plates; this feature and the form of the girdle distinguish this species from *O. lenticularis*. The 2007 rainy season was one of the wettest in recent years, and seems to have affected the benthic vegetation in the bay, as witnessed by lower diversity and abundance of the macroalgae and an increase in suspended particles. After the rains, the structure of the benthic dinoflagellate community changed radically, and *Ostreopsis ovata* (203.1 1 cells g^{-1} wet weight) and *Prorocentrum lima* (93.7 cells g^{-1} wet weight) were the dominants; *Ostreopsis lenticularis* was present in lower numbers(6.2 cells g^{-1} wet weight).

In July 2008 in the western part of the province, *Prorocentrum concavum* (150.5 cells g^{-1} wet weight) was dominant, together with *Prorocentrum emarginatum* (8.2 cells g^{-1} wet weight) on *Padina* sp, in a rocky biotope influenced by waves, with browsing sea urchins and molluscs such as the cigua (*Cittarium pica*). On the same date in Cienfuegos Bay, *Prorocentrum mexicanum* (3.1×10^3 cells g^{-1} wet weight) was recorded on the filamentous brown algae *Feldmannia irregularis* and *Hincksia mitchelliae*; these macroalgae bloom at the end of the dry season (April), and disappear when the heavy rains begin. Other dinoflagellates found at the end of the dry season in 2008 were *Ostreopsis ovata*, *Prorocentrum concavum*, *Ostreopsis lenticularis* and *Gambierdiscus toxicus*, all new records, the last two in the canal which links the bay to the Caribbean.

With the exception of *Prorocentrum lima*, the species recorded during these surveys are new records for the south central part of the island. These are the first records of *Ostreopsis siamensis*, *O. ovata* and *Prorocentrum emarginatum* in Cuba. The presence of this wide variety of toxic benthic species may be linked to outbreaks of ciguatera in the region, and refute the belief that the south of Cuba is unaffected by this syndrome.

Despite the need to augment these data, the results of this survey combined with epidemiological studies [4] provide evidence of a high incidence of ciguatera in the south central part of the island during the dry season (November–April), in contrast to the north where most episodes occur during the rainy season (May–October) [5]. Everything seems to indicate the existence of more estuarine conditions in the south, where the main river basins occur, and affect the stability of benthic communities during the rainy season. But due to climate changes occurring worldwide, there is now great variability in the frequency and intensity of the rainy periods. For example, the last years of the 20th century and the first four years of this one were a period of extreme drought in Cuba, while since 2005 several tropical storms and hurricanes have caused severe flooding in the island.

One feature which needs to be taken into account in future studies of ciguatera is the state of conservation of the coral reefs. Recent studies on the coast of La Habana, the densely populated capital of the country, have shown that the zones most affected by ciguatera coincide with the zones where the reefs are most deteriorated [6], a result of human activities. This deterioration also seems to be increasing as a result of the increased frequency

• India

First report of *Protoperidinium* bloom from India waters

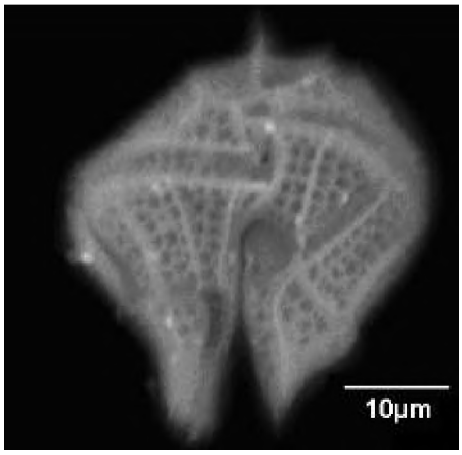

Fig. 1. Protoperidinium *sp (SEM)*

An extensive bloom of *Protoperidinium* was observed on 8th October 2008 during an RV *Sagar Sampada* cruise (Ministry of Earth Sciences, Govt. of India) along the west coast of India. The bloom occupied an area of about 3.2 nm circumference off Mangalore (12º 51.83'N & 74º 20.00'E); it was not monospecific, but a *Protoperidinium* species was dominant, with up to $5x10^8$ cells L^{-1}. Species such as *Protoperidinium oceanicum, Ceratium furca, C. fusus, C. trichoceros, Prorocentrum gracile* were abundant. This is the first report of a *Protoperidinium* bloom from Indian waters. The *Protoperidinium* is non-photosynthetic, so although the cell count was very high, the chlorophyll *a* concentration was (0.9 mg m^{-3}) low. At the time of the bloom, water temperature was 29ºC, salinity 34.64 psu, pH 8.34, and dissolved oxygen concentration 6.53 ml L^{-1}. Nitrate was below the detectable range while concentrations of phosphate and silicate were low (0.245 and 1.68 µm L^{-1}, respectively).

During the *Sagar Sampada* cruise, 53 stations were occupied on 10 transects (within 07°01.83 and 16°59.97 N latitudes) along the west coast of India. Algal blooms were observed at three stations. Besides the *Protoperidinium* bloom, a bloom of *Noctiluca miliaris* was observed off Goa at two successive stations (15º 30.50'N & 73º 36.82'E; 15º 30.28'N & 73º40.30'E) with $2x10^4$ cells L^{-1}. Surface discoloration of surface water was not conspicuous but cells appeared as loose patches. No fish kills or other mortalities were recorded in the bloom area.

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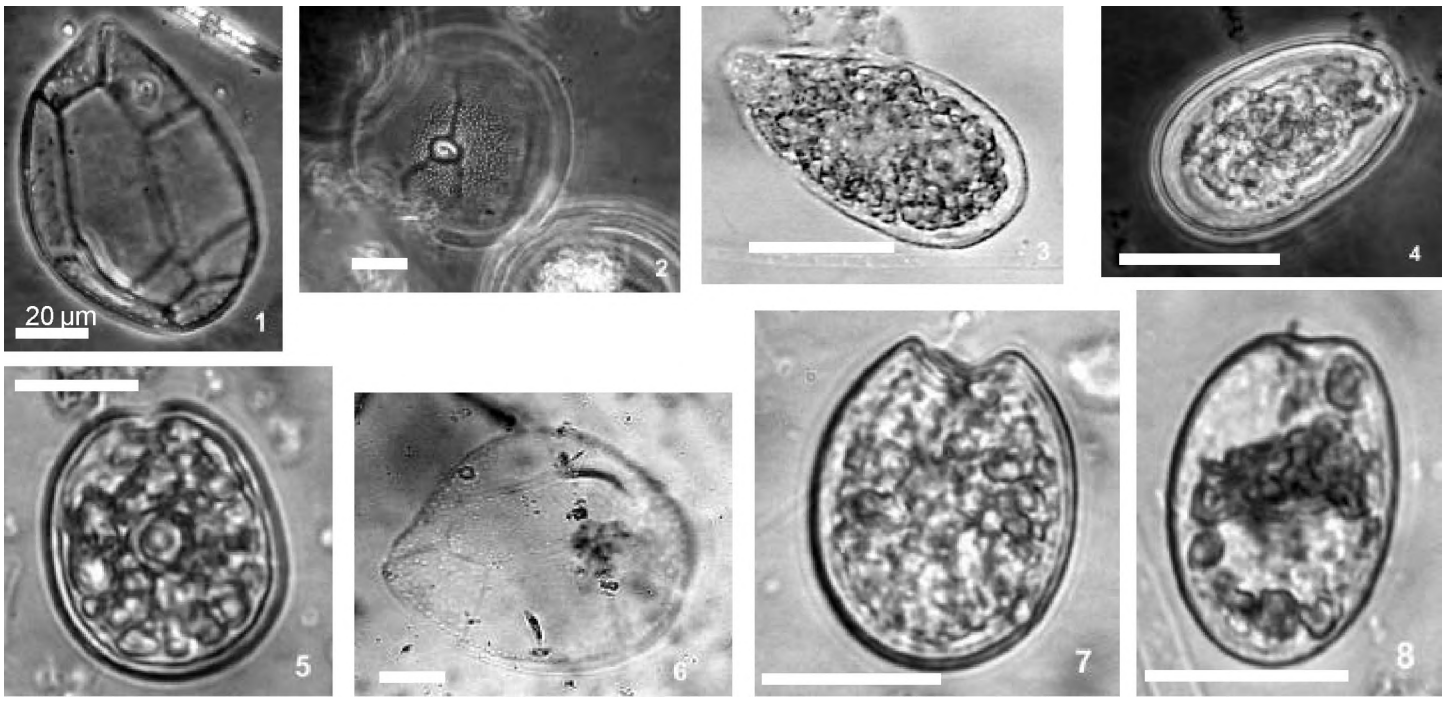

Fig. 2. 1) Ostreopsis siamensis, *2)* Gambierdiscus toxicus, *3)* Ostreopsis ovata, *4)* Prorocentrum lima, *5)* Prorocentrum concavum, *6)* Ostreopsis lenticularis, *7)* Prorocentrum emarginatum, *and 8)* Prorocentrum mexicanum *(provisional identification).*

and intensity of hurricanes in the region, all leading to the establishment of macroalgal mats in the reef areas.

Acknowledgements

To Beatriz Reguera (Centro Oceanográfico de Vigo, Spain) and staff of the IOC–IEO Science and Communication Centre.

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Marine Experts attend FerryBox 08

More than 60 marine experts from around the world attended **FerryBox 08** on 29 and 30 September – a two day conference at the National Oceanography Centre, Southampton UK co-hosted by Chelsea Technologies Group. The subject of the conference is one of the fastest growing areas of marine science – where sophisticated equipment is fitted to cargo and passenger ships to provide continuous monitoring of the world's oceans to improve our understanding of climate change.

Shipping companies are collaborating with oceanographers to measure what is happening in our oceans, seas and lakes on a day-to-day basis. Such information is essential if we are to understand and quantify both the causes and effects of our Earth's changing climate. Innovative sensor systems installed on the ships collect the data and a satellite communications system sends the numbers ashore for assessment. The oceanographic community is enthusiastic about expanding this interaction with the shipping industry and the shipping community is equally keen to find ways to partner.

Richard Burt (Chelsea Technologies Group) said, 'Chelsea was delighted to co-host the event as we have been supplying real time oceanographic monitoring systems for both military and commercial vessels for many years, and have built up a broad understanding of the design, installation and operation of such systems'. This knowledge has been further advanced through involvement in the European Union 'FerryBox' programme which led to the development of the Chelsea AquaLine FerryBox System. At the heart of the AquaLine System are a suite of sensors, proven in some of the harshest environments in the world, a passenger display system and automatic data transfer to a shore facility.

FerryBox 08 heard from users worldwide including Akira Harahishma (National Institute for Environmental Studies, Japan) about studies in the Seto Inland Sea and Herman Ridderinkhof (Royal Netherlands Institute for Sea Research), on how the shortest route to Texel has changed understanding of sediment transport into the Wadden Sea. Ute Schuster (UEA) presented the North Atlantic study by the EU's Science Framework 6 project *CarboOcean* on the marine carbon cycle, and the International Ocean Carbon Coordination Project.

David Mills (CEFAS) led discussion on further development of the FerryBox concept as part of integrated observing systems, and described the new European Marine Ecosystem Observatory (Emeco) initiative. Emeco will support and make measurements over decades at coastal and regional scales to quantify variability in physical, chemical and biological properties.

The Swire Group Charitable Trust recently pledged over £300,000 to the National Oceanography Centre, Southampton to support ocean monitoring with sensors fixed to a cargo ship. With sensors on Swire's MV *Pacific Celebes*, NOC scientists have been able to capture data about remote areas where ocean- atmosphere interactions are largely unknown. Dr David Hydes (NOCS Ferrybox programme) commented " the North Atlantic plays a major role in controlling the amount of carbon dioxide taken up by the oceans each year ... we now have the ability to monitor how the uptake of carbon dioxide changes from year to year, with a precision that can be used to quantify the results of reduced emissions of carbon dioxide when these are achieved."

David was pleased to report that the EuroGOOS Office in cooperation with SMHI, Sweden will be hosting next FerryBox Meeting in March 2010.

For more details contact: E.Keegan, Chelsea Technologies Group, Email: ekeegan@chelsea.co.uk.

D. Hydes, National Oceanography Centre, Southampton. Email: djh@noc.soton.ac.uk

ISSHA
International Society for the Study of Harmful Algae

From the ISSHA Vice-president

What makes a HAB society are its people. We all have our heroes. We all have had our mentors. We all end up being influenced by meeting inspiring personalities at critical times in our lives or by having read their ground breaking papers. For many HAB scientists, their backgrounds and personalities, the environments they worked in, and the HAB organisms they encountered explain much of the science they did. Inspired by the initiative by the Phycological Society of America who prepared a newsletter series on phycological "Trail Blazers", in my capacity as **Chair of the ISSHA Publications and Dissemination Committee**, I started to prepare a series of short (2-3 page) biographies on HAB trail blazers, covering their education, professional career, awards, key contributions, personal anecdotes, whom they mentored, and including 10 key publications. I commenced my coverage of our recent ISSHA "Yasumoto life-time achievement" awardees Taylor, Hasle, Smayda, Anderson, Steidinger, but I also am treating other key HAB people that we honoured at some of our conferences, Yasumoto, Okaichi, Margalef, Braarud, Balech, Dale. For people still alive we



HAB trail blazers: "Max" FJR Taylor

Education

Max was born in 1939 in Cairo, Egypt, only son of a South African-born officer serving in the Royal Air Force and an Australian mother. His parents dragged him around the world, from Dar es Salaam, Tanzania to Northwood, Middlesex and Leuchars, Scotland and then back to South Africa. Max received most of his education in a village near Sydney, Australia. He did a double major in Zoology and Botany and was awarded his PhD from the University of Cape Town in 1965. His thesis was a contribution to the International Indian Ocean Expedition and dealt with phytoplankton communities in the SW Indian Ocean, including the Agulhas Current. His thesis was rather large - as he put it, "he didn't have time to write a shorter one!"



Honours

2000 Yasumoto Lifetime Achievement Award by 9th Int Conf Harmful Algal Blooms (Hobart); 1995 Paleontological Society Golden Trilobite Award; 1997 elected Fellow of the Royal Society of Canada; three teaching awards while at University of British Columbia; etymology of *Alexandrium taylori, Amphisolenia taylori, Blepharocysta taylori, Gonyaulax taylori, Strombidiniopsis taylori.*

Professional Career

"Max" FJR Taylor joined the faculty of the University of British Columbia at the end of 1964 at the age of 24, having been offered a job even before his PhD thesis was formally approved. He was promoted to full Professor at the age of 35. Max joined the UBC Institute of Oceanography, then headed by George Pickard.

Key contributions

At UBC Max continued work for the Indian Ocean Expedition, using material collected by the R.V. "Anton Bruun", from a much wider area encompassing the Bay of Bengal, Andaman Sea, Arabian Sea and the whole western Indian Ocean. This material formed the basis for his beautifully illustrated monumental 1976 *Indian Ocean Dinoflagellate Atlas*. This work also included many of the earliest scanning micrographs of dinoflagellates. Max's students primarily explored the phytoplankton ecology of the coastal waters of British Columbia while he continued to develop his specialty: the ecology of red tides. Max has been doing research in this field longer than anyone else, having published his first paper on a mass mortality in False Bay, near Cape Town, in *Nature* in 1962. Shortly after arriving in British Columbia in 1965 he

will get them to check the accuracy of what we write about them and seek their permission to publish their portrait photographs. The idea is to initially put up these stories on our web-site but by the time we have written up 40-50 people, we will consider publishing them in an ISSHA booklet, perhaps embellished by HAB conference summaries. We may even want to consider in future a collection of HAB cartoons or HAB poetry! Let me know if you are willing to contribute to this initiative. I hope you will find reading these stories on the lives of our HAB heroes as stimulating as I am enjoying collating them.

Gustaaf Hallegraeff, School of Plant Science, University of Tasmania, Australia.
Email: Hallegraeff@utas.edu.au.

From the ISSHA Secretary: Dear present and future ISSHA members

As newly elected secretary of ISSHA I would like to introduce myself and the on-going work on the construction of a new interactive ISSHA web-site. I am currently a professor in Limnology at Lund University, in southern Sweden. Although I am a freshwater ecologist by training, I work with both limnetic and marine systems. My research focus has been on dinoflagellate life cycles, allelopathic interactions, and lately, genetic diversity in microalgal populations. Since December 2008, I have been in charge of leading the work to create a modern web-site for ISSHA. It is our strong belief, that an attractive website is central to the functioning and development of our organization. The new web-site will include the following features: quick on-line membership applications and membership payments through



PayPal, a member discussion forum, an events calendar, possibility for members to upload articles/reports, etc

Karin Rengefors.
Email: Karin.Rengefors@limnol.lu.se

investigated with Anand Prakash, a case of human death due to paralytic shellfish poisoning (PSP). It was the first such case anywhere in which the causative dinoflagellate was caught still at the scene. Dinoflagellates always held Max's closest attention, and he edited and wrote nearly a third of *The Biology of Dinoflagellates* for Blackwell, before teaming up with palynologists to combine the classifications of living and fossil dinoflagellates, an effort that won them the Paleontological Society's Golden Trilobite Award in 1995. In 1979 he recognized that the PSP causative organism in BC did not fit into *Gonyaulax* and he coined the new genus name *Protogonyaulax*, which he subsequently gracefully withdrew in favour of the resurrection of the older name *Alexandrium*. Along his diverse career path, Max worked on cryptomonad endosymbionts in *Mesodinium*, the feeding mechanism in *Protoperidinium*, the motility of the dinoflagellate transverse flagellum, the causative dinoflagellate of ciguatera, and bloom prediction of the fish-killing *Heterosigma carterae*(=*akashiwo*). Max always had a strong on-going interest in cell evolution. In 1974 he formalized the Serial Endosymbiosis Theory that in eukaryotic cells the mitochondria and chloroplasts had a symbiotic origin from bacteria. These were old independent proposals revitalised and combined by Lynn Margulis in the 1970s, but highly unpopular at the time even though they made complete sense to Max, who published further papers as evidence accumulated. It was largely this body of work which earned him a Fellowship in the Royal Society of Canada in 1997. Max still mourns the lack of interest that greeted what he believes was his most significant discovery which resulted from a sabbatical at Oxford University in 1986-87. Although the codons (the triplet bases that determine which amino acids will be incorporated in proteins) were claimed by Nobel prize winner Francis Crick to be randomly assigned but unchanging, Max and David Coates found that it is the most highly ordered data set they ever encountered.

Max is an intensely social person and an extraordinary raconteur with an anecdotal memory for details. These skills put him centre stage at numerous international conferences, whether as the cofounder in 1975 of the International Society for Evolutionary Protistology, the Founding President of ISSHA in 1998 or conferences on Fossil Dinoflagellates, and he produced numerous insightful state-of-the-art conference overviews.

Notable students

Alan Cembella, Greg Gaines, Paul Falkowski, Juan Saldarriaga.

10 Key publications

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Based partly on: SCOR Newsletter 15, May 18, 2005; EOS Alumni newsletter 8 (2005); portrait by G.Hallegraeff.

GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

Second Open Science Meeting on HABs and Eutrophication: 18-21 October 2009 Beijing, China

Eutrophication is recognized as one of the factors contributing to the increasing proliferation of harmful algal blooms in coastal areas worldwide. After the first Open Science Meeting on "HABs in eutrophic systems" held in Baltimore in 2005, the second OSM meeting is designed to bring experts together from around the world to review the state-of-the-art knowledge on eutrophication and HABs, and to take steps in designing the next phase researches to address this critical global issue.

Topics include: land-based nutrient pollution and coastal eutrophication, dynamics of HABs in eutrophic coastal waters, cyanobacteria blooms and eutrophication in freshwater, physiology of HAB causative species in eutrophic systems, comparative studies of HABs in eutrophic systems, monitoring, modeling and prediction of HABs in eutrophic systems, and impacts of HABs in eutrophic systems and management strategies.

The meeting will include keynote oral and poster presentations. Abstracts should be 200-300 words in length and should be submitted by email to the local secretary (or On-line) as an attachment. Abstracts will be collected for distribution in the meeting program book.

Important deadlines: Abstract submission: 1 Aug 09; Early registration: 15 Aug 09; Registration: 15 Sep 09; Venue registration: 18 Oct 09.

Limited funding may be available to support young scientists and scientists from the developing countries, please check the meeting website www.geohab-osm-bj.ac.cn for detail.

Saxitoxins in Argentinian inland waters

Saxitoxins from cyanobacterial algae were detected in the River Salado, Chaco Province (Argentina) in summer 2006. This is the first report of saxitoxin production in a freshwater environment in Argentina. River Salado is an alternative drinking water source for Castelli, a town with 36.000 inhabitants located at 25º56'S y 60º37'W. Seven sampling stations were placed along 14km of the river (Fig. 1). The river had a maxima depth of 2m, and several closures along its course, stopping water flow.

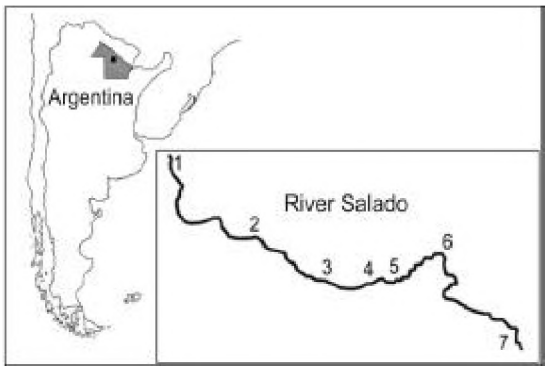

Fig. 1. Location of River Salado showing sampling stations. 1) Northern closure; 2) Km. 3; 3) Pampa Argentina; 4) Middle closure; 5) Native Colony; 6) Mereles; 7) Parra Bridge.

This study was carried out from late 2005 to 2006, during a long drought. Water samples after the drought period were analyzed during January and February 2007.

Physical, chemical and biological parameters were analyzed according to the Standard Methods for the Examination of Water and Wastewater [1]. Mouse bioassays were made at the Laboratory of Legal Chemistry of the National University of La Plata (Argentina), and toxin analysis by HPLC at the Federal University of Rio Grande (Brazil) on samples from February 2006.

Potentially toxic algae were abundant, causing troubles in drinking water treatment. The average species concentrations were: *Raphidiopsis curvata* 1.83 x 10^6 cell mL^{-1}, *Raphidiopsis mediterranea* 1.49 x 10^6 cell mL^{-1}, *Planktothrix agardhii* 0.49 x 10^6 cell mL^{-1}, and *Cylindrospermopsis raciborskii* 0.20 x 10^6 cell mL^{-1}. The highest algae abundance was detected between northern closure and Mereles, quickly decreasing at Parra Bridge. In November 2005, dominance of both *Raphidiopsis mediterranea* and *Raphidiopsis curvata* were detected, and represented 42-56% and 14-18% of biomass, respectively. Total phytoplankton reached 15.0 x 10^6 cell mL^{-1} (Fig. 2).

Biomass averages showed that *R. mediterranea* decreased from Pampa Argentina to Native Colony and increased from Native Colony to Parra Bridge. *R. curvata* behaved in inverse manner, with the highest value of 92% of total biomass at Native Colony (Fig. 3). The maximum algal concentration was 6.64 x 10^6 cell mL^{-1}.

During late 2005 and 2006 total rain was less than 200 mm. Water samples taken in November 2005 showed high levels of pH, turbidity, nitrates and chlorophyll-a. During early 2007 total rain reached 250 mm. Water samples taken in February and March showed improved water quality compared with the previous period, decreased nutrients, algal biomass and other parameters (Table 1).

Mouse bioassays showed the possible presence of paralytic shellfish poison (saxitoxins). Toxin analysis by HPLC from raw water confirmed the presence of saxitoxin (STX), decarbamyl saxitoxin (dcSTX), neosaxitoxin (NeoSTX) and gonyautoxins 1 and 5 (GTX1 and GTX5), reaching a total of 105.33 µg L^{-1} STX-eq. (Table 2). The guideline value of this toxin in drinking water is 3 µg L^{-1} [2, 3]. No other toxins were detected. The water had 1.84 x 10^6 cell mL^{-1} of *Raphidiopsis mediterranea*, 0.59 x 10^6 cell mL^{-1} of *Planktothrix agardhii*, 0.53 x 10^6 cell mL^{-1} of *Raphidiopsis curvata*, and 0.42 x 10^6 cell mL^{-1} of *Cylindrospermopsis raciborskii*.

It is known that *Raphidiopsis mediterranea* can produce the neurotoxic alkaloids anatoxin-a and homoanatoxin-a [4], and that *Raphidiopsis curvata* and *Cylindrospermopsis raciborskii* can generate the hepatotoxic alkaloids cylindrospermopsin and deoxicylindrospermopsin [5]. *C. raciborskii* can also produce saxitoxins [6]. *Planktothrix agardhii* can produce hepatotoxic peptid microcystins and the alkaloid anatoxin-a [7].

Saxitoxins were the only toxins detected, and were present at high concentrations. This could belong together with the presence of *Cylindrospermopsis raciborskii* that would be the responsible for the presence of saxitoxins, just as it happens in Brazil [8, 9].

River Salado under drought conditions behaves as a lake, with a closure each end of the sector studied. The reduction of water flow together with high temperatures, lack of rains and sufficient nutrients, generated ideal conditions for optimal growth of algae.

Under these conditions, toxins removal must be assured, to do not overcome the guide values for drinkable water.

Acknowledgements

I thank Dr. D. Andrinolo (National University of La Plata, Argentina) for his work for mouse bioassays, and Dr. J. Sarkis Yunes (Federal University of Rio Grande, Brazil) for toxin quantification.

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9th Session of the IOC Intergovernmental Panel on HABs

The Ninth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB) was held at UNESCO Headquarters, Paris, 22–24 April 2009. The Session was attended by representatives from: Brazil, Canada, Chile, Cook Islands, Croatia, Denmark, Greece, Italy, Malaysia, Mexico, Morocco, Namibia, New Zealand, Oman, Peru, Slovenia, Spain, Sweden, Thailand, Tunisia, United Kingdom, United States of America, Uruguay, IAEA, PICES, UNEP, and the International Society for the Study of Harmful Algae (ISSHA).

The purpose of the Panel is to develop international cooperation in training, research and management of harmful algae.

The Panel reviewed activities on HAB developed and implemented by IOC and partners during 2007–2009. The Panel noted with concern the reduced financial support for the IOC HAB programme, and stressed that the IOC HAB Programme will only be able to develop and be implemented in 2010-2011 if there is additional extra-budgetary support from Member States to fund programme staff and activities. The major achievements reported include: (i) the development of the integrated IPHAB–IODE Harmful Algae Information System; (ii) developments within GEOHAB including the launch of the GEOHAB Research Plans for the Core Research Projects in Stratified Systems and the development of a plan for GEOHAB Asia; (iii) development of the regional activities ANCA, FANSA, HANA; (iv) the implementation of twelve training courses and training-through-research projects; (v) the continued publication of the IOC Harmful Algae News; (vi) results from the ICES-IOC WGHABD; (vii) the IOC co-sponsorship of international HAB conferences; and (viii) provision of HAB literature to developing countries.

The Panel decided on a work plan and budget for 2010–2011 which has its main focus on regional HAB Programme development, capacity building, GEOHAB, the development of the Harmful Algal Information System, Task Teams on 'Biotoxin Monitoring, Management and Regulations', 'Algal Taxonomy' and 'HAB Monitoring within the Global Ocean Observing System'. The Panel also adopted guiding principles for capacity enhancement with respect to mitigating the effects of harmful algae, a draft strategy for IPHAB, and a focus for activities on the transfer and introduction of HAB species by human activity such as shipping (ballast water). The Panel made recommendations to assist the IOC Ocean Science Section in the development of a plan for integrated coastal research. Dr. Leonardo Guzman (Chile) was re-elected as Chair and Phil Busby (New Zealand) was re-elected as Vice-Chair.

More details: www.ioc.unesco.org/hab under Documents, IPHAB-IX

Future events

JUNE 2009

IOC/WESTPAC Workshop on Marine Invasive Species and Management in the Western Pacific Region

This will be held at Chulalongkorn University, Bangkok, Thailand, 4-5 June 2009. Presentations will cover the regional status of marine invasive species, their impacts, data quality, methods to confirm occurrence of bio-invasion; transport mechanisms of invasive species; factors that facilitate their establishment, and counter-measures. A group discussion will refine WESTPAC-BIODIVERSITY projects, work plans and research priorities, publish a current status report, and other actions related to invasive species in the WESTPAC region.

For further information contact Dr. S. Chavanich, Chulalongkorn Univ. at: suchana.c@chula.ac.th.

Pacific NW AOAC Meeting

This will be held June 17-18, 2009, at the Univ. of Puget Sound, Tacoma, WA, USA, with seminars and presentations on phycotoxins, and dietary supplements; and training sessions on method validation (AOAC). We now have poster presentations including student participation.

Workshops are listed at www.aoacpacnw.com, along with registration forms and more AOAC meeting details.

For additional information contact J. Hungerford, Chair of AOAC Task Force: James.Hungerford@fda.hhs.gov

OCTOBER-NOVEMBER 2009

A special course, ***Taxonomy of Recent Dinophyceae***, will be held from 26 Oct–05 Nov 2009 at the Wattenmeerstation Sylt of the Alfred Wegener Institut, in List/Sylt, Germany. It is restricted to 10 participants to assure individual attention. Welcome are all people who already have basic knowledge of dinoflagellates.

The programme includes an introduction to the group and its nomenclature, new taxa described since 2000, microscopical work on participants, an introduction to benthic and coccal Dinophyceae, and to aberrant forms.

Accomodation will be provided in the station guest-house. No funding is available from the German side.

To register, please contact M. Elbrächter (melbraechter@awi-bremerhaven.de) by 15 August 2009

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

www.ioc-unesco.org/hab No. 40

• France

Alexandrium catenella in Thau lagoon (France) is not a recent introduction from Asia?

In Thau Lagoon (France), cells belonging to the *Alexandrium tamarense* species complex were observed first in July 1995 by the French national monitoring network of phytoplankton and phycotoxins (REPHY) [1]. The first major bloom responsible for a paralytic shellfish-poisoning (PSP) outbreak occurred in 1998 [2]. Since then, recurrent toxic blooms have been observed over spring and autumn [3]. Studies using genetic markers revealed that the species associated with toxic events recorded during autumns 1998, 2004 and 2007 was the toxic dinoflagellate *Alexandrium catenella* belonging to the Temperate Asian clade within the *A. tamarense* species complex [4–6].

The sudden occurrence of *A. catenella* in Thau lagoon, its geographical disconnection from other Mediterranean populations [7], and relationships established with Temperate Asian populations had supported the hypothesis of introduction by human-mediated transport, e.g. the transfer of resting cysts from Temperate Asia through ships ballast waters [4] or the export of living molluscs [5].

Within the framework of the first study based on microsatellites [6], we aimed at resolving the origin of *A. catenella* from Thau, using hypervariable nuclear markers able to describe its genetic diversity in Temperate Asia. Samples were collected: (a) in Thau lagoon (n=74 samples); (b) along Japanese coasts (n=411), i.e. Pacific coasts, Seto Inland Sea and Sea of Japan; and (c) along the Zhejiang coast in the East China Sea (n=166).

Monoclonal strains were established by single cell isolation based on seawater or sediment sampled between 2003 and 2009. Seven polymorphic microsatellites were used to score the genotypes of 651 monoclonal strains. Primers and PCR conditions are given in [8] for the primer pair *ACAT14*, *ACAT16*, *ACAT49* and *ACAT50* and in [9] for *FMAC16*, *FMAC54* and *FMAC55*. Allele sizes were determined from electrophoresis patterns using the real-time system GelScan 3000 (Corbett Robotics) according to a size marker (GeneScan-350, Applied Biosystems). Factorial correspondence analysis (FCA) and

(Cont'd on p. 2)

• Mexico

Raphidophytes in Bahía de La Paz, Gulf of California

Raphidophyceae, golden-brown algae, are named for their extrusive organelles, or trichocysts, that under light microscopy, resemble the raphides (thin crystals of calcium oxalate) of plants. Raphidophytes are mainly marine, single-celled, flagellated, and typically range in size from 30–90 µm, and are considered naked flagellates because they lack cell walls and scales. Raphidophytes are associated with fish kills in coastal waters, and some produce toxins that pose a threat to human health. The mechanism causing fish kills is still debated. Brevetoxins, hemolysins, hemaglutinins, reactive oxygen species (ROS), and free fatty acids are present in raphidophyte species.

Five species of bloom-forming raphidophyceans have been reported in the Gulf of California [1–5]. They are *Chattonella marina*, *C. ovata*, *C. subsalsa*, *Fibrocapsa japonica*, and *Heterosigma akashiwo*. A bloom of *Chattonella marina* occurred in Bahía de La Paz, but fish mortalities were not reported [2]; since then, raphidophytes have been monitored in the bay. Samples were taken from December 2008 through May 2009 every two weeks at the petroleum port north of the city of La Paz (24°13' 19" N; 110°19'4" W). Seawater samples were collected at the surface, and surface and vertical net phytoplankton hauls (20-µm mesh) made.

(Cont'd on p. 4)





Caption on p. 4

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

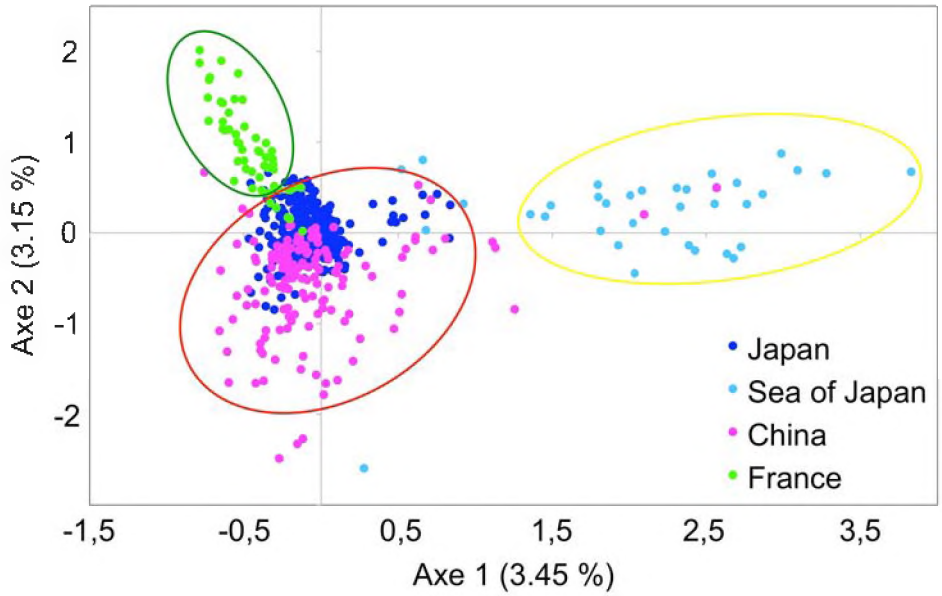


Fig. 1. Factorial correspondence analysis based on 651 Alexandrium catenella *samples and 7 microsatellite loci. Here, samples were represented according to axes 1 and 2. Green circle: France; red circle: China and most of Japan samples (including Pacific coasts and Seto Inland Sea samples); yellow circle: Sea of Japan. (For a full color version see the online issue).*

(Cont'd from p. 1)

pairwise F_{ST} estimate (significance tested through 10000 permutations) were conducted using the software GENETIX® 4.05 [10]. Assignment analyses were performed using the software STRUCTURE® 2.2 [11].

According to the FCA, the majority of the 651 samples (= individuals) could be divided into three distinct clusters (Fig. 1): i) French; ii) Chinese and most of the Japanese samples (i.e. samples from Pacific coasts and Seto Inland Sea); iii) Sea of Japan. Pairwise F_{ST} estimations evidenced highly significant differentiation among French, Chinese and Japanese populations; showing a clear genetic structuring (Table 1). Pairwise F_{ST} values varied widely and allowed us to define a range of values. The lowest values were observed among sites composing Japanese (including Pacific coasts and Seto Inland Sea samples) and among Chinese clusters, suggesting the possible existence of limited gene flows. However, as described above, the Sea of Japan population emerged as genetically differentiated from the Japanese group. This isolation may be due to some ecological barriers. Finally, highest and significant values were observed between Thau and all other Temperate Asian populations, suggesting the absence of gene flow, either through natural or artificial migrations.

Cluster analysis by STRUCTURE indicated that the four clusters best explained the uppermost hierarchical level of genetic structuring in *A. catenella* samples. When K=4, individuals were divided into populations from the Pacific coasts and Seto Inland Sea of Japan as Cluster 1, the Zhejiang coast populations as Cluster 2, the Sea of Japan population as Cluster 3 and Thau lagoon population as Cluster 4 (Fig. 2). In the Sea of Japan and Thau populations, most individuals were assigned with high probability to one of the clusters. On the other hand, admixtures of clusters were detected in other Japanese and Zhejiang coast populations, suggesting a mix of populations from different origins or the limited capacity of the software to discriminate recently diverging populations. More *loci* should be involved to overcome this problem.

Alexandrium catenella population blooming in Thau lagoon strongly diverges from the current Temperate Asian populations (according to microsatellite analyses), whereas both origins are gathered together according to the analyses of ITS1-5.8SrRNA gene-ITS2 sequences [4–6]. Despite the limited number of Asian samples analysed in this study, the genotypic data of *A. catenella* by microsatellites were globally collected in the area between latitudes 20–45°. Possibly the microsatellite data disprove the hypothesis of a recent introduction through human-mediated transport from Temperate Asian.

However, we have still some concerns and questions about: *(1) Is our sampling effort sufficient in Temperate Asia?* It does not provide a thorough description of the genetic diversity in Temperate Asia. However, it permits us to describe a large range of diversity, and to ascertain if the Thau lagoon population diversity overlapped those of Temperate Asia populations. *(2) Is our genotyping effort sufficient?* Yes for a large structure description, but more *loci* should be tested for an accurate description of close Asian populations; *(3) Did A. catenella originate from Japan-China area?* Our results clearly exclude an Asian origin from 20–45° latitude. More northern and southern regions should be investigated; *(4) Is A. catenella from Thau an indigenous population, which has been distributed as part of a hidden flora?* The existence of a fossil gene flow has been suggested to explain the current distribution of the *A. tamarense* species complex in the Mediterranean Sea [12]. This hypothesis assumes a shared origin between Mediterranean and Asian populations, which could explain genetic links between Thau and Temperate Asian populations observed through analysis of the ribosomal operon.

In conclusion, the recent expansion of HABs species could be explained not only by human-mediated transport, but also by the long-term presence of populations. If we accept this hypothesis, which needs to be investigated further, these possibly

Table 1. Pairwise F_{ST} *estimated (10000 permutations) for 6 comparisons between 3 populations of* Alexandrium catenella *(total of 651 samples) based on 7 microsatellite loci. Significant* F_{ST} *values, obtained after sequential Bonferroni correction [13].*

	Japan	Sea of Japan	China	France
Japan	----			
Sea of Japan	0.14305***	----		
China	0.12181***	0.18086***	----	
France	0.17160***	0.31127***	0.24444***	----

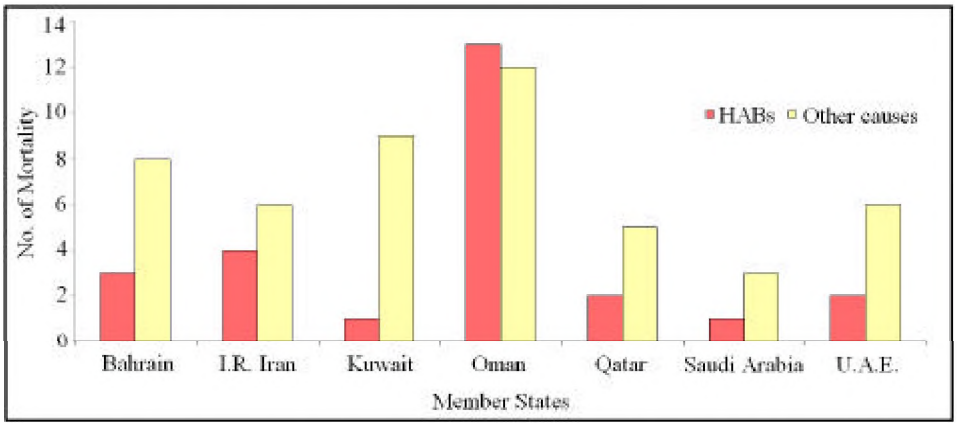
*** *P<0.001*; ** *P<0.01*; * *P<0.05*, significant after sequential Bonferroni corrections for 6 multiple tests.

• Kuwait

ROPME-IOC Regional Symposium on HABs

The Gulf region has in recent years been severely affected by harmful algal events. HABs have affected fisheries, aquaculture, operation of desalination plants, and tourism, and caused significant economic losses. The first of these events took place in 1999, as a result of a bloom of *Karenia selliformis*, and caused death to at least 130 types of fish, as reported Dr. Salah Al-Mudh'hi, Chairman of the Board and Director General of the Kuwait Environment Public Authority (EPA). More recently, blooms of *Cochlodinium polykrikoides* in particular have caused problems.

Nutrients enrichment from land-based human activities is thought to be one factor which plays a major role in triggering HAB outbreaks in the region. Dr. Abdul Rehman Al-Awadi, Executive Secretary of the Regional Organisation for the Protection of the Marine Environment (ROPME) commented that a fundamental challenge is that people tend not to understand the relationship between human behaviour and its effect on the environment.



Number of marine mortality events during 1976-March 2009 in the ROPME Sea area.

To strengthen regional cooperation in observing and mitigation these events, the Kuwait Environment Public Authority (EPA) hosted a Regional Symposium on Harmful Algal Blooms from 12 to 14 October 2009. The Symposium was organised as a joint effort of the ROPME and the IOC of UNESCO.

About 50 experts from ROPME member states attended the symposium. The presentations by representatives of research and monitoring agencies in the region provided an overview of HAB events and of the needs for capacity building in their institutions.

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(Cont'd from p. 2)

ancient settlements could have recently become visible (bloom) because of the modification of their environment (anthropogenic pressures, global warmth) or increased monitoring efforts.

Acknowledgements

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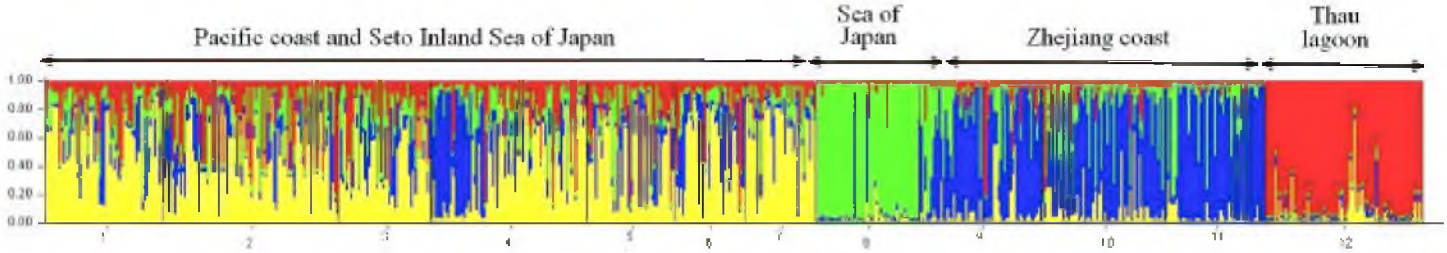

Fig. 2. Population structure based on 7 microsatellite loci of Alexandrium catenella *estimated by genotype clustering by* STRUCTURE. *Assignment of 651 individuals to K=4 genetically distinguished groups; 3 runs. For each run, we used a burn-in period of 50.000 generations and 200.000 iterations.*

(Cont'd from p. 1)

Live cells of *Chattonella* and *Fibrocapsa* species were identified in unfixed samples. For cell counts, water samples were fixed using non-acid Lugol and analyzed by the Utermöhl technique. Fixed cells were deformed and shrunken, making it difficult to identify species without studying live cells. Manipulation of live cells provoked them to become round-shaped. We observed that light passing through the microscope also deforms the cells and chloroplasts migrate to the center of the cell.

SST from December through May ranged from 18.5 °C to 22.5 °C. Four raphidophyte taxa were observed from Dec 2008 through May 2009. *Chattonella marina* was found in net hauls collected in Dec 2008 and Feb-Mar 2009. Cells of *C. marina* vary in size and form (Plate 1; Figs. 6, 7), from 40–55 µm × 24–28 µm (n = 10). *Fibrocapsa japonica* was found in May 2009 (Plate 1; Figs. 8, 9), for the first time in Bahía de La Paz. The most distinctive characteristic of this species is the group of mucocysts in the posterior part of the cell; *F. japonica* cells ranged from 22–31 µm × 14–18 µm (n = 22).

Two cells of an unidentified raphidophyte were also found in samples collected in January 2009 (Plate 1, Fig. 5). *Chattonella ovata* was found from early winter (Jan 2009) to the end of May; abundance ranged from 3000 in February to 37000 cells L^{-1} in March 2009 in surface samples; cells vary greatly in size and shape (Plate 1; Figs. 1–4), from 30–85 µm × 30–47 µm (n = 45). Cells of *C.* cf *ovata* have been observed in Bahía Kun Kaak, Sonora (28° 52' N, ~112° 04' W) and in Bahía de Mazatlán [1, 3]. In Bahía de La Paz, no dead fish or shellfish were observed during the first bloom of *C. marina* [2]. The low densities of *Chattonella* and *Fibrocapsa* species found during this study may explain the absence of mortality.

Identification of live plankton was an opportunity to observe several heterotroph dinoflagellates (such as *Noctiluca scintillans*) and ciliates [Plate 1, Fig. 10] feeding on *C. ovata*. *N. scintillans* is one of the most common dinoflagellate predators in the Gulf of California. A bloom of *Cylindrotheca closterium* (1.24 × 10^6 cells L^{-1}) occurred on 19 Mar 2009 (Plate 1, Figs. 3) together with *Chattonella ovata*. Dividing cells of *C. ovata* and *F. japonica* indicate conditions were favorable (Plate 1; Figs. 2, 9, respectively).



Plate 1. Light photomicrographs of Raphidophytes collected in Bahía de La Paz from December 2008 through May 2009. Live cells Chattonella ovata *(1–4). Unidentified raphydophyte cell (5).* C. marina *(6–7).* Fibrocapsa japonica*; arrow indicates presence of mucocysts in the posterior of the cells (8–9). Ciliate full of* Chattonella *cells; arrow indicates a* Chattonella ovata *cell (10).*

Raphydophytes, like other flagellates, are very delicate and deform or disintegrate in formalin; samples preserved with lugol change morphology, but can be recognized by a well-trained eye. Studies of live phytoplankton lead to identification of species otherwise omitted in traditional phytoplankton monitoring. We suspect that raphidophytes, as well as naked dinoflagellates, have not been detected in Mexican coastal waters because traditional methods used for sampling and preserving phytoplankton destroy identifying characteristics. For more accurate monitoring of harmful algae, unpreserved samples should be used to observe delicate phytoplankton forms. Some raphidophytes appear to have differences in morphology, but are genetically the same, as demonstrated with *C. marina*, *C. antiqua*, and *C. ovata* [6]. Cloned cultures of *C. ovata* from the bloom here sampled were isolated to evaluate toxicity and confirm their identification with molecular tools.

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PHYCOTOXINS

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• Uruguay

First report of *Pseudo-nitzschia multistriata* in Uruguay – January 2009

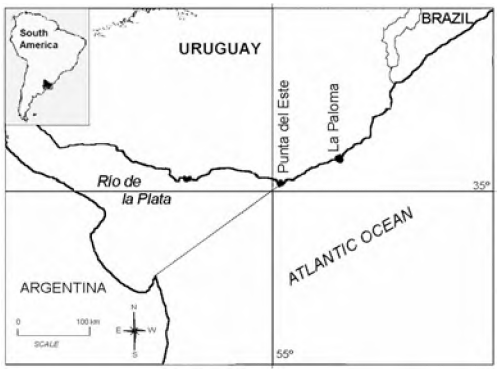

Fig. 1. Map showing the study area with location of samples in Punta del Este and La Paloma (Uruguay), where P. multistriata *and* P. multiseries *were detected.*

A long-term phytoplankton monitoring program has been conducted in Uruguay since 1980 by the National Management of Aquatic Resources (DINARA, DIrección NAcional de Recursos Acuaticos). This programme extends over about 250km of shoreline (Fig. 1). It includes weekly samples of phytoplankton, biotoxins and environmental factors measurements. Several toxic phytoplankton blooms have affected these estuarine and coastal waters almost every year, mainly with Paralytic Shellfish Toxin (PST) above the regulatory limits and the presence of Lipophylic Shellfish Poisoning (LSP). The species associated with PST outbreaks are *Gymnodinium catenatum* and *Alexandrium tamarense*. LSP producing species reported in the area are *Dinophysis acuminata*, *D. caudata*, *D. acuta,* and *D. rotundata*. The only Amnesic Shellfish Toxin (AST) event reported in mussels from Uruguay was in December 2001, below the regulatory level, caused by *Pseudo-nitzschia multiseries*. PST and LSP in mussels were determined by official bioassay and Domoic Acid (DA) by the standard HPLC method [1–3].

Qualitative and quantitative phytoplankton samples were analyzed under Light Microscope (LM), Leitz Diaplan and inverted microscope (Leitz Labovert FS), using the Uthermöhl method, respectively. In later studies at the University of La Plata, samples were analyzed using Leica DM 2500 LM and scanning electron microscope (SEM) Jeol JSM-6360 LV. Cells were washed with distilled water and treated using conventional methods, material not cleaned and cleaned of organic matter was mounted on permanent glass slides using Naphrax medium, and examined with phase contrast LM, equipped with Leica DFC420 digital camera.

Examination of natural water samples (January 2009) fixed with Lugol, from Punta del Este and La Paloma (Fig. 1), showed blooms of potentially toxic diatom *P. multistriata*, and another *Pseudo-nitzschia* species [4, 5]. There was no associated toxicity in local mussels. This is the first report of *P. multistriata* in shelf waters of Uruguay.

In field samples from Punta del Este and La Paloma (Fig. 1), a *P. multistriata*

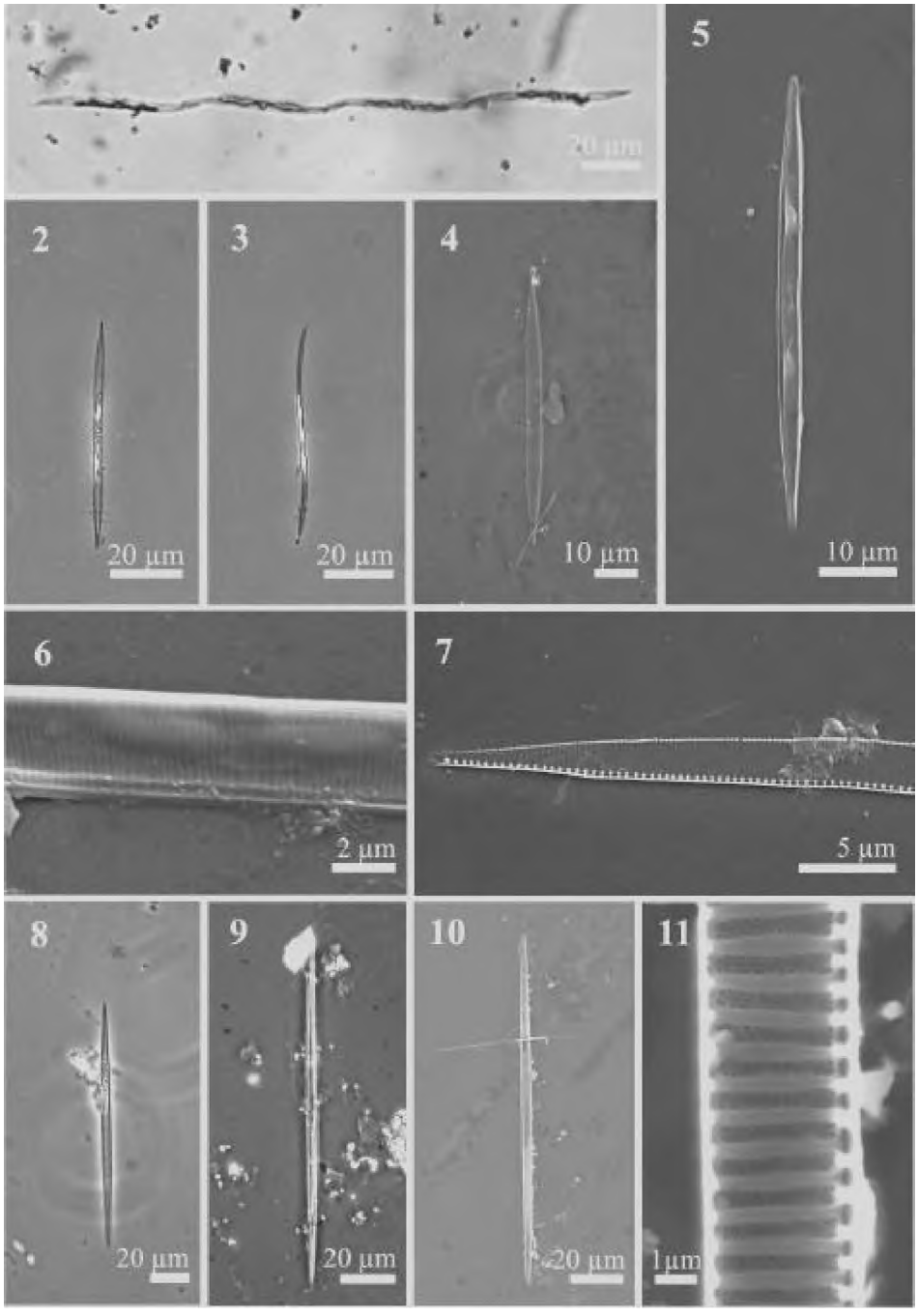

Fig. 2. Images of P. multistriata *1-7, (1–4 LM, 5-7 SEM) and* P. multiseries *8–11, (8–9 LM, 10-11 SEM), from Uruguayan samples, January 2009.*

• Mexico

Pseudo-nitzschia subcurvata (Bacillariophyceae) in the Gulf of Mexico?

Species of the genus *Pseudo-nitzschia* are commonly characterized by linear to spindle-shaped narrow valves; only one species with curved valve outlines has been described, *Pseudo-nitzschia subcurvata* (Hasle) G. Fryxell. This species has been traditionally considered to be restricted to the cold waters of Antarctica [1] but there are now records from the Northern Hemisphere as well as tropical and subtropical waters (Table 1). This species may represent one of the most important fractions of the phytoplankton assemblage in the Antarctic Ocean [2, 3].

In routine observations (using light microscopy) of phytoplankton samples collected during a one-year period (2007–2008) in the National Park Sistema Arrecifal Veracruzano, a coral reef system in the southern Gulf of Mexico facing the state of Veracruz, Mexico (19°02'–19°16'N, 95°46'–96°12'W), a diatom species resembling *Pseudo-nitzschia*, with valves with

(Cont'd on next page)

(Cont'd from p. 3)

bloom was observed simultaneously with *P. multiseries*. *P. multistriata* formed chains of sigmoid shape in girdle view (Fig. 2, 1). The distinctive shape of the colonies in girdle view is a character that can be easily observed under LM, however it was identified under SEM (Fig. 2, 5–7).

Cells of *P. multistriata* overlap at their ends for about one-eleventh the frustule length and the valve are slightly asymmetric, with one side straight and the other convex, tapering rapidly towards rounded ends (Fig. 2, 1–5). Valve width is 2.5–3.3 µm and length 52–63 µm. With SEM, valve surface, show 25–28 fibulae in 10 µm and 35–40 striae in 10 µm, each striae biseriate, rarely with one or three rows of circular small poroids. The poroids number 11–13 in 1 µm. There is no central interspace between the two central fibulae (Fig. 2, 5–7).

The number of poroids is the same as material from the Atlantic coast of France [6], the Gulf of Naples, Italy [4] and Peter the Great Bay, Russia [7]. Although this differs from the original description [8] of 5–6 poroids per 1 µm, Larsen & Nguyen [9] noted that this number is probably an error, since Takano's indication of 5–6 poroids is in conflict with his published photographs.

P. multistriata is recorded from Japan [8], New Zealand [10], Italy [4], Spain [11], Vietnam [9] and France [6]. This is the first report of *P. multistriata* from Uruguay and the second report for South America since it has only been found in Brazil before [12, 13].

P. multiseries was analyzed with LM and SEM, and is characterized by

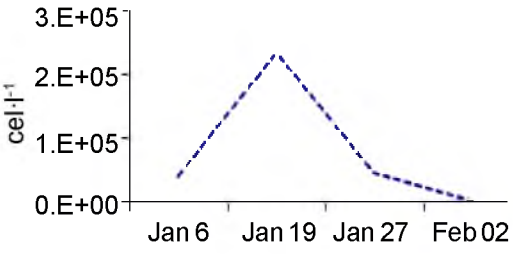

Fig. 3. Pseudo-nitzschia multistriata *in Punta del Este, Uruguay, January 2009.*

symmetrical frustules, linear to lanceolate in girdle as well as in valve view, with rounded ends. The length of the valve is 90–140 µm and width 3.5–4 µm. (Fig. 2, 8–9). With SEM the number of interstriae is 12–15 in 10 µm and fibulae 13–15 in 10 µm. Each striae has three rows of poroids. The number of poroids per 1 µm is 4–5.5. The valve shows no central interspace (Fig. 2, 10–11).

Field samples showed that the bloom started in early January, reached the maximum concentration of 236 x 10^3 cells L^{-1} in January 19 at Punta del Este (Fig. 3); lower concentrations of 200 cells L^{-1} were observed at La Paloma in early February.

Environmental conditions during the bloom were characterized by salinity between 32.5 and 34.4 and water temperature 19–23°C. The reported salinity is considered high for this region. Summer environmental data from this monitoring programme were studied and the average salinity and temperature (January–February 1991–2009) was 26.7 and 22.1°C respectively.

The presence of *P. multistriata* in Uruguayan waters may be related to high salinity values reported during the bloom after several months of drought. We think this environmental condition could be a limiting factor in accordance with studies by Villac [12] who found salinity to be a growth factor for this species under experimental conditions.

Acknowledgements

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Table 1. Morphometric data of Pseudo-nitzschia *spp. with curved cells.*

Species	Valve shape	Apical axis (µm)	Transapical axis (µm)	Stria in 10 µm	Fibulae in 10 µm	Distribution	Comments	Reference
Pseudo-nitzschia granii var. *curvata* Hasle	Rhomboid valves, curved cells probably in girdle view	25-79	1.5-2.5	44-49	12-18	Northern cold waters to temperate? Subarctic Pacific Ocean	Solitary, associated with *Phaeocystis*	Hasle 1964 [4] Hasle & Syvertsen 1997 [1] Taylor and Water 1982 [8]
Pseudo-nitzschia subcurvata (Hasle) G. Fryxell in Fryxell *et al.*	One side straight or slightly concave, the other convex	47-113	1.5-2.5	44-49	12-18	Southern cold water regions Bearing Sea Central and southern Great Barrier Reef Artic sea ice	Solitary, abundant in Antarctic zone	Hasle 1964, [4] Hasle & Syvertsen 1997 [1] Flint *et al* 2001 [3] Crosbie & Furnas 2001 [9] Ikavalko 2003 [10] Scott & Thomas 2005 [11]
	One margin straight, the other convex	47-113	1.5-2.5	44-49	12-18	Antarctic	Solitary or in chains	Almandoz *et al* 2008 [5]
	One side sharp or slightly concave, the other convex	48-86	1.3-1.8	43-55	12-22	Weddell Sea	Mostly solitary, rarely cells in pairs	
Pseudo-nitzschia cf. *subcurvata*	Both sides of valves curved (one concave, the other convex)	28-36	1.9- 2.5	?	?	Veracruz Reef System, Gulf of Mexico	Cells in pairs, rarely forming chains 3-6 cells	This report

markedly curved outlines was detected (Fig. 1). This species was rare, and few cells were observed in net hauls (mesh 30 µm). The cells were encountered commonly in pairs and rarely in chains of 3 to 6 cells. Cells were joined by their valve surfaces overlapping 1/7 to 1/8 of the cell length. The valves were narrow, attenuated towards the tapering apices, and both sides of the valves were parallel curved. No valve structures were resolved under the light microscope. General morphology, i.e. valve outline, was consistent with that described for *P. subcurvata*, but the length of the apical axis was less than the range recorded for *P. subcurvata*. According to Hasle 1964 [4], the fibula can be observed with a light microscope; we were unable to resolve it in our specimens using an oil-immersion 100x/ 1.25 Ph objective. Almandoz *et al.* 2008 [5] in their study of *Pseudo-nitzschia* species in the Weddell Sea gave a description of *P. subcurvata* and showed a picture of two cells. This picture resembles the paired specimens we observed in our samples from the Gulf of Mexico. We are aware of the morphological similarities between various species of the genus [6], and complementary techniques (electron microscopy, cultures and molecular studies) must be used for accurate species identification. However, the conspicuous curved outline of the valve, the records in the Northern Hemisphere, and the record of *P. subcurvata* in tropical areas make us believe that we may be observing a *P. subcurvata* morphological variation or a closely related species. *Pseudo-nitzschia* species have shown to be potential producers of domoic acid, and even when they may not be detected in routine phytoplankton counts, they can be "hidden" in the environment [7]. Our future efforts must be directed to the detection of this morphotype during the monitoring programs of harmful algal blooms that we (Unidad de Investigación de Ecología de Pesquerías in collaboration with the Acuario de Veracruz) are implementing in the Veracruz coastal waters to initiate cultures of local species and to collaborate with the investigators working with the molecular biology of the genus.

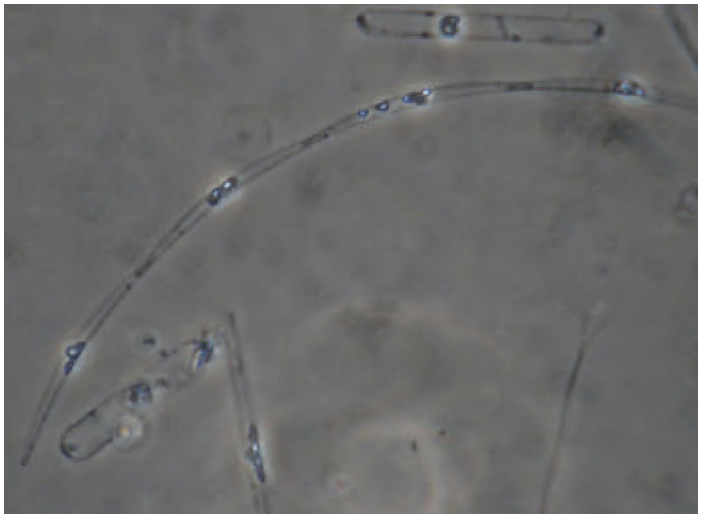

Fig. 1. Light micrograph of Pseudo-nitzschia *cf.* subcurvata *found in the southern Gulf of Mexico.*

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• Mexico

Cochlodinium polykrikoides and *Gymnodinium catenatum* in Bahía de Acapulco, Mexico (2005–2008)

Microalgae blooms are frequent and periodic throughout the year along the Pacific coast of Mexico. A recent bibliographic study reveals the occurrence of more than 175 blooms along the coast [1]. Species with the greatest increase are the ciliate *Myrionecta rubra* and the dinoflagellates *Noctiluca scintillans* and *Gymnodinium catenatum* [1]. One the most studied areas in the Pacific region is the Gulf of California [1, 2]. HABs are common events along the coasts of Guerrero, particularly in Bahía de Acapulco. The predominant species in this bay are: *Akashiwo sanguinea, Ceratium balechii*, *Hemiaulus hauckii*, *Noctiluca scintillans*, *Prorocentrum gracile*, *Pyrodinium bahamense* var. *compressum*, and *Takayama* sp. [1, 3–4]. In the last four years, naked, chain-forming, icthyotoxic dinoflagellate *Cochlodinium polykrikoides* and the paralytic shellfish toxin producer *Gymnodinium catenatum* are two of the most recurring species. *C. polykrikoides* is characterized by a conical epitheca and a bi-lobed hypotheca with single cells being ellipsoidal with a displaced cingulum. Although the first blooms of *C. polykrikoides* in Bahía de La Paz were found in 2000 and 2001 [5], blooms of this species were reported in Bahía de Mazatlán since 1979 as *Cochlodinium* sp. [6] and later as *C. catenatum* [7] in Bahía Banderas. *C. polykrikoides* was originally described by Margalef and commonly occurs in warm-temperate and tropical waters, and is broadly distributed along the Pacific coast of Mexico [8] and also *G. catenatum* are prominent in blooms [1, 9–10]. Sometimes, these two species seems to occur simultaneously in our coasts [5, this study].

This report describes the blooms of *C. polykrikoides* and *G. catenatum* that were documented at sampling stations inside Bahía de Acapulco from 2005 through 2008 (Fig. 1). Samples were taken with a plastic bucket and preserved with Lugol's solution. Temperature was recorded with a bucket thermometer. In some cases, a sub-sample was taken for live phytoplankton analysis. Live samples of the red tides were used for identification of *C. polykrikoides* (Figs. 2 and 3) and *G. catenatum* (Figs. 4 and 5). Abundance was estimated using a Sedgwick chamber under an inverted microscope [2].

The first record of *G. catenatum* in Bahía de Acapulco had a low density (7–78 × 10^3 cells L^{-1}) in March 1999 [11, 12]. The first bloom of *C. polykrikoides* was observed with a bloom of *G. catenatum* in December 2005, through February 2006. The highest density of *C. polykrikoides* ranged from 1264 × 10^3 cells L^{-1} at Punta Bruja to 8228 × 10^3 cells L^{-1} at Playa Icacos. Highest density of *G. catenatum* ranged from 141 × 10^3 cells L^{-1} at Punta Bruja to 1604 × 10^3 cells L^{-1} at Playa Icacos. Estimated density of *C. polykrikoides* during January and February 2006 ranged between 980–1600 × 10^3 cells L^{-1}. Cells counts of *G. catenatum* ranged between 4200–10,000 × 10^3 cells L^{-1} (Table 1). Blooms of *C. polykrikoides* but not *G. catenatum* occurred in June 2007. Density of *C. polykrikoides* varied from 44.8 ×10^3 cell L^{-1} in the entrance of the bay to 2088 × 10^3 cells L^{-1} at the Muelle Base Naval. In December 2007, the two species flourished together, with C. *polykrikoides* reaching 532 × 10^3 cells L^{-1} and *G. catenatum* reaching 1942 × 10^3 cells L^{-1}. Temperatures were within a narrow range of 26 to 27 ºC during this bloom. During 2008, several blooms of *C. polykrikoides* occurred. Lowest counts were (39–58 × 10^3 cells L^{-1}) in early January and the highest counts were (1100–1250 × 10^3 cells L^{-1}) in late January. Temperatures were 23–27 ºC. Another bloom occurred in April, with C. *polykrikoides* at 1160–6000 × 10^3 cells L^{-1} at several sampling stations. Temperatures were 25–29ºC. In October of the same year, a bloom of *C. polykrikoides* occurred at Playa de Icacos with highest density of 2680 × 10^3 cells L^{-1} (Table 1).

Although a monitoring program identifies and quantifies toxic and potentially toxic species, such as *C.*



Fig 1. Sampling stations in the shore of Bahía de Acapulco, Acapulco, Guerrero, Mexico.









Figs. 2–5. Light photomicrographs of live four cells chains Cochlodinium polykrikoides *(1, 2) and* Gymnodinium catenatum *(3). Lugol's fixed cells of* Gymnodinium catenatum *(4) from Bahía de Acapulco, Acapulco Guerrero.*

Table 1. Abundance (x 10^3 cells L^{-1}) of Cochlodinium polykrikoides *and* Gymnodinium catenatum *at five sampling stations in Bahía de Acapulco, Acapulco, Guerrero, Mexico*

Date	Species	Punta Bruja	Casa Díaz Ordaz	La Bocana	Muelle Base Naval	Playa de Icacos
06/12/2005	*G. catenatum*	0	0	0	1104	141
	C. polykrikoides	2	6	0	6432	1692
15/12/2005	*G. catenatum*	6	4	0	16	1604
	C. polykrikoides	86	8	0	241	8288
22/12/2005	*G. catenatum*	48	0	0	8	32
	C. polykrikoides	1264	94	0	4	54
		0	0	0	0	0
10/01/2006	*C. polykrikoides*	0	0	0	1100	1600
	G. catenatum	0	0	0	10000	7600
14/02/2006	*C. polykrikoides*	0	0	0	980	1400
	G. catenatum	0	0	0	5000	4200
28/06/2006	*C. polykrikoides*	0	0	0	232	246
	G. catenatum	0	0	0	130	136
		0	0	0	0	0
12/06/2007	*G. catenatum*	0	0	0	0	0
	C. polykrikoides	1792	44.8	1292	2088	110
06/12/2007	*C. polykrikoides*	17	62	192	30	130
	G. catenatum	27	257	1942	120	3
18/12/2007	*C. polykrikoides*	0	0	0	532	0
	G. catenatum	458	487	0	8	1083
		0	0	0	0	0
09/01/2008	*C. polykrikoides*	0	0	0	39	58
31/01/2008	*C. polykrikoides*	0	0	0	1100	1250
02/04/2008	*C. polykrikoides*	0	1370	0	2070	6000
08/04/2008	*C. polykrikoides*	0	0	0	1160	3150
29/10/2008	*C. polykrikoides*	0	0	0	36	2680

polykrikoides and *G. catenatum*, new programs, including hydrological and phytoplankton monitoring must be improved. These programs are necessary to prevent future mass kills of marine animals, particularly caged species of economic value. No fish mortalities were observed in Bahía de Acapulco during the blooms of *C. polykrikoides*. Toxicological studies of blooms revealed high toxicity levels. In March 1999, toxicity was 120–209 µg STXeq 100 g^{-1} [11, 12] and similar levels occurred in December 2005 (25–217 µg STXeq 100 g^{-1}) [1]. During the blooms of *G. catenatum* here reported, catches of marine mollusks were prohibited to avoid health public problems. Monitoring of other PSP toxin-producing species, such as *Alexandrium tamarense*, which was recorded in the bay [3] and *Pyrodinium bahamense* var. *compressum* must be addressed because this last species have generated public health problems in the past [4]. Adequate water quality is necessary to achieve sustainability of coastal ecosystems. Insights into the mechanisms that determine lethal species composition are needed to predict the effect of water quality. On the other hand, examination of live phytoplankton has identified other bloom-forming dinoflagellates, such as *Gyrodinium*, *Karenia*, and *Takayama* [1].

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SCOR Visiting Scholars for 2010

In 2009, SCOR initiated a program of SCOR Visiting Scholars and named one scholar, Paulo Relvas (Portugal), to teach a physical oceanography course in Guatemala. SCOR's program covers all areas of ocean science, but not observations or modeling, which are included in a POGO program (see: www.ocean-partners.org/index.php?option=com_content). SCOR is looking for both individuals interested in serving as Visiting Scholars in 2010 and institutions in developing countries who are interested in hosting a SCOR Visiting Scholar. If you or your institution are interested in participating in the program, please visit: www.scor-int.org/capacity. Requests are accepted by 31 January 2010.

• India

Microcystis aeruginosa bloom in a high altitude lake in the Western Ghats of India



Fig. 1. Map showing the location of the Lake in the Western Ghats.

Toxin producing Cyanobacteria in lakes and reservoirs form a threat to humans, bird and fish as well as various other forms of aquatic life. Increased detection of cyanotoxins in water bodies generates a complex challenge for water resource managers all over the world; among these toxins, microcystins are potent liver toxins and tumor promoters produced by several common freshwater Cyanobacteria including *Microcystis* [1].

An algal bloom was first observed in 2005 in Mullaperiyar Lake in the Western Ghats of India (Fig. 1), coincident with a mass death of tadpoles. Itching of the skin among fishermen and forest officials was also reported. A study to determine the presence of toxic algae in the bloom was carried out. The Mullaperiyar Dam was constructed across the Periyar River in 1895, and has a surface area of 26 km^2.

Water samples were collected from the Lake between 15th and 20th of every month of 2005 in sterilized polythene bottles, from different locations in the Lake. The samples were analyzed in the laboratory for the presence of toxic algae; the blooms were dominated by *Microcystis aeruginosa* (Fig. 3). During the pre-monsoon (February to March) and northeast monsoon (October to January) of 2005, *M. aeruginosa* flourished at different locations in the Lake. *Microcystis aeruginosa* is an S-strategist, i.e. stress tolerant, with low growth rate, high nutrient storage capacity, and enhanced resistance to sinking and grazing losses, and apparently characteristic of waters with phosphate fluctuations, is a specialist in phosphate storage and efficient in regulating its density [2]. *Microcystis* is widely distributed, dominates the phytoplankton community in nutrient rich lakes and is the main microcystin producer of lakes [3–4]. The gut analysis of the tadpoles revealed the presence of *Microcystis* cells. Thus the cause of the tadpole deaths could have been "mycrocystin". Hence presence of this alga in the lake poses a serious threat also to wildlife in this sanctuary (especially to the endangered tiger population of the area); the degree to which they may be affected by this alga should therefore be monitored. 'Cyanotoxins' in general are hepatotoxins, known tumor promoters, and have been associated with high rates of primary liver cancer in people drinking water with high concentrations of microcystins. The presence of this toxic bloom was reported during 2008 by one of the authors in Chalakudy River originating from the southwestern Ghats. This should be seriously considered since the water is the sole source for drinking in five districts of the neighboring state of Tamil Nadu.

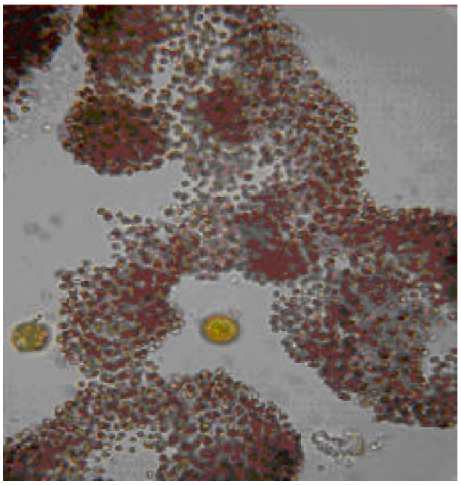

Fig. 3. Microcystis cells *x 45.*



Fig. 2. Mullaperiyar Lake.

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14th International Conference on Harmful Algae

First Announcement - June 2009

The International Society for the Study of Harmful Algae (ISSHA) is pleased to announce that the 14th International Conference on Harmful Algae will take place in Hersonissos, Crete, on 1–5 November 2010, with the support of the Hellenic Centre for Marine Research (HCMR).

Aims & Topics

The Conference will address the following topics:

- Population dynamics of HABs
- Time series of HAB events: Climatic and anthropogenic induced impacts
- Impact of HABs on marine food webs and ecosystem structure and function
- Biological interactions: allelopathy, mixotrophy, parasitism, symbiosis, bacteria and viruses
- New regional HAB events
- Inland Seas and HABs
- Introduction of alien species and HABs
- Cyanobacterial ecology, physiology and bioactive compounds
- Genomics and genetic diversity of HABs
- Toxins: chemical structure and synthesis, detection and analytical methods
- Novel sensor technologies for bio-sensing applications in HAB research and monitoring
- Harmful Algae culture collections
- Management, mitigation and public outreach for HABs

The official language of the Conference is English.

The Conference proceedings will be published under the auspices of the International Society for the Study of Harmful Algae (ISSHA) and the Intergovernmental Oceanographic Commission (IOC).

Abstract submission

You may submit your abstract(s) online, by 1 June 2010. Further details on abstracts and topics to be covered by the Conference will be available soon. If you have any questions please contact: info@hab2010.gr



Important dates

Deadlines, for Early Registration 1 June 2010, for Late Registration 15 Sep, for Full Paper Submission 30 Nov.

About Crete

Crete is the largest Greek island, one of the thirteen regions of Greece and the fifth largest island in the Mediterranean. Heraklion, the capital, is a dynamic city, the fifth largest in Greece. Crete is renowned for its natural beauty and diverse landscape. Spectacular mountains are sliced by impressive gorges that spill out to the sea, while infinite plateaus and fertile plains form its interior, while stunning endless beaches and isolated coves comprise its 1046 km coastline. Crete's traditional villages, cultural heritage and significant ancient history; as the centre of Europe's most ancient civilization; the Minoan; are some of the reasons why Crete is one of the most popular tourist destinations in Europe. Hersonissos, a traditional and picturesque town in northern Crete, is situated 26 km east of Heraklion. It offers many accommodation options, two sandy beaches, traditional and international cuisine restaurants, souvenir shops and a famous nightlife.

Transportation

Direct flights from several European cities to the N. Kazantzakis international airport in Heraklion, Crete or connecting international flights via the Athens El. Venizelos international airport operate daily. Alternatively, ferries operate both ways between the Heraklion and Piraeus.

Participation in social events

During the Conference, participants may choose from a selection of tours in the area. These include tours of the Knossos Palace —the largest Bronze Age archaeological site on Crete and what was probably the ceremonial and political centre of the Minoan civilization —and the Cretaquarium— a site of marine science and aquaculture, a meeting point where science, discovery and recreation are equally provided.

More info will be posted on the 14th ICHA website at: www.hab2010.gr

ICHA2010 Secretariat

Hellenic Centre for Marine Research (HCMR), Institute of Oceanography, 47 km Athinon-Souniou, 19013 Anavyssos, Attiki, Greece. Tel: +30 229 10 76466, Fax: +30 229 10 76323. Email: info@hab2010.gr

Tentative registration fees

All payments must be in EUR.

Please visit the ISSHA website (www.issha.org) and/or www.hab2010.gr to keep up to date with conference information.

Early, until 1 June 2010		Late, from 1 June to 15 Sep 2010		After 15 Sep 2010 and On-site	
Participant	**Fee**	**Participant**	**Cost**	**Participant**	**Cost**
ISSHA Member	330 €	ISSHA Member	400 €	ISSHA Member	450 €
Non ISSHA Member	430 €	Non ISSHA Member	500 €	Non ISSHA Member	550 €
Full Delegate		Full Delegate		Full Delegate	
Students	165 €	Students	200 €	Students	225 €
Accompanying	165 €	Accompanying	200 €	Accompanying	225 €
Day Card	100 €	Day Card	125 €	Day Card	150 €

• Mexico

Gambierdiscus toxicus in the southeastern Gulf of Mexico

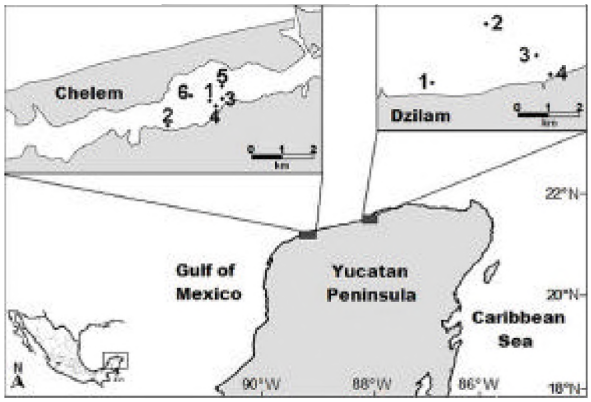

Fig. 1. Sampling sites at the northern coast of Yucatan Peninsula, Mexico, in 2008-2009.

Gambierdiscus toxicus Adachi et Fukuyo is known in Gulf of Mexico waters: e.g., the Florida Keys [1; M. Parsons, unpublished data]; Mexican coastal waters [2], and the northwestern Gulf [3]. This species most likely causes ciguatera, and affects fisheries and human health in the gulf. The first written report of ciguatera fish poisoning in Florida was in 1918 [4]. It is estimated that 1300 cases of ciguatera occur annually in just four counties in Florida (Dade, Broward, Palm Beach, and Monroe) [5].

Ciguatera is also common in Mexico, where 291 incidents were reported in the 1980–1990s from Baja California Sur in the Pacific, and from the Mexican Gulf states, Quintana Roo and Yucatan [6–7]. It is still unknown if *G. toxicus* is endemic or a recent invader to the Gulf of Mexico. *G. toxicus* was rare in the southern region of the Gulf of Mexico, where we recorded it in Veracruz reef zone waters in biweekly samples in 2005 [2].

Epiphytic microalgae were monitored at 10 stations in two localities near the northern Yucatan Peninsula, Mexico, between May 2008 and May 2009, (2008–May 19, 21; August 20, 26; November 25, 28: 2009–March 3, 6; May 19, 21). About 250 samples of macrophytes with associated microalgae were taken at Dzilam de Bravo (6 sites) and in the lagoon El Ojo de Agua near Chelem (4 sites) to obtain data on annual dynamics of epiphytic dinoflagellates (Fig. 1). Samples were collected manually during free diving to a 4-m depth. Water temperature (20.5–32.7°C), salinity (26.5–38.6 psu), dissolved oxygen (2.27–12.6 mg/L), turbidity (36.73–63.8 ntu), nitrates (0.15–26.56 µM), nitrites (0.01–0.73 µM), ammonium (0.02–6.96 µM), phosphates (0.13–1.76 µM) and silicates (1.65-139.96 µM) were measured. Dinoflagellates were identified and counted using Olympus BX51 and CKX41 microscopes and a JEOL JSM-5310LV scanning electron microscope (Fig. 2-F).

Macrophyte beds were dominated by the seagrasses *Thalassia testudinum, Syringodium filiforme, Halodule wrightii* and *Rupia maritima* and by algae of the genera *Acetabularia, Batophora, Caulerpa, Chaetomorpha, Chondria, Eucheuma, Gracilaria, Halimeda, Laurencia, Penicillus* and *Udotea.*

While pennate diatoms usually prevailed as epiphytes, dinoflagellates were common and present in almost all samples. *Prorocentrum* spp. (at least 6 species of which are potentially toxic), *Coolia monotis, Gambierdiscus toxicus, Amphidinium* cf. *carterae, Bysmatrum caponii, Pileidinium* sp., *Sinophysis microcephala, S. ebriolum, S. stenosoma, Ostreopsis* cf.

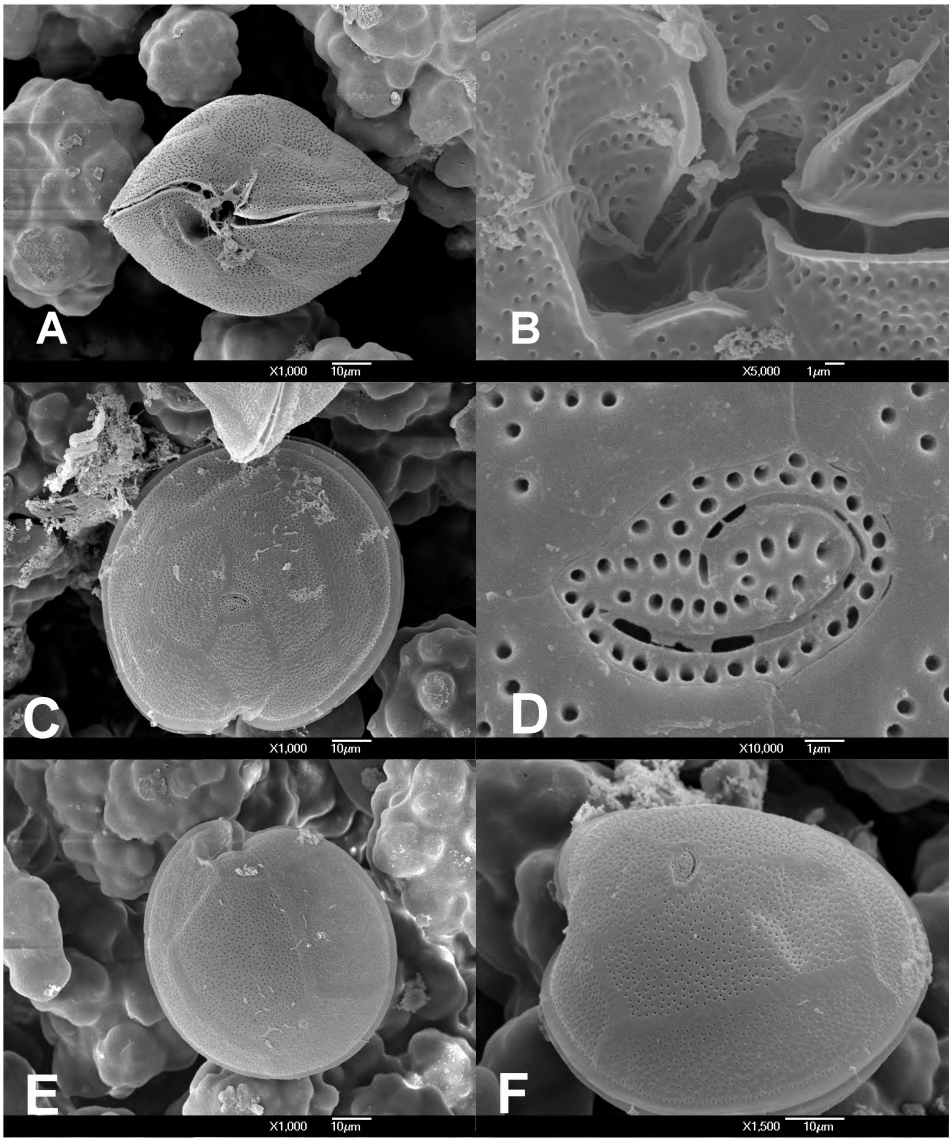

Fig. 2. Gambierdiscus toxicus *from Yucatan: a) ventral view, b) sulcul area, c) apical view (epitheca), d) the pore plate, e) antapical view (hypotheca), f) apical-left-side view.*

heptagona and some dubious taxa preliminarily identified as *Durinskia* sp., *Cabra matta* and *Togula*-like species contributed to the epiphytic associations. Frequently, *Prorocentrum mexicanum* was the dominant dinoflagellate.

In comparison with the Veracruz epiphytic associations, the almost complete absence of *Ostreopsis* spp. and the relative abundance of *G. toxicus* were characteristics of the Yucatan samples. Yucatan specimens of *Gambierdiscus* correspond well with the original description and figures of *G. toxicus*, although showing a rather narrow range of size variability: 52.5–70 µm long (58.59 ± 4.34), 65–95 µm wide (78.52 ± 6.83) and 63–87.5 µm deep (72.96 ± 6.75), width/length ratio 1.28–1.62 (1.39 ± 0.06) and width/depth ratio 0.71–1.21 (1.03 ± 0.16) (n = 100). All measured cells are smaller that those reported from the Mexican Caribbean, 97–102 µm wide [8]. Cells are lenticular, considerably anterior-posteriorly compressed and slightly compressed ventrally, with smooth and porous theca. Cingulum is narrow and deep, in general circular but ascending on ventral side, forming a sigmoid line around the sulcal area. Sulcus is deep, bordered posteriorly with a V-shaped rim. Apical pore complex is ovoidal and somewhat angulated, especially in its narrower part, which is directed to the left side of the cell, with a fishhook-shaped apical pore; the pore plate is 6.23–8.61 (7.32 ± 0.75) µm long and 4.56–5.38 (5.03 ± 0.24) µm wide, length/width ratio 1.27–1.63 (1.47 ± 0.14) (n = 8). The 2' plate is about 1.5 times larger than the 3' plate. Precingulars 1" and 7" are extremely small and do not contact with the apicals 2' and 4'. The 4" plate is almost symmetrical in relation to the longitudinal axis of the cell. The 1"" plate is about 1.5-2 times smaller than the 2""plate. Compared to the original illustrations [9], the cells from Yucatan have a relatively wider 1' plate. Nevertheless, we identify them as *G. toxicus*.

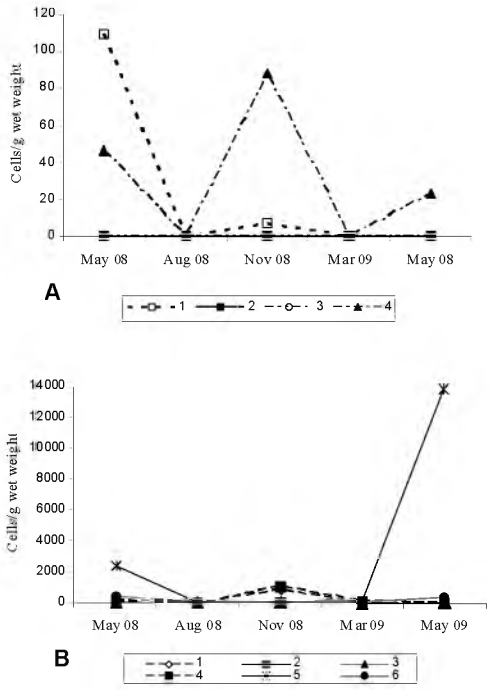

Fig. 3. Annual cycle of Gambierdiscus toxicus *at Dzilam at 4 stations (A) and Chelem at 6 stations (D), Gulf of Mexico. Mean values averaged from all the samples for each station are shown.*

At Dzilam (Fig. 3A, 4A) *G. toxicus* was sporadically found only in May and November 2008, in low numbers at stations 1 and 4 close to the coast (Fig. 1). Near Chelem (Fig. 3B, 4B) it was present year round, but only in one sample in August 2008. The highest numbers of *G. toxicus* were encountered in May and November (Table 1). The absence of *G. toxicus* in early March 2009 at Dzilam, and very low concentrations (maximum 17 cells/g wet weight) at Chelem, may have been related to comparatively low water temperatures (20.5–22.7°C). Curiously, the total absence of this species in early March samples at Dzilam coincided with the minimal registered nitrate, nitrite and ammonium concentrations. In general, the distribution of abundance of *G. toxicus* in the two areas was very heterogeneous (Fig. 3A-B).

G. toxicus can easily be overlooked when present in low densities. We recommend sampling with a hand net in shallow waters, because *G. toxicus* is sometimes rather abundant in horizontal net hauls; besides, net sampling provides a cleaner material for scanning electron microscopy. It is obvious that *Gambierdiscus* cells can be transported along the coast to new sites.

Table 1. Maximal abundances of Gambierdiscus toxicus *found at the northern Yucatan coast in 08-09 (in cel/g substrate wet weight).*

Substrate	Month	Site	St.	Cells/g
Hydroid	May-09	Chelem	5	9988
Halodule wrightii	Nov-08	Chelem	5	3962
Halodule wrightii	May-08	Chelem	5	1733
Batophora oerstedii, B. occidentalis	Nov-08	Chelem	6	1255
Enteromorpha spp.	May-08	Chelem	5	647
Halodule wrightii	Nov-08	Dzilam	4	532
Rhodophyta gen. spp.	Nov-08	Chelem	4	425
Gracilaria sp.	May-08	Chelem	6	318
Caulerpa paspaloides	Nov-08	Chelem	1	224

There is no commercial fishery in the areas studied. Most of fish are carnivorous. The omnivorous pinfish *Lagodon rhomboides* (Sparidae), typical in shallow subtropical estuaries, is the dominant species. In Florida waters,older pinfish consume increasing amounts of plant material in addition to animal prey, and their foraging activities are capable of altering the composition of estuarine epifaunal seagrass communities [10]. Although the pinfish is not consumed by local Yucatan communities, the consumption of the great barracuda *Sphyraena barracuda* (Walbaum) should be considered.

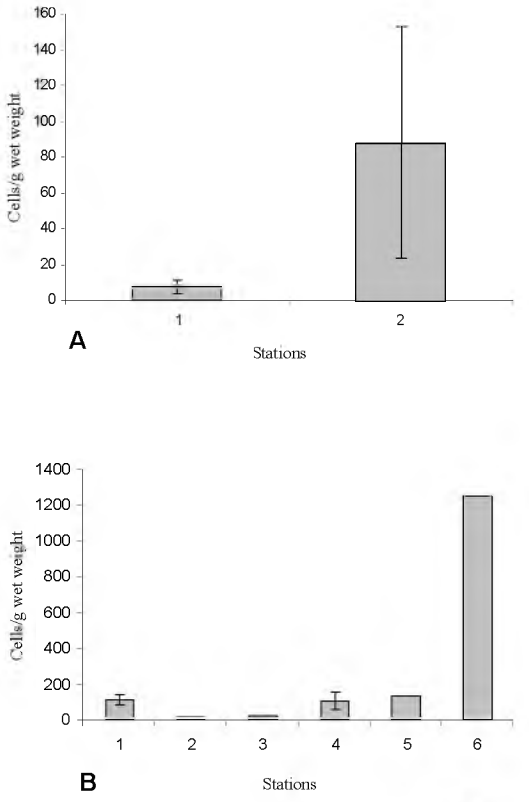

Fig. 4. Abundance of Gambierdiscus toxicus *in November 2008 at Dzilam (A) and Chelem (B), Gulf of Mexico (rectangulars present mean values per station and a standard error bars are shown in case of more than one sample per station); at sts. 3 and 4 at Dzilam the species was not detected.*

First record of *Gymnodinium* cf. *catenatum* and other potentially toxic planktonic dinoflagellates in southern Cuba

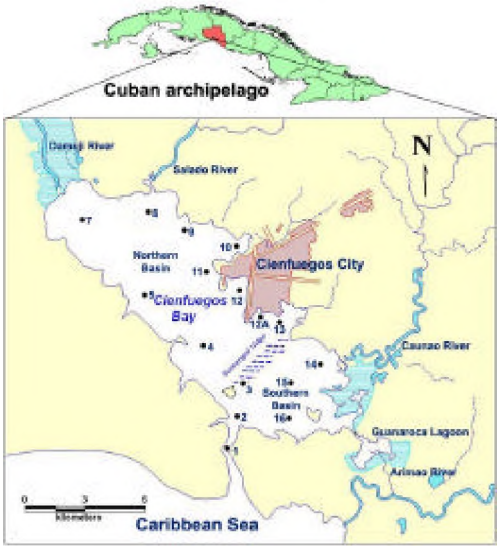

Fig. 1. Sampling stations in Bahía de Cienfuegos.

Cienfuegos Bay (southern Cuba) is a semienclosed embayment of 90 km^2 and a mean depth of 14 m. The bay is divided in two parts by a 1.5 m-deep submerged bank. Four rivers, Arimao, Caunao, Damují y Salado (Fig. 1) enter this bay subject to two distinct seasons: a dry season from November to April—average salinity of 33.8 psu and temperature of 27.5 °C— and a rainy season, from May to October—mean salinity of 23.7 psu and temperature of 29.8 °C [1]. The northern part, with sewage input from the city of Cienfuegos (106 504 habitants), as well as industrial waste and the inflow from rivers Damují y Salado, is under stronger anthropogenic impacts than the southern part, that receives the inflow of rivers Caonao and Arimao and has a natural park (Laguna Guanaroca) for the protection of migratory birds.

Figure 1 shows the location of the 16 stations that are sampled every 3 months as part of a monitoring programme for water quality control that includes estimation of physico-chemical parameters and phytoplankton composition. In June 2009, at the beginning of the rainy, species diversity and abundance, mainly of diatoms (*Rhizosolenia hebetata* f. *semispina*, *Dactyliosolen fragilissimus*) and dinoflagellates (*Prorocentrum compressum* and *P. micans*) usually increase. Some potentially toxic dinoflagellates were found in these communities, including *Gymnodinium* cf. *catenatum*, potential producer of PSP toxins and first time reported in southern Cuba.

Gymnodinium cf. *catenatum* was mainly found in the southern cleaner waters of the Bay. The maximum concentration was detected at stations 14 (3.3 x 10^4 cel. L^{-1}) and lower levels at station 13 (8.3 x 10^3 cel. L^{-1}), 16 (7.2 x 10^3 cel. L^{-1}) and 12 (9.0 x 10^2 cel.L^{-1}). Four-celled chains were predominant, but pairs, triplets and 6 to 8 celled chains were also observed (Fig. 2). Cells suffered deformation with the fixatives but still their characteristics incline us

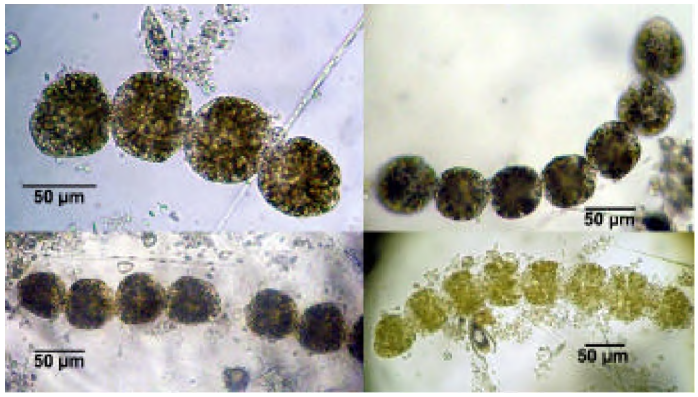

Fig. 2. Gymnodinium *cf.* catenatum *(lugol-preserved), chains of 4, 7 and 8 cells.*

(Cont'd from p. 13)

The relative abundance of *G. toxicus* near Chelem, especially in May (both 2008 and 2009) and November, and the presence of a ciguateric food web, including rich seaweed beds in an extensive shallow zone around the Yucatan Peninsula with fine bottom sediments, indicate a potential risk for a future ciguatera outbreak. Monthly monitoring of epiphytic and benthic dinoflagellates coupled with routine toxicity analyses are urgently needed to study seasonal changes and to minimize or prevent toxicity to humans.

Acknowledgements

We thank A. Aguilar-Trujillo for technical support, J.M. Hernández de Santillana for the data on fish and fishing (both from CINVESTAV, Merida), and K.A. Steidinger for her help. Financial support (FOMIX CONACYT-Yucatan project "Analysis of the causes, dispersion and environmental consequences of the red tides in Yucatan") to JAHS is appreciated.

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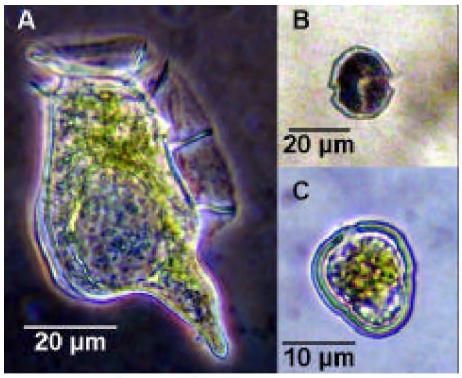

Fig. 3. A. Dinophysis caudata. *B.* Alexandrium *cf.* minutum. *C.* Prorocentrum minimum.

to identify it as *Gymnodinium catenatum*, a species with larger and more elongated cells (53 x 39 µm) in the extremes of the chain than the morphologically close species *Gymnodinium impudicum*. Some of the inner cells were shorter (49 x 44 µm) and most of them isodiametrical (43 x 43 µm), and were full of plastids. The conditions observed in the area where *Gymnodinium* cf. *catenatum* bloomed were: temperature 30.6 °C, salinity 31.4 psu, dissolved oxygen 7.06 mg · L^{-1} and chlorophyll *a* 3.12 µg · L^{-1}.

In the Caribbean Sea, *Gymnodinium catenatum* associated with PSP outbreaks was first reported in Venezuela [2] and low concentrations of the species (4-8 x 10^2 cel L^{-1}) were reported by Leal *et al.* [3], who considered them as part of the cryptic flora, in the archipelago Sabana-Camaguey in northeast Cuba.

A small dinoflagellate species of *Alexandrium*, *Alexandrium* cf. *minutum* (Fig. 3A), potential producer of PSP toxins, was observed in moderate concentrations (10^2–10^3 cel L^{-1}). In comparison with *Gymnodinium catenatum*, this species was more scarce but with a wider spatial distribution (present at most sampling stations). Maximum levels of *Alexandrium* cf. *minutum* were observed in shallow areas by the city.

The potential PSP producer *Pyrodinium bahamense* was first reported in the Cuba. Cells were loose (Fig. 4) and not in chains, the latter a characteristic of the non-toxic *Pyrodinium var. bahamense* that occurs in the neighbour island of Puerto Rico (tropical western Atlantic). *Pyrodinium bahamense* was observed off the Bay (concentrations up to 6.3 x 10^3 cel L^{-1}) in the eastern littoral region under estuarine influence, in the beginning of the dry season (Nov 2008).

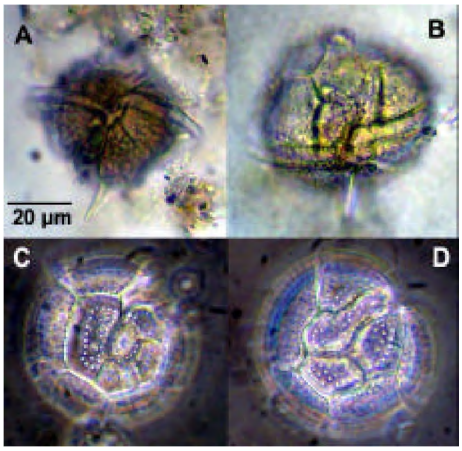

Fig. 4. Pyrodinium bahamense. *A-B. Ventral view; C. Apical view. D. Antapical view.*

Within the potential DSP-toxin producers, the most frequently observed species (concentrations up to 10^2 cell L^{-1}) was *Dinophysis caudata* (Fig. 3A), that was most abundant in areas subject to anthropogenic and riverine inputs. *Dinophysis rotundata* is a very rare species. *Dinophysis tripos* has been detected in previous samplings by the mouth of the Damují river, an area with high concentrations of electrolites [4]. In May 2009, *Dinophysis* cf. *ovum* (Fig. 5) was detected in periphyton samples at the entrance of the Bay. This is the first report for Cuban waters of this species that was recently associated with a toxic outbreak in Texas (Gulf of Mexico).

Prorocentrum minimum (Fig. 3C) is a species the toxicity of which needs to be confirmed. Its concentration increases in the beginning of the rainy season (10^2 –10^3 cel L^{-1}) at the stations under the influence of sewage and industrial waste and river outflow.



Fig. 5. Dinophysis *cf.* ovum.

It is more abundant in the southern part, where maximum concentrations of 10^4 cel L^{-1} in previous years. It has been observed, together with *Dinophysis caudata*, in multispecific patches (red tides) during the dry season (i.e. January 2005) when water stability is highest.

There is no shellfish cultivation in the Bay of Cienfuegos and harvesting from natural banks is very limited. Nevertheless green mussels (*Perna viridis*) (Fig. 6), an invasive species from southeast Asia, was first detected in the area in May 2004 [5]. Initially settled in the northern part of the Bay (stn. 10), where it caused damage in the cooling system of the electric plant, is currently expanded through the whole area and has displaced other autochthonous species, such as the "ostión" (*Crassostrea virginica*). The possible recollection of green mussels by local consumers with a potential contamination with PSP and DSP toxins pose a serious threat for human health in the Bay of Cienfuego that should be taken into account in future monitoring efforts.

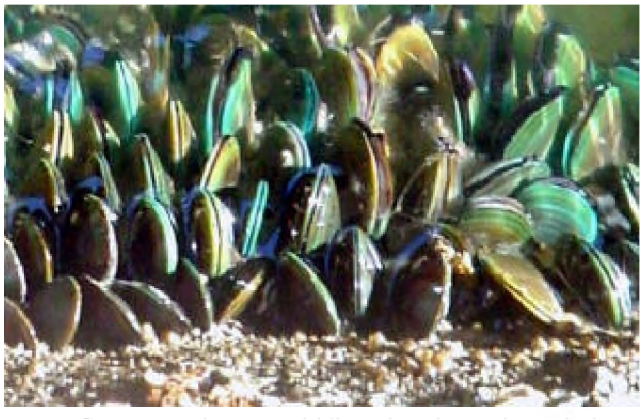

*Fig. 6. Green mussels (*Perna viridis*). Photo by Raul Fernández.*

Acknowledgements

To C. Alonso and the Water Quality laboratory (CEAC) for their support.

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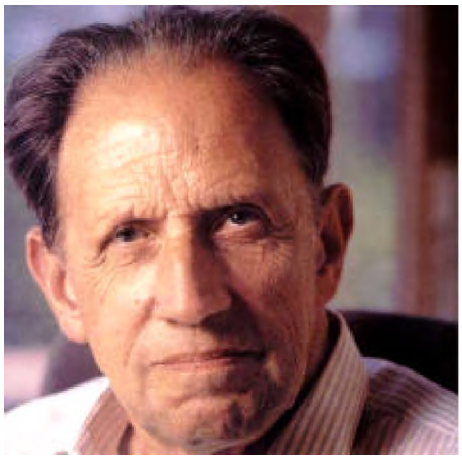
ISSHA's Corner

ISSHA
International Society for the Study of Harmful Algae

HAB trail blazers

Ramon Margalef (1919-2004)



Education

Margalef was born in Barcelona in 1919. His education was interrupted by the Spanish civil war in 1938, when he was recruited by the Republican army. After Franco's victory he was forced to do three more years of military service. He then worked as a messenger in Barcelona's Botanical Institute, and as an insurance clerk until, thanks to the help of several scientific personalities who appreciated his intellectual potential, he got a scholarship and managed to obtain his BSc from the Universitat de Barcelona (1949). He finished his PhD only two years later in 1951.

Honours

2002 CSIC Gold Medal, Spain; 1995 Excellence in Ecology Prize, Germany; 1990 Alexander von Humboldt Award,Germany; 1984 Ramon y Cajal Award, Spain; 1980 Huntsman Prize,Canada; 1972 Prince Albert medal, France. Margalef became the most cited Spanish researcher, sharing with Santiago Ramón y Cajal and Severo Ochoa the fact of being the three most relevant Spanish scientific workers in the life sciences out of 95 researchers in the world. The book Perspectives in Ecological Theory (1968) and the articles "*On certain unifying principles in ecology*" (1963), "*Life-forms of phytoplankton as survival alternatives in an unstable environment*" (1978) and "*From hydrodynamic processes to structure (information) and from information to process*" (1985) are citation classics, and the first is considered to be one of the two classical articles in the life sciences of the 20th Century.

Professional Career

After finishing his PhD, Margalef started to work in the newly created Institute for Fisheries Investigation in Barcelona, an institution which he later presided over from 1965 to 1967. In 1967 he became Spain's first professor of ecology, a position he held at the Universitat de Barcelona until his retirement 20 years later. As is to be expected in a man gifted with a prodigious curiosity, his involvement in active research never vanished, and he continued to visit his tiny office in the Ecology Department of the Universitat de Barcelona until a few weeks before his death in 2004.

Key HAB contributions

Margalef was one of the founding fathers of modern ecology and one of the most distinguished Spanish scientists of the twentieth century. His contributions have been extremely fertile in fields as diverse as limnology, oceanography, and theoretical ecology. Margalef was a prolific writer and he authored over 400 scientific papers and books. His first studies, published mostly in Spanish in the 1940s and 1950s, focused on the organization of planktonic communities in continental and oceanic waters. It was not until the late 1950s, with the translation into English of his inaugural lecture as a member of the Barcelona Royal Academy of Arts and Sciences "*Information Theory in Ecology*", that he gained a worldwide audience. Another groundbreaking article, "*On certain unifying principles in ecology*", published in American Naturalist in 1963, and his book "*Perspectives in Ecological Theory*" (1968), based on his guest lectures at the University of Chicago, consolidated him as one of the leading thinkers of modern ecology. His studies have greatly contributed to our understanding of the spatio-temporal structure of ecosystems, the relationship between diversity, biodiversity, stability and connectivity, the role of external energy in biological productivity, and the interplay between ecological succession and evolution. He proposed a conceptual model for the ordination of major phytoplankton life-forms as a function of availability of nutrients and external energy, later called "the phytoplankton mandala", that provided a valuable framework for the ecological understanding of red tides. In his lectures at the Universitat de Barcelona, or in the numerous invited courses and seminars elsewhere, he always promoted creative thinking and transmitted in a fresh and challenging fashion his views on how nature works, prompting students to "get out and discover nature" for themselves. His views were summarized in two monumental textbooks: "*Ecología*" (1974) and "*Limnología*" (1983). Ramon Margalef exemplified one of those rare cases in which an outstanding intellect coexists with equally exceptional personal qualities. The scope of his knowledge, his humane nature, his modesty, his honesty and his sense of humour gave him a human dimension well beyond his scientific qualities. Much is to be learned from his approach to science and to life in general, in which an insatiable, childlike curiosity sprang from the intimate pleasure he found in observing the world around him. Some of his views have proven to be wrong, but they have been always original and inspiring and often truly revolutionary. In a scientific world dominated by reductionism he always was one of the few minds capable of seeing forests where most saw only the trees. He was also very much interested

in the public appreciation of science and always advocated for the engagement of scientific rigour in environmental policy. "If God has put us on Earth, we have the right to make use of it but we might as well do so with a modicum of intelligence". We owe to him the introduction of information theory to the study of ecological diversity, arguably one of the major inflection points in the history of ecological thinking.

Mentored

Martha Estrada, Dolors Blasco, Rosa Miracle.

10 Key HAB publications

Margalef R., 1997. Red Tides and ciguatera as successful ways in the evolution and survival of an admirable old phylum. *In* B. Reguera, J. Blanco, M.L. Fernandez and T. Wyatt (eds). *Harmful Algae*, Proc. of the VIII International Conference on Harmful Algae Vigo, Spain 25–29 June 1997. Xunta de Galicia and Integovernmental Oceanographic Comission of Unesco; **Margalef R.,** M. Estrada and D. Blasco, 1980. Functional morphology of organisms involved in red tides, as adapted to decaying turbulence. *In*: *Toxic Dinoflagellate Blooms*. Elsevier. North Holland; **Margalef R.,** 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica Acta*, 1(4): 493–509; **Margalef R.,** 1978. Phytoplankton communitties in upwelling areas. The example of NW Africa. *Oecologia Aquatica,* 3: 97–132; **Margalef R.**,1968. *Perspectives in ecological theory*. University of Chicago Press. 111 pp; **Margalef R.**, 1968. Diversity and stability: A practical proposal and a model of interdependence. *Brookhaven Symp. Biology,* 22: 25-37; **Margalef R.,** 1967. Some concepts relative to the organization of plankton. *Oceanogr. Mar. Biol. Ann. Rev.* 5: 257–289; **Margalef R.,** 1963. On certain unifying principles in ecology. *American Naturalist,* 97: 357–374; **Margalef R.,** 1962. Succession in marine populations. *Advancing frontiers of plant sciences,* 2: 137–186; **Margalef R.,** 1958. Temporal succession and spatial heterogeneity in natural phytoplankton. *Perspectives in marine Biology*. Univ. California Press.

Adapted from Margalef biography by Jordi Martínez Vilalta (2004) and comments by M. Estrada.

One of a series of articles prepared by Professor Gustaaf Hallegraeff, ISSHA's Vice-President, for the ISSHA Website (www.issha.org).


The Skibbereen & West Carbery Eagle:
OR, SOUTH WESTERN ADVERTISER.

An early *Karenia* bloom off southwest Ireland?

The Skibereen & West Carbery Eagle newspaper began publication in 1857, the year before the foundation of the Irish Republican Brotherhood and the Fenians. The year 1865 marked the end of the American Civil War … Skibereen was the epicentre of the Great Famine which had sent 2 million Irish refugees to emigrate, most of them to USA. Some returned in 1867 to participate in the first Fenian uprising, one of the many precursors of Irish independence.

From the *Skibereen & West Carbery Eagle*, Saturday, 6th May, 1865:

Many reports have reached us that a streak of coloured matter has shown itself in the sea water along this coast for the last two weeks. These reports vary as to the length, breadth, appearance and nature of this very extraordinary phenomenon. Putting them all in a collected form and adding what we have ourselves seen, the facts seem to be these: - On last Wednesday week we visited Castletownsend, and the evening being a lovely one took a boat to enjoy a row along that picturesque harbour. The sea was smooth as glass, and we were tempted beyond our usual limits and boldly pulled outside the harbour. Scarcely had we reached the open ocean when we at once noticed that the usually clear, transparent waters of the great deep were now muddy and forbidding to look upon. Rowing along we saw that all the ocean waters were not so tinged but that the colour was confined to a streak or river, as it were, in the sea, and along the rocks where no tide swept for about 20 to 30 feet the water was clear as ever. We happened to be fishing for pollock, and found that no fish took where the colour was great; but whether this was owing to the fact that the fish could not see the bait or that the colouring matter was poisonous, we are not prepared to say. We went down two or three times since and saw the same appearance, but the colour seemed more diffused, and less obnoxious to the fish, as they took the bait when it showed itself. Several people have told us about a similar appearance all along Lough-Ine, Baltimore, Cape Clear, and Crookhaven. Some say it is the blood of American patriots that has been borne down her mighty rivers into the Gulf of Mexico, and has thence been carried by the great Gulf Stream to these our sympathising shores. Others (and with more probability) ascribe it to some volcanic eruption or upheaval which has taken place beneath our waters, and has thrown up mud, scoria, and ashes, thus to show us that we are not beyond the reach of this mighty scourge of some of the fairest portions of our earth. We do not attempt to decide between these rival theories. We leave our readers to visit the coast, and having seen for themselves, judge for themselves.

GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

HABs and Eutrophication: A Core GEOHAB Project

The relationship between HABs and eutrophication is complex and of global concern. The complexity arises from many factors. Both high-biomass forming HABs and those that cause harm even when representing a small proportion of the total plankton assemblage occur in eutrophic environments. Additionally, the physiology of these diverse organisms not only varies between species (and even strains) but may include both autotrophy and heterotrophy, the degree of which may change with nutrient cell status or availability of nutrient form. Although there is growing awareness of mixotrophy in the nutrition of many HABs, our overall knowledge of the flux of organics, their bioavailability and the propensity for individual species, which may include prey for HABs, to be promoted under changing organic flux conditions is very poorly known.

Adding to the above is the problem of determining how nutrients may affect biological processes such as motility, release of mucus, and allelopathy, among other processes. The relationship between HABs and eutrophication is also complex because although eutrophication is occurring globally, nutrient export from coastal watersheds is not evenly distributed, and in many regions of the world is changing rapidly. Changes in nutrient loads are occurring due to population growth, changes in sewage treatment - both overall increases and in some cases improvements to treatments – as well as other changes in land use. Animal husbandry operations are resulting in more animal wastes reaching some coastal areas. Changing compositions of fertilizer in many agricultural areas is also altering the composition of nutrient loads.

Also, without doubt, the expanding aquaculture industry in many parts of the world is altering localized nutrient loads and concentrations. Nutrient loads in one form are also rapidly transformed through biochemical process and tropic interactions in the water column and in the benthos, so the form of nutrient actually "seen" by a HAB community may be different from those that were discharged from land. Lastly, changes in nutrient loads to coastal watersheds are also changing in many areas coincident with other changes related to climate, so that differentiating the effects due to eutrophication from those due to climate is difficult; in some cases these changes are synergistic. Thus, it is a formidable challenge to relate nutrient loads to HAB occurrences.

Promoting research that addresses this complexity is the objective of the Core Research Project of the GEOHAB Programme on HABs and Eutrophication. GEOHAB, The Global Ecology and Oceanography of Harmful Algal Blooms Programme, has as its central mission the fostering of international cooperative research on HABs in ecosystem types with common key species or common processes (including anthropogenic) that affect their population dynamics. The goal of GEOHAB is to improve our predictive capability of HABs and their impacts by integrating biological, chemical, and physical studies supported by enhanced observational and modeling techniques.

The goal of promoting international cooperation and collaboration on the topic of HABS and eutrophication was recently advanced through the Second GEOHAB Open Science Meeting on HABs and Eutrophication, held in Beijing from October 18-21, 2009. Over 130 participants from 23 countries attended this event. It was particularly

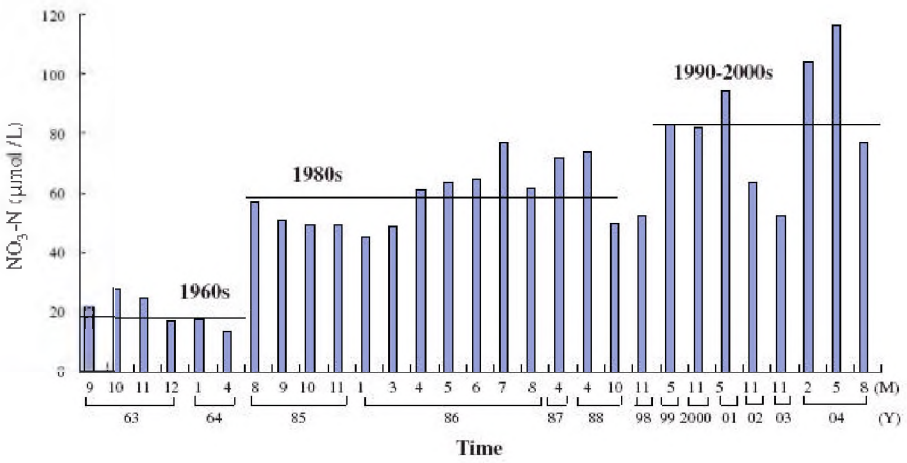


appropriate that this meeting was held in China, as the rate of nutrient loading to Chinese coastal waters has increased rapidly over the past several decades with the rapid industrialization of this country, and eutrophication-related HAB events are now common along the Chinese coast. As noted by Mingjiang Zhou, co-convener of the conference, "The international gathering of scientists in Beijing for the GEOHAB HABs and Eutrophication conference will help to inform local managers of the importance of this issue for China."

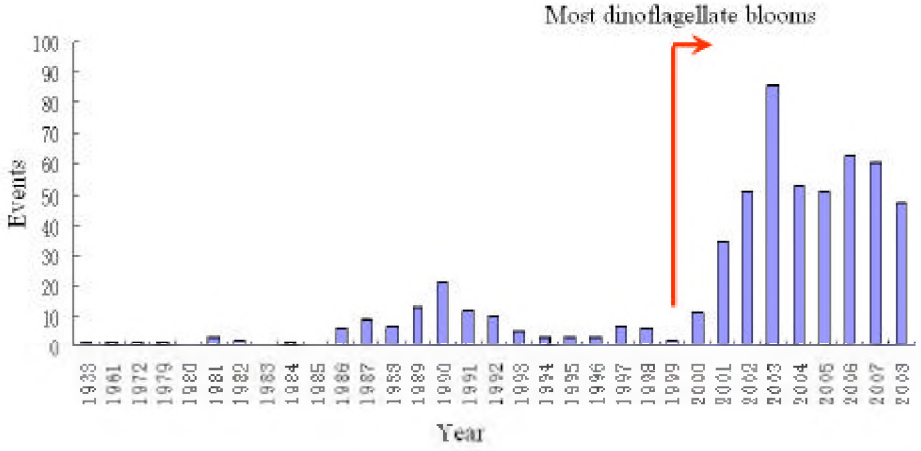
The conference featured seven keynote presentations, with reviews of the rapidly expanding HAB events and their impacts throughout Chinese waters in the past decade (Mingyuan Zhu), global estimates of the expanding distribution and impacts of *Nocticula scintillans* (Paul Harrison), and nutrient discharges from expanding aquaculture operations and their impacts (Lex Bouwman and Sandor Mulsow). Participants also heard reviews of eutrophication related HAB events in the Yucatan Peninsula, Mexico (Jorge Herrera Silveira), and the Gulf of Oman (Adnan Al-Azri). Bioengineering approaches to the problems of cyanobacterial blooms in the Baltic Sea were also addressed (Wilhelm Graneli). Thus, the goal of global networking was achieved without question.

Contributed talks described a broad range of topics, underscoring many aspects of the complexity of the relationships of HABs with nutrients described above. Among the common themes were the impacts of sewage discharge in the US and in Hong Kong, the effects of varying stoichiometric ratios in many regions and with respect to the growth of numerous species, factors promoting the expansion of cyanobacterial blooms, and interactions of anthropogenic nutrients and regional and global climate change. Many new approaches and technologies were also highlighted.

The local organizers did an outstanding job of preparing for this conference, with no detail left unattended. All attendees were able to enjoy wonderful meals and tremendous hospitality in beautiful Beijing along with excellent science. The city of Beijing has been transformed in the past few years, in large part because of the 2008 Olympics. During the entire conference blue skies prevailed, demonstrating the reduction in air pollution in this region.



Increase of nitrate concentration at the Changjiang River estuary in 40 years, (From Zhou et al 2008)



Red tides increase at the Changjiang River estuary and its adjacent sea areas (Zhou redrawn from SOA data)

To further the dialogue and the sharing of information on this important topic, a special issue of the *Chinese Journal of Oceanology and Limnology* is being prepared to capture the highlights of this meeting. Two types of papers will be accepted: regular manuscripts and notes. Please contact Pat Glibert (glibert@umces.edu) if you wish to contribute to this special issue.

Information will also be available on the conference website (www.geohab-osm-bj.ac.cn) as will the any photos of the conference that document so well what an excellent symposium this was. The Core Research Project on HABs and Eutrophication aims to continue the tradition of Open Science Meetings, first conducted in Baltimore, MD, USA, in 2005, through a Third Open Science Meeting in several years- most likely in Europe. Follow the GEOHAB website (www.geohab.info) for information about this meeting as it develops in the coming years. Given the rapidly of advances in knowledge and application of new techniques and models, and the expanding impact of eutrophication globally, it can be projected that there will be much to report in a few years' time.

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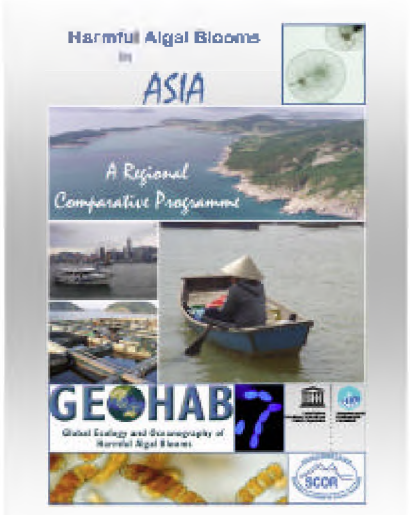
GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

Open Science Meeting on Benthic Harmful Algal Blooms

Tentative dates 21-23 June 2010, Hawaii, USA

The Programme will focus on Intoxications, Biodiversity and Biogeography including biogeographical species distribution, global analyses/data sets, methodology for sampling to abundance and distribution, methodology for taxonomic identification; Ecological Factors such as methodology for evaluation/ comparison of habitats, anthropogenic forces and/or stimuli, bottom-up and/or top-down control, controls such as nutrients and grazing, relationships with macroalgae and/or substrates, and effects of toxins on marine foodwebs; Adaptive Strategies including physiological characteristics and life cycle traits; Phylogenetic Characteristics; and Modelling.

Detailed info will appear at www.geohab.info. Contact: P. Bienfang (bienfang@soest.hawaii.edu)



The Science Plan for GEOHAB in Asia will be launched in December 2009 and will be available on line at: www.geohab.info.

ISSHA

International Society for the Study of Harmful Algae

NEW ISSHA website to be launched by mid-December: www.issha.org

Future events

Yasumoto to be Honored in Spain

Vigo, Spain, January 28, 2010

Professor Takeshi Yasumoto will be awarded with the distinction of "Doctor Honoris Causa" by the University of Vigo, Spain, in an academic ceremony. The award recognizes his many achievements and leadership in the marine toxins field. The following day, on January 29th, a one-day seminar entitled "Marine Biotoxins Analysis and Toxicology" will be held, followed by a round table discussion: "Are New Limits on Seafood Biotoxins Necessary?". Invited speakers will include: J. Lawrence, M. Quilliam, R. Dickey, J. Hungerford, Y. Oshima, P. McNabb, P. Burdaspal, S. Franca, A. Tubaro, etc.

The aim of these events, besides presenting gratitude and respects to Prof. Yasumoto, is to gain new insights into this exciting and crucial field of research by joining together world experts on both analysis and toxicology.

The venue for all events is the campus of the University of Vigo and the organizers are Ana Gago Martínez and Dr. José A. Rodríguez Vázquez. In case you are interested in participating, please contact Ana Gago (anagago@uvigo.es) by 1st January 2010.

VI International CONyMA'2010 Workshop on Pollution and Environmental Protection

Havana, Cuba, 1–5 March 2010

The workshop, focused on ciguatera, a potential risk in the Caribbean, will cover the following topics: ciguatera: ecotoxicology & socioeconomic aspects; marine pollution; biodiversity; environmental management; preservation and protection of mangroves; toxic and harmful algae; environmental education; marine biotechnology and pharmacology; database use and implementation; management of coastal ecosystems; trophic dynamics; and process modelling. Papers are accepted until Jan 15, 2010.

Further info: conyma@cip.telemar.cu.

Harmful Algae News

Previous issues of HAN and newsletters of the IOC HAB Programme can be downloaded at http://ioc.unesco.org/hab/news.htm

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HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

www.ioc-unesco.org/hab No. 41

• France

Pseudo-nitzschia australis on French Atlantic coast - an unusual toxic bloom

The occurrence of the toxic diatom *Pseudo-nitzschia australis* was first suspected in a water sample from Bay of Brest, France, in 1995 (Fig. 1). At this time, and because of very low number of cells, the identification could not be confirmed by Scanning Electron Microscopy (SEM). In autumn 2004, this species was again reported, and in association with *P. multiseries*, suspected to be the source of domoic acid (DA) in king scallops (*Pecten maximus*) from the Bay of Seine (English Channel) [1]. Since 2006, it has been encountered every year at low concentrations in either spring or autumn along the Atlantic coast of Brittany.

This year, it was first observed in early March in the Bay of Douarnenez and bloomed a week later in the "Pertuis Charentais" area with densities exceeding 10^6 cells L^{-1} (Fig. 1). It then for more than a month remained noticeable (from 10^5 to 10^6 cells L^{-1}) in several areas of the Atlantic coast (Fig. 1). At the same time, the monitoring network (REPHY of Ifremer) detected domoic acid far above the EU-regulatory limit of 20 mg DA/Kg tissue in shellfish (up to 10 times the safety threshold), leading to the prohibition of harvesting and sale of scallops during several weeks.

In contrast to other years where toxic blooms were dominated by species of the *Pseudo-nitzschia pseudodelicatissima/cuspidata* complex [2] as defined by Lundholm *et al.* [3], this year was notable for a species of the *Pseudo-nitzschia seriata* complex *sensu* Hasle [4]. An identification key using features observable in Light Microscope (LM) led us to think that it was *Pseudo-nitzschia australis* with asymmetrical lanceolate valves, a width varying from 7.0 to 8.9 µm, apices slightly rostrate and a 23-31% overlap of cells in chains (Fig. 2). SEM examination of valves showed that there was no central

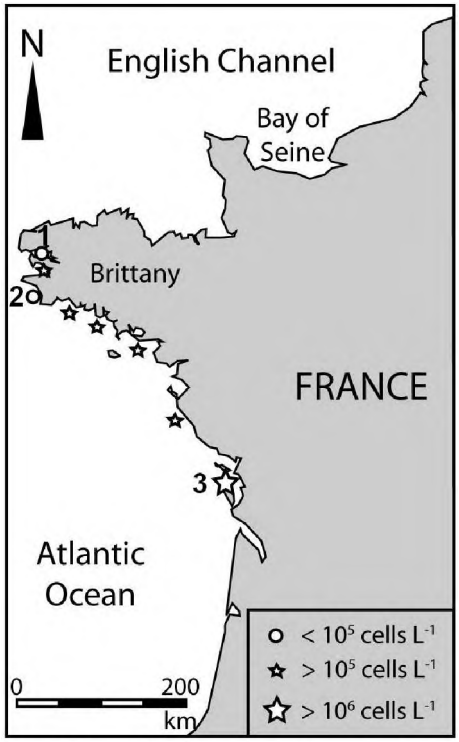

Fig. 1. Map with location of Atlantic areas with Pseudo-nitzschia australis *in spring 2010. 1) Bay of Brest. 2) Bay of Douarnenez. 3) "Pertuis Charentais".*

(Cont'd on p. 2)

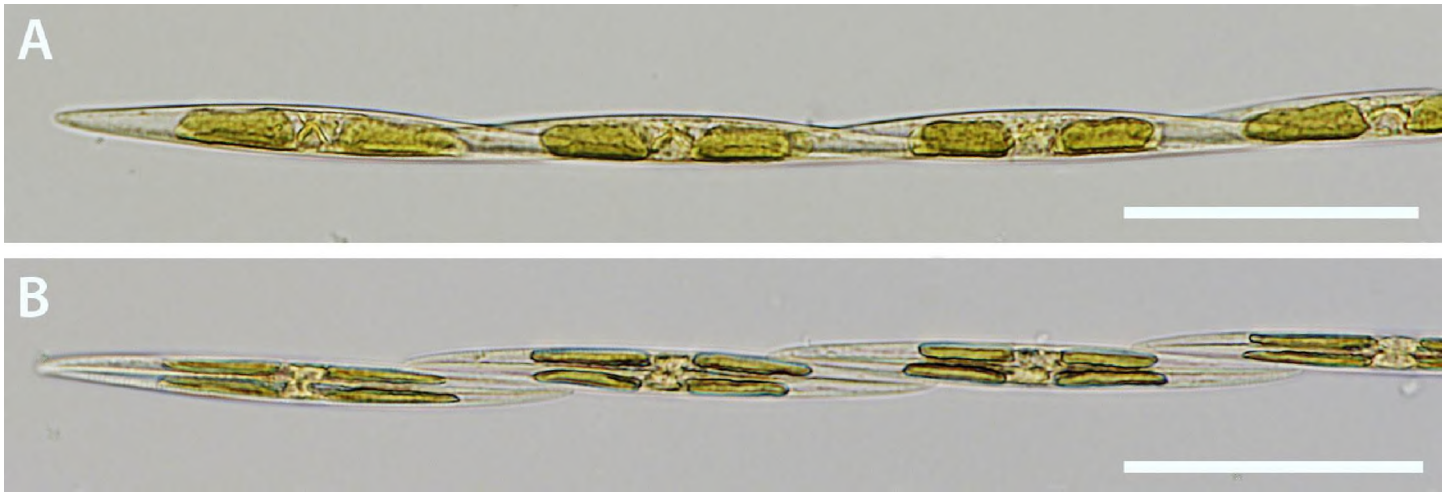

Fig. 2. Light micrographs of Pseudo-nitzschia australis. *A) chain in valve view. B) same chain in girdle view showing large overlap of cells. Scale bars = 50 µm.*

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

interspace (Fig. 3 A), and that interstriae and fibulae were usually distributed in equal numbers (12–14 in 10 µm). The striae were composed of two rows of poroids (4–5 in 1 µm) (Fig. 3B). Moreover, the valves were heteropolar (Fig. 3C & D). Otherwise, the structure of the girdle revealed the presence of three types of bands with 17–19 band striae in 10 µm (Fig. 3E). The identification of *P. australis* was thus confirmed, excluding the hypothesis of *P. seriata*, the closest congeneric species [5–6]. Molecular analysis (PCR amplification and sequencing) was undertaken on individual chains isolated from field samples. The two resulting ITS (ITS1-5.8S-ITS2) rDNA sequences were compared with other sequences of *Pseudo-nitzschia* available in GenBank. They were identical (100% similarity) with all ITS sequences of *P. australis* of both European and Californian origin (Table 1).

This unexpected outbreak happened after the "Xynthia" storm on the 28 february 2010 which severely affected the coasts of the "Pertuis Charentais". Many reared bivalves from this area were contaminated with high toxicity levels. At present, the degree of contamination of king scallops is unknown due to closure of the harvesting sites. But we can be anxious considering the kinetics of contamination and depuration of these shellfish.

Table 1. List of ITS rDNA sequences of Pseudo-nitzschia australis *available in GenBank and used for a comparison with our two studied isolates.*

Accession number	Strain designation	Origin
AM118029	PLY1St.24D	Scotland
AM118030	PLY1St.27E	Scotland
AM118031	PLY1St.33A	Scotland
AM118032	PLY1St.37A	Scotland
AM118033	PLY1St.37B	Scotland
AM118034	PLY1St.54C	Scotland
AM118035	PLY1St.56B	Scotland
AM118036	PLY1St.68C	Scotland
AY257842	ØM1	Aveiro, Portugal
AY452527	PLYSt19A	Scotland
AY452528	PLYSt54B	Scotland
DQ062661	au43	Monterey Bay, California
EF564717	Pau1	Bay of Seine, France
EU523100	C1	France
EU684233	Delta 2	Portugal

Acknowledgements

We thank our colleagues Sylvie Genauzeau, Mireille Fortune and Jacky Chauvin from coastal laboratories for providing field samples and Karine Chèze of the Muséum National d'Histoire Naturelle of Concarneau for sequencing.

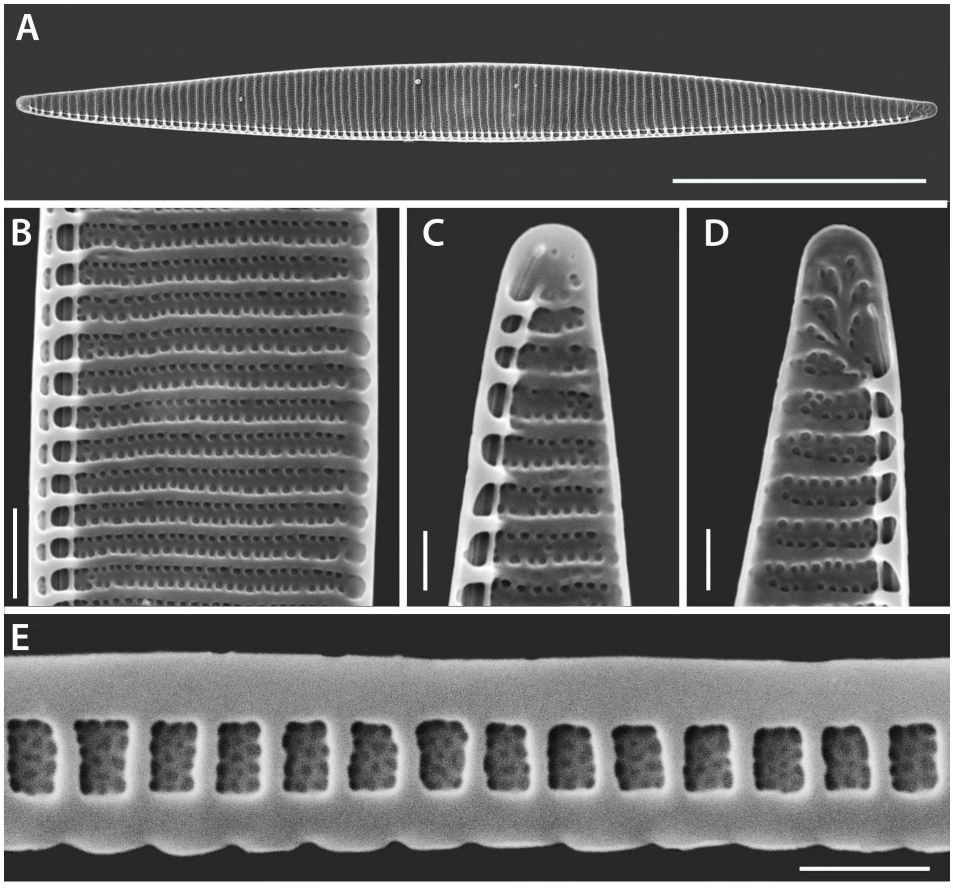

Fig. 3. Scanning electron micrographs of Pseudo-nitzschia australis. *A) Valve illustrating asymmetrical valve outline and absence of central interspace. B) Higher magnification of central part of valves showing the two rows of poroids per striae. C, D) same valve with apices exhibiting the heteropolarity. E) Detail of a girdle band. Scale bars – 20 µm (A), 2 µm (B), 1µm (C-E).*

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5. Fehling J *et al* 2004. *J Phycol* 40: 622–630
6. Hasle GR & Lundholm N 2005. *Phycologia* 44: 608–619

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PHYCOTOXINS

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Archives are located at www.agr.ca/archives/phycotoxins.html

• Canada

Multi-species raphidophyte blooms in British Columbia coastal waters

The official records of major fish-killing blooms of *Heterosigma akashiwo* in British Columbia in the 1980s and 1990s did not include unidentified raphidophytes, but this did not mean that they were not present. Records of total phytoplankton species collected for the purpose of tracing water movements in the region [1] show that *Heterosigma* was often accompanied and sometimes outnumbered by one or more of three unidentified raphidophytes, inlet dwellers with a preference for water in the 10–15°C range. Two of them almost certainly belonged to the same unnamed genus and the third was a species of *Chattonella* morphologically similar to *C. marina* but with a different water temperature preference.

In the first week of October 1990, a bloom containing all four of them and two unidentified species of *Prymnesium* was blamed for severe losses of penned salmon at a B.C. Packers fish farm in Simoom Sound, off the southern end of Queen Charlotte Strait. On this occasion *Heterosigma* was outnumbered 100:1 by the larger of the unnamed raphidophytes, but there was no official report of the bloom because none of the probable causal organisms was a named toxic species. B.C. Packers had chosen this location in the hope that its cold water would prevent *Heterosigma* from forming a bloom. It did, but the one that took its place was just as bad, and meant that the fish farmers' options were even more limited than previously thought. The result of not reporting it was that the fish-killing *Chattonella* bloom at Grieg Seafood's salmon farm in Esperanza Inlet in September 2002 took the company completely by surprise. Another result was that it was automatically reported as *C. marina*, setting a new record for the area which had to be dropped after it was compared with drawings of the 1990 *Chattonella*. The latter is described here in enough detail and with enough additional information to upgrade it to *C.* cf. *minima*, and hopefully the two unnamed raphidophytes are described well enough to be recognisable. Here are the toxic and suspected toxic flagellates found in the bloom, and their maximum concentrations in the samples:

Raphidophyte 1 = F.J.R. Taylor's undescribed chloromonad [2]	1.1×10^6 cells L^{-1}
Raphidophyte 2	2.2×10^5 cells L^{-1}
Chattonella cf. *minima* = *Chattonella* sp. [1]	2.0×10^4 cells L^{-1}
Prymnesium spp.	5.5×10^4 cells L^{-1}
Heterosigma akashiwo	9.7×10^3 cells L^{-1}

Water temperature was 13°C and salinity 26 ppt when they were collected at the height of the bloom. With a total phytoplankton count of 2×10^6 cells L^{-1}, it was not a particularly heavy one. However, if all the above species were toxic to fish, the combination of different kinds of toxins would have had an exacerbatory effect (J.C. Green, pers. comm.) The cells were preserved in acid Lugol's iodine, and the illustration shows them as they appeared under phase contrast illumination, though internal detail is missing. The cells of Raphidophyte 2 from oceanic stations were more squat than those from coastal stations and are included. The scale is 1cm:10 µm.

The following descriptions are from light microscopy only:

Species 1 is Raphidophyte 1. Samples were sent to anyone in the area who might be able to identify it, but the live cells were very difficult to transport and mine had rounded off and lost their flagella. Having no culture facilities with which to revive them I had to rely heavily on a pers. comm. from Edward Black who had found strong similarities to the fish-killing Flagellate X [3–4]. A rumour that it was a new *Olisthodiscus* meant that it could be E.M. Hulbert's presumed brown *O. luteus* [5], and Hulburt's Fig. 3 closely resembles Droop's Fig. 5 #1 [3]. The cell length was ~18 µm in both cases. Reconstructing the collapsed cells of Raphidophyte 1 assuming the same degree of shrinkage as Raphidophyte 2, the live ones were no more than 23 µm. The rounded live cells were dorso-ventrally flattened with 6–18 brownish-yellow, peripherally-arranged discoid chloroplasts and many peripheral extrusomes whose presence only became apparent when they discharged their contents. The unusual way that the cells collapsed in the fixative suggests that they had a posterior invagination. A posterior invagination like that of *Haramonas* would have been easy to miss.

When the rounded cells were examined under a coverslip, it was found that the extrusomes had discharged their contents in the form of discrete, slender threads. Edward Black, who had seen motile and non-motile cells with their extrusomes discharged, found similarities to drawings of Flagellate X in the same condition (Figs. 5 #2 & 3 in ref. 3; Fig. 3A in ref. 4).

Raphidophyte 1 was recorded from stations on Canadian Weathership Line P in the 1970s (Table 8 in ref. 6), and subsequently [1] from Saanich Inlet, Esquimalt Lagoon and offshore plumes from Barkley and Kyuquot Sounds. Its highest recorded concentration was 9.1×10^6 cells L^{-1} in a Barkley Sound plume in July 1985, water temperature 14.4°C, salinity 31.5 ppt. Its recorded water temperature and salinity ranges were 9.5–15.5°C and 25.0–32.4 ppt.

Species 2 is Raphidophyte 2. F.J.R. Taylor and I had this species in culture in the 1970s, and a sketch of a live cell [6] shows that in ventral view it is bilaterally symmetrical and ovoid or sub-pyriform. It also shows what appears to be a posterior invagination. The cells had 4–6 brownish-yellow, discoid, non-overlapping, peripherally-arranged chloroplasts and many peripheral extrusomes which discharged their contents when a coverslip was applied. At first sight they looked like trichocysts, but they remained attached. The cells were dorso-ventrally flattened, 8–12 µm long, 6–9 µm wide, 4–5 µm thick, concave on the ventral face and convex

on the dorsal. Two sub-equal, heterodynamic flagella much longer than the cell arose from the ventral surface about a third of the way down from the anterior end. The swimming motion was a glide while slowly turning over and over, or maybe over and back again, with the longer flagellum extended forwards and the shorter one trailing. Asexual reproduction was by binary fission in the motile stage.

Raphidophyte 2 was recorded from coastal and oceanic stations on Line P in the 1970s (Table 8 in ref. 6), it was subsequently recorded [1] and in and off Barkley Sound, from Saanich Inlet and Esquimalt Lagoon, the coastal waters adjacent to Quatsino Sound, Nootka Sound and Esperanza Inlet, and from Hecate Strait and Station P. Its numbers at Station P were surprisingly high, up to 2 x 10^5 cells L^{-1} at a water temperature of 6.5 °C. The highest concentration recorded was 1.4 x 10^6 cells L^{-1} in the mouth of an inlet on the west coast of Moresby Island in the Queen Charlottes in July 1983, water temperature 14°C, salinity 31.74 ppt. Its recorded water temperature and salinity ranges were 5.4–16.2 °C and 26.0–33.65 ppt.

Species 3 is *Chattonella* cf. *minima*. Until I saw it alive in 1990 I did not know what it was and recorded it as a raspberry shape (see also 7). The cells in the 1990 bloom were 20–55 µm long, and very variable in shape. The few fully motile cells in a live sample were 30–35 µm long and obovoid or oblong (cf. Hulbert's *O. magnus*). Most cells were globular (cf. Fig. 2(3) in ref. 7) and slow-moving or non-motile. The big round one in the drawing which appears to have burst open was fully intact but behaving like an amoeba, and as these cells deteriorated further they became plasmodial (cf. Fig. C of *C. globosa* in ref. 8; Fig. 3 in ref. 9). The motile cells had numerous brownish or greenish-gold ellipsoid chloroplasts sometimes situated just beneath the cell membrane. Their minimum and maximum dimensions were 1.5 and 3.4 µm, and their basal pyrenoids were just visible. There were numerous minute lipid globules immediately beneath the cell membrane, staining black in osmium tetroxide, and these

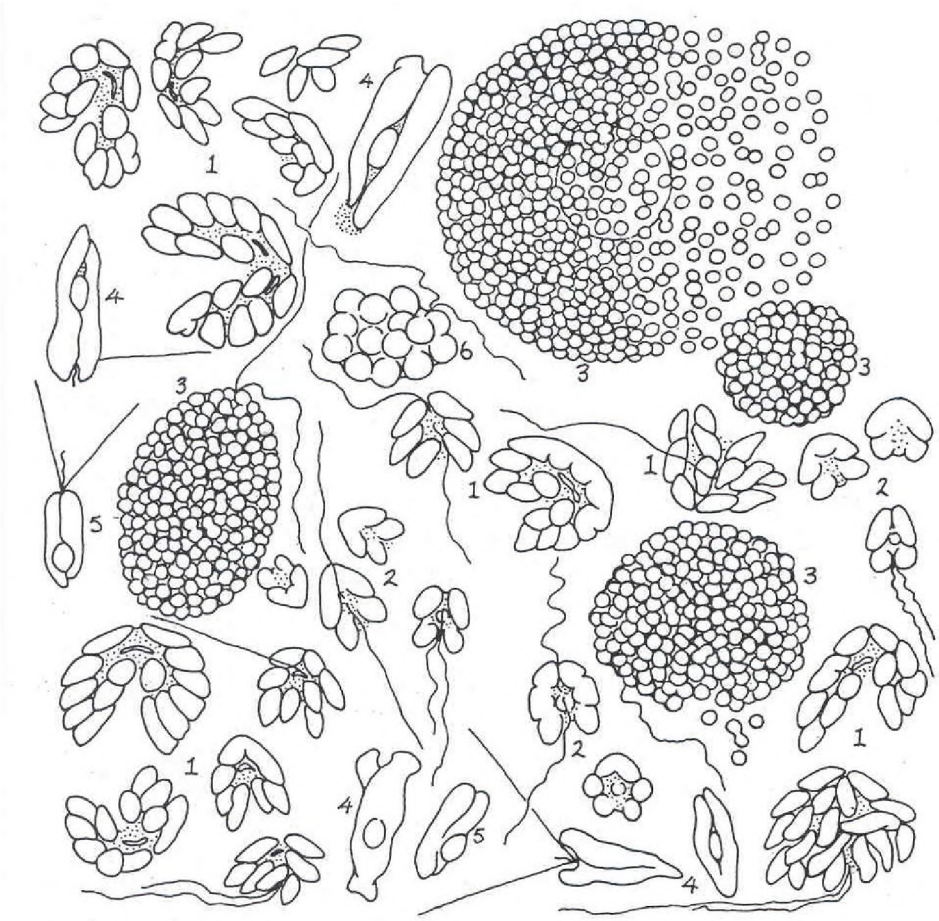

Fig. 1. Sketches of raphidophytes from British Columbia coastal waters.

were interspersed with spherical vesicles approximately 0.5–1.5 µm in diameter. When the cells lysed they became surrounded by many tiny fibrils, but there was no sign of mucocysts. Two sub-equal, heterodynamic flagella arose from an anterior depression which looked quite deep in the big globular cells. On the basis of detailed colour drawings and a photograph of the live cells, Y. Hara (pers. comm.) suggested that it was a type of *Chattonella* often seen in the Inland Sea of Japan when the water temperature was 10–15°C. He said that it was toxic to farmed yellowtail but did not form red tides, unlike *C. marina* which preferred 24–27°C water, and that he was preparing its taxonomy for publication. It turned out that he was referring to *C. minima* [9], already provisionally named by Fukuyo *et al* [8].

Chattonella was recorded by A.G. Lewis and myself from Jervis Inlet in June 1977 (unpubl.) and later [1] in and off Barkley Sound and Kyuquot Inlet. The highest concentration found was in the 1990 bloom. It was seen less often than the other two raphidophytes, possibly because it could not survive for long in the higher salinity water outside the inlets and only two of the cruises included an inlet. Its recorded water temperature and salinity ranges were 9.9–16.5 °C and 26.0–32 ppt.

Species 4 and 5 are *Prymnesium* spp. The larger one was yellow-brown and dorso-ventrally flattened, 16–24 µm long, and the other was brown and cylindrical, 11–17 µm long. The larger one was previously recorded from Esquimalt Lagoon and the smaller one off Barkley Sound [6]. They were rarely seen outside the inlets.

Species 6 is *Heterosigma akashiwo*. It was not always as easily distinguished from Raphidophyte 1 in Lugol samples as the illustration implies.

Raphidophytes 1 and 2 were found in good condition in the offshore plumes from the inlets. The plumes were known to have come from the inlets because they contained unusually high numbers of inshore algae such as *Tetraselmis* and *Eutreptiella*, and dead freshwater algae such as *Tabellaria* and *Scenedesmus*. The July 1985 Barkley Sound plume was a *Heterosigma* bloom containing high

numbers of Raphidophyte 1 (up to 3.9 x 10^7 cells L^{-1} and 9.1 x 10^6 cells L^{-1}, respectively). As this is quite a low ratio, it is likely that Raphidophyte 1 contributed to the fish-killing *Heterosigma* blooms in Barkley Sound from 1988 to 1990 [2]. The plume also contained the other two raphidophytes, and the four of them were there again in June, July and August 1986 and in a plume from Kyuquot Sound in August 1989. The full data [1], cover a water temperature range of 5.4–19.7°C, show that 7.2 x 10^4 cells L^{-1} was the recorded maximum for *Heterosigma* at or below a water temperature of 13 °C. They also show that Raphidophyte 1 can be mistaken for *Heterosigma* in Lugol samples. In Waters *et al* [6], a combination of Lugol material and a mistranslation of German led to a complicated mix-up between the harmless *Cochlodinium citron* and the fish-killing *C. polykrikoides*. This was an avoidable mistake, but I hope that in the foregoing I have made a case for including pictures of Lugol's-preserved cells in guides to toxic flagellates.

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Yasumoto honored by the University of Vigo



On January 28, 2010 at the University of Vigo, Spain, Professor Takeshi Yasumoto was given special recognition for his leadership in marine toxin research and his assistance to many countries over the years. Prof Yasumoto was awarded *Doctor Honoris Causa*, to recognize a deserving scientist who has devoted much of his life to the study of marine biotoxins, with an exemplary attitude of support and collaboration with many scientists worldwide. He was accompanied by his wife Tomiko and surrounded by many colleagues and friends, who came from many different countries to support him and acknowledge his many contributions. The Ceremony was chaired by the University Rector, Professor Alberto Gago.

Professor José Antonio Rodríguez Vázquez, Director of the Analytical Chemistry Department at the University of Vigo, presented Professor Yasumoto as the most recognized scientist in the field of marine biotoxins. In this presentation Prof. Rodríguez-Vázquez summarized the intense activity of Professor Yasumoto in the field, spanning several aspects, from the identification of many new marine algal toxins, to providing toxin standards to laboratories around the world, and assisting countries around the world to establish methods.

To further honour Professor Yasumoto, a seminar entitled: ***Marine Toxins: Analysis and Toxicology*** was chaired by Dr. Ana Gago-Martinez, Professor in the Department of Analytical and Food Chemistry at the University of Vigo and Director of the European Reference Laboratory of Marine Biotoxins. The seminar was held on January 29, the day after the *Honoris Causa* ceremony. In the Chair's introduction, she shared her experiences regarding the remarkable personality of Professor Yasumoto, both his personal warmth and his scientific excellence. Professor Gago-Martínez expanded on these aspects of Yasumoto's character through accounts of his other activities in the field of marine biotoxins, including his involvement and collaboration with different Institutions worldwide, in particular in Galicia (Prof Yasumoto has often described Galicia as his second home).

Included in the seminar programme were presentations by leading scientists in the marine biotoxin field who attended to honour Prof. Yasumoto on the occasion of his award and also to share their own knowledge and experience with other scientists. The Seminar covered several topics in the analytical and toxicological aspects of marine

Cont'd in back page

• Nicaragua

First report of PSP on Pacific coast of Nicaragua associated with *Pyrodinium bahamense*

Studies of marine phytoplankton and harmful algae on Nicaraguan coasts are very scarce. As historical antecedents, there were red tide alerts on the Pacific coasts of Guatemala and El Salvador in December 2000 and of Costa Rica in September 2001. During those events, the Nicaraguan health ministry (MINSA) detected neither red tides nor shellfish toxicity.

In early November 2005, reddish patches were seen off the north Pacific coast of Nicaragua, due to a bloom dominated by *Pyrodinium bahamense*. This event was associated with 50 cases of PSP toxicity, including one death, following consumption of mangrove cockles (*Anadara tuberculosa*).

This event had a strong economic impact in six municipalities (Corinto, El Realejo, Chinandega, El Norte Viejo, Posoltega and León) whose main sources of income are the harvesting and commercialization of molluscs. The health authorities sought help from the Nicaraguan Aquatic Resources Research Centre of the National Autonomous University in Managua (CIRA/UNAN), to identify the phytoplankton species potentially responsible for the toxic outbreak.

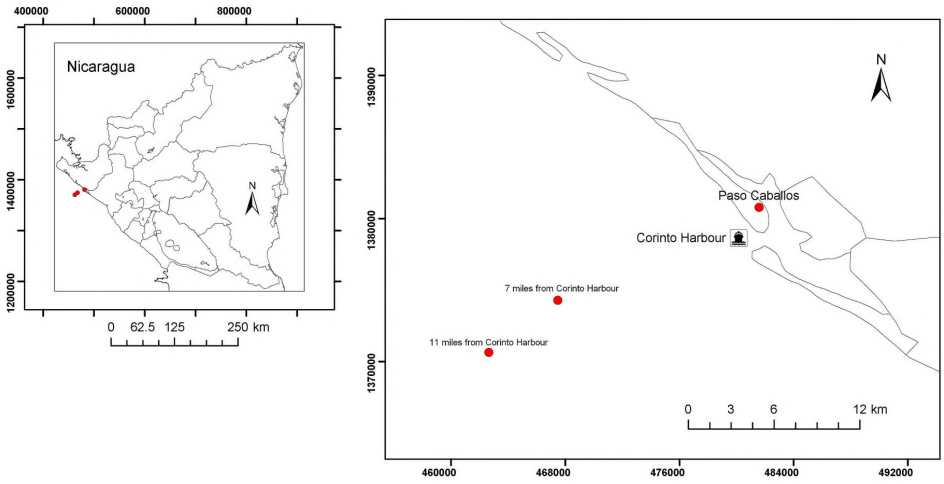

Fig. 1. Sampling stations on Pacific coast of Nicaragua

Water samples for phytoplankton analysis were taken on 11 and 15 November 2005 at three coastal stations off Corinto (Chinandega) selected by MINSA (Fig. 1), at Paso Caballos (11 November) where the cases of poisoning occurred, and from two stations 7 and 11 miles from Corinto (15 November), where reddish patches had been seen the previous day during overflights. Oblique hauls for qualitative analysis were taken with a 10 µm mesh net and transported to the laboratory live, quantitative samples with a 2.5 L van Dorn at 0.5 m depth, and fixed with Lugol's iodine. Counts were made with an Olympus CH40 inverted microscope following the Utermöhl method. Both diatoms and dinoflagellates were common at the Paso Caballos and 11 mile stations, but the patch detected from the air the previous day was a practically monospecific bloom of *Pyrodinium bahamense* (Fig. 1), which reached concentrations of 17.3 x 10^6 cell L^{-1}, compared with 72 x 10^3 cell L^{-1} and 14 x 10^3 cell L^{-1} at Paso Caballos and the 11 mile station respectively.

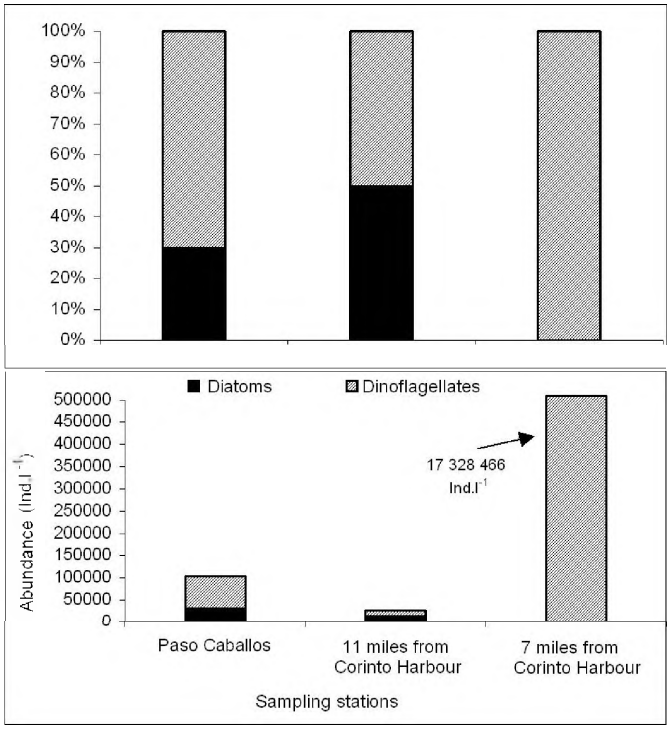

Fig. 2. Percentage contributions and numerical abundance of phytoplankton groups at the three stations

Epidemiological results are summarized in Fig. 2 and 3. Symptoms were muscular weakness (76%), oral numbness (56%), and vomiting (50%). Mouse bioassay indicated a maximum saxitoxin concentration of 1265 µg STX eq 100 g^{-1} of meat; toxicity was undetectable one month later.

Pyrodinium bahamense var. *compressum* (Fig. 4) is presently considered a harmful species in the tropics due to its high toxicity, and has been associated with intense episodes of PSP on the Pacific coast of Mexico and Central America which have included fatalities. In November 1989, 99 people were poisoned (three fatally) in the Gulf of Tehuantepec, Mexico [1]; 187 people (26 fatalities) were affected in July 1987 in Guatemala [2]. In El Salvador, between August 2001 and January 2002, there were 41 cases [3], and in Costa Rica, in 1999 and 2000, there were about 70 cases [4].

This is the first report of a PSP event in Nicaragua, accompanied by observations of plankton patches and standard methods of toxin analysis, identification of the suspect species, and a parallel epidemiological study coordinated by MINSA. This event indicates the need for a phytoplankton

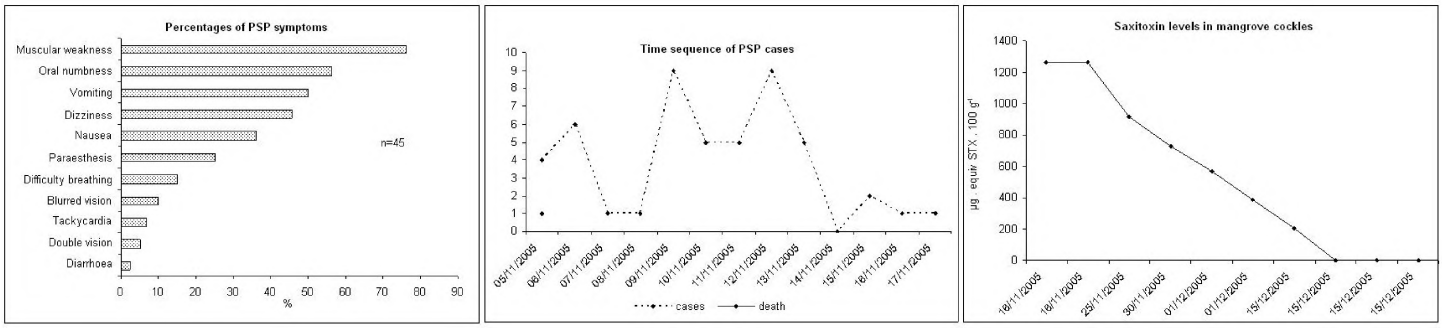

Fig. 3. Percentages of different symptoms in people poisoned, temporal distribution of cases and saxitoxin levels (µg equiv. STX · 100 g^{-1} of meat) estimated by mouse bioassay in Anadara tuberculosa *during toxic event on Pacific coast Nicaragua, November 2005. (Data of SILAIS, Sistema Local de Atención Integral de Salud).*

monitoring program on the Pacific coast of Nicaragua, and for testing toxicity in shellfish destined for human consumption. This type of program would prevent the occurrence of human poisoning, identify periods and zones at risk, and mitigate economic losses to the mariculture industry.

There is also a need to establish links for technical cooperation and information exchange among Central American countries, especially those which share common *P. bahamense* events. It is possible that blooms of *P. bahamense* reflect mesoscale events over a wide zone of Pacific Central America, and that a communication network between countries affected would improve prediction of the development of these events, and improve the analytical capacity of national laboratories responsible for toxin detection and phytoplankton taxonomy.

Finally, it is of great importance that consumers be informed of the risks associated with shellfish consumption exposed to these toxins by the state institutions (INETER; ADPESCA) to deal with the problem in a coordinated manner.

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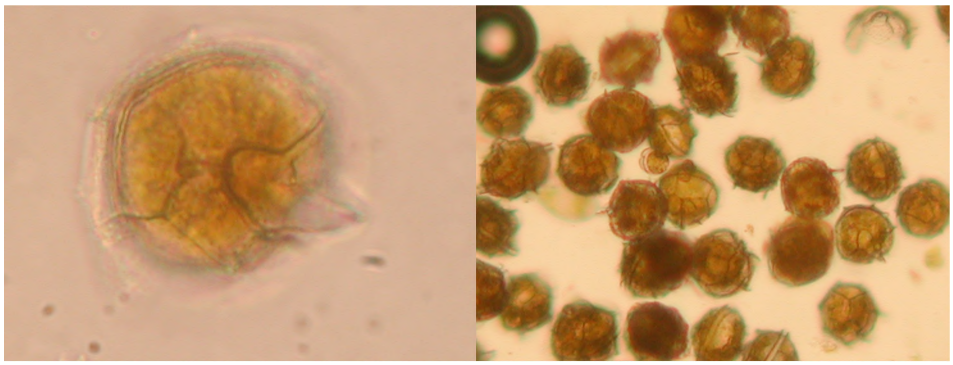

Fig. 4. Photograph of Pyrodinium bahamense *(40x and 10x).*

14th International Conference on Harmful Algae

Alexandrium catenella during 2009 in Chilean waters, and recent expansion to coastal ocean

In Chile, historical data show that blooms of the toxic dinoflagellate *Alexandrium catenella* have increased in frequency, duration, extent and intensity in recent decades [1–2]. In 1972, *A. catenella* was recorded in Estrecho de Magallanes for the first time [3]. Subsequently, in 1981 a new bloom of this species was described in the Magallanes region [4]. The first record of the species in Churrecue Island, Region XI, was in 1992 [5].

The presence of this paralytic shellfish poison (PSP) producer was first detected in Region X in quantitative samples between January and June 2002; it caused an intense bloom which covered a wide area of the Chiloé Archipelago. This outbreak occurred off Quellón, Cailín Island, including Guapiquilán, in the southern part of the Chiloé archipelago, and lasted from 22 January 2002 to April of the same year; it advanced northwards and reached Dalcahue channel [2]. Since then, *A. catenella* has recurred off Chiloe Island in the years 2003, 2004, 2005, 2006 and 2009.

A. catenella outbreak 2009

The 2009 outbreak has been one of the most important in terms of abundance and distribution. The outbreak covered a wide geographical area from 46º to 43º 45' S during the first half of March [2] (Fig. 1). *A. catenella* cells were first detected on 15 January in the Izaza area (44º 34' S), King Channel. For several weeks, cell concentrations did not exceed 40 cells mL^{-1}, until 23 February when the first major outbreak was detected off Ester Island (45º 09' S), reaching a maximum of 2230 cells mL^{-1} to a depth of 5 m. Later, from 4 to 10 March, the dinoflagellate reached maximum abundance, and we observed several peaks in the area of the Guaitecas archipelago, reaching concentrations of 1200, 1500, 1800, 3000 and exceeding 6000 cells mL^{-1} in the areas of Cuptana (44º 32' S), Canalad (44º 33' S), Angostura (45º 36' S), Ester (45º 09' S) and Allan (45º 45' S), respectively (Fig. 1).

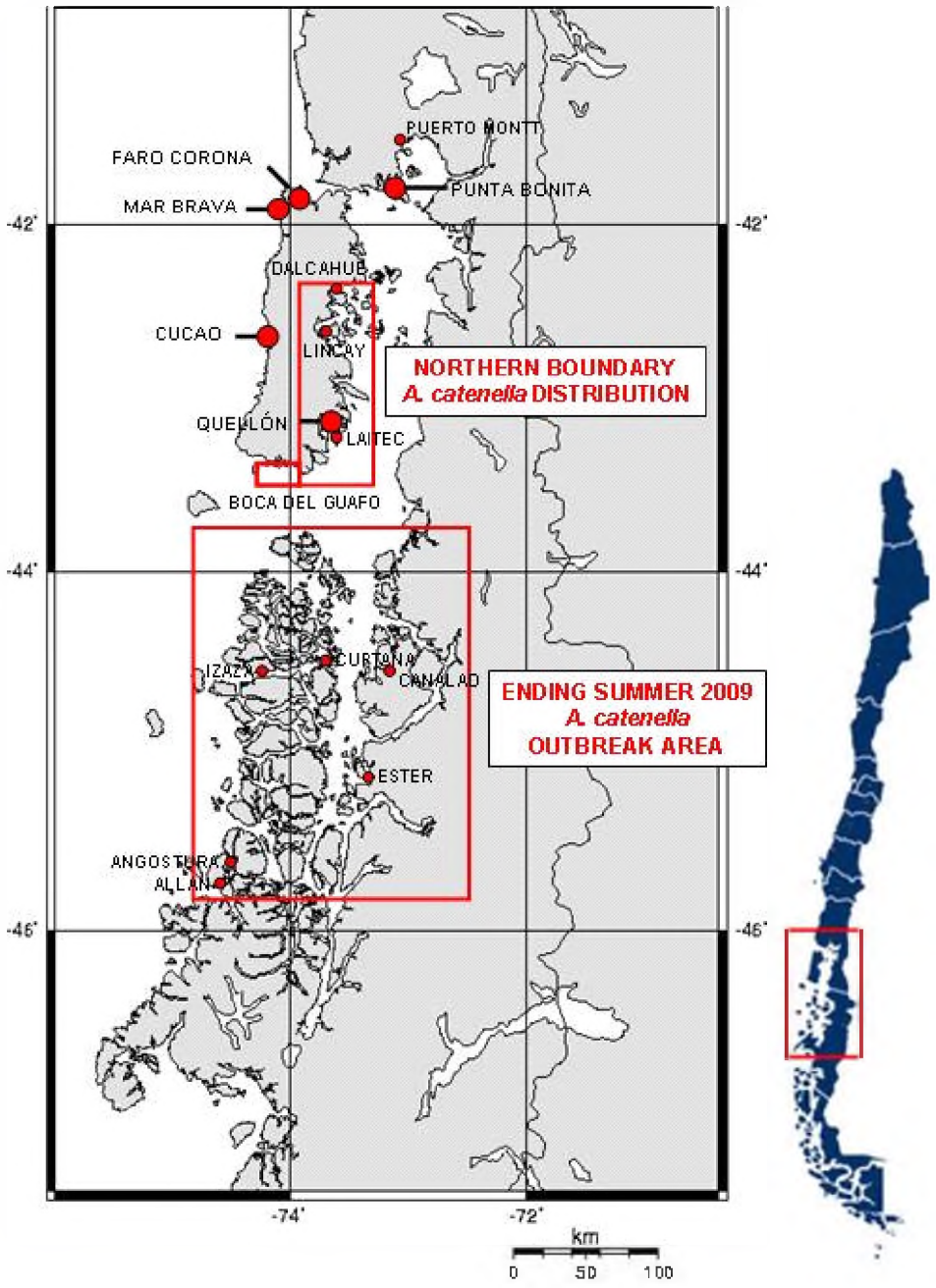

Fig. 1. Distribution of A. catenella *during 2009.*

During this period, the high cell concentrations generated intense reddish discoloration of the water, and bioluminescence, with cell chains that were visible to the naked eye due to their long length (Fig. 2a).

Recent expansion of *A. catenella*

During the first fifteen days of March 2009, *A. catenella* was detected only in the Guaitecas south area (43 º 50'S), but on 17 March 2009 it occurred off Chiloé Island, Laitec zone (43º 13'S) where it persisted until April 15; it was next detected off central Chiloé, in Lincay zone (42° 38' S) and then further north in Huyar, Quinchao Island (42° 23' S) on July 6.

Prior to 2009, *A. catenella* was confined to the northern edge of the Chiloé inland sea [2]; it was found in net samples (23 µm) in the Faro Corona area northwest of Chiloe on 30 Oct 2009, and in Mar Brava and Cucao areas on 3 and 4 Nov 2009 respectively.

This report of *A. catenella* is important due to the following issues;

- it shows a northward expansion of the species range
- it is the first record of the species in SE Pacific coastal oceanic waters, off Chiloé Island.

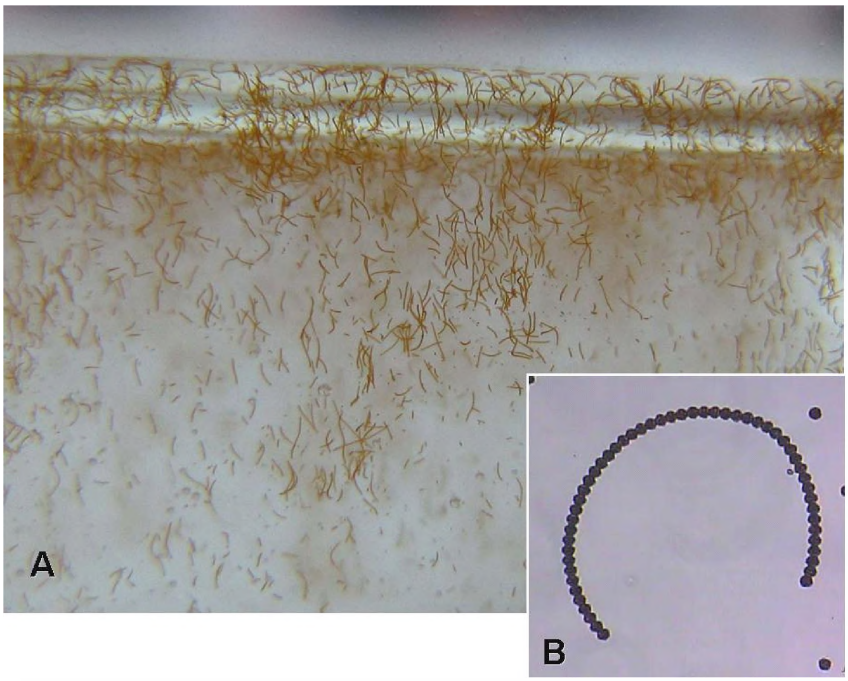

Fig. 2. Images of A. catenella. *Ester Island Summer 2009. a) Assemblage in a laboratory flask, ca. 4500 cell mL^{-1}. (photo: E. Minte). b)* A. catenella *chain of 52 cells (photo: J. Mardones).*

This new distribution of *A. catenella* suggests cell transport to inland waters through the Chacao Channel. Cells can reach Chiloé waters and further north, not only from the fjords and channels south of 43° S, but also from water masses of coastal oceanic origin.

On 4 Nov 2009, *A. catenella* was detected in Punta Bonita (41° 44' S) Calbuco area; this is the most northerly record of the species so far. During this period, *A. catenella* was not observed off the northeast coast of Chiloe Island, suggesting that these cells reached the area via the Chacao channel, in agreement with previous records of the species in the Mar Brava and Faro Corona areas (Fig. 1).

We thank Instituto Tecnológico del Salmón (INTESAL) for data support.

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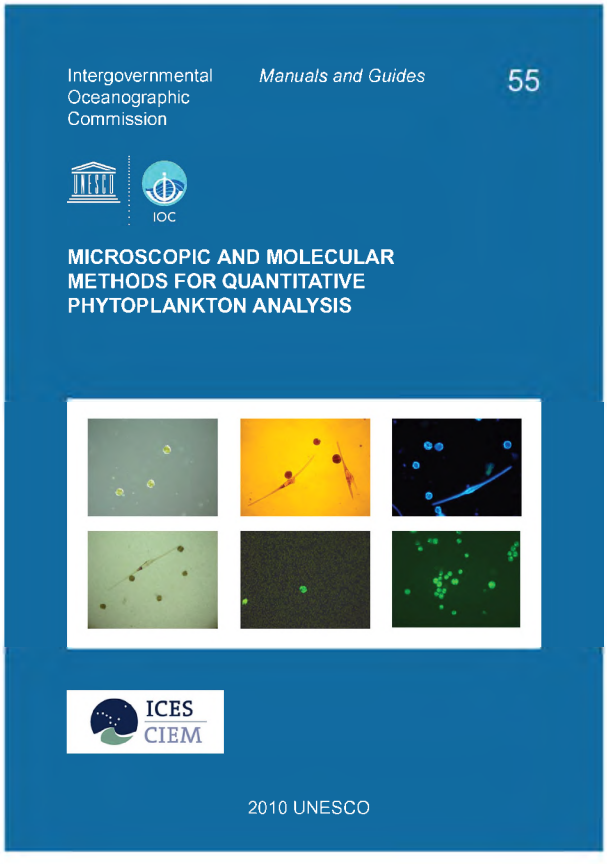
New manual available

Microscopic and molecular methods for quantitative phytoplankton analysis

A new manual on microscopic and molecular methods for quantitative phytoplankton analysis is available from the IOC in cooperation with ICES. The manual includes illustrated step by step instructions on how to carry out the methods. The manual can be downloaded as a pdf-file from www.ioc-unesco.org/hab.

The manual complements the existing IOC Manual on Harmful Marine Microalgae published in 2003.

Intergovernmental Oceanographic Commission of ©UNESCO. 2010. Karlson, B., Cusack, C. and Bresnan, E. (editors). Microscopic and molecular methods for quantita-tive phytoplankton analysis. Paris, UNESCO. (IOC Manuals and Guides, no. 55.) (IOC/2010/MG/55), 110 pages.(English only)



Dinoflagellate blooms in eutrophic zones of Bahía de Cienfuegos, Cuba

In recent years, increasing supplies of nutrients to water bodies by human activities has been associated with growth in the predominance of algal blooms. Increased nutrient fluxes to estuarine and coastal areas result from agriculture, aquaculture, and discharges of industruial and domestic wastes. The resulting blooms lead to hypoxia, fish mortalities, toxicity of food organisms, and damage to benthic communities resulting from reduced light penetration and resulting alterations in trophic interactions throughout the ecosystem [1].

Bahía de Cienfuegos, one of the most valuable natural resources of the province of the same name, is distinguished by its extent and its beauty (Fig. 1). The variety of developments in the surroundings and exploitation of the bay itself have impacted both the water and the coastal zone, and for these reasons it has been chosen for study within the framework of the Ramal Scientific-Technical Program, "Cuban Environmental Protection and Sustainable Development". Estimates indicate that direct nutrient discharges from urban areas contribute 15,1% to the total discharges from the river systems [2].

At the beginning of the rainy season, July 2009, about 50 to 100 dead fish were observed near domestic waste discharged from Cienfuegos city, near station 12 (Fig. 1). According to local dock-workers, the fish had reached the shore showing signs of lack of oxygen, and red patches had been seen repeatedly that week. The monitoring the day after showed these coincided with blooms of *Heterocapsa*. Cell shape and the position of the nucleus and pyrenoid coincide with the description of *H. circularisquama* [3]. The patch covered about 500 m^2.

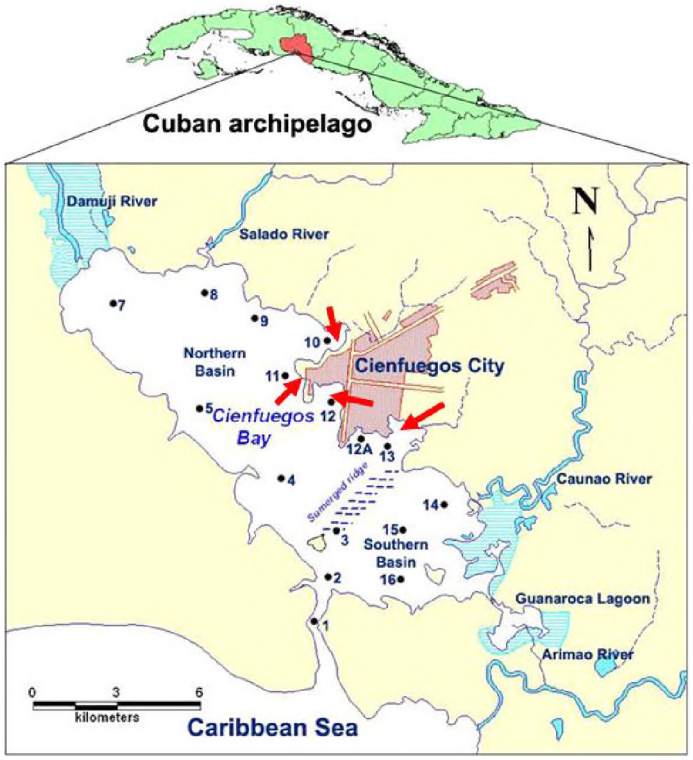


Fig. 1. Bahía de Cienfuegos. State monitoring network. Arrows show main discharge zones of domestic and industrial wastes.

Heterocapsa circularisquama has caused mass mortalities of bivalves in Japan [4]. Cells of this dinoflagellate were abundant in the fishes gills, but cells of the benthic *Amphidinium* cf. *carterae* (Fig. 3), which produces haemolytic ichthyotoxins, were also found there. *A. cf. carterae* was present in the plankton samples, but in low concentrations. Its presence in the gills might indicate that it was relatively abundant in the nearby benthos. One other benthic species, *Prorocentrum rathymum*, also appeared in moderate numbers in the plankton samples. In the areas close to the domestic discharges, substrates for benthic microflora are abundant, with flourishing filamentous macroalgae and hydrozoans, and carpets of cyanobacteria, most of them toxic.

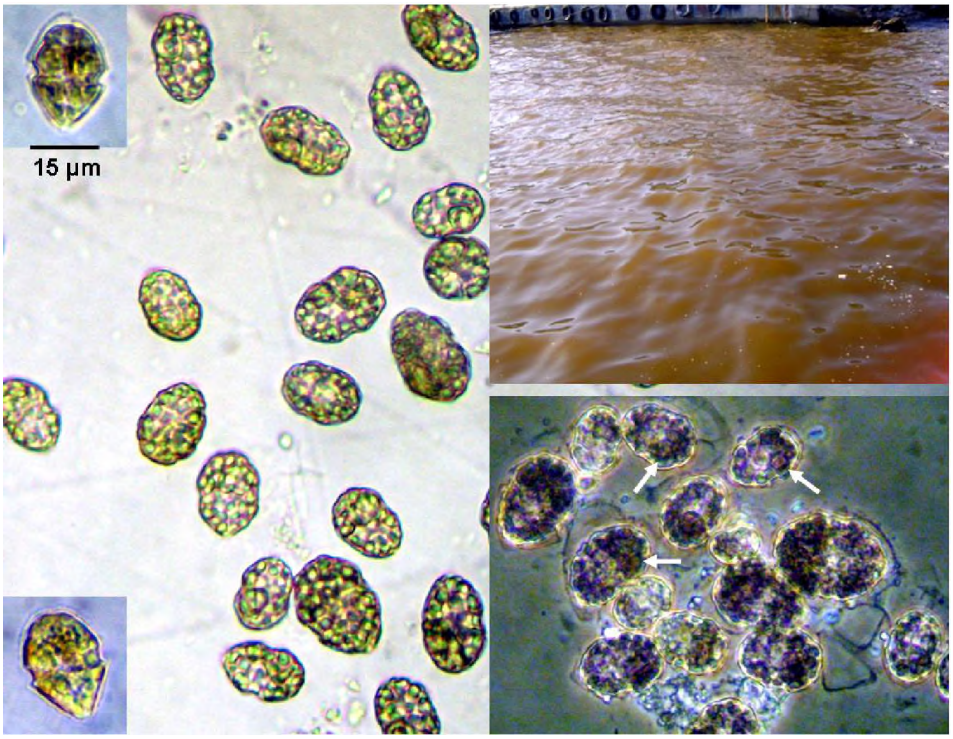


Fig. 2. Heterocapsa *cf* circularisquama *in Lugo preserved sample. Arrows indicate location of pyrenoid.*

Meteorogical conditions at the time were calm with high air temperatures (35–36º C) typical of summer in Cuba, and scarce rainfall.

Other toxic or noxious species (Fig. 4) have been seen accompanying blooms in other parts of the bay, and seem to be established components of the microflora here. Amongst these are *Gymnodinium* cf. *catenatum*, a potential source of paralytic toxins, *Cochlodinium* cf. *polykrikoides* with ichthyotoxic proerties, *Dinophysis caudata* and *D. ovum*, producers of



Fig. 3. Amphidinium *cf* carterae

diarretic toxins, *Prorocentrum minimum*, producer of neurotoxins, and *Gonyaulax polygramma* y *G. spinifera*, associated with ichthyotoxic events.

Blooms of nontoxic dinoflagellates also occur, mainly at the end of the dry season and beginning of the rainy season, when temperatures increase. These include *Gyrodinium instriatum*, *Gymnodinium estuariale*, *Peridinium quinquecorne* and *Prorocentrum compressum* (Fig. 5) close to stations 10 and 12, contributing to decline of the density and biomass of *Halodule wrightii*, the most abundant phanerogam in the bay, as well as the large rhodophytes (*Gracilaria*, *Acanthophora*, *Hypnea*) typical of this ecosystem. Blooms of *Gymnodinium estuariale* close to the outlet of the River Salado and near the industrial sector (stations 8 and 9) increase chlorophyll *a* concentrations up to 38.17 µg L^{-1}. These blooms of nontoxic dinoflagellates and the abundant epiphytes of the filamentous macroalgae and hydrozoans reduce light levels in the marine meadows and lead to their deterioration.

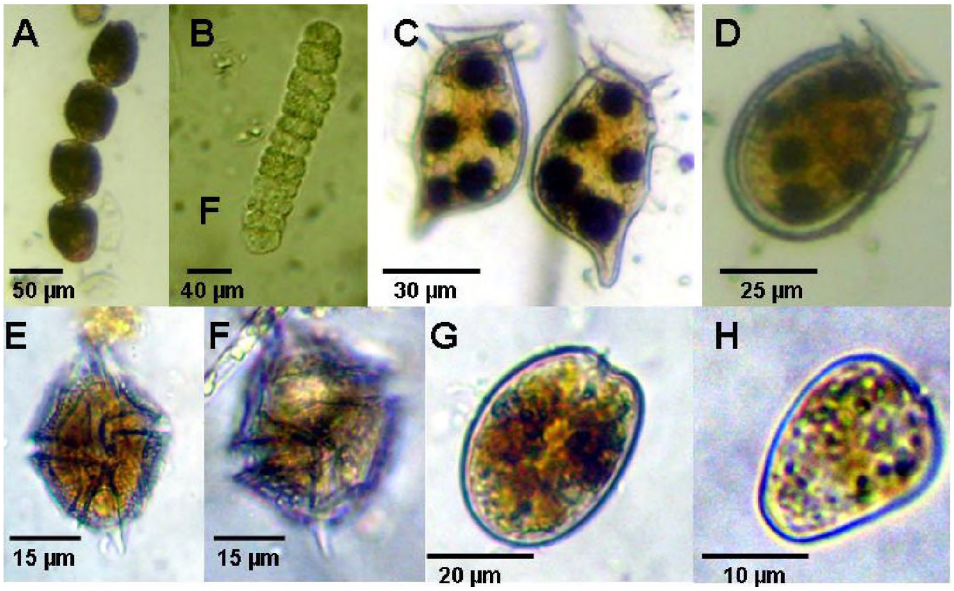


Fig. 4. Other toxic or noxious species associated with blooms in waste discharge areas; A) Gymnodinium *cf.* catenatum, *B)* Cochlodinium *cf.* polykrikoides, *C)* Dinophysis caudata, *D)* D. ovum, *E)* Gonyaulax polygramma, *F)* G. spinifera, *G)* Prorocentrum rhathymum, *H)* P. minimum

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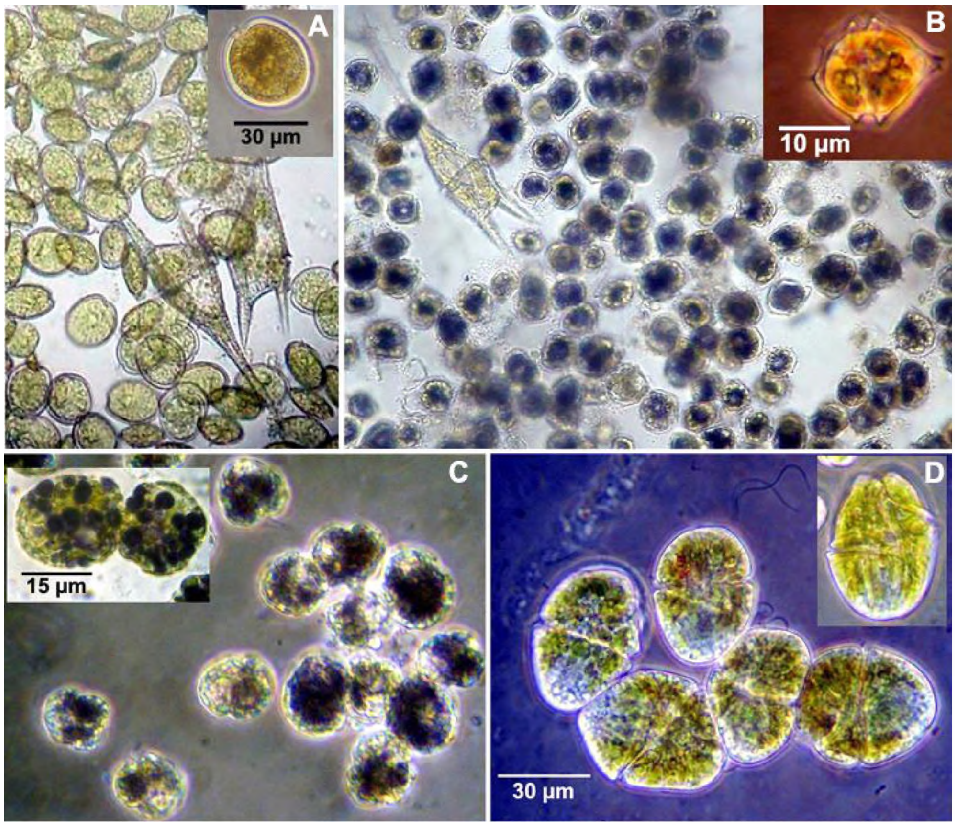

Fig. 5. Nontoxic species which form blooms in discharge areas; A. Prorocentrum compressum, *B.* Peridinium quinquecorne, *C.* Gymnodinium estuariale, *D.* Gyrodinium instriatum

Harmful Algae News

Previous issues of HAN and newsletters of the IOC HAB Programme can be downloaded at http://ioc.unesco.org/hab/news.htm

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Prorocentrum levis and *Coolia monotis* in Abruzzo coastal waters (W Adriatic)

The first appearance of *Prorocentrum lima* (Ehrenberg) Dodge in Abruzzo coastal waters (Western Adriatic) was reported in 2007 [1]. The ichthyotoxic *Fibrocapsa japonica* had been detected earlier [2]. Since then, other allochthonous toxic microalgal species have been observed, such as *Ostreopsis* cf. *ovata* in summer 2008 [3], and *Prorocentrum levis* and *Coolia monotis* in 2009. These investigations were carried out in four areas, harbours and rocky banks during June, July, August and September 2007, 2008 and 2009 (Fig. 1).

These species are known to produce toxins that can be dangerous for human health. *F. japonica* produces neurotoxic (fibrocapsin), haemolytic, haemo-agglutinating compounds and oxygen radicals. The high toxicity of *F. japonica* is proposed to result from the production of a secondary metabolite named fibrocapsin [4]. It was described in detail and named by Toriumi and Takano in 1973 after dangerous blooms along Japanese coasts [5]. *P. lima* produces DSP toxins (Diarrhetic Shellfish Poisoning) that can contaminate filter-feeding shellfish and cause gastrointestinal illness in humans [6]. The complex of DSP toxins produced by *P. lima* includes okadaic acid (OA), dinophysistoxins (DTX-1, DTX-2), prorocentrolide and fast acting toxin [7]: this species is distributed world-wide, often associated with coral reefs, or attached to floating detritus in mangrove habitats [8].

Ostreopsis ovata produces palytoxin-like compounds [9]. The production of putative palytoxin with other palytoxin-like compounds by *O. ovata* from Italy has recently been confirmed [10]: this species has generally been associated with tropical areas but has recently become relevant in temperate climates, including the Mediterranean Sea [11].

Prorocentrum levis was first isolated in 2002 from the Belize barrier reef system [12]: it was initially confused with *Prorocentrum concavum*. Its populations are often associated with floating mangrove detritus and sediments. The first report of this species in the Mediterranean, in 2004, was from Greek waters [13]. It was first reported in the Adriatic in 2006 [14], and on the Abruzzo coast in summer 2009. *P. levis* is considered toxic and produce DSP toxins okadaic acid and DTX2 [12].



Fig. 1. Research Area.

P. levis is photosynthetic with brown-golden chloroplasts (Fig. 2a). Cells in culture forms chains, as also described by Aligizaki [13], of two to many cells, usually in hyaline envelopes. In culture, it forms spots attached to the flask walls (Fig. 2b).Cells measured 44 ìm in length and 37 ìm in width, a length to width ratio of 1.17 (n = 20) (Fig. 2c). The periflagellar area of the right valve is shallow depressed, bearing about eight platelets (Figs. 2d and 2e).

Coolia monotis is considered toxic [15] and produces cooliatoxin, a neurotoxic analog of yessotoxin [16]; it has been found in plankton from brackish habitats and tidal pools, as well as mangrove environments. This species is most common in warm shallow waters of the Caribbean Sea and the Pacific Ocean [17], but during the last decade, has been reported from the Mediterranean [18]. In the Adriatic, this species has been observed in the Gulf of Trieste 19] and Conero Riviera [20] and now on the Abruzzo coast (summer 2009). *Coolia monotis* is a planktonic, benthic and epiphytic species [21]; it is photosynthetic, with many golden-brown discoid chloroplasts. The cells are compressed, round and lens-shaped. Cells measured 36 µm in length and 35 µm in trans-diameter (n = 20) (Fig. 3a). The cingulum is equatorial, narrow, and enclosed by lists with a smooth edge (Fig. 3b).The sulcus is narrow with a deep chamber-like appearance with straight walls (Fig. 3c). The apical pore plate, Po, is located off-center on epitheca (Fig. 3d).

In this study, four confined areas were monitored during the summers 2007, 2008 and 2009: Pescara harbour; Ortona harbour; San Vito and Fossacesia rocky point respectively. These stations were selected to localize epiphytic species like *P. lima*, *C. monotis*, *O. ovata* and *P. levis* since they live on macroalgae, on sediments and in stagnant environments. Chemical - physical parameters of seawater were measured to understand their relationship with the presence/absence of tropical harmful species. At each

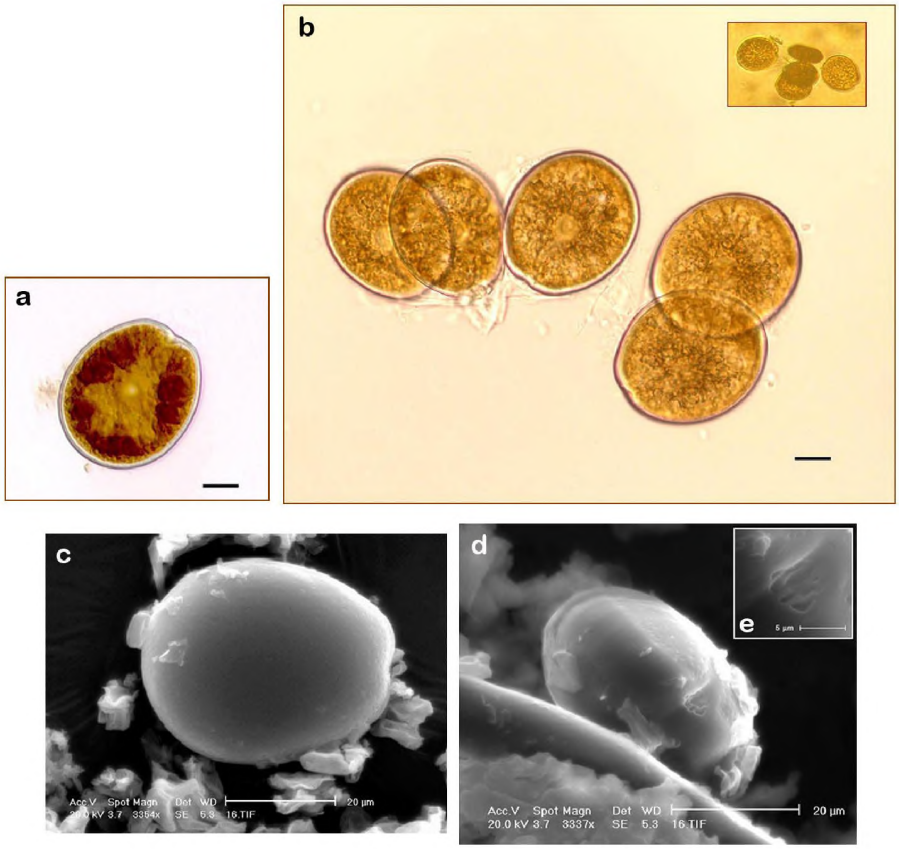

Fig. 2. Light microscopy photographs of Prorocentrum levis *at 1000x magnification scale bar = 10 µm: a) single cell with a centrally located pyrenoid and golden brown chloroplasts; b) Cells in chains stick on Petri plate. Scanning electron micrographs of* P. levis *from isolated strain; c) Left valve, scale bar = 20 µm; d) The periflagellar area of the right valve is shallow depressed with pore along the valve margin, scale bar 20 µm; e) bearing about eight platelets, scale bar 5 µm.*

station, one seawater sample was taken at 0.5 m below the surface with a pump. Cell concentrations are expressed as cells L^{-1}, reading the entire well using a volume of 50 mL.

Algae observations were effected by the use of the light microscope (ausJENA Telaval 3) at 200x, 400x and 1000x magnification. For taxonomic determination and plate tabulation of *O. ovata* and *Coolia monotis* it was used a light microscope epifluorescent using a Zeiss Axiophot microscope at 200x, 400x and 1000x magnification following the Calcofluor method of Fritz & Triemer, 1985 using fluorescent brightener 28 (Sigma – Aldrich). For SEM analysis of *O.* cf. *ovata*, *P. lima*, *Coolia monotis* and *Prorocentrum levis* isolated cultures, cells were fixed in 4% formaldehyde. After stored for three days at 4 °C, cells were washed three time into distilled water eliminating the supernatant at each washing. They were successively dehydrated in alcohol series up to 100%, dried with CO_2 critical point (CPD), sputter coated with Au/Pd and examined on Philips XL–30–CP electron microscope according to Zingone *et al.* 1990.

F. japonica has always been detected for the entire period of monitoring with a bloom phenomenon on August 2009 along the entire coast of study (7.1 x 10^5 cells L^{-1} mean abundances; 24 ± 1 °C mean water temperatures).

The first appearance of *P. lima*, instead, was recorded on summer 2007 inside Ortona harbor. Its cellular abundance has been detected for the entire season (June, July, August and September) with a mean concentration of 1.9 x 10^5 cells L^{-1}. During summers 2008 and 2009 *P. lima* was revealed not only at Ortona harbor but also at San Vito rocky bank, with mean concentrations of 6.9 x 10^3 cells L^{-1}.

The first appearance of *O.* cf. *ovata* was at the end of August 2008 at Ortona harbor and Fossacesia rocky bank. Its mean abundance was 3.6 x 10^3 cells L^{-1} (28 ± 1 °C mean water temperatures), while on summer 2009 (at the beginning of September) it was recorded also at San Vito rocky bank (1.5 x 10^3 cells L^{-1} mean concentrations).

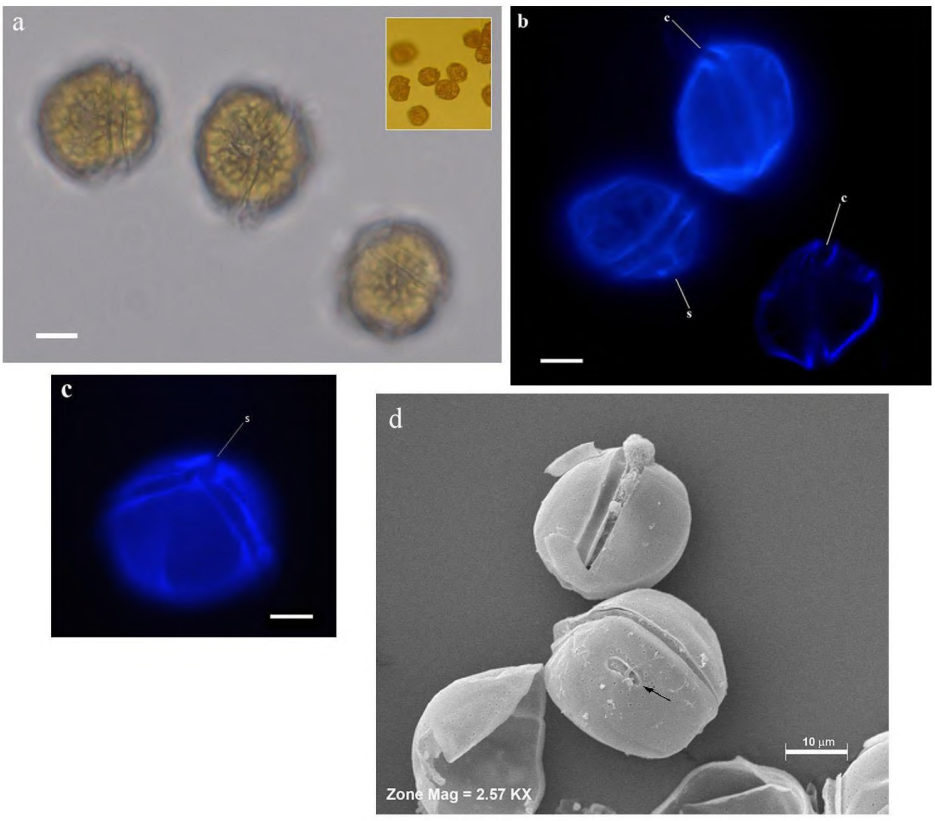

Fig. 3. a) Light microscopy photographs of Coolia monotis. *Cells are round and lens-shaped with many golden-brown discoid chloroplasts, 1000x magnification scale bar = 10 µm. LM epifluorescence of* C. monotis, *1000x magnification scale bar = 10 µm; b) Ventral and dorsal view of the cells where is possible to observe the equatorial cingulum and narrow; and c) deep sulcus. Scanning electron micrographs of* C. monotis *from isolated strain d) Dorsal view with apical pore plate Po on epitheca (white arrow), scale bar = 10 µm.*

P. levis and *C. monotis*, at last, have been detected for the first time during this last summer (August and September 2009) only at Ortona harbor, with 1.5 x 10^3 and 3.0 x 10^2 cells L^{-1} mean concentrations respectively.

The increase of sea water temperature and the expand of ships traffic and their ballast waters have caused the entry of several tropical harmful microalgae. In fact, the Ortona harbor point, characterized by a major ship traffic, has revealed the entire community of the species in study among the four sampling stations. Besides, the highest temperature values of the three years of monitoring were always measured on August. During these periods, the greatest contemporary presence of the harmful species has been registered. As a matter of fact, water temperature trend has been positively correlated with harmful algae abundances ($p = 0.001$; $n = 96$).

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GEOHAB updates and news

The *GEOHAB Implementation Plan* (GEOHAB, 2003) specifies the formation of Core Research Projects (CRPs) related to four ecosystems types: upwelling systems, fjords and coastal embayments, eutrophic systems and stratified systems. In 2010, a fifth working group will be initiated, on Benthic HABs. These CRPs are initiated through small, focused open science meetings. GEOHAB also supports infrastructure activities, including development of modeling, and interaction with other international efforts such as the International Ocean Colour Coordinating Group (IOCCG). GEOHAB has made significant progress in many of these core areas, as highlighted below.

New GEOHAB Release—Fjords & Coastal Embayments

Harmful algal blooms are a frequent and important problem in coastal environments, particularly in fjords, inlets and embayments where conditions for growth are often favorable (cell retention, availability of nutrients, etc.). Recognizing this, SCOR and IOC's International Programme on Global Ecology and Oceanography of Harmful Algal Blooms, GEOHAB, supported an Ocean Sciences Meeting in Viña del Mar (Chile), to foster the development of international collaboration on this topic. The Report of this Meeting has now been published as GEOHAB's Report n°. 6, entitled "GEOHAB 2010. Global Ecology and Oceanography of Harmful Algal Blooms, GEOHAB Core Research Project: HABs in Fjords and Coastal Embayments", edited by Allan Cembella, Leonardo Guzmán, Suzanne Roy and Jorge Diogène. It is available on the Web at www.geohab.info, or by contacting Ed Urban (Ed.Urban@scor-int.org) or Henrik Enevoldsen (h.enevoldsen@unesco.org). This Report contains six major sections that describe the GEOHAB approach, an overview of harmful algal blooms in fjords and coastal embayments, the identification of research priorities, a description of framework activities and a list of planned activities. Seven key questions have been highlighted, including adaptive strategies that characterize HAB species in these environments, the importance of life history transitions and cysts, the role of physical dispersion and aggregation, the relative contribution of nutrient flux and supply ratios to HAB dynamics, the importance of spatial scale and retention time in the expression and effects of allelochemicals, and the effects of anthropogenic activities (e.g. aquaculture or ship transport through ballast water) and of global climate change on the dynamics of harmful algae in embayments and fjords.

New GEOHAB Release—GEOHAB Asia

Harmful algal blooms pose serious impacts on both coastal ecosystems and human activities in Asia, including aquaculture, public health and coastal

management, and damages caused by blooming of these species lead to huge economic losses. Frequency and severity of HAB events are increasing, and furthermore, their distribution within the region appears to be expanding. In this situation, the importance of exchange of information and cooperative research is a priority for researchers and managers working on HABs in Asia. In this context, two Asian GEOHAB Meetings were held, the first one in Tokyo, Japan in 2007, and the second one in Nha Trang, Vietnam in 2008. During the both meetings, the importance and usefulness of the comparative ecosystem approach were well recognized. The Report of these two meetings has now been published as GEOHAB's Report no. 7, entitled "GEOHAB 2010. Global Ecology and Oceanography of Harmful Algal Blooms, Harmful Algal Blooms in Asia", edited by Ken Furuya, Patricia M. Glibert, Mingjiang Zhou and Robin Raine. It is available on the Web at www.geohab.info, or by contacting Ed Urban (Ed.Urban@scor-int.org) or Henrik Enevoldsen (h.enevoldsen@unesco.org). This Report contains five major sections: 1) an introduction on the process of developing GEOHAB Asia, 2) an overview of Asian HAB problems, 3) regional background and challenges for advancing the understanding of Asian HABs, 4) implementation of GEOHAB Asia following GEOHAB programme elements, and 5) programme advancement and capacity building. Key objectives and many questions have been highlighted for national and regional research in Asia, and targeted HAB species identified for co-operative comparative studies in GEOHAB Asia are suggested.

GEOHAB Special Issue in the journal Progress in Oceanography

The Canary, California, Humbolt and Benguela comprise the main eastern boundary upwelling systems of the world. Located on the western margins of continents, on each side of the equator, trade winds combined with the rotation of the earth generate coastal upwelling, bringing nutrient-rich water to the surface, thereby fueling high primary productivity. These upwelling systems are susceptible to Harmful Algal Blooms (HABs) owing largely to this high productivity and the consequent high biomass blooms characteristic of these environments. This special issue of *Progress in Oceanography*, dedicated to HABs in eastern boundary upwelling systems, is an outcome of the scientific programme GEOHAB initiated by SCOR and the IOC to foster international co-operative research on HABs in ecosystem types sharing common features (GEOHAB, 2001). Based on the knowledge and understanding of HABs as presented at the Open Science Meeting, eight key questions were developed to provide research foci for the Core Research Project. These key questions were to address the adaptive strategies of HAB species within upwelling systems, specifically their seeding strategies; the role of small-scale physical processes, and the influence of nutrient type and ratios on the growth and dispersion of HABs; the role of genetic predisposition versus environmental conditions in toxin production for a given genus or species; the importance of coastal morphology and bathymetry, and across-shelf and alongshore advection in the development and distribution of blooms; and the use of climate indicators as predictors of HAB events in upwelling systems (GEOHAB, 2005). This series of review papers, edited by Grant Pitcher and Stan Pillar, provides a comparative assessment and update of the status of our knowledge of HABs in upwelling systems and also serves as a measure of our progress in addressing the above questions formulated at the Open Science Meeting in 2003.

GEOHAB Special Issue in Journal of Marine Systems

The GEOHAB Science Plan defines Observation, Modelling and Prediction as one of GEOHAB's five central elements. To foster better integration of modeling efforts within GEOHAB and its Core Research Projects, a Modeling Workshop was held on 15-19 June 2009 at the Martin Ryan Institute, National University of Ireland. Primary objectives included stimulation of modeling activity in GEOHAB Core Research Projects; engagement of researchers, particularly students, in HAB modeling; improve understanding of HAB processes through linkage of models, *in situ* observations, and remote sensing; and integration of HAB modeling and monitoring with the broader community of biogeochemical, population dynamics, and ecosystem modeling. Primary outcomes from this workshop include the forthcoming publication of both a GEOHAB report and a special issue devoted to HAB modeling in the *Journal of Marine Systems*.

New Working Group on HABs and Ocean Colour

The International Ocean Colour Coordinating Group (IOCCG) and GEOHAB have initiated an international working group on harmful algal blooms and ocean colour. The group will provide a guide to ocean colour-based harmful algal bloom methods, summarising the state of knowledge with regard to research and operational applications. Case studies from around the globe will be used to examine best-practice ocean colour application to harmful algal bloom observation across a variety of ecosystems. There will be a strong "consumer guide" focus: identifying uncertainties associated with ocean colour applications in the optically complex coastal zone; contextualising application with regard to the ecological role of potentially harmful algal blooms in different ecosystem types; and examination of the suitability of available ocean colour techniques within an ecosystem framework. The group will be composed of international ocean colour and harmful algal bloom scientists, and will begin work with a meeting in Cape Town in the third quarter of 2010. The working group convener is Stewart Bernard from the Council for Scientific and Industrial Research in South Africa.

GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

• Mexico

Karenia sp. "Mexican hat" first bloom in Mexico

On 2 January 2010, north of the port of Veracruz, Mexico, large amounts of beached dead fish were reported by fishermen. Some water samples were obtained from the sea in front of this site to investigate if a HAB was the cause of the mortality. *Karenia* spp. were found in high concentrations.

Historically, Veracruz has suffered from HAB events for at least two centuries. The first record for fish kills dates back to 1797, and *K. brevis* was later thought to be the causative agent [1]. This species is known to be toxic and brevetoxins have been detected in molluscs from Veracruz [2–3]. The last two records of HAB events in the State of Veracruz, namely, in the vicinity of the port of Veracruz, were in 2001–2002 and 2003. In December 2001–January 2002, cell densities reached 2.3 x 10^7 cells L^{-1} [4]. In August 2003, cell densities were 5.0 x 10^4 cells L^{-1} [5]. Since then, no occurrence of HABs caused by this species has been recorded.

In the period 2003 to 2009, numerous blooms (more than 50) occurred near the port of Veracruz, and the non-toxic dinoflagellate *Peridinium quinquecorne* was found to be responsible [6]; this species was recorded as the cause of a HAB for the first time in 2002 [7]. A few diatom blooms have also occurred, mainly of *Chaetoceros* spp. (especially, *C. curvisetus*) and *Thalassiosira* spp. Several *Pseudo-nitzschia* spp., including potentially toxic ones, have been detected in high concentrations in samples from the National Park Sistema Arrecifal Veracruzano, the zone lying in front of the port. The filamentous cyanobacterium *Trichodesmium erythraeum* formed a bloom in October 2007 [8], and more recently has been observed forming blooms in coastal Veracruz waters.

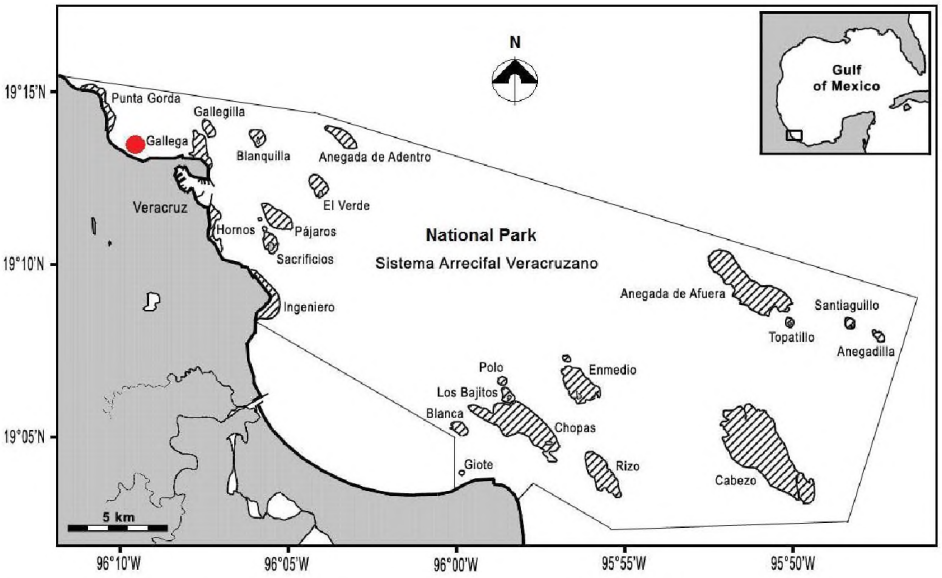

Fig. 1. Red circle indicates location of a HAB event in early January 2010.

To date, more than a dozen species of *Karenia* have been described. The formally described species of *Karenia* occurring in the Gulf of Mexico are as follows: *K. brevis, K. mikimotoi, K. papilionacea, K. selliformis,* and *K.* cf. *longicanalis*. Four of these can be separated molecularly, but all five can be morphologically differentiated even in fixed samples. In addition, other morphologically distinct unnamed *Karenia* species are known to inhabit the Gulf waters [9–10]. One of them is characterized by having a carina in form of a "sombrero" and it is known as "Mexican hat" [10–11].

Microscopic analysis of two surface samples taken near Playa Norte, 19° 15' 25.38" N, 96° 11' 54.7" W (Fig. 1) revealed 92% by cell counts of *Karenia* sp. "Mexican hat" (Fig. 2) and 8% of *K. brevis* (Fig. 3) on January 2, and around 50% of each on January 4. The cells of *Karenia* sp. ranged from 20.5 to 32.5 µm (26.0 ± 2.4; n = 180) in length (L), from 27.5 to 45.0 µm (34.55 ± 4.23; n = 224) in width (W), from 15.0 to 20.0 µm (17.47 ± 1.34; n = 22) in depth (D), W/L ratio (1.32 ± 0.11; n = 177), W/D ratio (2.01 ± 0.19; n = 22). The nucleus is located in the low left hypotheca, closer to the centre of the cell. The carina is prominent in most cells, always slightly tilted to the left, and its sides are angular (in ventral-dorsal view, the line between the shoulders and the carina are even more pronounced due to the curvature of the right and left ventral lobes of the epitheca. Numerous peripheral chloroplasts (about 15–20) were clearly seen only in understained cells. In general, our cells correspond very well with the photograph of a cell collected in the northern Gulf of Mexico [9]. In contrast, *K. brevis* cells were smaller and less compressed anterior-posteriorly. The measurements were as follows: 21.0–30.0 µm (24.98 ± 2.05) length, 22.5–35.0 µm (26.12 ± 2.70) width, W/L ratio (1.01 ± 0.22) (n = 29). Statistical differences were significant in length and width between the species

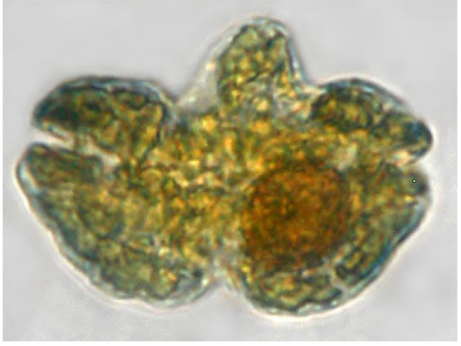

Fig. 2. *Karenia* sp. "Mexican hat".

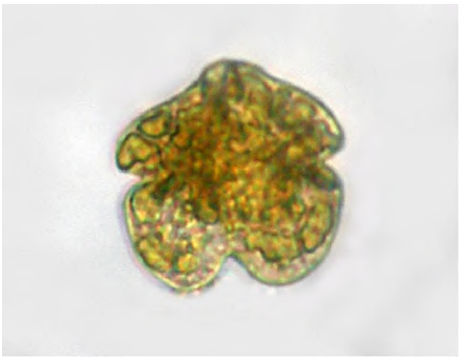

Fig. 3. *Karenia brevis*.

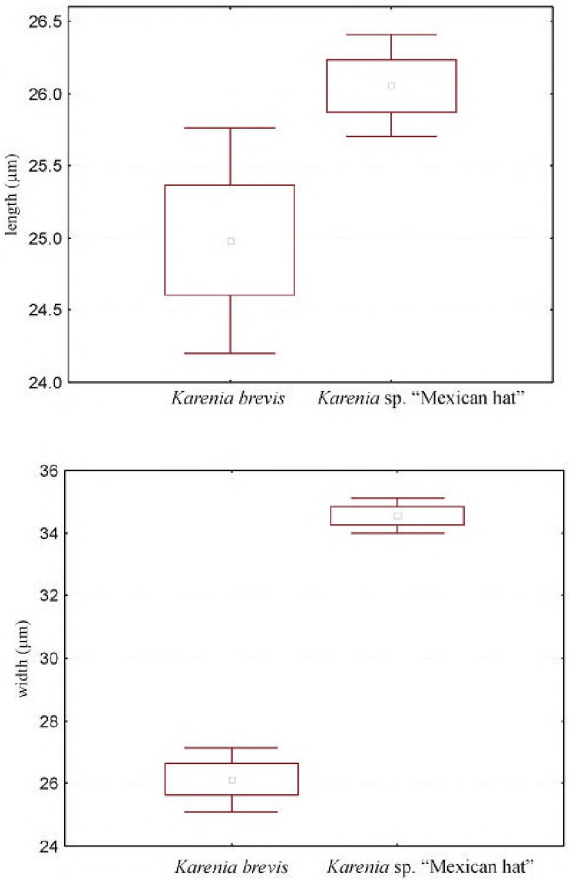

Fig. 4. Mean values of length and width of Karenia brevis *and* Karenia *sp. "Mexican hat". Boxes represent standard error, and whiskers show 0.95 confidence intervals.*

(F = 5.14, p = 0.024; F = 108.7, p < 0.005), so in addition to shape, they can be separated by size (Fig. 4).

We do not know exactly when the mixed bloom event of these two *Karenia* species began, but fishermen reported they had seen dead fish in the sea since 20 December 2009. The HAB was not visually evident until the dead fish were cast on the beach on January 2 (water temperature was 19°C). Fishermen reported they had experienced eye and respiratory irritation during the sampling period. Our count of *Karenia* that day was 1.17×10^5 cells L^{-1}, a high concentration for this taxon, considering that species of the genus had rarely been found in phytoplankton samples since 2003 in the State of Veracruz. Another phytoplankton sample obtained from the same site on January 4 was dominated by the diatom *Asterionellopsis glacialis* (1.16×10^5 cells L^{-1}), with *Karenia* still in high concentration (5.6×10^4 cells L^{-1}). This suggests that the *Karenia* HAB was ending when we detected the bloom.

According to the Sanitary Department of the State of Veracruz, almost one ton of dead fish was recorded. We estimated dead fish concentrations of up to 7 individuals m^{-2}. Scaridae (herbivores; especially, redtail parrotfish *Sparisoma chrysopterum*) dominated. Among others, the following taxa were identified: Atlantic thread herring *Opisthonema* sp. (planctivore), red or rock hind *Epinephelus* sp. (piscivore), moray *Gymnothorax* sp. (piscivore), surgeonfish *Acanthurus* sp. (herbivore), triggerfish *Balistes* sp. (benthophagous) and Gerreidae (detritivores).

During the years 2003 to 2010, among *Karenia* species, only *K. brevis* had been recorded, rarely, in phytoplankton samples from Veracruz waters, It has however caused HABs in the states of Tamaulipas (to the north) and Tabasco (to the southeast) [2–3, 12–13]. We believe that *Karenia* blooms in Veracruz have been displaced by *Peridinium quinquecorne*, and the northerly winds during the last one or two weeks in December 2009, specifically, the northerlies numbers 19, 20 and 21 [see: http://smn.cna.gob.mx], might have favoured higher nutrient concentration as a result of turbulence in this area, which has a permanent load of organic matter derived from a waste treatment plant. Previously, *Karenia* sp. "Mexican hat" has been reported from Florida and Louisiana coasts, blooming several times off the Naples area of the west coast of Florida [9]. The bloom described here, to our knowledge, is the first HAB event of this species in the southern Gulf of Mexico.

Acknowledgements

Financial support (DGI-UV project "Algae of the Veracruzan reef zone, Gulf of Mexico, with emphasis on red algae, diatoms and dinoflagellates" 2007–2009) to YBO is appreciated.

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ISSHA President's Corner

Route map of activities before the 14th ICHA Conference

Dear HAB experts and managers:

The time for the 14th International Conference on Harmful Algae (Crete, Greece, 1-5 Nov 2010) is approaching, and we are pleased to invite you to participate in important decisions/ activities that have now become part of our traditions during the ICHA Conferences. We invite you to become an ISSHA member if you are not still so. Your contributions will help to facilitate the participation of young PhD students and/or experts from developing countries/economies in transition.

The issues for which we need your help are:

1. Nominations for the Yasumoto Award and the Young Scientist Award
2. Travel Awards to attend the 14th International Conference on Harmful Algae
3. Donations for the ISSHA Auction 2010
4. Bids to host the 16th ICHA Conference (2014)

1. Nominations for the Yasumoto Award and the Young Scientist Award

ISSHA members are invited to submit nominations for the Lifetime Achievement Award and the Young Scientist Award. Further information about the awards can be found at www.issha.org; click on Awards.



Fig. 1. Rosa Figueroa, Young Scientist 2008 awardee, with her present from ISSHA. A beautifully carved Gymnodinium catenatum *by Prof. Takayama. Photo by K. Rengefors.*

The Yasumoto Lifetime Achievement Award

This award is offered in recognition of a long and outstanding record of contribution to harmful algal research by an ISSHA member (see the membership list at www.issha.org). Previous recipients: Dr. F.J.R. (Max) Taylor (2000), Dr. Greta R. Hasle (2002), Dr. Theodore Smayda (2002), Dr. Donald M. Anderson (2006) and Dr. Karen Steidinger (2008).

The Young Scientist Achievement Award

This award is offered in recognition of outstanding achievement in any aspect of harmful algal research by scientists early in their career (i.e. within ten years of receiving their PhD). The award is intended for an exceptional or "break-through" contribution to an area of harmful algae research, rather than a cumulative record of achievement. The nominee should be an ISSHA member (see the membership list at www.issha.org). Previous recipients: Dr. Chris Scholin (2002), Dr. Rosa I. Figueroa (2008).

Any ISSHA member in good standing may submit nominations for either achievement award, which should include a description of the nominee's contribution (not more than one page). Please, make sure that you and your nominee have renewed membership for the period 2010-2011. Those of you who paid your membership at the beginning of 2008, or at the 13 ICHA Conference in Hong Kong in 2008, paid for the 2008 and 2009 fee.

Nominations should be sent by e-mail to Jennifer Martin (Jennifer.Martin@dfo-mpo.gc.ca), Chair of the Committee on Achievement Awards, **to be received before May 15th 2010. Please write 'ISSHA Achievement Awards 2010' in the message subject**. Nominations will be considered by the ISSHA Council, and eventual awards will be presented at the XIV HAB Conference in Crete (Greece), 1-5 November 2010.



Fig. 2. Barrie as auctioneer at the Copenhagen 2006 conference. Photo by M. Lion.

2. Travel Awards to attend the 14th International Conference on Harmful Algae

ISSHA will offer a limited number of travel awards for students and postdoctoral investigators to attend the *14th International Conference on Harmful Algae*, 1-5 November 2010, in Hersonissos, Crete, Greece.

Awards are usually limited to a maximum of $1,000 US.

Successful applicants will need to demonstrate that other sources of funds are available to supplement their award. Among the criteria for selection will be the following:

- Membership in ISSHA (if you are not a member, please join before the applications are reviewed; students get a 50% reduction in their fees)
- Availability of "matching" funds
- First time award applicants will receive priority over those who have received travel support in the past
- Commitment to an oral or a poster presentation
- Quality or unique nature of science to be presented
- Demonstrated financial need

To apply for funds, please send the **application letter (forms will be available on the ISSHA site as of June 1), as a PDFile,** to Clarisse Odebrecht, Chair of Committee on Travel Awards (doclar@furg.br)

following the format that will be available on the ISSHA website in due course. Please write **"ISSHA Travel Awards 2010"** in the message subject. Applications not submitted this way may be rejected.

The deadline for receipt of applications is **July 3, 2010**, with decisions announced approximately one month thereafter.

Additional information about the 14th ICHA can be found at www.hab2010.gr.

3. Donations for the ISSHA Auction 2010

ISSHA auctions during past International Conferences on Harmful Algae have been a tremendous success and are becoming a tradition!! It is an opportunity for the conference participants to bid on donated items. The range of items is broad and may include books, signed reprints, photos, jewellery, T-shirts, paintings, algae-related items, liquor, and scientific equipment. The auction items are donated by ISSHA members from around the world and are used to benefit ISSHA members. Funds collected during the auction are an important income for ISSHA and may represent up to 25% of the total income of the Society. ISSHA was able to offer partial travel support to more than 25 students to attend the Conference in Hong Kong (3-7 Nov 2008).

Barrie Dale has so far been serving as the auctioneer and provocateur, ensuring the evening has been exciting, fun filled and a bit over the top! Please help us make the 14th Conference auction a success.

We need donations for the auction and only your imagination limits what can be donated. Please contact the responsible person for the auction, Anke Kremp (anke.kremp@ymparisto.fi) about your donations for the auction and the way you are going to transport it. **Please, write "ISSHA Auction 2010" in the message subject**. You may also include a picture of your item if you wish. We intend to present a list of items on the ISSHA site (www.issha.org).

We look forward to hearing from you.



Fig. 4. Satisfied ISSHA members with their bargains at the ISSHA auction in Hong Kong 2008. Photo by L. Velo-Suárez.

4. Request for Bids to Host the 16th International Conference on Harmful Algae (ICHA)

During 2010, ISSHA members will be asked to vote for which site will host the 16th ICHA (2014) that will follow the 15th ICHA in Korea (2012). The chosen site will be announced during the ISSHA General Assembly held during the 14th ICHA (Crete, November 2010).

Now is the time to ask for bids to host the 16th ICHA, so that voters will have the time to examine the different options and make up their mind. For the first time, ISSHA members in good standing who cannot attend the Conference will have the opportunity to vote by e-mail. **Deadline for e-mail votes will be 15 October 2010**.

Bids should be submitted before **31 July 2010**, and should include the following information:

· Scientists and organizations who will serve as local hosts, including evidence of their prior expertise in the organization and financial management of similar-sized international conferences.

· Description of the meeting venue and capacity of meeting space for a 5-day meeting, including a plenary room, 2 rooms for simultaneous sessions and several small rooms for administrative use and other necessities. Estimated participation is between 400 and 600 people, but sometimes there are surprises, as was the case during the 10th ICHA (St Pete Beach, Florida), with around 800 participants.

· Availability of accommodations at a range of costs, including the number of rooms, distance from the meeting location, and room costs for high and low seasons. If university residence or other inexpensive accommodation is available for students, this will be considered favorably.

· Distance of meeting and lodging facilities from major airport(s) and cost of transportation between airport and meeting and lodging facilities.

· Availability of local public transportation.

· Expected cost of lunches per day.

· Availability and distance to restaurants for lunches.

· Expected level of local support and associated funding, including ability to provide meeting space, audiovisual support, and staff support, at no cost to the international organizers.

· Dates when the facility would be available in 2014.

· Plans for mid-week excursions and accompanying persons' activities.

· HAB scientists who would be responsible for editing the ICHA proceedings[1], including evidence of their prior experience in producing English-language conference proceedings.

Enquiries and bids should be e-mailed to the President of ISSHA. Bids should be limited to 2-3 pages, plus supplementary illustrations and web links. Information on the previous ICHA venues can be found at the ISSHA website (www.issha.org), by clicking on the "Conferences" navigation button on the left-hand column. Bids will be examined by the ISSHA Council and posted on the ISSHA website.



Fig. 3. Excited ISSHA members inspecting items before the auction in Copenhagen 2006. Photo by M. Lion.

[1]Traditionally, ICHA proceedings have been edited by a group led by the head of the local organizing committee. If the local hosts are not willing to continue this practice, they can ask the ISSHA Committee on Publications and Dissemination to take it over, BUT the money earmarked for the proceedings (in the registration fee) would have to be transferred to ISSHA for the printing and dispatching of the books.

B. Reguera, ISSHA President.
Email: beatriz.reguera@vi.ieo.es

Cont'd from page 5

biotoxins. Prof Yasumoto's talk focused on his more recent research in the field. He discussed alternative methodologies to the mouse bioassay based on protein phosphatase inhibition for OAs, as well as competitive receptor binding assays. He also presented his work on the development of modern chemical alternatives such as MALDI-TOF Mass Spectrometry for the analysis of emergent toxins such as Palytoxin and Ostreocin D. Other invited speakers included Dr. James Lawrence from Health Canada, Ottawa; Dr. Michael Quilliam from the CNRC, Halifax, Canada; Dr. Robert Dickey from FDA, Dauphin Island, GA, USA; Dr. Pedro Burdaspal from AESAN, Madrid, Spain, Dr. Susana Franca from Instituto da Saúde; Dr. Ricardo Jorge, Lisboa, Portugal, and Dr. Aurelia Tubaro from University of Trieste, Italy. Among the invited speakers, Drs. Lawrence, Quilliam, Dickey, and Burdaspal focused their discussions primarily on the importance of developing and improving analytical methodologies as alternatives to animal assays, the need for validated methods for existing and emergent



toxins, and the critical need for the development of certified reference materials to achieve these goals. The toxicology of the marine toxins and their impact on public health were also discussed by Dr. Tubaro and Dr. Franca. The seminar ended with a round table, with the invited speakers responding to questions from the audience. The majority of these discussions related to the applicability of modern analytical techniques versus the mouse bioassay, the advantages and disadvantages of these modern techniques, and concluded with the speakers restating the need for fully validated alternative methods able to efficiently and reliably determine marine biotoxins.

Congratulations to Professor Yasumoto on behalf of the Marine Biotoxins Community!

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Photos by M. Lion

AUGUST 2010

8th International Conference on Toxic Cyanobacteria (ICTC8)

Istanbul, Turkey, 29th of August – 4th of September 2010

ICTC8 will take place in Istanbul hosted by the Istanbul University, Faculty of Fisheries, Department of Inland Waters and Association of Aquaculture Engineering.

The Conference will once again bring together researchers from all over the world for five days of intensive scientific meeting with the aim to examine the whole of Toxic Cyanobacteria, that extends our knowledge, improves on our practice, and widens our friendship networks.

Important dates:

· Deadline for Abstracts Submission: May 15, 2010
· Information for Accepted Abstracts: June 30, 2010
· Deadline for Early Registration: May 31, 2010

More info at: www.cyano2010.org

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HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

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First records of *Ostreopsis heptagona*, *O.* cf. *siamensis* and *O.* cf. *ovata* – in the Azores archipelago, Portugal

During summer 2008, surveys were carried out around São Miguel island in the Azores archipelago (36–39ºN, 25–31ºW) (Fig. 1). The sampling area is located between the main paths of the Azores Current (AC) and North Atlantic Current. The average SST around the island, was above 21ºC, denoting a greater influence of the AC. Seawater samples were collected with a Niskin bottle, and preserved with neutralized formalin, for phytoplankton observations and cell counting. Species of *Ostreopsis* were morphologically characterized with an Olympus BX50 equipped with epifluorescence, following Penna *et al.* [1]. The three *Ostreopsis* species were found together in the water, exclusively on the north side of the island where the waters were warmer. The highest concentration of *Ostreopsis* spp. was 90 cells L^{-1}.

The measurements (Table 1) and plate patterns observed suggest the presence of three species of *Ostreopsis*: *O. heptagona* Norris, Bomber et Balech, 1985 (Fig. 2), *O.* cf. *siamensis* Schmidt, 1982 (Fig. 3) and *O.* cf. *ovata* Fukuyo, 1981 (Fig. 4).

Since sampling was not focussed on

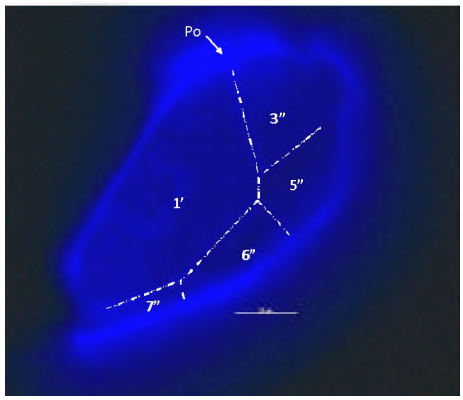

Fig. 2. O. heptagona, *LM, epithecal view (scale bar = 20 µm). The distinct feature of plate 5", pentagonal, in contact with 1', can be observed.*

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First records of *Ostreopsis* cf. *siamensis* in Moroccan Atlantic upwelling waters

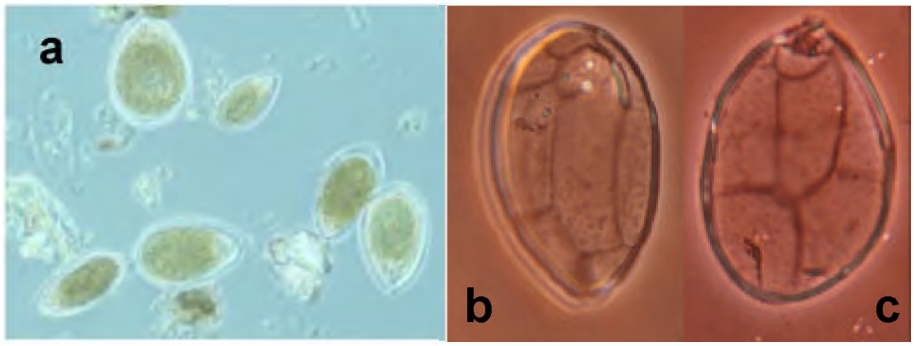

Fig. 1. Ostreopsis *cf.* siamensis *from a sample off Cape Ghir. Light microscopy– phase contrast (a). Epitheca (b) and hypotheca (c) views. (right).*

We report the first records of *Ostreopsis* cf. *siamensis* Schmidt detected in the NE Africa Atlantic upwelling system by the Moroccan HAB and phycotoxins national monitoring programme. The species was identified by phase contrast (Fig. 1) and epifluorescence microscopy during the Morocco-Portugal project of scientific cooperation on HABs and marine biotoxins. Identification was confirmed by sequencing the 5.8S –ITS and LSU ribosomal genes [1].

Ostreopsis blooms were first observed in seawater samples from the Cape Ghir region (30°N, 9°W) on 5 October 2004, reaching 3.7 x 10^3 cell L^{-1}. Blooms recurred in the following years, and increased in concentration and duration, reaching 9.8 x 10^3 to 1.2 x 10^4 cell L^{-1} in 2008, and a maximum of 10^5 cell L^{-1} observed in August 2009. In 2007, *O.* cf. *siamensis* cells were observed from late summer to early autumn, in 2008 from early summer to late autumn, and in 2009 from spring to late autumn. The blooms occurred with surface temperatures ranging from 20 to 24°C, with maxima recorded in August and September. In parallel, the detection of toxins by mouse bioassay in mussels collected from the same area indicated the presence of neurotoxins.

Four monitoring stations were sampled in the Agadir area (Fig. 2). Highest *Ostreopsis* concentrations were always observed in samples from Cape Ghir and a station further north, at Tamri, both sites located on a rocky coastline highly exposed to northerly (upwelling favourable) winds and waves

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The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

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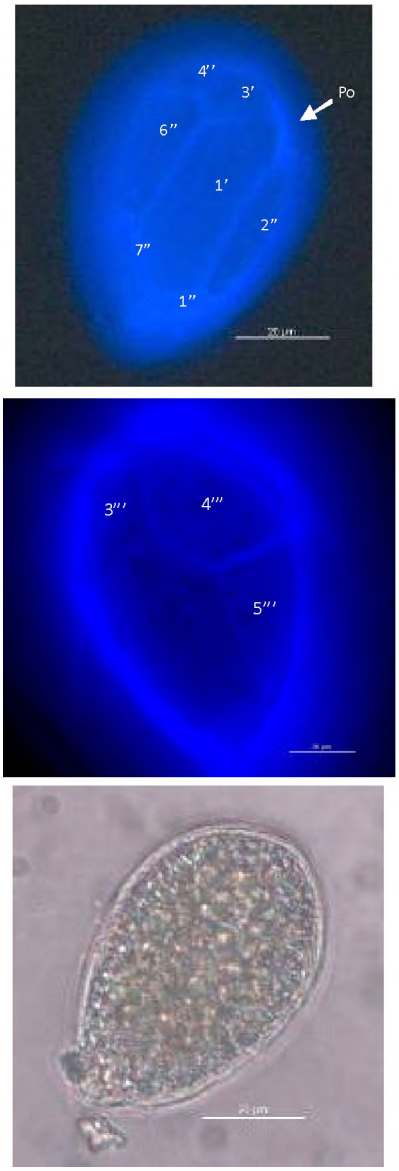

Fig. 3. O. *cf.* siamensis, *LM, epithecal (top) and hipotechal views (center) (scale bar = 20 µm).*

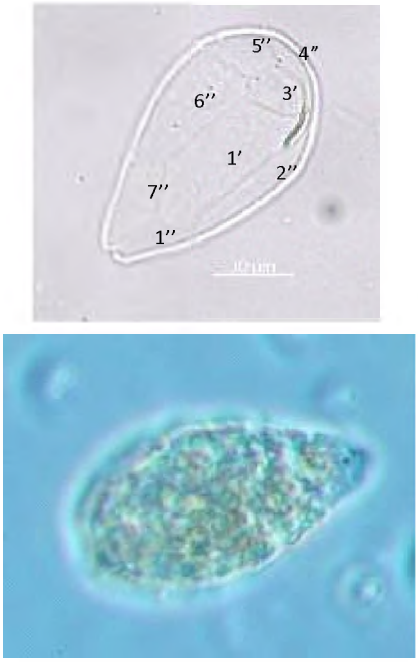

Fig. 4. O. *cf.* ovata, *LM, epithecal view (top) (scale bar = 10 µm).*

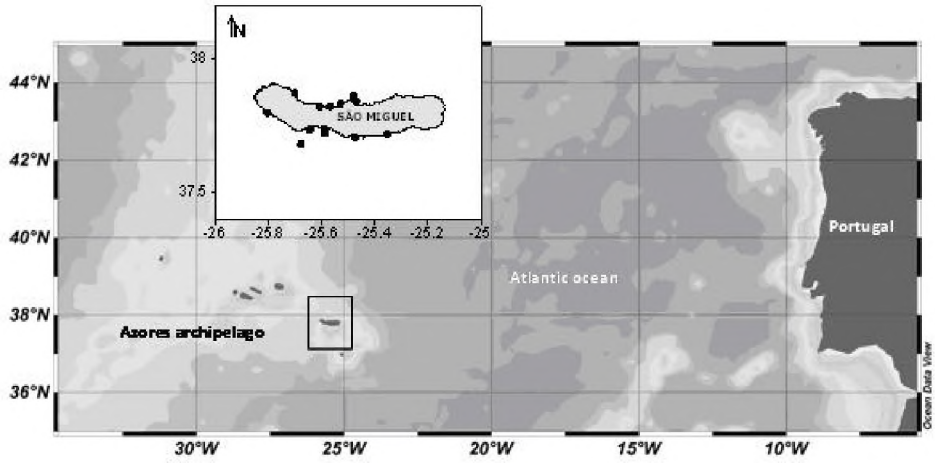

Fig. 1. The Azores archipelago: São Miguel (oriental group).

Table 1. Dorsoventral and transversal measurements.

	Dorsoventral diameter (µm)	Transverse diameter (µm)
O. heptagona (Fig. 2)	80–83	53–55
O. cf. *siamensis* (Fig. 3)	60–70	34–42
O. cf. *ovata* (Fig. 4)	49–51	22–30

the study of this genus, the fixative used did not allow complementary phylogenetic analysis. Future research will comprise genotyping *Ostreopsis* strains by PCR based assay.

This preliminary work in Azores waters is expected to contribute to the biogeographical distribution of HAB species, and to clarify their role as environmental tracers in Atlantic waters and as climate indicators.

Acknowledgements

V. Veloso for technical assistance with the epifluorescence and photography. R. Ferreira Patarra, N. Álvaro and A. Prestes for the field sampling, SRAM/DROTRH, Governo Regional dos Açores for financial support.

Reference:

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14th International Conference on Harmful Algae

See you in Hersonissos, Crete, in November!

(Cont'd from p. 1)

(Fig. 2). In contrast, other dinoflagellate species were favoured at stations south of Agadir, in the shadow of the Cape, on a sandy coast with calmer waters. This suggests that turbulent conditions north of the cape favoured resuspension of this epiphytic species in the water column although not dispersing the cells. Villa *et al.* [2] also pointed out that *Ostreopsis* sp. prefers more turbulent conditions than other benthic dinoflagellates such as *Coolia monotis*. In contrast, the abundance of *Ostreopsis ovata* in the Adriatic Sea was significantly higher in sheltered sites compared with exposed ones, indicating a major role of hydrodynamism in regulating bloom dynamics [3].

According to a recent study of the phylogeography of *Ostreopsis* species based on several strains isolated from the Mediterranean and Northeast Atlantic [1], *O.* cf. *siamensis* was, until now, only observed in the Mediterranean, while *O.* cf. *ovata* was found both in the Mediterranean and NE Atlantic, *e.g.* in Madeira and the Canary Islands. However, neither species was observed on the upwelling coasts of Iberia, such as the Galician *rías* (Santiago Fraga, pers. com.), or NW Africa where the species was not investigated before. Simultaneously, during the Morocco blooms, a strain of *O.* cf. *siamensis* was isolated from macroalgae off the SW coast of Portugal [4]. It is possible that this species is actually spreading in this region of the Atlantic due to an exchange between the Atlantic and the Mediterranean basins or even to a warming of these coastal upwelling waters [5]. However, it is not yet clear whether this apparent biogeographical expansion is real since HAB monitoring only became routine on the Agadir coast after 2002, and there is still no monitoring programme for microphytobenthic communities along the Moroccan coast. After these *Ostreopsis* blooms and due to their annual re-occurrence, the HAB monitoring programme has included this genus among the potentially harmful species of the Atlantic and Mediterranean coasts of Morocco.

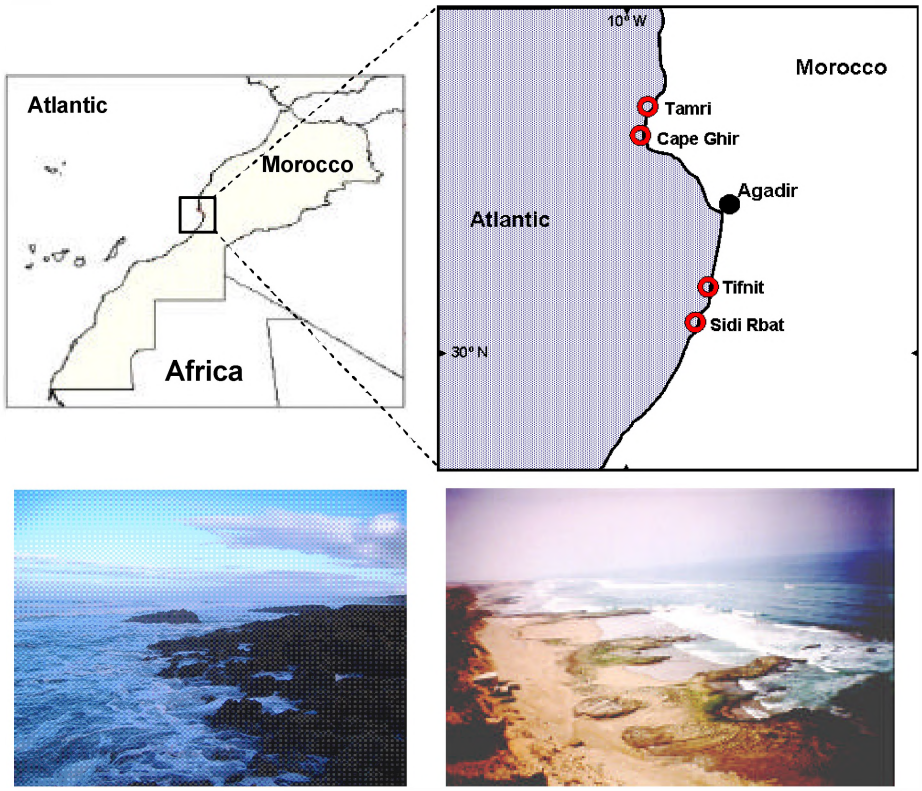

Fig. 2. Location of Moroccan monitoring stations in Agadir region and photo of Cape Ghir (lower left) and Sidi Rbat (lower right) monitoring sites.

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PHYCOTOXINS

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Ostreopsis cf. *siamensis* proliferations in coastal water of Bizerte, Northern Tunisia



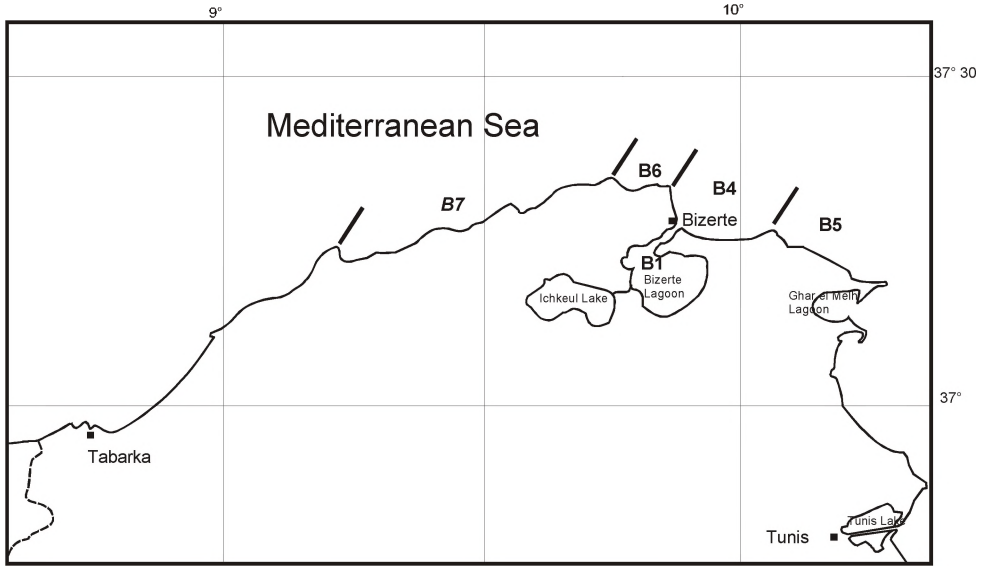

Fig. 1. Sampling stations in the Bizerte region.

As part of national monitoring network for bivalve molluscs and sea urchins for Northern Tunisia (Fig. 1), detection of potentially toxic microalgae, including *Ostreopsis* cf. *siamensis* (Fig. 2) were carried out in Lake Bizerte, a coastal lagoon. Sampling was also carried out on coasts of the Bizerte region. Lake Bizerte is the only mollusc culture site in Tunisia, with 6 farms producing mussels and oysters. The coastal waters of Bizerte support a sea urchin industry. Seawater was sampled weekly from Lake Bizerte near station B1, and every month at 4 coastal stations, B4, B5, B6 and B7 (Fig. 1). The samples were taken at the surface and at a depth of 5 m. Counting of *O. siamensis* was carried out after sedimentation.

From 2007 to 2009 and for the first time in Bizerte coastal waters, we recorded the dinoflagellate *Ostreopsis siamensis*. Maximum concentrations were found in July 2007 (3.75×10^4 cells L^{-1}) as well as during August and November 2008 at stations B4 and B7 (Fig. 4).

Monitoring of plankton microalgae was carried out between 2004 and 2008 in Lake Bizerte at station B1 where maximum concentrations for *O.* cf. *siamensis* and *Coolia monotis* growing mainly as epiphytes or as part of the benthos, were about 24.7×10^4 and 17.5×10^3 cells L^{-1} in July 2006 and May 2008, respectively (Fig. 3).

The monitoring of phycotoxins in the flesh of urchins collected in the Bizerte region (B4 to B7) allowed us to record contamination of these organisms by DSP-type toxins during the following periods: April–May 2007, early January and the beginning of March and August 2008 (Fig. 4).

Discussion

The morphology of the *Ostreopsis* cells suggests that there are two distinct species in the Mediterranean: *O.* cf. *siamensis* and *O. ovata* [1]. *Ostreopsis ovata* was first observed in the Mediterranean in 1972 at Villefranche-sur-Mer [2].

During the last 10 years, several blooms have been reported along the coast of Tuscany (northern Tyrrhenian

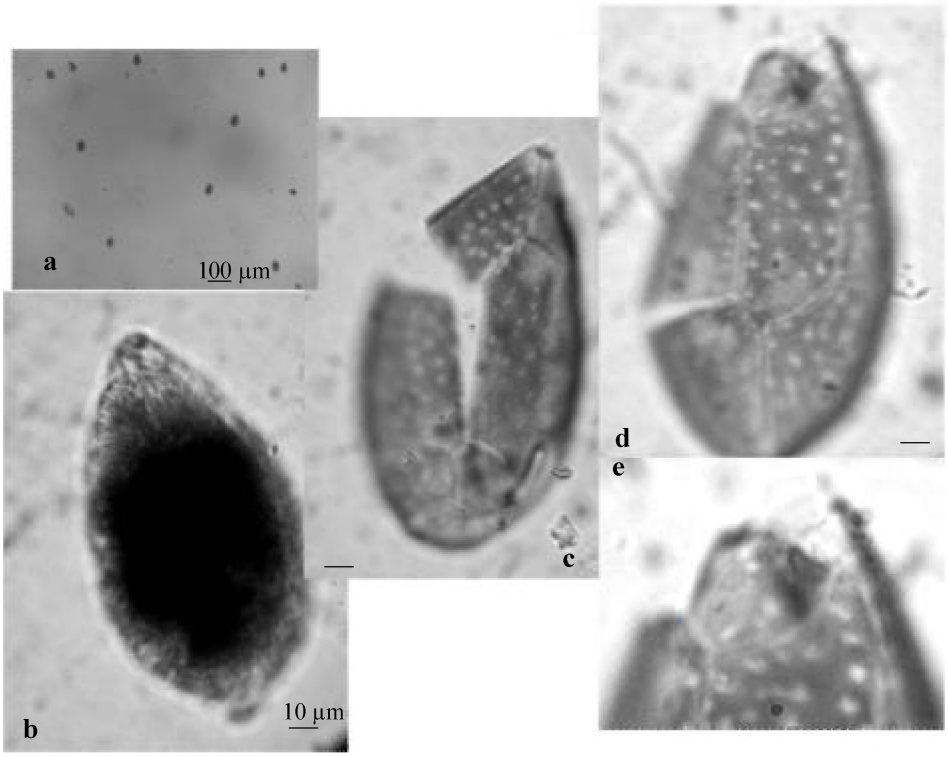

Fig. 2. Micrographs of the dinoflagellate Ostreopsis siamensis*: Cells of* O. siamensis *(a), fixed in Lugol's, (b) epiphytic (c), hypotheca (d), detail of the antapex.*

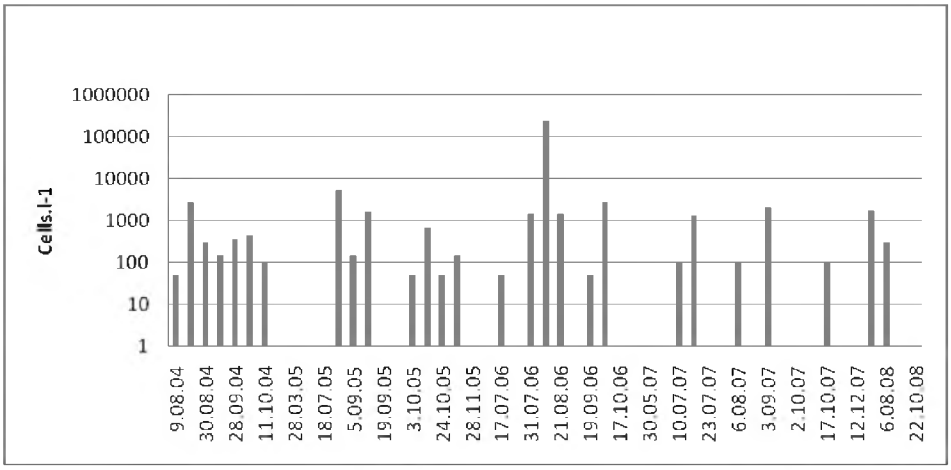

Fig. 3. Densities of the dinoflagellate Ostreopsis siamensis *in Lake Bizerte at station B1.*

Sea). They have been associated with mortalities of marine fauna [3]. The most probable period for blooming in this alga on the Tyrrhenian Sea coast (Province of Campania) is June and July [2]. Tognetto *et al.* [4] observed *Ostreopsis* in the Tyrrhenian Sea from August to October 2004 and found a link between the concentration of this alga and water temperature, with a maximum in August around 28°C.

In Tunisian waters, high concentrations of *O. siamensis* were recorded in *Posidonia* meadows in the Gulf of Tunis, with maxima of about 3.5 x 10^5 cells L^{-1} from July to August [5] as well as in waters of the Gulf of Gabès, with a concentration around 300 cells L^{-1} in October [6]. In the Northern Lake of Tunis, considerable growth of *C. monotis* was recorded in April 2005 following clearance of aquatic vegetation on the banks of the lake [7]. Maxima were around 5 x 10^5 cells L^{-1} in the southern part of the lake.

Ostreopsis produces palytoxins that, with ciguatoxins, are amongst the most toxic natural toxins known. Palytoxins occur in the Mediterranean taxa of both *O.* cf. *siamensis* and *O. ovata.*

At Genoa in 2005, following cases of human illness, water samples were taken from five beaches, every day for the first week, then twice a week, then once a week. The first samples showed maximum concentrations of *Ostreopsis* of 33,000 cells L^{-1} in the water column, and 182,000 cells g^{-1} on macrophytes [8].

The analyses of the water, plankton and macrophytes showed the presence of "putative" palytoxin, the term "putative" being used because the test can show positive for several isomers of palytoxin [9].

In Bari, southern Italy, in two separate events in 2003 and 2004, three days after recording the first symptoms, water samples were taken, in which *Ostreopsis* was found to be abundant, with over 1 million cells L^{-1} [10]. During the blooms, gastropods and crabs appeared unaffected (which does not mean that they did not accumulate toxin, but rather that they seemed not to be susceptible to it), while mussels, limpets and sea urchins seems markedly affected (many empty shells, and loss of spines) [11].

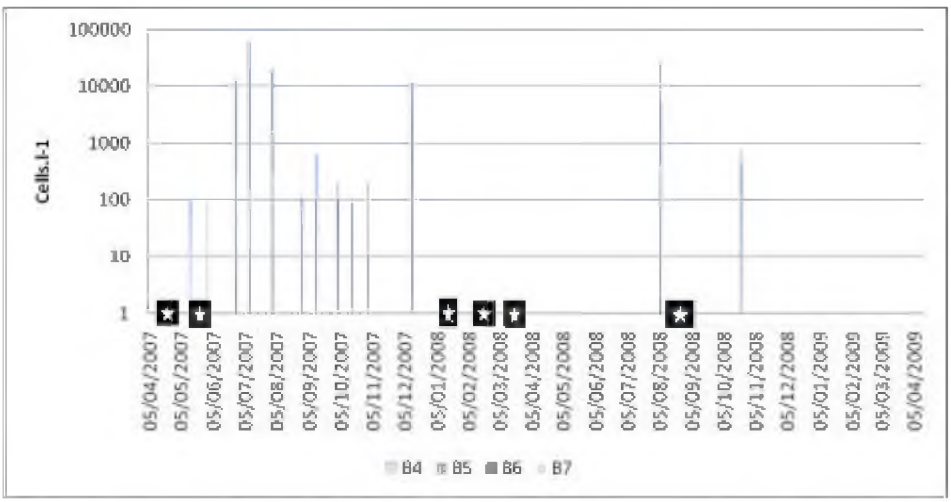

Fig. 4. Densities of the dinoflagellate O. siamensis *in Lake Bizerte at stations B4 to B7 in the Bizerte region (☆: presence of DSP toxins in sea urchins. Source: DGSV, 2009).*

The presence of *Ostreopsis siamensis* in Mediterranean waters should be further investigated, integrating the water column, the sediments and vegetative cover. Such work will permit hypotheses to be proposed and tested about the dispersion and the introduction of phytoplankton species, and will help in understanding the causes of blooms. It will allow water-colonisation dynamics and processes to be better understood for the complex of invasive species. Projects targeting the ecology of toxic algae and the genetics of populations of the different species of *Ostreopsis* will help understanding of the causes of blooms on Mediterranean coasts.

Acknowledgements

We thank Dr Hichem Ben Jannet and the Vetinerary department of the CRDA of Bizerte (DGSV/Ministry of Agriculture, Water Resources and Fisheries) for help.

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First detection of *Ostreopsis* cf. *siamensis* in Portuguese coastal waters

The coast of Portugal is located in the warm temperate/sub-tropical biogeographical transition of the North Atlantic. It is part of the major discontinuity in the eastern boundary of the NE Atlantic, being affected both by seasonal upwelling and water mass exchange between the Mediterranean and Atlantic basins [1].

In October 2007, following repeated reports of nuisance blooms associated with *Ostreopsis* species in the Mediterranean Sea (www.bentoxnet.it), a sampling programme aimed at early detection of *Ostreopsis* species along the Portuguese coast was initiated. The first survey targeted coastal lagoons along the south coast (Ria Formosa and Alvor), under influence of the Mediterranean outflow and where the environmental conditions are more likely to favour the development of dinoflagellate assemblages with warm-temperate to tropical affinities (Fig. 1). The survey was repeated in July 2008. Samples were collected by harvesting macroalgae, such as *Dictyota dichotoma* (Phaeophyceae), *Codium decorticatum* and *Ulva rigida* (Chlorophyta) and mixed microalgae mats attached to pontoons and other surfaces, eel grass and floating organic debris. Plankton net tows (20 µm) were also collected. From 2008 onwards, 2 recreational marinas on the west coast were sampled (Fig. 1). Samples were collected from floating pontoons, at depths never deeper than arms' length, by detaching macroalgae from pontoon walls and buoys. Prior to analysis samples were kept in plastic flasks with seawater from the collection site and transported to the laboratory under cool conditions.

Samples were vigorously shaken and the water poured into plastic Petri-dishes to be checked under an inverted microscope (Olympus IX70). Single cells of *Ostreopsis* sp. were isolated by micropipette and transferred to culture wells with filter-sterilized seawater from the sampling site. When cell numbers were observed to consistently increase, cells were transferred to f/50-Si medium (35 psu) and finally to f/20-Si medium (35 psu), where they were routinely maintained. Cultures were kept at 19ºC ± 1ºC with overhead illumination of 40 µmol $m^{-2} s^{-1}$, supplied with cool white fluorescent lamps, and a light:dark cycle of 14:10 (L:D).

From all the samples analysed, specimens attributable to *Ostreopsis* were only detected in Sines marina (37º 57' 2 N; 8º 51' 53 W) (June 2008, October 2008 and September 2009). On June 2008, the species was associated with *Colpomenia peregrina* (Phaeophyceae). Three cultures were established and are now kept in the culture collection of the Oceanography Centre, University of Lisbon (ALISU strains IO96-01, IO96-02 and IO96-03). Strain IO96-02 was analysed for species-specific genotyping. Approximately 10 mL of exponentially growing culture were harvested by centrifugation (4000 rpm, 5 min). The pelleted cells were rinsed twice with filter sterilized (0.22 µm) artificial seawater. Total genomic DNA was extracted as described in [2]. PCR amplification on the 5.8S rDNA and ITS regions and direct sequence reactions were conducted as described in [3]. The 5.8S rDNA-ITS sequences obtained in this study were aligned in BLAST silico platform and using the confidential ribosomal sequence database at the Department of Biomolecular Sciences, University of Urbino.

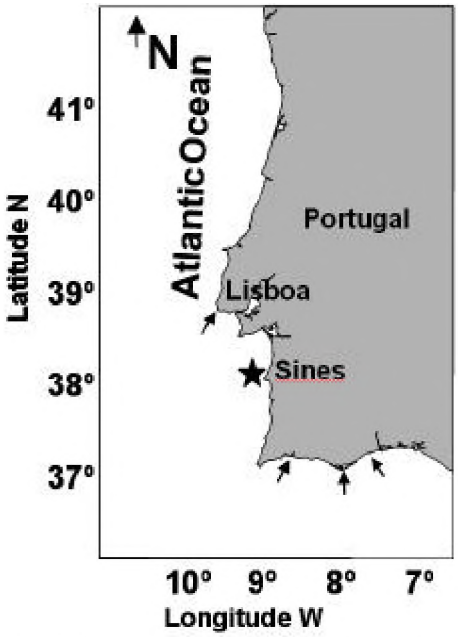

Fig. 1. Location of sampling stations (arrows) with indication of site where Ostreopsis *cf.* siamensis *was identified (star).*

The morphological analysis of isolated cells and cultures was done under the light microscope, using bright field and epifluorescence after staining with Calcofluor White [4], and by SEM (JEOL JSM-5200LV) (Fig. 2). The cells

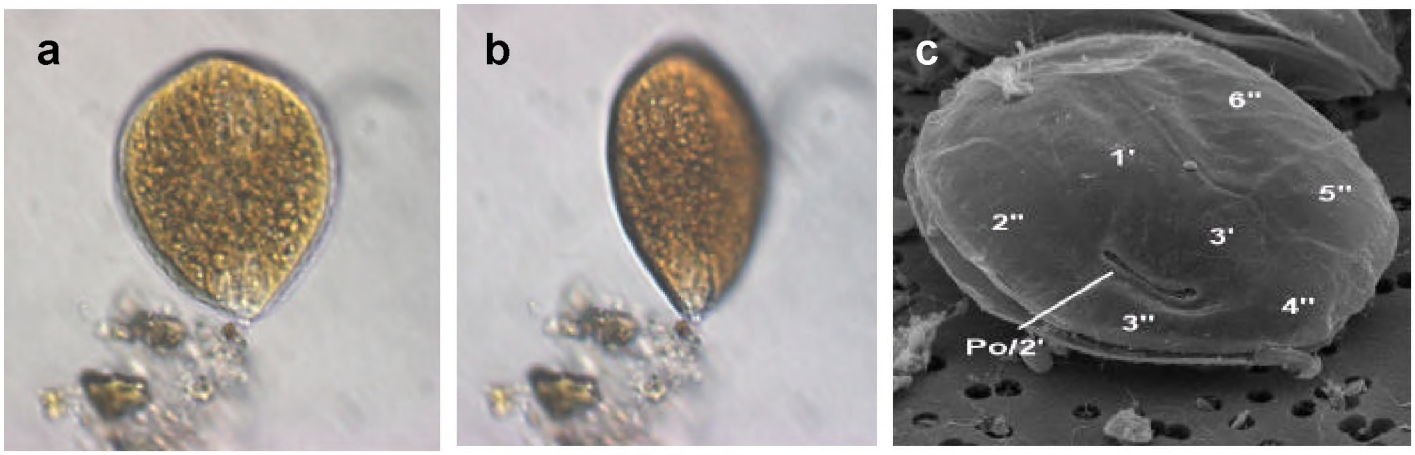

Fig. 2. Ostreopsis *cf.* siamensis. *Specimen from field sample attached by the ventral pore to particles in apical/antapical view (a) and in lateral view (b). Epithecal view of cultured specimen (SEM) (c).*

analysed have the characteristic morphology and plate formula of *Ostreopsis* (Fig. 2). Cells are oval, markedly anteroposteriorly compressed, attaching to surfaces by the ventral pore (Fig. 2a and b). The morphological characteristics agree well with the description of *Ostreopsis siamensis* by [5] and *Ostreopsis* cf. *siamensis* by [2]. The wall is smooth covered with scattered pores of only one type (Fig. 2c). The dorso-ventral diameter varied between 58–61.2 µm (n=12) (61.2 µm in field specimen) and the width between 48–50 µm (n=12) (48.9 µm in field specimen). In side view, the cells did not show any evident undulation. When grown in f/2 medium, cells showed gross malformations and were difficult to interpret.

The final alignment of the ribosomal sequence of the tested *Ostreopsis* strain IO96-02 with all ribosomal sequences of *Ostreopsis* species available in the database used in this study revealed total identity (100%) with *O.* cf. *siamensis* sequences belonging to the Mediterranean clade [2]. Because of the lack of molecular data associated with strains from the type locality, we follow the recommendations of [6], and the species name we assign to the strain isolated from the Atlantic coast of Portugal is *Ostreopsis* cf. *siamensis*.

These results extend the geographical distribution of *O.* cf. *siamensis* outside the Mediterranean basin to 38º N, representing the highest latitude at which species of *Ostreopsis* have been reported from the Atlantic. For marine protists with no fossil record it is very difficult to assess whether previously unreported species are native cryptogenic species that have been overlooked or the result of a recent introduction. Species of the genus *Ostreopsis*, although occasionally reported from the water column, are more commonly described as benthic-epiphytic [7]. Methods used in phytoplankton surveys do not aim at sampling benthic communities and these will only be detected on rare occasions when blooms develop or cells are re-suspended. Previous results on the phylogeny of *O. ovata* recognised several geographical clades and identified the temperate-tropical Atlantic and the Mediterranean basin as a single geographical unit [6]. The total identity of the analysed sequences from the Atlantic strain of *O.* cf. *siamensis* with the Mediterranean strains reported in the present study suggests a similar phylogeographical unit for *O.* cf. *siamensis*. Further studies on the phylogeography of this species based on more regional strains and other global populations will be needed to clarify the phylogenetic and phylogeographic position of *O.* cf. *siamensis* in Atlantic waters.

So far populations of *O.* cf. *siamensis* have not been detected in high numbers in Portuguese waters, nor have toxic events been reported. The site of Sines marina represents an artificial sheltered environment on an otherwise highly hydrodynamic coast influenced by seasonal upwelling. The geographical distribution of *Ostreopsis* spp. is generally associated with low energy systems and described as inter tropical and from warm waters, as in the Mediterranean Sea [6–7]. However, the recent report of planktonic blooms of *O.* cf. *siamensis* along the upwelling coast of Morocco [8] should be considered as an early warning, and highlights the need for more studies on the distribution and ecology of *O.* cf. *siamensis* along the Portuguese coast.

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EFSA scientific opinions on marine biotoxins

In July 2006, the European Commission asked the European Food Safety Authority (EFSA) to provide a scientific opinion to assess the current European Union (EU) limits with regard to human health and methods of analysis for marine biotoxins as established in the EU legislation, including new emerging toxins.

From that moment until now, EFSA's Panel on Contaminants in the Food Chain (CONTAM Panel) has developed and adopted a series of scientific opinions related to marine biotoxins: **okadaic acid** (OA) and analogues (2008), **azaspiracid** (AZA)-group toxins (2008), **yessotoxin** (YTX)-group toxins (2008), influence of processing on the levels of **lipophilic** marine biotoxins in bivalve molluscs (2008), **saxitoxin** (STX)-group toxins (2009), **pectenotoxin** (PTX)-group toxins (2009), **domoic acid** (DA) (2009), summary on **regulated marine biotoxins** (2009), **palytoxin** (PlTX)-group toxins (2009), **ciguatoxin** (CTX)-group toxins (2010), **cyclic imines** (CIs) (spirolides, gymnodimines, pinnatoxins and pteriatoxins) (2010), and **brevetoxin** (BTX)-group toxins (2010), and also the most recent statement on **shellfish consumption figure** (2010).

All these reports have been printed in the EFSA Journal and are available on-line at: www.efsa.europa.eu/en/panels/contam.htm

Beyond Guam: Current status of research on the neurotoxic amino acid BMAA by cyanobacteria

The production of toxins by cyanobacteria is well documented, and new compounds continue to be isolated and described. Observations on and reports from the Island of Guam in the 1940's and 1950's described an increased incidence of a neurodegenerative disease among the Chamorro people there. This disease termed *lytico-bodig* by the indigenous people was described as an Amyotrophic Lateral Sclerosis-Parkinsonism Dementia Complex (ALS-PDC) with an incidence 50-100 times greater than the world average. Research concerning possible causes and mechanisms has been ongoing and the only statistical correlate identified with the disease was diet [1].

Studies on Guam isolated a number of toxic compounds from cycads, including a novel neurotoxic amino acid â-N-methylamino-L-alanine (BMAA). Since that time investigators have examined the occurrence of this neurotoxin, from its initial isolation from cycads through to the potential occurrence of this compound worldwide. Even though BMAA has been studied for over 40 years, new insights and discoveries are still being made. In order to summarise the current status of the research on BMAA in cyanobacteria and its association with human disease, a special supplement of the journal *Amyotrophic Lateral Sclerosis* (November 2009, vol. 10, supplement 2) was recently devoted to this topic.

The supplement contains 20 contributions from environmental scientists, analytical chemists, neurologists and ethnobotanists. The supplement begins with a contribution from **Walter Bradley** and **Deborah Mash** outlining the cyanobacterial/ BMAA hypothesis with its various strengths and weaknesses. The late Arthur Bell then provides a discussion on the discovery of BMAA, with examples of protein incorporation and biomagnification. **Peter Nunn**, one of **Arthur Bell**'s colleagues examines various phases of BMAA research, considering the acute neurotoxicity of BMAA in experimental animals, followed by acute neurotoxicity in primates with a final discussion of the broader impacts of BMAA in toxicology.

Three submissions to the supplement consider human dietary exposure to BMAA. The first considers the diet of the Chamorro people, BMAA concentrations and the link with ALS-PDC (**Banack** and **Murch**). This is followed by an analysis of BMAA in cycad flour, a staple of the Chamorro diet, and underestimates due to a lack of analysis of protein-bound fractions (**Cheng** and Banack). Finally a new potential route of exposure to BMAA in the human diet is considered by Roney *et al.* They examined FaCai *Nostoc* soup, finding BMAA present within this foodstuff and not in fake FaCai made from starch.

Five papers consider the toxicity of BMAA following dietary exposure. Vyas and Weiss examine the toxicity of BMAA to motor neurons, which is followed by a contribution on the molecular mechanisms of BMAA neurotoxicity (**Lobner**). The remaining three papers consider the toxicity to invertebrates (*Drosophila*, **Zhou** *et al.*; *Artemia, Nassula*; Purdie *et al.*), *Danio rerio* (**Purdie** *et al.*) and avian vacuolar myelinopathy, a disease associated with epiphytic cyanobacteria on aquatic macrophytes (**Bidigare** *et al.*).

Human exposure to BMAA through the aquatic environment is then examined, with the need to monitor water resources (**Metcalf** and **Codd**), the use of HILIC-MS to monitor Dutch drinking waters (**Faassen** *et al.*) and cyanobacterial BMAA in the marine environment of South Florida (**Brand**). Small ponds and seeps in the Gobi Desert were also examined as these are water resources for the Gobi Bear, a critically endangered species and both BMAA and the related toxin 2,4-diaminobutyric acid (DAB) were identified in water resources where cyanobacteria were present (**Craighead** *et al.*). Researchers in New Hampshire have identified clusters of ALS patients around lakes with cyanobacterial blooms and their current findings are also reported in the supplement (**Caller** *et al.*).

The incidence of ALS among veterans serving in the 1st Gulf War has increased compared to those not deployed [2]. Consequently, Cox and colleagues examine desert cyanobacterial crust and identify both DAB and BMAA in crust, and hypothesise that desert borne dust containing BMAA may have contributed to this increased incidence of disease. Finally, Walter Bradley discusses potential ALS therapies based on the BMAA/cyanobacteria hypothesis, and Paul Cox discusses the various tenets of the BMAA hypothesis. Submissions to this supplement are based on research presented at a special session of the 19th International Symposium on ALS/MND in Birmingham, UK in November 2008. As BMAA in cyanobacteria is currently a topic of great interest and concern, this supplement provides a definitive summary of the current state of this research.

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Patrick Gentien In Memoriam

Patrick was born in Saint Pol de Leon (Brittany, France) in 1950. He was a chemical engineer (HEI, Lille) and doctor of physical oceanography. His early professional days (1980-1982) started at the Australian Institute of Marine Science (AIMS). There with John Andrews, occasional upwellings on the central Great Barrier Reef were discovered (Andrews & Gentien 1982), a paradigm shift in the understanding of nutrient budgets for the reef. This finding has influenced reef research to this day. Patrick joined IFREMER in 1982 as a chemical researcher and hydrologist at the department of Coastal Environment and Marine Environmental Management. He joined a team tasked with making an ecological survey off Cap de Flamanville, where Electricité de France was building a nuclear power station. The survey lasted the three years (1983-1986) before the power station became operational. A dominant summer species in this region is *Karenia mikimotoi*, which became one of Patrick's lasting interests.

Karenia mikimotoi was first reported (as *Gyrodinium aureolum*) in European waters in 1966, and characteristically forms thin layers (another lasting interest) in the pycnocline as well as surface blooms. Vertical migrations of up to 15 m range take place when stratification is not too marked, but with increased stability, persistent non-migrating populations form in the pycnocline (the *magic carpet* of Bjoernsen & Nielsen 1991). Why? We don't know, but the question was one of many which challenged Patrick, and he often came up with ingenious explanations. Allelopathy exerted by *K. mikimotoi* may have been playing an effective role at the onset of the population development. An obvious problem facing cells which secrete toxins is how to protect themselves, whence the invocation of bioconvection cells ... by playing with the time scales of these convection cells, the secretion rate of the toxin, and its decay rate, Patrick argued that high concentrations of cells can exist without poisoning themselves. Ingenious, although how the toxin would then reach a sufficient concentration to affect its intended target remains a mystery. Blooms of *Dinophysis* leading to massive and long-lasting shellfish harvesting closures due to diarrhetic shellfish toxins is another major problem in Brittany. As an engineer and expert on hydrodynamics, one of Patrick's passions was, in close cooperation with his colleague Michel Lunven, to develop appropriate high resolution instruments to hunt these cryptic, patchy and unpredictable creatures throughout the Bay of Biscay, and find their hiding place when they had apparently disappeared. Years later, and with the same (improved) equipment and colleagues Patrick performed multiple cruises in the Baltic Sea, Irish Sea, Galician Rías...and even in the Chilean fjords, always looking for fine resolution aggregates, thin layers of dinoflagellates, and the special hints that would explain the presence of a species bloom in a given place.



Photo Sonsoles González-Gil

Beginning in the early 1990's, Patrick became deeply involved in international cooperation on harmful algal blooms research and management, while retaining his responsibilities at IFREMER. For the last 15 years he was the face of France at the ICES-IOC Working Group on HAB Dynamics (Chair 1996-1999), the Scientific Steering Committee of GEOHAB (**G**lobal **E**cology and **O**ceanography of **H**armful **A**lgal **B**looms IOC-SCOR, www.geohab.info) (founding Chair, 1999-2003) and the IOC Intergovernmemntal Panel on Harmful Algae Blooms. His unique way of species-specific targeted cruises and models was widely applied in diverse ecosystems and European projects, networks and others (*LIFEHAB*, *NEMEDA*, *HABIT*, and the forthcoming *ASIMOUTH*) often in cooperation with US researchers.

Patrick passed away on Sunday 9 May. He had been fighting lung cancer for three months, but still his sudden death caught us all by surprise. Until the very end, he kept in touch with his projects and collaborators and continued work whenever the chemotherapy and accompanying exhaustion gave him a break. He leaves a wife, four children and two grandchildren. He was a key person in the establishment of links between HAB biologists and physical oceanographers and modellers. Those of us who worked with him had the opportunity to enjoy his sharp and acid sense of humour, his unique sense of criticism, and his always generous attitude to share original ideas and go to the core of scientific problems.

Here we present brief extracts from the numerous condolences we received from all over the world. The full texts written in their original languages (English, French, Spanish, Portuguese) will be posted on the ISSHA website (www.issha.org).

Beatriz Reguera

Tim Wyatt

Appreciations

On est impressionné, admiratif sans doute, devant le courage et la volonté dont tu as fait preuve ces dernières semaines, toujours aux commandes, toujours plein d'idées, toujours débordant d'initiatives, et te préoccupant de la relève pour laquelle tu t'es battu, sans jamais évoquer ta maladie, évitant l'inutile, et programmant l'avenir **(Pierre Le Hir, France)**



Photo Mónica Lion

Dans le domaine des efflorescences microalgales Patrick s'est montré un visionnaire ... Toujours intéressé par la nouveauté, les découvertes dans tous les domaines l'ont passionné: depuis les performances instrumentales (avec Michel Lunven et Michel Lehaître: granulomètre laser utilisé dans de nombreuses mers, les échanges physicochimiques en zones stratifiées, ... les particularités de la viscosité... (avec toi Ian) ... Il faut également souligner le réalisme et le sens critique de Patrick, et sa sensibilité tant humaine qu'artistique. Tout ceci constituait un personnage attachant, auquel je pense encore parfois en me disant souvent: "je devrais en parler à Patrick", car j'oublie qu'il n'est plus **(Geneviève Arzul, France)**

entusiasmo contagioso ... me enseñó, con su mirada crítica a no tomar nada por sentado e ir despacio por el camino largo de las respuestas ... Patrick sabía reirse de si mismo y con los demás. Era capaz de sacarle punta a un alfiler y le gustaban los juegos de palabras. No tenía pelos en la lengua y decía siempre lo que pensaba... una semana antes de su muerte... en sus ojos, la chispa de su genio y el afán de lucha seguían estando presentes. Para mi, Patrick sigue estando entre nosotros porque sus ideas y sus consejos no han muerto con él **(Lourdes Velo, France)**

Patrick was one of the most intellectually honest and insightful scientists I have been privileged to know. His spirit was generous, as were his practices... his wonderfully dry humor. Patrick's twinkling eyes were clear signals that he was engaged in an exchange that pleased, provoked and challenged everyone in the conversation. His enigmatic smile signaled he was already a step ahead of exchange and waiting for us around the turn **(Pat Tester, USA)**

Un gran sentido del humor el tuyo... recuerdo tus sonrisas y tus ojillos risueños entre la nube de tu cigarro... Ay, este humo, ... te fuiste con él, trabajando hasta el final... con esa actitud amigable que hace que la ciencia sea lo que debe ser ante todo: vocación y humanidad **(Elisa Berdalet, Spain)**

When we could just sit and chat we'd get all churned up with his excitement over thin layer sampling, somehow very infectious and challenging, with glasses of wine or other assorted liquids stimulating



Photo Mónica Lion



Photo Robin Raine

wild 'flights of fancy' over what we could learn from these new technologies. His quick joke or sly, slightly sarcastic quip with that smile and god-awful heavy cigarette smoke rising from his lips were all parts of our dialog **(Kevin & Stella Sellner, USA)**

He was a true scientist in that he never grew tired of asking questions. he was a great proponent of conducting HAB science within the "bigger picture" of mainstream phytoplankton ecology and oceanography **(Grant Pitcher, South Africa)**

Era tambem das pessoas com quem adorava conversar de ciencia e de ir beber um copo e falar de musica. Estava toda entusiasmada por irmos trabalhar em conjunto com ele pois era um excelente critico para o nosso projecto com modeladores. Mas não largava o cigarro... Eu bem lhe dizia e ele punha sempre aquele sorrizinho malandro! **(Teresa Moita, Portugal)**

You have well described Patrick's character: a man of integrity, very generous and very willing to understand scientific questions, always helping and encouraging his co-workers **(Michel Lunven, France)**

We have lost one of the few who always cut through the BS and posturing in science—never afraid to point out when the "high-flyers" were over-stretching their science, and their own importance—always worth sharing ideas with, and very good company **(Barrie Dale, Norway)**

Un gran orientador científico... particle size analyzer... Lo hicimos funcionar a bordo del barquito Melipulli de la Universidad de Los Lagos, con grandes dificultades de habitabilidad, pero Patrick solo se reía y estaba tranquilo, pero a veces se enojaba un poquito, ya que se nos enredaba el CTD y PSA en la mallas de las jaulas de salmoneras **(Alejandro Clément, Chile)**

For me Patrick was a pioneer, a firebrand, a mentor, an intellectual, a sometime comedian, a generous spirit and a loveable rogue but mostly he was a friend **(Pauhla McGrane, Ireland)**

Any successful organization needs visionary members who are passionate about the outcome of the mission, and that describes Patrick very well … A warm, gallant individual with a wicked sense of humor who would not hesitate to express his opinion **(Raphael Kudela, USA)**



Photo Don Anderson

Late night 'discussions' often heated, were always inventive and stimulating. Patrick disliked dogma, courted controversy and made you see the world in a new light (from HABIT website)

His frankness and feed back was refreshing and helpful in handling both science and people issues **(Henrik Enevoldsen, Denmark)**

Su agudeza mental, su claridad, sencillez y serenidad para expresarse, mostrando siempre un rostro generoso, afable y también, por qué no, porque compartíamos el mismo deseo irrefrenable de fumar **(José Carreto, Argentina)**

He loved the science and he made it fun to talk about. There were always new results, ideas, and connections in his considerations… His leadership of committees and workshops was fundamental, all performed with grace, competence and elegance. But for all his contributions, it is still his presence, twinkling eyes, and sometimes sardonic smile that I miss the most **(Tom Osborn, USA)**

His infectious enthusiasm drove the project [HABIT], and was reflected in his excitement during a chase of a tiny, yet high density, patch of Dinophysis drifting along the Irish coast. He encouraged and was patient with the team of students from Ireland, who on more than one occasion (and not entirely to his displeasure) remarked on his similarity to 007 Sean Connery! **(Robin Raine, Ireland)**

His glasses are slightly lowered on his nose, his head is tilted, and legs are crossed. The ash from his unfiltered cigarette is about to drop to the ground. He smiles with that most pleasant smile that creates a dimple on one side of his face. With a twinkle in his eye and that wonderful French accent, he greets me with his smoky voice … His support, encouragement and complimentary, friendly demeanor fill me with an understanding of how collaboration forms friendships and how science among friends is one of the best aspects of our jobs **(Vera Trainer, USA)**

… his clear vision and razor-sharp intellect … He pushed his colleagues to develop unique techniques fit to test clearly formulated scientific hypotheses, such as the sampling of thin layers, and he had inspired a generation of colleagues and students in his area. Patrick was astonishing in his way to go to the end of things (he was working with all his colleagues to the very last day) **(Phil Hess, France)**

Patrick was a great mind, a true researcher, always looking for something new. His fascination for the interplay between physics and the biota has motivated most of his research, and his contributions to this still developing field are remarkable … it was with contagious enthusiasm that he showed me his new microlayer sampler … These memories make me realise that I lost not only an immensely knowledgeable colleague, but also a very good friend **(Maurice Levasseur, Canada)**

… one phrase comes to mind above all others – "scientific rigor". Patrick held himself (and all of us) to very high scientific standards **(Don Anderson, USA)**



Photo Sonsoles González-Gil.

Patrick was an inexhaustible information source, always open for discussions … Availability and enthusiasm for science well define him **(Laure Guillou, France)**

… in 1997 … I met Patrick among the other "grandparents" of the discipline … he added a bit of healthy banter and scientific jousting to the occasion, and perhaps also a bit of smoky French charm **(Martina Doblin, Australia)**

He was one of the coolest people I have had the pleasure of meeting in our field. He was fantastic, a great mind, and taught me so much … Declan will really miss him too - he loved hanging out with him. They both loved telling stories and having the craic **(Carolina Cusack, Ireland)**

Eutreptiella marina (Euglenophyceae) bloom causes significant fish kills in Banderas Bay, Jalisco, México

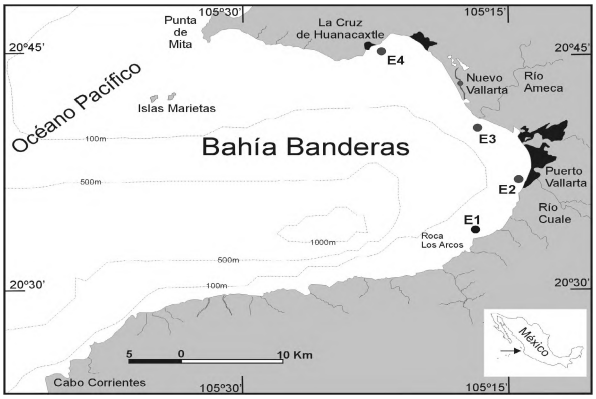

Fig. 1. Location of sampling in Banderas Bay, Jalisco México.

Phytoplankton analyses in April 25 and 27, 2009, in the southern part of Banderas Bay (Bahía de Banderas), Jalisco, Mexico (Fig. 1), revealed the presence of a potentially harmful bloom of *Eutreptiella marina*. Samples were stored in a 50 ml plastic bottle, and preserved with Lugol-acetate solution. Phytoplankton abundance was determinate by the standard Utermöhl technique [1], and algal quantification was made with 100x magnifications in aliquots of 10 ml using a 1mm^2 Whipple disc with double cross pattern of 100 µm. Temperature was recorded with HOBO temperature/light data logger and salinity with a salinometer. During the days prior to event, gusty winds occurred, with a temperature range of 24.5 to 25ºC and 35 psu, as well green sea water discoloration, the colour of anti-freeze (Fig. 2); bright plumes were also seen. Hundreds of dead fish were observed on the beach and at the surface of Banderas Bay, possibly due to oxygen depletion following the bloom. On the other hand, Zimba *et al* [4] identified neurotoxin production by two similar species, *Euglena sanguinea* and *E. granulate*; both species grow well in eutrophic environments. Increased nitrification of wetlands, wastewater treatment systems, and runoff from urbanization, increase suitable habitat for growth of these algae in Banderas Bay, and there has been a rapid shift in phytoplankton community composition. On May 11th the dinoflagellate *Prorocentrum micans* caused an enormous bloom in the vicinity with 10.13 x 10^6 cells L^{-1}; this abundance has never been recorded before in this area. Two days later, on May 13th, an *Akashiwo sanguina* bloom with densities between 760 x 10^3 and 566 x 10^3 cells L^{-1} replaced the *Prorocentum micans* population. The *Eutreptiella marina* bloom reached a maximum concentration of 2.21 x 10^6 cells L^{-1} (Table 1) with 94% dominance, enough to be lethal with a temperature range of 24.5–25 °C and 35 psu (Table 2). These *E. marina* cells are cylindrical to elongate oval, with a variable number of chloroplasts, 15–80 µm long, 3–15 µm wide, and swim rapidly [3], (Fig. 3A–E). According to Bravo [2], *Eutreptiella gimnastica* Schiller (=*E. marina* Cunha, 1914) is the only euglenophyte in Mexican Pacific, which he describes as coastal in polluted areas with high organic contamination, with no outbreaks or associated toxicity. No harmful bloom of *E. marina* has been seen on the Jalisco coast before, and this is the first record for the Mexican coast. No human health effects or intoxication were associated with this event, only hundreds of dead fish (Fig. 4A-B).

Table 1. Phytoplankton abundance during the bloom in Banderas Bay.

Species	Cells · ml^{-1}	%
Eutreptiella marina	2208	94.52
Pseudoguinardia recta	2	0.09
Chaetoceros sp.	4	0.17
Dictyocha californica	48	2.05
Peridinium quinquecorne	8	0.34
Rhizosolenia sp.	2	0.09
Diatomea central N.I. cadena	10	0.43
Diatomea pennada N.I.	2	0.09
Diatomea central N.I.	2	0.09
Proboscia SP.	12	0.51
Thalassiosira sp.	10	0.43
Prorocentrum micans	2	0.09
Prorocentrum gracile	8	0.34
Pseudonitzschia sp.	10	0.43
Ceratium balechii	2	0.09
Ceratium fusus	6	0.26
	2336	**100.00**



Fig. 2. Greenish water in the bloom area of Eutreptiella marina.

The affected fish included balloon fish, Mexican needlefish, snapper, moray, and the Californian salema. Table 3 shows the affected organisms in several localities of Banderas Bay. The salema (*Xenistius californiensis*) were washed ashore in hundreds, and many were also found dead in open waters; this species was the most affected, with specimens 24 to 26 cm long. During the *E. marina* bloom, the sea water was described as unusually turbid, and the heaviest concentration of dead fish were in the southern part of the bay. Fish were also observed gasping at the surface with erratic behaviour; up to 90% had with lesions, bleeding in the internal organs, but with gills, eyes, skin and fins healthy.



Fig. 3. Forms of Eutreptiella marina*: a) elongated and thin forms; b) showing its two unequal flagella: 1- short, 2- long; c) wide form; d) rounded form; e) drop-shaped*

Table 2. Temperature, salinity and deep of sampling stations.

Coordinates		Continental reference	Temp. °C	Salinity psu	Deep (m)
Latitude	Length				
20° 39' 07"	105° 13' 45"	Dársena Portuaria	25	35	11.0
20° 37' 57"	105° 14' 10"	Hotel Sheraton	25	35	36.0
20° 36' 26"	105° 14' 44.7"	Boca de Tomatlán	24.5	35	39.0
20° 31' 00"	105° 16' 05"	Yelapa	24.7	35	40.0

Table 3. Fishes affected by Eutreptiella marina *bloom in Banderas Bay, Jalisco, México.*

	Scientific name	Common name	Abundance
1	*Diodon holocantus*	Balloon fish	Few
2	*Tylosurus crocodilius*	Mexican needlefishes	Few
3	Family Haemulidae	Snapper	Few
4	*Muraena argus*	Moray	Few
5	Family Cupleidae	Pacific sardines	Hundreds
6	*Xenistius californiensis*	Californian salema	Hundreds

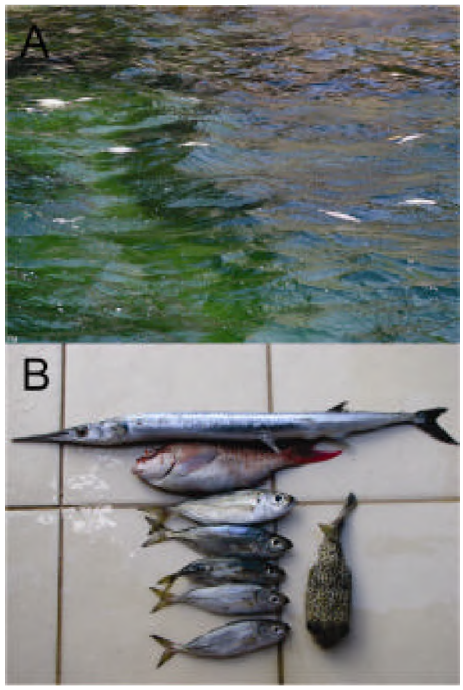

Fig. 4. a) Fish kill in the greenish water. b) Some fish samples, the reference square is 30 cm per side.

Acknowledgements

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11th IOC-AECID-IEO Course on HAB Taxonomy

The 11th IOC-AECID-IEO Course on Taxonomy of Harmful Phytoplankton was held in Vigo, Spain, in June 2010. As in previous years, this course was jointly sponsored by the Intergovernmental Oceanographic Commission (IOC) of UNESCO, the Spanish Agency for International Cooperation and Development (AECID) and the Spanish Institute of Oceanography (IEO).

The course was intended for Latin-American and African experts with experience in microalgae identification, responsible for setting up or strengthening their national or regional harmful marine microalgae monitoring programmes.

For the second time in Vigo, the course had an e-learning part which participants were to complete before attending the classes held in Vigo.

This e-learning was supported by the IOC Science and Communication Centre on Harmful Algae in Copenhagen. Jacob Larsen was in charge, and used the Absalon e-learning platform at Copenhagen University. This part of the course comprised approximately 45 hours of individual learning.

The second part of the course was held in Vigo from 2nd to 18th June. It comprised 97 hours of lectures and practical sessions on the identification of harmful algae and cysts, as well as introductory lessons on biotoxins, monitoring and management of harmful events, chemical taxonomy and molecular tools, ecology, and sampling at sea and in the laboratory. Participants were also able to report on their national HAB events, exchanging impressions on their experiences.

14 scientists from Angola, Chile, Colombia, Cuba, Mexico, Peru and Spain attended the course.

This year we had Jacob Larsen and Celia Villac as guest teachers, as well as our usual "local collaborators" for these courses: Santiago Fraga, Isabel Bravo, Beatriz Reguera, Francisco Rodríguez and Pilar Rial from the IEO, Vigo; José Franco and Pilar Riobó from IIM-CSIC, Vigo; Covadonga Salgado, Yolanda Pazos and Angeles Moroño from INTECMAR; María Rodriguez-Velasco from the EU-CRLMB, Vigo; and Monica Lion from the IOC-IEO SCCHA, Vigo.

Participants contributed actively to the classes and we enjoyed a superb work atmosphere.

If you are interested in this kind of IOC training activities, please check our web site http://ioc.unesco.org/hab/ for complementary information.

Course photos on page 15.

Dinophysis norvegica: First report of the toxic temperate water *Dinophysis* in Manori creek of Mumbai water

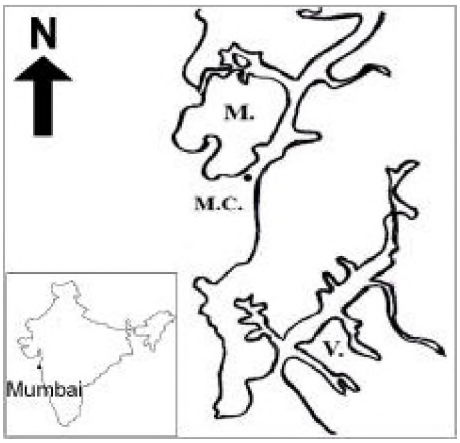

Fig. 1. Manori Creek (MC) of Mumbai coast. The dot at the mouth of creek is the exact location of sampling.

Dinophysis is a group of unicellular microscopic eukaryotic algae. The genus *Dinophysis* is the largest genus under family Dinophysiaceae of order Dinophysiales. Like other dinoflagellate algae, some members of *Dinophysis* contain toxins that get accumulated in bivalve shellfishes, while the later feed on the algae. The toxins cause diarrhea in human who consume the infected shellfishes. The disease outbreak is known as Diarrhetic shellfish poisoning (DSP). Unlike Paralytic shellfish poisoning (PSP), another type of algae-originated poisoning, DSP cases is rarely fatal. DSP outbreaks have long been overlooked because the symptoms DSP are often mistaken for bacteria caused diarrhea [1]. The DSP causing algae have drawn little attention in India, even though they have been reported at a potentially alarming density in Indian waters [2]. We specifically studied this important group of algae in Manori creek of Mumbai water. We are reporting the presence of *Dinophysis norvegica,* a toxic member of *Dinophysis* group, known to be a temperate water species. This assumes significance because there is a possibility of algal species being transferred through ballast water of ships.

The sampling site is located at northern most side of Mumbai in Manori creek (Fig. 1) that opens to Arabian Sea on the west side. Manori creek has a number of *Meretrix meretrix* beds contributing to seasonal fishery all throughout the year, except during monsoon. There are few beach resorts, salt pans and water recreational activities along the creek. The bottom is muddy with high organic load. This creek is also a ferry site and has rich mangrove vegetation (*Avicennia marina*).

Monthly sampling was done at the mouth of Manori Creek from June 2005 to May 2006 during high tide for qualitative and quantitative analysis of *Dinophysis*. Sampling was not done in August due to rough weather and heavy rain.

The phytoplankton samples were collected by filtering 20 L of surface water with plankton net of 10 mm mesh size. The filtrates (50 ml) were then passed through 200 mm net to remove large zooplanktons. The plankton concentrates were fixed immediately with lugols iodine [3] as well as with formaldehyde. Live samples were also collected by similar means, but brought to the laboratory without fixing. The observations were made within 2 hours of collection. Sea surface and atmospheric temperatures were recorded in the field using mercury thermometer. Water samples fixed with Winkler's solution were brought to lab for Dissolved Oxygen (DO) analysis. Water samples were also collected in glass bottle for nutrients analysis. Other water quality parameters like pH, salinity and Biological Oxygen Demand (BOD) were estimated in laboratory. Phytoplanktons were identified by their morphology, cell size and thecal plate patterns, using inverted epifluorescence microscope. Cells were counted by loading 1 ml of iodine fixed sample in Gridded Sedgwick Rafter Slide (wild life supply company, USA). A total 300 chambers were counted for enumerating the plankton number.

During the study period, seventeen samplings were done. Total plankton count, total dinoflagellate count and count of *Dinophysis* were done during the analysis. Though dinoflagellates like *Alexandrium* spp and *Gymnodinium* spp were present in almost all the samples, *Dinophysis* groups were present only in few samples. Among *Dinophysis* members *D. caudata*, *D. norvegica*, *D. dens*, *D. rotundata* and *D. aciminata* were observed sporadically at low cell concentrations [4]. Among all the *Dinophysis* species recorded, *D. norvegica* drew our attention because it is a bloom-forming toxic species associated with DSP events and is widely distributed in cold to temperate coastal waters of the Northern Hemisphere [5]. *D. norvegica* is the producer of dinophysistoxin-1 and okadaic acid, the two toxins implicated in DSP cases. DSP is not fatal but may cause stomach tumor on prolonged exposure [6].

Out of seventeen samples collected, *D. norvegica* was present only in three samples. *D. norvegica* was identified by morphology of the left sulcal list & the ribs supporting it, shape & size of the cingular lists and the size of epicone. The morphological characteristic of this species (Fig. 2) matched completely with the description of Dodge [7] and Tomas [8].

The cell concentration of *D. norvegica* was 114 cells L^{-1} on 18th July 2005, 46 cells L^{-1} on 30th November 2005 and 81 cells L^{-1} on 8 December 2005. Though another member of the same genus, *Dinophysis fortii*, has been reported to cause poisoning at a concentration of 200 cells L^{-1} [9], the concentrations with respect to *D. norvegica* has been in the order of 0.5×10^6 when associated with

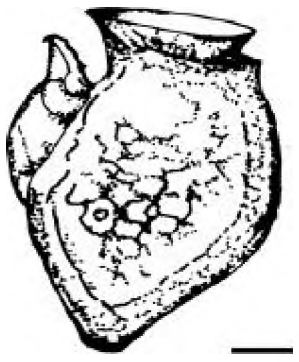

Fig. 2. Dinophysis norvegica. *Scale bar = 10 µm (lateral view).*

Table 1. Nutrients concentration in surface water at the time of presence of D. norvegica.

Date	Sea surface Temperature (°C)	Salinity (PSU)	Dissolved Oxygen (mg L^{-1})	Biological oxygen demand (mg L^{-1})	NH_4-N (µg L^{-1})	NO_3-N (µg L^{-1})	NO_2-N (µg L^{-1})	PO_4-P (µg L^{-1})
18/7/2005	32.0	23	4.9±0.2	3.80±0.49	15.7±4.6	27.6±5.1	108.7±7.1	155.4±37.4
30/11/2005	26.0	36.0	7.8±1.67	13.4±1.1	52.3±6.1	33.7±7.0	126.6.0±4.1	240.5±59.9
8/12/2005	25.0	34.5	0.44±0.17	8.15±0.85	45±3.9	19.3±4.0	149.4±5.2	169.4±18.2

outbreaks [10]. Therefore, the concentrations recorded in Mumbai water are not very alarming. Moreover, the minuscule proportion of the total phytoplankton *D. norvegica* constituted does not indicate a blooming situation.

Interestingly, being a temperate water species, *D. norvegica* was recorded over a wide range of temperature and salinity. The average surface water temperature ranged between 25°C–32°C, while salinity ranged between 23–36 PSU on the sampling days, when it was recorded. The DO ranged between 0.44 ± 0.17 mg L^{-1} to 7.8 ± 1.67 mg L^{-1} and BOD between 3.8 ± 0.49 to 13.4 ± 1.1 mg L^{-1}. BOD values were found to be high indicating high organic pollutant in water. Concentration of different nutrients on the days when *D. norvegica* was recorded is given (Table 1). As stated, *D. norvegica* was recorded in July, November and December. The physico-chemical parameters are expected to change significantly over this time period. Understandably, the density of cells of *D. norvegica* did not correlate with any of the environmental as well as hydrological parameters. To our knowledge *D. norvegica* is not reported from Mumbai waters of India.

Presence of this temperate water toxic species in tropical climate proves the theory of aggravation of HAB events by ballast water and ocean currents. Since Mumbai has a major port, there is a possibility that this species might have entered by ballast water discharge. It is now commonly accepted that ships while taking and discharging ballast water can cause transfer of planktonic organisms between different geographical areas. Though it is hard for a temperate water species to survive and establish in a new environment, presence of *D. norvegica* in July as well as November and December indicate that the survivability is probably not temporary. Even though, their number is very less, we need to take it as a signal that under favorable environmental conditions it has potential to cause bloom and human intoxication.

Acknowledgement

The authors are thankful to ICAR and CIFE for providing fellowship to the first author during her studentship at CIFE.

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Snapshots from the 11th IOC-AECID-IEO Course.
See page 13.



Green discolorations in Nuevo Gulf, Chubut Province, Argentina

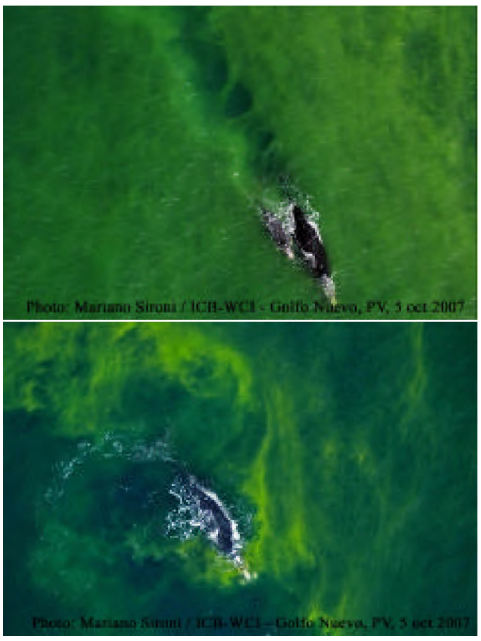

Fig. 1. Green blooms cover large part of Golfo Nuevo. Southern Right Whales are observed.

Intense green discolorations were produced in the waters of Nuevo Gulf in the spring of 2007 and 2008 (Fig. 1). The appearance of these large green blooms in the Gulf coincided with an apparent increase in mortality of the Southern Right Whale (*Eubalaena australis)*, particularly among calves [1–2]. This species of dinoflagellate has been observed along our coasts since 1998, with increased abundance in the last two years. The aim of this study was to identify the organism producing these algal blooms and analyze the potential production of toxins.

Península Valdés (between 42ºS and 43ºS) is situated on the Atlantic coast of Patagonia where the San Matías, San José and Nuevo Gulfs coincide. These bodies of water are commonly referred to as the North Patagonian Gulfs (Fig. 2). Nuevo Gulf (from 42º 29' S to 43º S and 65º 03' to 64º 03' W) is a semi-closed ecosystem limited by a mouth of 12 km which minimizes water exchange with the open sea [3]. This Gulf has an area of approximately 2,200 km^2 and average depth of 90 m and maximum of 170 m. Nueva Bay is situated to the extreme west of Nuevo Gulf (42º 45' S and 65º 02' W) and is characterized by a semi-open morphology, with an approximate surface area of 5.8 km^2 and volume of 1.1 km^3. Scarce information on current velocity in the interior of the bay suggests extremely weak flow (~2.5 cm seg^{-1}) [4]. Puerto Madryn city is the only urban development located in front of the Bay.

Live samples of the green algal bloom were taken from Nueva Bay and were observed under an optic microscope both with and without phase contrast. Photomicrographs of the observed phytoplankton cells were taken and the cells were measured. Quantitative analyses were carried out using an inverted microscope following Utermöhl's technique.

Microscopic observations showed both algal blooms were produced by a small dinoflagellate gimnodinioide with green chloroplasts accumulated on its surface (Fig. 3). The dinoflagellate was identified as *Lepidodinium* sp., whose morphological characteristics include: Subglobular cell, slightly dorsoventrally compressed; Length: 20–25 µm; Width: 10–12.5 µm; Subconic episoma equal or slightly shorter than the hiposoma; Cingulum descendent and slightly displaced.

The dinoflagellates observed under the light of the microscope were shown to quickly lose shape, secrete mucilage and aggregate together forming cell masses (Fig. 4).

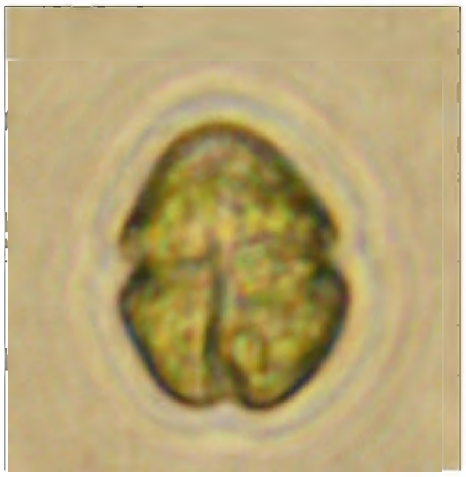

Fig. 3. Living cell of Lepidodinium *sp.*

Claquin *et al.* [5] has shown that this species produces ten times more transparent exopolymeric particles (TEP), compared with seven other species of cultured microalgae.

Richter *et al.* [6] studied the vertical diurnal migration of *L. chlorophorum* in the water column and the impact of solar radiation on its motility. They found that solar radiation resulted in a slight but significant decrease in swim velocity and in percentage of mobil cells after five hours of exposure to all radiation treatments. However, although cells were distributed more or less homogeneously throughout the water column, a slight but significant response was observed at midday when cells migrated to deeper parts of the water column to avoid high radiation.

Observations under the optic microscope were unable to distinguish between *Lepidodinium chlorophorum* (= *Gymnodinium chlolrophorum*) [7–8] and *L. viride* [9], whose defining characteristics can only be observed with an electron microscope. These two species contain both chlorophyll *a* and chlorophyll *b*, uncommon pigment in dinoflagellates. These species are also characterized as the only organisms possessing an

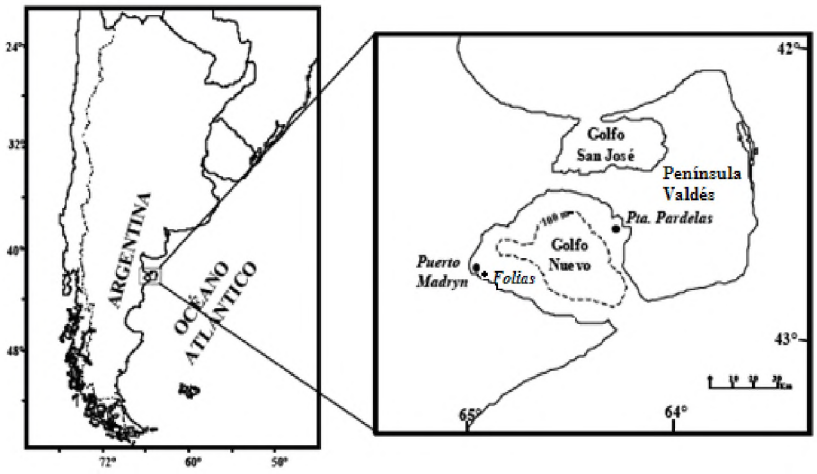

Fig. 2. Location of North Patagonian Gulfs

vestigial eukaryotic endosymbiont of prasinophytic lineage [7, 9–10].

MODIS Aqua satellite images taken from September 30 to October 7, 2007, show high values of chlorophyll in the entire Nuevo Gulf region (more than 30 mg/m^3) (Fig. 5). However, quantitative analysis of phytoplankton samples of two coastal stations, Folías and Punta Pardelas, observed 5,080 cells/L (12% total phytoplankton) and 61,448 cells/L (51% total phytoplankton) for the same time period. In October 2008, during the second discoloration event, Punta Pardelas station registered 53,760 cells/L (26% total phytoplankton).

Neither of these values are representative of a phytoplanktonic bloom. Thus, it can be inferred that the bloom did not reach coastal areas.

Similar green dinoflagellate blooms have been observed in other seas around the world, such as the Adriatic Sea [11], the North Sea [7] and western coasts of France [12] and Spain [13].

Chromophotographic analysis of paralyzing toxins carried out by the Laboratory of Chromotography of the Department of Environmental Health, Chubut Province, showed negative results coinciding with results found in other seas, thus ruling out its presence as cause for whale mortality (G. Marino, pers. comm.). However, *L. chlorophorum* is cited as a harmful species that causes anoxia, endangers marine life (especially sedentary species) and obstructs fish gills [14–15].

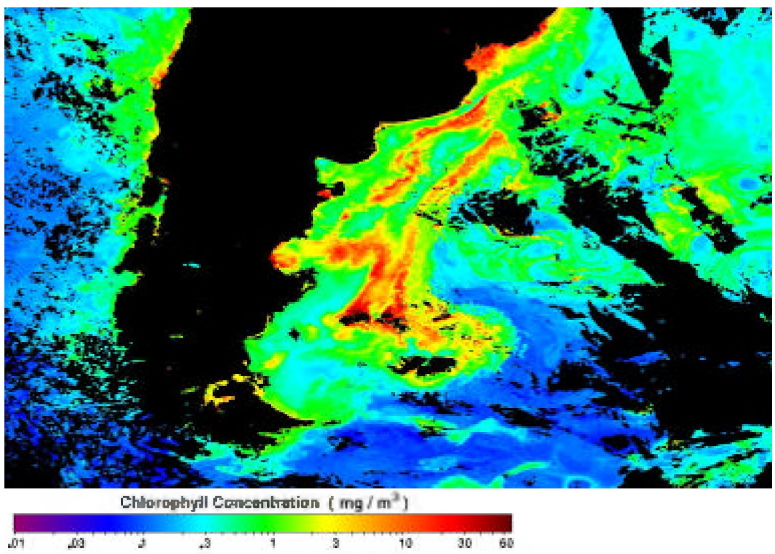

Fig. 5. MODIS Aqua satellite image showing chlorophyll concentration

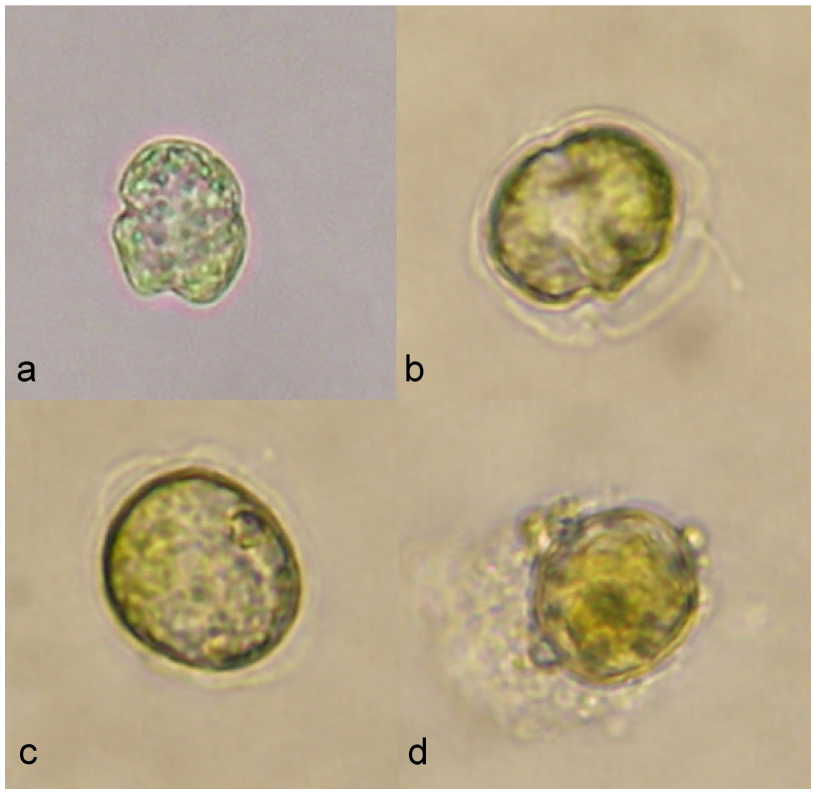

Fig. 4. a, b, c and d: The dinoflagellate showing different cellular shapes.

Acknowledgements

The authors would like to thank Drs. A. Chirife, M. Uhart, M. Sironi, and Lic. N. Mohamed and other team members of the Southern Right Whale (*Eubalaena australis*) Health Monitoring Program of the Península Valdés, Chubut, Argentina for their collaboration in the collection and shipment of samples. We would also like to especially thank Dr. M. Sironi for providing aerial photographs of the algal bloom.

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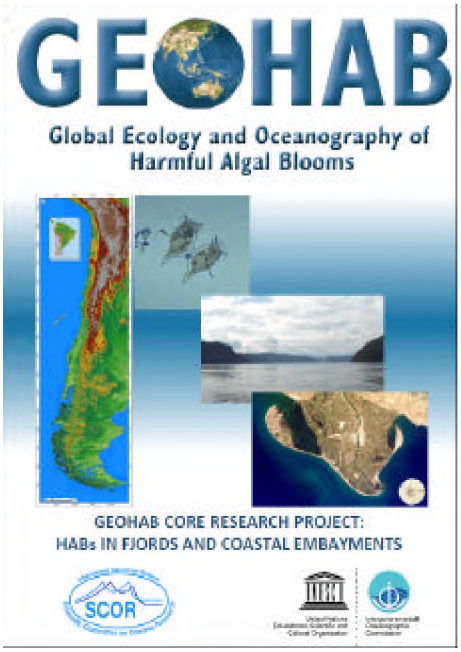
The GEOHAB Core Research Project on HABs in Fjords & Coastal Embayments launches its research plan

This recently published research plan summarizes the Open Science Meeting (OSM) held in Chile in April 2004 and provides the proceedings, progress and synthesis of research efforts on the study of HABs in fjords and other coastal embayments. This report also discusses a wide variety of research topics related to HABs in these coastal systems, and identifies key research questions. These include the influence of embayment morphology, bathymetry and hydrodynamics on HAB dynamics, particular adaptive strategies of harmful algae in these systems, the importance of life history transitions and cyst distribution, the role of physical aggregation and dispersion mechanisms, the relative contribution of nutrient flux and supply ratios, the importance of spatial scale and retention time in the expression and effects of allelochemicals and toxins in semi-confined systems, and the influence of anthropogenic activities such as aquaculture and climate change on HAB dynamics. Our understanding of and ability to predict HABs in coastal embayments over the next 5-10 years will reflect the extent to which this GEOHAB CRP can answer these questions. The practical implementation of Core Research activities in coastal embayments was initiated in 2005. A sub-committee for this CRP was formed recently; it includes Drs Allan Cembella (Germany), Leonardo Guzmán (Chile), Marina Montresor (Italy) and Suzanne Roy (Canada). An Open Science Meeting is planned for 2011, focusing on life history transitions, particularly the connection between benthic cysts and blooms in coastal embayments.



Contact Dr. Suzanne Roy: suzanne_roy@uqar.qc.ca

More info at: www.geohab.info

A Regional Plan for HAB Research Focus and Cooperation in Asia



Many countries in Asia face problems associated with harmful algal blooms, especially those that border the China Sea and western Pacific. Blooms are formed by toxic species that cause human intoxications, kill wild and cultured fishes, and others that are noxious but non toxic. In some areas noxious blooms are also formed by macroalgae. Several of the HAB species affect more than one country in the region. These include *Pyrodinium bahamense*, *Alexandrium* spp., *Cochlodinium polykrikoides*, *Noctiluca scintillans*, and *Phaeocystis globosa*. Early on, many researchers in Asia realized the value of establishing cooperative and comparative studies on HABs in the region based upon the GEOHAB model. This approach would harmonize research plans and methods, facilitate exchange of information and expertise, and provide access to technologies that may not be available in some countries.

In order to produce a science plan for GEOHAB Asia, two meetings were convened. During the first meeting on 15-16 March 2007 held in Tokyo Japan participants presented HAB-related research carried out in their respective countries. In the second meeting from 31 Jan to 1 Feb 2008 held in Nha Trang Vietnam the presentations were more focused on identifying key questions and research components to be included in the GEOHAB Asia science plan. The meetings were also attended by some members of the GEOHAB Scientific Steering Committee, representatives from the North Pacific Marine Sciencies Organization (PICES), and some prominent HAB scientists from outside Asia. The output of these meetings is a research plan entitled "Harmful Algal Blooms in Asia: A Regional Comparative Programme". The planis available for perusal and download from the GEOHAB homepage. It is hoped that starting in 2011 some studies outlined in the research plan will be implemented by researchers in the region. Success of the two planning meetings was facilitated by generous support of the Japanese government, the Danish International Development Agency (DANIDA) and IOC/WESTPAC.

Contact Dr. Gires Usup: giresusup@gmail.com

The GEOHAB Core Research Project on Benthic HABs is launched



Ciguatera is caused by a type of dinoflagellate (*Gambierdiscus spp.*) that grows epiphytically on macroalgae in coral reef environments. These and other dinoflagellates such as *Ostreopsis*, *Prorocentrum*, and *Coolia* are consumed by herbivorous fish, beginning processes of bioaccumulation through the food web as herbivorous fish are consumed by carnivores and ultimately by humans. Collectively such organisms may be referred to as Benthic Harmful Algal Blooms (BHABs). Because of their significant threat to human health, fisheries resources, and marine ecosystems, attention has been focused on international coordination of research on BHABs. The importance of such toxin-producing, benthic organisms throughout tropical areas worldwide led the Scientific Steering Committee of the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) program to create a Core Research Project (CRP) for BHABs. GEOHAB is co-sponsored by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of the United Nations Education, Scientific and Cultural Organization (UNESCO). Establishment of a CRP begins with an open science meeting to bring together the international science communities studying these organisms to create structure for future research collaborations.

Recently an open science meeting for BHABs was organized and convened in Honolulu, Hawaii, USA. This meeting attracted an international group of over sixty scientists from more than twenty countries to share their insights regarding BHABs in various environments. An accompanying training workshop for twenty researchers was designed to harmonize protocols for sampling and taxonomic identification of BHABs to be applied by researchers throughout the world. Subsequent to these activities, the organizing committee for the meeting is leading the production of a conceptual research plan to provide the foundation for collaborative research on oceanographic and ecologic controls on BHAB species to be presented at the 14th International Conference of Harmful Algae in Crete later this year. *Gambierdiscus* spp. are responsible for more human illness than all other HAB-species combined, and it is important to have assembled the international communities studying these organisms to erect an international structure for future research collaborations in comparable systems.

GEOHAB Training Workshop: Taxonomy challenges and identification of benthic dinoflagellates

In conjunction with the GEOHAB Open Science Meeting on Benthic HABs held 21-23 June 2010, Hawaii, USA, was held a training workshop from 24 to 27 June on 'Taxonomy challenges and identification of benthic dinoflagellates'. The training workshop included microscopy and molecular techniques and trainers included Drs. Jacob Larsen, Wayne Litaker, Pat Tester and Mona Hoppenrath. Local microscope distributor PAC/RIM Medical Technology & Supplies Corp. provided the microscopes. Participants worked with both samples from cultures and the field that had been identified. Known species could be compared with the drawings and micrographs and it was a good mix of fresh samples from Hawaii, known species samples from around the world and the samples the participants had brought with them for examination/ identification. As always training workshops provide the opportunity to network and build relationships and not the least for taxonomists, who are often not well supported, this is also a major outcome of a workshop.



Photo by N.N. Lam

Future events

SEPTEMBER 2010

Oceanography and ecology of HABs: physical/biological interactions

Nantes, France, 20–24 Sep

Session at the ICES Annual Science Conference 2010.

More info at: www.ices.dk/iceswork/asc/2010

AOAC Annual Meeting

Orlando, Florida, 26–29 Sep

There will be a Marine and Freshwater Toxins Taskforce meeting and also the following symposia:

· Marine toxin control – Modern methods, validations, animal use reduction, and international standards.

· Issues and criteria for validation of analytical methods for cyanotoxins detection and global suitability.

· Natural toxins impact and methods needs in the developing world.

For further info visit: www.aoac.org/meetings1/124th_annual_mtg/main_2.htm, or contact J. Hungerford (James.Hungerford@fda.hhs.gov).

NOVEMBER 2010

Taxonomy of Recent Dinophyceae by Malte Elbrächter

List/Sylt, Germany, 2–8 Nov

The course, that will be held at the Wattenmeerstation Sylt of the Alfred Wegener Institut für Polar- und Meeresforschung.

Registration deadline: 15 Aug

Registration: Malte.Elbraechter@awi.de

More info at: www.ioc-unesco.org/hab/index.php?option=com_oe&task=viewDoclistRecord&doclistID=71

Baltic Sea Phytoplankton Species Identification Course

Tvärminne, Hanko, Finland, 14–26 Nov

Nordic Marine Academy Awarded this course that will be held in Tvärminne Zoological Station.

Teaching by Ø. Moestrup, H.Kuosa, S. Hällfors, J. Larsen, A. Kremp, H.Annadotter, H. Hällfors, M.Majaneva, J.Blomster and J-M.Rintala.

The course corresponds to 5 ECTS-credits and it is primarily meant for researchers/students at the Postdoctoral and Ph.D.-level with previous experience in Baltic Sea phytoplankton identification.

Participants should be actively working on different aspects of phytoplankton ecology, physiology and/or taxonomy.

Application deadline: 31 Aug.

For additional info contact: J.-M. Rintala (janne.rintala@helsinki.fi).

JANUARY 2011

Aspen Ocean Symposium: Physical microenvironments modulating biological interactions in the Ocean

Aspen, Colorado, USA, 17–21 Jan

The meeting will address how physical microenvironments affect marine organisms, focusing on seven 'microscale' themes bringing together biologists, chemists, ecologists, physicists, engineers, and mathematicians for a highly interactive workshop. A mixture of invited talks, contributed short talks, posters and discussion sessions, as well as ample time for informal interactions, will foster novel views and collaborations at the interface between physics, biology and chemistry.

For more info: web.mit.edu/aspenoceansymp

APRIL 2011

IPHAB-X: Tenth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms

Paris, France, 12–14 Apr (tentative dates)

Session will be held at UNESCO Headquarters.

More info at: www.ioc-unesco.org/hab

JUNE 2011

8th International Conference on Molluscan Shellfish Safety (ICMSS)

Charlottetown, Prince Edward Island, Canada, 12–17 Jun

The Canadian Food Inspection Agency and the Prince Edward Island Department of Fisheries, Aquaculture and Rural Development are pleased to announce that the 8th ICMSS will be held at the University of Prince Edward Island located in Charlottetown.

Scientists from all over the world will gather in Charlottetown to discuss issues such as microbiology, chemistry and marine biotoxin risks, analytical methods, molluscan shellfish harvest area management and more.

The conference will provide the international community an excellent forum to advance molluscan shellfish safety science and advocate the soundness in its application. It also provides a good opportunity for the science and academic community, industry participants and regulators to establish and maintain contacts around the globe.

More info can be found at: www.gov.pe.ca/ICMSS2011



HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

www.ioc-unesco.org/hab

No. 43

Special issue: the 14th International Conference on Harmful Algae, Hersonissos, Crete



Photos by M. Lion (top) and K. Pagou (bottom).

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

Tangles and Threads

14th International Conference on Harmful Algae

Plate tectonics informs us that "Crete rides, as if on the back of a bull, at the point where Africa burrows under Europe."[1] The Dhikti Mountains which form the southern backdrop to Hersonissos are part of the bull's back. Zeus, head of the Olympian pantheon, was said to have been born in a Dhiktean cave. A local tradition had him buried under Mount Yioúhtas, casting doubt on his immortality. Some say this is the origin of Epimenides paradox, "All Cretans are liars"; he was himself a Cretan, hence the paradox. Zeus in the form of a bull carried the Phoenician goddess Europa off to Crete, whence the name of the western end of Eurasia. In Crete, Europa gave birth to Minos; those ICHA 14 participants who went to Knossos saw what remains of the palace named after him. It was in Knossos that Minos kept the bull-headed Minotaur in a labyrinth, and it was Minos' daughter Ariadne who showed Theseus how to escape from it, after killing the monster, by unravelling a *thread*.

Archaeologists think the Minoans may have reached Crete from Anatolia, present day Turkey. Whatever the truth, Cretan (and European) society was burrowed into from the east, in the ever ongoing process of cultural tectonics, as Europa's fate indicates. The music and dance at the conference dinner was one result of this hybridization. Inspiration for the Greek alphabet was Phoenician, and even the names of the Greek a-b-c-, alpha-beta-gamma-, are Semitic. By this route Pythagoras learned of the theorum we now know by his name, and we inherited the Babylonian sexagesimal base of their mathematics, which still survives in the degrees, minutes, and seconds of our circles and our time keeping.

With what threads can we navigate the labyrinthine imbroglio of ICHA 14, with its more than 500 contributions? **Linda Medlin** provided one thread, a review of the evolution of dinoflagellates, a dazzling cataract of erudition; she called them "kings of symbioses" (miniature minotaurs), spoke of their complex evolutionary history, of plastids gained and lost multiple times, of the phylogeny of peduncle feeding, and much more. Some details of plastid origins in *Dinophysis* were explored by **Nicolás Raho**; plastid maintainance following their acquisition by *D. acuminata* was addressed by **Jennifer Wisecaver**. Peduncle feeding, a trait shared with euglenids, was described in *Gymnodinium aureolum* by **Kyung Ha Lee**, and in *Karlodinium armiger* by **Terje Berge**. Like prokaryotes, dinoflagellate chromosomes are always condensed, their nuclear envelopes persist during mitosis, and they lack histones and nucleosomes; but their cell cycles are eukaryotic, as are the nucleus



and nucleolus, the repetitive noncoding DNA sequences, the way ribosomal genes are arranged, and other features. To the outsider, navigating these giant genomes looks as perplexing as understanding turbulence. Genes are in a constant state of flux, from Barbara McClintock's jumping genes to unequal crossing over, DNA slippage, gene conversion, Gabriel Dover's molecular drive, evolution by genomic flux, and other dark matters. No wonder there is so often mismatch between the old names of classical taxonomy and the new trees of parsimony analysis. Add more genes and the trees change. Toxin profiles too might contribute to understanding phylogeny and distinguishing clones, suggested **Takeshi Yasumoto**. How long will the old names maintain their status as the *gold standard*? **Shauna Murray** provides an insiders view of some of the genetical perplexities unravelled here (see p. 10).

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Gustaaf Hallegraeff provided a second thread and summarized some anticipated responses of marine ecosystems to projected climate changes. He is well aware of the pitfalls of trying to discern the future of complex systems, for which he used the word *tangles* – an echo of Darwin's *tangled bank*? Some of his main points were, that if climate does change in the sense projected by IPCC models, we can expect phytoplankton to respond with changes in the ranges, abundance, and seasonal timing, that these changes will impact ecosystems through match-mismatch mechanisms, and that they will challenge our abilities to manage the harmful ones. A variety of contributions at the conference explored some of these expectations experimentally, but it is not clear so far whether what we may be seeing in field data are responses to *climate change* (in what now appears to be the canonical and, unfortunately, very politicized meaning of the phrase) or to *climate variability*. Or to neither! Several powerful voices attribute imputed trends in coastal phytoplankton ecology to eutrophication. Gustaaf's summary of conference contributions to the climate debate appears on page 11.

Protagoras asserted that there are two sides to every question. Thus **Barrie Dale** challenged us to view the high frequency fluctuations or trends we detect in monitoring data (with decadal to secular time scales) in the context of the *deep time* of geology. Whether we are in fact dealing with change or variation is of course a question of time scales, but the problems of even securely identifying trends in phytoplankton communities are not trivial[2]. In terms of management however, it would be Olympian indeed to think we could do anything about Barrie's larger scales. An alleged major cause of the destruction of Knossos in



the 12th century bce was a marked increase in aridity which persisted for some centuries; more recent droughts have impacted human history in regions around the world[3]. Some IPCC projections threaten some regions of the planet with a similar fate within the next few decades, and emphasize that the *rates* of likely change are unprecedented. In fact even higher rates of change in temperature have been identified …

To confound Protagoras, **Judit Padisak** took a third view, and reminded us that as oxygen breathers, we would not be here but for the labours of *harmful* cyanobacteria, partly responsible for oxygenating the atmosphere - the "Great Oxidation"[4]. But this change was inimical to organisms adapted to anoxic conditions. That was back in the Precambrian. Even today, prokaryotes are thought to represent about 70% of surface water planktonic biomass[5], and as Judit pointed out, they are "integral parts of healthy functioning ecosystems" and play essential biogeochemical rôles. "What strange creatures they are!", she said, quoting a Czech fairy tale. "Algae to the rescue" wrote David Swinbanks[6], of cures proposed by Tadashi Matsunaga and Shigetoh Miyachi to alleviate increasing atmospheric CO_2 concentrations.

Climate is always changing. If we only examine a brief period of warming, that warming is of course unprecedented. So, Gustaaf's editorial rule of thumb, that accounts of *change* based on time series shorter than 30 y should be rejected, is acceptable to a point. A longstanding and less arbitrary rule says that we need time series about 2 – 3 times as long as the signals we are seeking, so that a 30 y series might allow us to identify decadal signals like those related to sunspots, to ENSO or to the NAO, but is a little short to identify the 18.6 year tidal signal found by Alan White in Bay of Fundy PSP records[7]; the more extended data set now available might usefully be analysed to see if the signal found by White has persisted. Longer time-series are sometimes available but not used: *Phaeocystis* was considered a problem in the 1920s and 1930s in the North Sea, and there is quite an extensive literature on it from those decades; but many analyses of long-term trends in the species in the more recent literature use 1950s data as a base-line – a special issue of *Harmful Algae* (vol 4, issue 5) on *Phaeocystis* refers to the earlier work only once.

There is general agreement that climate has warmed a little (about 1°C) in the last 100 years (but not monotonically, and not by equal amounts in all regions), and that greenhouse gas concentrations in the atmosphere have risen, CO2 for example from about 280 ppm in 1750 to 380 ppm at present. Agreement about what these facts mean is less widespread. It is often forgotten that the rate of temperature change in the period from the 1920s to 1940s was as high (in the North Atlantic) as it was after 1980, and that regional changes in species ranges in that earlier period were as extensive as those described more recently from CPR surveys (some of the latter presented at this conference by **Stephanie Hinder**, and by **Chris Reid**). Chris referred to the "*tangle* of feedback mechanisms" which make interpretation so difficult. Trends analogous to those in the Atlantic are hinted at by tropicalization of the East China Sea as indicated by increased occurrence of warm water dinoflagellates, fish, and macrophytes around Jeju Island, Korea, attributed to strengthening of the Tsushima Current (**Joon-Baek Lee**).

A few contributions based on multi-annual data sets identified changes in species abundance. Sediment trap results from the Santa Barbara Channel, NE Pacific, covering the years 1993 to 2007, indicate an increased frequency of *Pseudo-nitzschia* blooms after 1998 (**Emily Sekula-Wood**), attributed to changes in upwelling and in the chemistry of the California Undercurrent. A short time-series, 2000 to 2009, from lagoons in Texas seems to show that winter *Prymnesium parvum* blooms are strongly influenced by flushing rates there (**Daniel Roelke**), hence on varying rainfall. Flushing may be responsible too for the decline of *Karlodinium veneficum* blooms in the Canning estuary, Western Australia (**Alice Gedaria**). A comparison of early 20th century plankton records (1903-1911) with more recent ones (1993-2005) from the Baltic suggests there have been marked changes in the occurrence of *Skeletonema costatum* (more likely *S. marinoi*) and *Thalassiosira baltica*, that *Dinophysis* spp may have increased in abundance in Baltic coastal waters, while the bloom forming *Aphanizomenon flos-aquae* and *Nodularia spumigena* of open waters have not (**Heidi Hällfors**); an earlier comparison of Baltic ecology over the



same time period found that pelagic energy flow had increased by 30-70%, and pelagic fish catches more than ten times[8].

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Detailed attention to hydrographic data in short time-series can often unmask the unique conditions that promote blooms of selected species in particular years; **Beatriz Reguera** presented such an analysis for *Dinophysis acuta* in Galician coastal waters. A 20 y time-series off Aveiro, Portugal, yields evidence of a retention area for *Dinophysis*, focus of a project called HABSPOT (**Teresa Moita**), and potential source of the blooms farther north in Galicia. Conditions in the Yatsushiro Sea favour accumulation of *Chattonella* cysts (see below) and make it a "hot spot". The unique cysts of *G. catenatum* and its non-toxic congeners lead to some intriguing questions about the identity of subfossil deposits. It now appears that cysts of *G. catenatum* and *G. microreticulatum*, and possibly also *G.* cf *nolleri*, are found together in cores from the Portuguese shelf; *G. catenatum* cysts first appeared in southern Portuguese cores (off Mira) in the 1890s, farther north (off Douro) in the 1920s, and exhibited a major increase in abundance in the late 1960s (**Sofia Ribeiro**). A longer *G. catenatum* cyst record (~ 500 y), from the Gulf of California, published last year[9], shows a rather similar pattern for the 20th century, which the authors relate to upwelling rates and surface temperatures. Cyst counts from a core taken in Puget Sound, USA, have provided estimates of *Alexandrium catenella* abundance from 1878 to 2005 (**Kirsten Feifel**); counts were more closely related statistically to local conditions (SST and air T) than to large scale climatic indices (PDO, ENSO).

"Acidification" has provoked considerable attention. Seawater pH can vary from as high as 8.3 to as low as 7.8, daily in some coastal areas - so it is in fact always alkaline (pH > 7.0). Even the worst IPCC end-of-century scenario with pH ~ 7.6 does not forsee an acid ocean, only a less alkaline one. Experiments with bubbled cultures of *Lingulodinium* (**Christos Loukas**), *Karenia* (**Reagan Errera**), *Phaeocystis* (**Yan Wang**), *Alexandrium* (**Rhett Tabbada**), and mixed populations (**Lasse Nielsen**, **Edna Granéli**) have not yet provided any clearcut guides as to what might result from declining pH, even on the short time scales of these experiments. The well known impact of bubbling induced turbulence seems to have been ignored. Values of pH < 7.07 dissolve the spines of *Scrippsiella* cysts (**Hyeon Ho Shin**). Hallegraeff pointed out that physiological plasticity and strain diversity may significantly impact such experiments. As if in illustration, **Seung Ho Baek** reported highest growth rates of *Heterocapsa triquetra* in culture at 30‰/20°C; he nevertheless found blooms under ice in Lake Shihwa, Korea, and it blooms too in the highly eutrophic Golden Horn, Turkey, at a less extreme 13°C (**Seyfettin Tas**).



Most of us rarely face the real causes of the ecological changes we worry about, whether it be plankton communities or rain forests, namely our own increasing numbers, increasing expectations, and increasing wastes. Forests and swamps are destroyed by lumber companies, palm oil plantations, cattle ranching, marine ecosystems by overexploitation of various kinds. The corporate capitalist part of our nature is disembowelling the planet. Concern about overpopulation was already expressed in ancient times. In the Babylonian *Atrahasis*, Enlil asks for plague, famine and flood to control human numbers; and the same motif is found in the Greek *Cypria*. Fast forward to Malthus. But general concern about ecological change stems from the hypothesis that human activities have become so influential since the Industrial Revolution that they are now as effective as geological forces, and so have initiated a new geological era, the Anthropocene. In the sea, these new forces include the impacts of eutrophication, use of fresh water resources, dumping and dredging, fishing, aquaculture, as well as climate change. Some small scale instances reported at the conference relate the occurrence of blooms in response to changes in local flushing rates. Dams in Beijang River, China (**Fajin Chen**), spit growth at Juag Lagoon in the Philippines (**Joan Reotita**), construction of marinas along the Catalan coast, NE Spain (**Esther Garcés**) provide examples, and recall the changes at Alexandria Harbour, Egypt (Amany Ismael at 13th ICHA). Larger scale changes attributed to eutrophication were addressed by **Patricia Glibert**, who ascribes picoplankton blooms (*Aureococcus*, *Synechococcus*) to their competitive ability to thrive on reduced forms of nitrogen, as well as to live in anoxic conditions. She also pointed out that these picoplankton blooms favour dinoflagellates and raphidophytes over other grazers, and referred to such situations as *tangles*! The rôles of nutrients and their ratios were simulated in a box model of the Chesapeake (**Ji Li**), with uptake and assimilation rates derived from laboratory studies.

It is assumed that climate-linked rising surface temperatures will influence phytoplankton species composition. Higher temperatures reduce viscosity, which might select for smaller-celled more slowly sinking species, or for species able to increase viscosity by means of extracellular secretions. These matters come under the rubric of *thalassorheology* (**Ian Jenkinson**). The recent *Cochlodinium* blooms in NW Indian Ocean coastal waters produce some

kind of froth, not yet characterized, and mucilage events of the kind familiar in the Adriatic and other parts of the Mediterranean (*mare sporco*) now occur in the Sea of Marmara, associated with *Fibrocapsa japonica* (**Yelda Aktan**).

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Some elementary models of phytoplankton population dynamics provide nutrients to allow growth, and control algal numbers by grazing. In slightly more complex models, nutrients are regenerated as a result of grazing. In multispecies models, some species become dominant as a result of reduced grazing pressure and exploitation of regenerated nutrients. **Hong Ying** suggested that dynamics of this kind lie behind *Cylindrospermopsis* blooms in reservoirs in Queensland, Australia. We usually think of nutrients supplied by upwelling and runoff, but groundwater and atmospheric inputs can also be important. Groundwater was earlier invoked to account for brown tides (*Aureococcus*) in Long Island (NY) lagoons[10], and now for *Pseudo-nitzschia* blooms in a lagoon in the northern Gulf of Mexico (**Justin Liefer**). Desert dust may supply essential iron to surface phytoplankton, and was proposed as a source of iron for *Cochlodinium* blooms in the Persian Gulf and Omani waters (**Cemal Saydam**); the key feature of Cemal's model is biological reduction of iron by atmospheric microorganisms. **Savvas Genitsaris** has identified 63 algal taxa in air samples from Thessalonika, Greece; curiously, *Dinophysis acuminata*, common in the local waters, was absent from the air samples. The notion that N:P ratios are a key factor in red tide occurrences in Tolo Harbour, Hong Kong, was originally based on an 8 year time series (1982-1989); **Paul Harrison** has extended the series another 19 years (to 2008) and shown that the hypothesis fails.

Access to nutrients can also be enhanced by attacking the competition. *Karenia* outcompetes (allelopathy?) *Prorocentrum micans* in mixed cultures of the two (**Ji Xiaoqing**). *Alexandrium minutum* secretes a product which targets photosystem-II of *Chaetoceros* and reduces its chlorophyll content, hence growth rate (**Aurélie Lelong**). This study as well as several other presentations, especially from the Alfred Wegener Institute, indicate that the suite of secondary metabolites available to *Alexandrium* (and presumably most phytoplankton species) is much more varied than the earlier focus on PSTs might suggest.

Grazing is not the automatic filtration of particles once imagined. Grazers have their likes and dislikes, and can select from mixtures of phytoplankton. In the Senegal River, mixed zooplankton avoid eating *Microcystis aeruginosa* but graze *Cylindrospermopsis raciborskii* and *Anabaena* spp in variable amounts (**Mireya Mendoza-Vera**); *Fragilidium duplocampanaeforme* feeds only on *Dinophysis* (but avoids the more toxic *D. fortii*) when offered a mixture of prey (**Miran Kim**); *Mytilus galloprovincialis* preferentially selects *Dinophysis* and *Prorocentrum* from mixed phytoplankton (**Živana Ninèeviæ-Gladan**). Toxins in food webs provide evidence of trophic relations as well as essential epidemiological information. Azaspiracids (**Joe Silke** summarized the history of their discovery) are found in *Favella ehrenbergii* and *Protoperidinium crassipes* revealing that they feed on *Azadinium spinosum* (**Phillip Messtorff**); PST of *Pyrodinium bahamense* in zooplankton indicates that grazing impacts bloom dynamics (**Joseph Palermo**).

The numerical effects of grazing are reduced by prey species in a variety of ways. *Heterosigma akashiwo* can avoid grazing by *Favella* and *Oxyrrhis* by seeking water of lower salinity (**Elizabeth Harvey**). Many species, *H. akashiwo* included, can transit metazoan guts unharmed; ascidians provide an example (**Sandra Shumway**). Allelopathy to deter grazers was demonstrated, by *A. tamarense* against *Polykrikos kofoidii* (**Uwe John**); it is not the PSTs but lytic compounds (partially characterized by **Haiyan Ma**) which are active in this context, more evidence of the diversity of secondary metabolites.

Population growth rates are also reduced by infections. We learned of a virus that infects *Prorocentrum minimum* (**JinJoo Kim**), several bacteria which attack *Microcystis aeruginosa* (**Myung-Soo Han**) and other cyanobacteria (**Shingo Hiroishi**), and a chytrid fungus which parasitizes recently excysted *A. tamarense* (**Mineo Yamaguchi**). Chytrid infections of phytoplankton are well known in freshwaters, but have not received much attention in the sea. Bacterial control of *Microcystis* blooms may be a management option (**Ichiro Imai**). Infection rates of various dinoflagellates with *Amoebophrya* in Monterey Bay, California, were described by **Fernanda Mazzillo**, and the demise of a *Prorocentrum micans* bloom due to this parasite in Reloncavífjord, Chile, by **Catharina Alves-de-Souza**; it also infects *Dinophysis acuminata* in Galician waters (**Lourdes Velo-Suárez**). A fascinating account of how the swimming patterns of *Karlodinium veneficum* (host), and *Amoebophrya* (parasite) can interact and hence control infection rates was given by **Allen Place**.

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The rôle of cysts in population dynamics has been debated since the first conference in this series in 1974. Some studies reveal more or less clear relations between excystment and bloom initiation, and between encystment and bloom termination. One link or both are illustrated by *Alexandrium minutum* in Cork Harbour, Ireland (**Sarah Cosgrove**), by the extensive data set on *A fundyense* from the Bay of Fundy (**Jennifer Martin**), by the same species in a Cape Cod salt pond (**Emil Vahtera**), and by *Chattonella* in the Yatsushiro Sea (**Mineo Yamaguchi**). These links are less clear in two other studies, of *A. catenella* in the Chilean inland sea (**Patricio Díaz**), and of *Pyrodinium bahamense* in various Philippine bays (**Elsa Furio**); in these cases, a relationship between cyst abundance and blooms may be obscured by the hydrodynamics. In contrast, Japanese (**Ken-Ichiro Ishii**) and Korean (**Myung-Soo Han**) studies of several species suggests that excystment and vegetative growth are largely

uncoupled, so that resting stages are not necessarily bloom precursors. **Marta Estrada** presented a model of these aspects of population dynamics, and **Donald Anderson** brought us up to date on the sophisticated Gulf of Maine model for *Alexandrium* which is initialized with cyst counts. Cyst dynamics was a component of a model of the life history of *Pyrodinium bahamense* in Sorsogon Bay, Philippines (**Aletta Yñiguez**), in which transport, stratification, excystment and encystment, and grazing, each play their part in the generation and decline of blooms. Life cycle transitions and motility are also key components of a model of bloom dynamics presented by **Inga Hense**; the former are controlled externally, by nutrients, in Hense's model. A few contributions focussed on aspects of life cycle regulation – and hint at the existence of *chronogenes* whose transcription meters time. Internal control of life cycle transitions is suggested by reorganization of the transcriptome in *Karenia brevis* during the transition from logarithmic to stationary growth (**Jillian Johnson**).

Some important HAB topics received less attention than in earlier conferences. *Thin layers*, a core research area for GEOHAB, appeared only once, dealing with *Dinophysis* in the Galician rías (**Laura Escalera**); results like these have important consequences for sampling, modelling, and management. But some contributions on vertical migration relate to the theme. Distinct behaviour patterns of *Prorocentrum minimum* and *Heterocapsa triquetra* in different temperature and salinity gradients in the laboratory were reported (**Therese Jephson**), and, based on field data from Alfacs Bay in the NW Mediterranean, of *Pseudo-nitzschia* and *Karlodinium* in stratified and turbulent water columns (**Mireia Artigas**). Settlement patterns of *Heterosigma akashiwo* seem not to depend on changes in swimming behaviour during life history transitions (**Elizabeth Tobin**); their cysts apparently reach the sediment by passive sinking, so that their distribution there might depend on the local hydrodynamics.

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We end with a few further notes on some of the actors. The characterization of *Alexandrium tamarense* and its cousins continues to perplex. Toxic and nontoxic *A. tamarense* in Scottish waters appear to hybridize (**Jennifer Graham**), sequence data distinctions of *A. ostenfeldii* and *A. peruvianum* are blurred (**Anke Kremp**); within species variability is found wherever it is sought (**Pia Mäenpää**, **Daniel Varela**); all this is just as it should be in a Darwinian world. **Anna Godhe** presented a fascinating analysis of interactions between life histories and genetics in fjord and adjacent shelf populations of *Skeletonema marinoi*. The stx-genes are under scrutiny in cyanos and several dinoflagellates (see Murray's accompanying article), and the steps which lead from these genes to the toxins are being explored too (**Ralf Kellmann**; **Maria Wiese**).

Basic information on *Pseudo-nitzschia* was reported from Plymouth, UK (**Naomi Downes-Tettmar**), Atlantic Marocco (**Karima Chaira**), Lesvos, Greece (**Sofie Spatharis**) where there are large winter blooms, and from Nha Phu Bay, Vietnam (**Dao Viet Ha**), Sarawak (**S N-P Su**), Pacific Mexico (**David Hernández-Becerril**), and Uruguay (**Silvia Mendez**). Fourteen species of *Pseudo-nitzschia* occur together in Tongyeong waters (**Weol-Ae Lim**), a taxonomist's nightmare now being resolved with real-time PCR probes; similar species richness is found in Mexican Pacific waters (**Sonia Quijano-Scheggia**). Studies of the genetics of *Pseudo-nitzschia* from the South China Sea (**Peng Wang**, **Junrong Liang**), Malaysian waters (**Hong-Chang Lim**), and the Gulf of Naples (**Sylvie Tesson**) were also presented. Tesson's results indicate the existence of sympatric populations of genetically distinct but sexually compatible strains of *P. multistriata*.

Persistent high concentrations of *P. bahamense*, from November 2009 to May 2010, occurred about 60 km off the coast of El Salvador, with lower numbers inshore (**Sergio Licea**), and were associated with dead turtles (*Chelonia* and *Lepidochelys*). Blooms of this species were also described in the Gulf of California (**Lourdes Morquecho**), in Oaxaca (Mexico) waters in July 2010, with maximum numbers 3-5 m below the surface (**Esther Meave**). **Florence Onda** described conditions favouring pellicle cyst formation in culture.

In Manila Bay, we normally imagine *Pyrodinium bahamense* blooms (**Rhodora Azanza**), but *G. catenatum* was found there in February 2010, and *Noctiluca scintillans* too was widespread (**Teresa Escobar**, **Garry Benico**). *G. catenatum* and *N. scintillans* also occur together in the Gulf of California, where the latter grazes on the former (**Jose Bustillos-Guzmán**).



Extensive blooms of *Cochlodinium polykrikoides* now occur in the Northwest Indian Ocean, in the Persian Gulf, Gulf of Oman, with associated faunal mortalities (**Gilan Attaran-Fariman**, **Fereshteh Saraji**, **Anbiah Rajan**), and as far south as Masirah on the southern Oman coast (**Adnan Al-Azri**), and to the east in Karachi waters (**Sonia Munir**). In the Pacific, the species now blooms frequently in the Pearl River Estuary, China (**Yuzao Qi**). *Noctiluca scintillans* and other species accompanied the blooms in Omani waters (**Hamed Al Gheilani**). Experiments show that can control its competitors allelopathically, kill potential grazers, and acquire nitrogen in a variety of forms (**Christopher Gobler**). With such a well-equipped armoury, one must wonder why it waited until the 1980s before causing problems, or is our conceptual grasp of phytoplankton

variability way off target? Are sporadic appearances and disappearances the norm?

Ostreopsis cf. *ovata* is attracting a lot of attention in Mediterranean laboratories (**Adriana Zingone**, **Cristina Ingarao**). It is found both in the plankton and on the surfaces of rocks and seaweeds (**Stefano Accoroni**, **Magda Vila**), and may move into the water column on a daily basis (**Rodolphe Lemée**). On the bottom it ejects thrycocyst nets (or tangles!) (**Giorgio Honsell**) and secretes mucilaginous material in which it sometimes attains attains very high cell concentrations (**Oriana Blasutto**), sharing its bioingeneered space with a variety of other organisms (**Roberta Congestri**). Mediterranean and Atlantic strains form a single panmictic population that differ from the Indo-Pacific one (**Antonella Penna**). A new genotype has recently been isolated from the Canaries and Eastern Mediterranean (**Katerina Aligizaki**). Some details of *Ostreopsis* life histories are emerging (**Isabel Bravo**). Several studies report the preferences of *O. ovata* in culture, for temperature, light, and nutrient conditions (**Marina Montresor**, **Rossella Pistocchi**, **Nayani Vidyarathna**), while other culture results are being used to interpret field data (**Veronique Sechet**). *Ostreopsis* cf. *siamensis* is recorded from Atlantic coasts, in Maroccan (**Asmae Bennouna**) and Portuguese waters (**Ana Amorin**), the latter in 38^{0}N, its most northerly occurrence so far known. Both *O. ovata* and *siamensis* are found along Japanese coasts, where ITS sequence analyses show that strains of Mediterranean-Atlantic populations of *O.* cf *ovata* are also found in those waters (**Shinya Sato**). Two new species of the related genus *Gambierdiscus* have been isolated from the Canaries and Eastern Mediterranean (**Santiago Fraga**), the former with both CTX-like and MTX-like toxicity, the latter lacking MTX-like toxicity (**Amandine Caillaud**).

Karenia mikimotoi practises phagotrophy when offered bacteria, *Isochrysis*, and even artificial microspheres (**Qingchun Zhang**) – so too does *Prorocentrum donghaiense* which can feed on *Skeletonema* (**Linjian Ou**). A suggestive correlation was found between *Karlodinium veneficum* and *Cryptomonas* in the Lesvos work mentioned earlier[11]. For many years, it has been assumed that *K. mikimotoi* was an introduction to European waters, since it was first reported in 1966. New evidence from genetic analysis of CPR silks now indicates that it was present in this region at least some years earlier (**Manal Al-Kandari**); *corrections* of this nature may appear more frequently in the future as such analyses become more widespread, and may need to be taken into account in time-series studies.

The most recently described harmful dinoflagellate, *Azadinium spinosum*, is now recorded in southwest Atlantic waters (**Rut Akselman**), and along Pacific Mexican coasts (**David Hernández-Becerril**): there are now three known species, *Azadinium obesum*, which does not produce azaspiracid, another new non-toxic species under description (**Urban Tillman**), with new challenges for monitoring operations. The only toxic species in the genus, *A. spinosum*, is hardly distinguishable from its congeneric non-harmful species, despite the presence of a very small posterior spine. Molecular probes (**Kerstin Töbe**) are being developed that will help monitoring, and clarify the biogeography of these tiny species.

James Lovelock[12] introduces the Greek earth mother Gaia as a metaphor of the biosphere, atmosphere, oceans, and soil, "the totality constituting a feedback of cybernetic systems which seeks an optimal physical and chemical environment for life on this planet." The key feature of Gaia is a set of *negative* feedback mechanisms which give the system the ability to home in on a stable state, in a modelling idiom, to prevent the system from *blowing up*.

Peter Ward[13] adds Medea to the new pantheon of ecology. While Gaia nurtures her children, Medea consumes them; Ward's thesis is anti-Gaian, it does blow up. Medea is a metaphor of the dystopian scenarios provoked by life itself. The key feature here is *positive* feedback. The *great oxidation* already mentioned is an example. Aided by the evolution of the typical tubular guts of triploblastic animals, which package wastes so that they sink instead of floating around in the neighbourhood (like the invention of drains!), the oxygen produced by photosynthesis was able to accumulate in the upper water column of ancient seas - positive feedback. The use of clay to coagulate and precipitate blooms of noxious algae imitates the packaging process (**Xihua Cao**, **Paolo Rivera**, **Kevin Sellner**, **Hyun-Jae Shin**, **Ting Wu**, and others).

We might add Niobe to this pantheon[14]; she loses all her numerous children, and can stand for oligotrophy and the end state of thermodynamics, perfect entropy or energy death. Gaia is the middle path between Medean and Niobean extremes. Other gods, like Helios and Vulcan, play the rôles of boundary conditions[15]. It may be possible to correct small disturbances which move ecosystems away from the Gaian ideal by appropriate management strategies, but larger disturbances towards either Medea or Niobe may alter our life support systems in ways that we can neither anticipate nor reverse.

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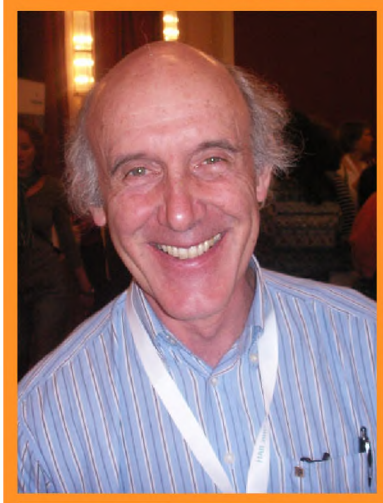


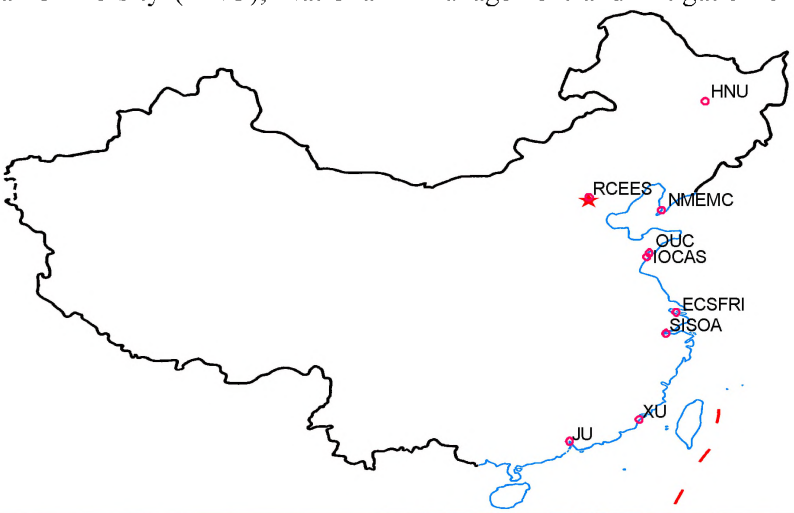




Chinese involvement in international HAB studies

14th International Conference on Harmful Algae

About 40 Chinese scientists and graduate students took part in the 14th International Conference of Harmful Algae held in Crete, Greece from November 1-5, 2010. They came from a variety of institutions and universities in China, including the Institute of Oceanology, Chinese Academy of Sciences (IOCAS) in Qingdao, Jinan University (JU) in Guangzhou, Ocean University of China (OUC) in Qingdao, Xiamen University (XU), Harbin Normal University (HNU), National Marine Environmental Monitoring Center (NMEMC) of the State Ocean Administration (SOA) in Dalian, The Second Institute of SOA in Hangzhou, East China Sea Fisheries Research Institute (ECSFRI) in Shanghai, Research Center for Eco-Environment Sciences, CAS (RCEES) in Beijing, as well as those working or studying abroad. Their study results on the genetics, biological interactions, population dynamics, impacts, management and mitigation of HABs were presented in oral or poster sessions.





China has experienced HAB problems in her coastal waters since the 1970s. Since then, many projects have been set up supported by the National Natural Science Foundation of China (NSFC), Ministry of Science and Technology (MoST) and Chinese Academy of Sciences (CAS). And a systematic HAB monitoring network has been established by the State Ocean Administration (SOA). After the beginning of the new century, an apparent "evolution" of HABs in coastal waters of China, with more harmful and extensive blooms, was observed. Large-scale dinoflagellate blooms of *Prorocentrum donghaiense* was first recorded in the sea area south to the Changjiang (Yangtze) River estuary, which became a recurrent phenomenon in this region in the last ten years. The extensive dinoflagellate blooms, sometimes affecting an area over 10,000 km^2, posed potent threats to the services and value of the local marine ecosystem including seafood safety. In the sea area north to the estuary of the Changjiang River, a bloom of green alga *Ulva* (*Enteromorpha*) *prolifera* first occurred in 2007 and then appeared every summer in the following years. The rapid expansion and evolution of HABs in coastal waters of China drew great attention from the Chinese government, scientific community and public. A group of research projects and monitoring programmes were launched, including a new national HAB research project - CEOHAB II under the support of MoST, focusing on the "evolution" of HABs along Chinese coasts. Chinese scientists are also actively involved in international HAB related research and management activities such as GEOHAB, EASTHAB, the ICHA conferences series and others.

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Genetics and genomics at ICHA 14



For the first time, this conference included an expanded section on the genetics and genomics of HAB organisms, as a result of the exciting technological advances that now allow us to ask deeper questions, gain a new perspective on factors underlying bloom dynamics and toxin dynamics, and routinely use genetic tools for monitoring purposes. As a taste of what was to come, **Linda Medlin** provided a comprehensive overview of what is known of the molecular evolution of dinoflagellates and what is yet to be determined; the genetic tools that have been applied to study intraspecific and interspecific diversity; and a summary of studies in phylogeography, plastid and mitochondrial genetics and evolution; genomics and the evolution of toxicity in dinoflagellates.

Perhaps the most exciting developments were studies on the genetic basis of toxin production, particularly saxitoxin, as these open up the possibility of new methods of studying HAB dynamics. **Allan Cembella** discussed an EST* sequencing project on *Alexandrium minutum*, that was then used to construct a microarray to determine genes that were differentially regulated. **Jeremiah Hackett** presented a comprehensive data set of Illumina and 454 sequencing of EST libraries from *Alexandrium tamarense*, *Pyrodinium bahamense* and *Gymnodinium catenatum*. Using this approach, sequences homologous to fourteen *sxt* genes from cyanobacteria were found, several of which appeared to differentiate evolutionary histories from one another. **Anke Stüken** showed that saxitoxin in dinoflagellates is clearly autonomously produced, as transcripts for *sxtA* from *Alexandrium fundyense* had polyA tails on their 3' ends and dinoflagellate specific spliced leader sequences on their 5' ends, as deduced from EST sequencing and RACE* studies of *Alexandrium* species. **Shauna Murray** found that *sxtA4* existed in multiple copies in *Alexandrium catenella*, and used the sequence to design a qPCR* assay, specific to *Alexandrium* strains which contained this gene. Using this approach, they detected *sxtA4* in a bloom of *Alexandrium catenella* that resulted in toxin uptake in Sydney rock oysters.

The search for genetic markers of bloom formation and decline were the subject of several presentations. In studies of *Karenia brevis* bloom decline, **Jillian Johnson** found that 29% of the genes assessed changed in their transcription patterns during changes from logarithmic to stationary growth phases, based on microarray and qPCR studies. Fourfold changes in gene expression in another kareniaceaen, *Karlodinium veneficum*, related to the light and dark periods, were the subject of a study by **Holly Bowers**. The genetic basis of cell cycle regulation, and its role in population growth in *Alexandrium fundyense*, was examined by **Yunyun Zhuang**, who sequenced full length transcripts of mitotic cyclin and examined their expression throughout the cell cycle. A study by **Allen Place** of the prevalence of the replacement of thymine by 5-hydroxymethyluracil in the genomic DNA of dinoflagellates found that it appeared to be restricted to the core dinoflagellates, and was absent in the closely related genera *Amoebophyra, Oxyrrhis* and *Perkinsus*.

Our understanding of population structure and phylogeography of HAB taxa continues to improve as new comprehensive studies of large numbers of cultures are undertaken, using methods such as AFLPs*, microsatellite markers and fast evolving rDNA regions. **Mindy Richlen** showed that local populations of *Alexandrium fundyense* in the Nauset Marsh System in Massachusetts, USA, were genetically isolated from those in the Gulf of Maine. **Pia Mäenpää** used AFLP markers to analyse 180 strains of *Alexandrium ostenfeldii* from the Baltic and found high genetic diversity. **Karin Rengefors** used AFLP analyses to study genetic differences in *Scrippsiella hangoei* and *Polarella glacialis* in Antarctic saline lakes. A statistical parsimony analysis of rDNA sequences was used to determine that populations of *Ostreopsis* cf *ovata* appeared to be limited to particular geographical regions by **Antonella Penna**. **Karen Lebret** examined population structure using AFLPs in the raphidophyte *Gonyostomum semen*. Microsatellite markers were used by **Anna Godhe** to analyse population structure of *Skeletonema marinoi* and determine the degree of genetic differentiation between benthic resting stages inside a fjord and in the surrounding open sea. **Sylvie Tesson** used a combination of microsatellite markers and ITS* sequences to determine that 2 genetically distinct population of *Pseudo-nitzschia multistriata* occurred in the Gulf of Naples. In this context, the finding by **Senjie Lin** of extensive within genome genetic polymorphisms in rRNA genes of clonal strains of *Alexandrium fundyense* is a reminder of the complexity of studying dinoflagellate intraspecific diversity.

Phylogenetics, particularly of newly discovered toxic species and strains, was the subject of a large number of studies. In line with the strong emphasis on benthic HAB-causing dinoflagellates, these tended to be from benthic habitats. **Lesley Rhodes** described the relationships of the pinnatoxin-producing peridinioid dinoflagellate *Portodinium honu.* Several new lineages of toxic *Gambierdiscus* species were discussed **(Mahfuzur Shah, Santiago Fraga)**, as well as new clades of *Ostreopsis* **(Shinya Sato, Mariangela Menezes, Atsushi Nakashima)** and *Bysmatrum* **(Cecilia Satta)**. New insights into the evolution and phylogeny of *Dinophysis* species and their kleptoplastids were discussed in several studies **(Nicolás Raho, Katerina Aligizaki, Sara Handy, Jennifer Wisecaver)**.

Newly developed qPCR detection tools were the subject of several studies, including for the detection of *Ostreopsis* cf *ovata* **(Cecilia Battocchi)**, six *Gambierdiscus* species **(Wayne**

Climate Change at ICHA14

14th International Conference on Harmful Algae

Greek mythology delivered us the concepts of Chaos, Gaia (earth), Ouranus (sky), Oceanus and Tethys. The Creta Maris resort in Hersonissos provided the HAB community with the perfect "summer camp" theatre to play out our usual suspects.

While climate change had occasionally been raised in previous HAB meetings (eg. Fraga & Bakun commenting on *Gymnodinium catenatum* in relation to Iberian upwelling; Barrie Dale's long-term dinoflagellate cyst core records from the Kattegat; both at the Rhode Island 1991 meeting), the ICHA14 in Crete for the first time delivered a solid body of 50 papers and posters on this topic. Climate change was represented by a plenary talk, but also featured in sessions on Cyanobacterial Ecology, Time Series of Harmful Algae Bloom events, and Population Dynamics of HABs. **Gustaaf Hallegraeff** presented an overview on the formidable challenge of predicting behavior of HABs in response to multifactorial climate change: increased temperature, enhanced surface stratification, alteration of ocean currents, sealevel rise, extreme weather events of rainfall and dust storms, elevated CO_2 stimulating photosynthesis but also causing ocean acidification etc. We need to design realistic laboratory simulation experiments incorporating complex factor interactions and allowing for adequate acclimation. Organism responses will be species or even strain-specific, and there will be winners and losers from climate change.

Beatriz Reguera explored *Dinophysis acuminata/acuta* time series in relation to thermal stratification in Iberian waters. **Pat Tester** alerted the audience to the fact that range expansions of benthic HABS including *Gambierdiscus* and *Ostreopsis* are the ones to watch. *Karenia brevis* is predicted to produce larger biomass blooms under increased CO_2 (**Reagan Errera**). Increasingly we are discovering climate feedback mechanisms (e.g., Antarctic ozone-hole driving circumpolar westerly winds enhancing deep ocean CO_2 venting), never predicted from first order principles. We should never lose the perspective that climate change is a matter of scale and time. Cyanobacteria evolved on our planet at 500,000 ppm atmospheric CO_2; on a geological scale the last glacial period was nothing but a "cold weekend", and many gonyaulacoid and protoperidinioid cyst taxa survived the K/T climate catastrophe without a blink (**Barrie Dale**). HABs have been studied for 40 years only, and strictly speaking we need at least 100 yrs of data before we can confidently speak of climate change (Dale). Crying wolf does not serve our discipline and we need to refrain from making unsubstantiated climate



predictions!

The best recent plankton archive available is that offered by the Plymouth based continuous plankton recorder (CPR) surveys (since the 1940s; **Chris Reid**). Even though initially focused on zooplankton, these formalin preserved plankton silks provide an unexpectedly well preserved phytoplankton record which can be interrogated using electron microscopy (Hallegraeff) and even molecular sequencing (**Rowena Stern**). **Stephanie Hinder** reported on changes in bloom window for *Dinophysis*, *Pseudo-nitzschia* and *Protoperidinium* in the North Atlantic which could lead to a mismatch with higher trophic levels. CPR records of calcareous coccolithophorids and

(Continued on p. 12)

Litaker), and *Pseudo-nitzschia* species (**Karl Andree**). Allele specific qPCR was used to detect specific populations of *Alexandrium tamarense*, that differed in their production of potential allelochemicals (**Uwe John**). Allelochemicals were found to have strong negative impacts on grazing pressure but positive intra-specific impacts, based on this method.

The detection of toxic species using microarray approaches, as part of the large scale EU funded MIDTAL (MIcroarrays for the Detection of Toxic ALgae) project, were the subject of a series of presentations (**Katrina Campbell**, **Jixen Chen**, **Jane Lewis**, **Johannes Hagstrom**, **Gary McCoy**) describing preliminary results and methodological development and testing.

An exciting development was the first metagenomic analyses of HAB taxa based on 454 sequencing of environmental samples (**Satoshi Nagai**). This approach detected the presence of more than 1500 unique sequences of 18S rDNA in the samples, comprising 21 algal classes.

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*EST = expressed sequence tag
RACE = rapid amplification of c DNA ends
qPCR = quantitative real time polymerase chain reaction
AFLP = amplified fragment length polymorphism
ITS = internal transcribed spacer

(Continued from p. 12)

Foraminifera in the North Atlantic show increased abundance in the past decades, in apparent contradiction to expressed concerns on impacts from ocean acidification.

Edna Granéli and **Lasse Nielsen** demonstrated results from ocean acidification experiments in swimming pools and plankton wheels, respectively. Coastal plankton appears to tolerate well pH changes predicted by the end of the century (pH=7.6). Escalating interest in the phenomenon of ocean acidification and climate models has brought to light our poor understanding of ocean biogeochemistry. Climate change offers the opportunity of significant new investment in more work on carbon and nutrient cycling.

Paul Harrison reviewed the impact of sewage diversion on Hong Kong red tides, only to conclude that hydrodynamic forcing (low wind events) plays a greater role than N, P and Si nutrients, with nutrient concentrations rather than ratios most important. **Jennifer Martin** showed an association between *Alexandrium* blooms in the Bay of Fundy and long periods of fog. **Dedmer van de Waal** predicted impact from climate change on C:N stoichiometry and hence microcystin speciation in freshwater cyanobacteria. Climate change also can influence extreme weather events such as heavy rainfall and dust storms. Increased humics can reduce Fe availability (**Granéli**), while UV enhances N and P availability from humics (**Patricia Glibert**). **Cemal Saydam** sought to explain why Sahara dust is a more potent HAB fertilizer, based on complex interactions between Fe speciation, oxalic acid from fungi, solar intensity and cloud cover. Precipitation thresholds also drive Texas *Prymnesium parvum* blooms (**Daniel Roelke**). Only with significant new infrastructure investment in global ocean observation systems (e.g. IMOS in Australia, OOI in US, IOC-UNESCO's GEOHAB) can we hope to quantitatively forecast ocean-related risks to human health and safety, and shed light on the impact of climate variability on marine life and humans in general. Phytoplankton monitoring (continuous plankton recorder, flowcytobot, ecogenomics) provides a sensitive early warning for climate-driven perturbations to marine ecosystems and should play an important role in these endeavours.

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ASIMUTH

A collaborative project entitled Applied Simulations and Integrated Modeling for the Understanding of Toxic and Harmful Algal Blooms: "ASIMUTH" held its initial partner meeting in Cork Ireland on the 14 and 15 December 2010. This FP7 funded project has 11 research and SME partners from Ireland, France, Spain, UK and Portugal. Through the ASIMUTH project, scientists and industry from these countries along Europe's Atlantic Margin will form a network to produce the first realistic HAB advisory and forecasting capability to the European aquaculture industry. The project will be an applied downstream service of the MyOcean core project of Global Monitoring for Environment and Security (GMES) which is a joint initiative of the European Commission and European Space Agency, which aims at achieving an autonomous and operational Earth observation (EO) capacity. Using Satellite observations, a variety of model products and the outputs of core monitoring programmes an early warning of severe blooms will allow fish and shellfish farmers to adapt their culture and harvesting practices in time, in order to reduce potential losses.



In recent years there has been much discussion of satellites being able to track surface algal blooms. Understanding biological phenomena in the ocean requires a complex approach, though there is some merit in using satellite derived chlorophyll images to delineate high biomass near surface algal blooms. Much cutting edge HAB research work has focused on subsurface profiles, where certain HAB species are present in thin layers of limited geographical extent often associated with strong density interfaces. Clearly, in order for a toxic/harmful algal bloom forecast to be realistic, physical factors including changes in water column structure and transport pathways are necessary. ASIMUTH is the next step towards providing an operational advisory service by integrating these physical drivers (derived from GMES downstream services) with all available biotoxin, phytoplankton count and bioassay data. A distributed advisory service and thematic experts distributed across the participating countries will network to provide regular advisory products and forecasts of impending toxic and harmful algal events. ASIMUTH brings together experts in the areas of Modelling, Earth Observation, HAB monitoring programmes and end users to assemble a number of key data sets and explore forecasting options.

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Parameters


Fish kill by *Dictyocha californica* in Banderas Bay, Jalisco, México

Microscopic examination of phytoplankton samples in April 2009 from Banderas Bay revealed widespread abundance of silicoflagellates: dense patches lasted for 5 days. High densities of *Dictyocha californica* Schrader *et* Murray dominated the silicoflagellate assemblages in the discoloured area. Surface phytoplankton samples were collected from several sites in the discoloured area on April 16, 17 and 18, 2009, stored in 50 ml plastic bottles preserved with Lugol-acetate solution. Living and preserved material was used for identification of species, and morphological details were observed using scanning electron microscopy (SEM); fine structure information was needed to validate the identification of some species. Phytoplankton abundance was determined by standard Utermöhl technique [1], and counts made with 100x magnification in 10 ml aliquots using a 1mm^2 scale Whipple disc, with double cross pattern of 100 µm. Temperature was recorded with HOBO a temperature/light data logger.

Information on silicoflagellates in Banderas Bay is scarce [2, 3] and the studies are mainly concentrated on the Gulf of California and some areas of the Mexican Pacific Ocean [4, 5]. This is the first record of a Dictyochales bloom in Banderas Bay, and was associated with sardine mortalities. Satellite imagery shows increased chlorophyll *a* at the time of the dense algal bloom (Fig. 1). The mean temperature was 19.7 ºC, and the chlorophyll *a* averaged 7.6 mg^{-3} (Table 1). No toxicity or human health problems have been reported in Banderas Bay linked to silicoflagellates, only hundreds of dead fish (this study).

Table 1. Data for each locality in the study area.

Station	Latitude	Longitude	Depth (m)	Temp ºC	Chl *a*	Secchi (m)
Bucerías	20º40'06"	105º16'40"	6.50	17.5-19.9	7.6	6.50
Nuevo Vallarta	20º40'05"	105º14'55"	7.0	17.5-19.9	7.6	5.00

Fish mortalities related to plankton blooms have been observed before in Banderas Bay, associated with dinoflagellates such as *Cochlodinium catenatum* [6], and the euglenophyte *Eutreptiella marina* [7]. This is the first report of fish kills, of sardines, associated with *Dictyocha californica* in the Mexican Pacific. Dead fish were observed in the north part of bay, and washed onshore (16 April was the peak mortality day). Fish were observed gasping at the surface as well freshly killed. Hundreds of sardine were seen along the Nuevo Vallarta and Bucerías a few meters from the beach, where the high density of *Dictyocha californica* was found (Fig. 2). Toxin profiles were not measured, but the dead sardines were found free from infections.

Concentrations in the *Dictyocha californica* bloom reached 809 x 10^3 cell L^{-1} (Fig. 3A y C). *D. californica* skeletons are relatively large and elliptical with two longer radial spines in the mayor axis, and the two other spines in the minor axis; spines and axis are covered by clusters of small spines (Fig.3D). Living cells have a single flagellum and numerous chloroplasts (Figs. 3B). Other silicoflagellates identified in this study were *Dictyocha fibula*, *D. fibula* var. *robusta* and *D. octonaria*, but in minor abundance. Our observations agree with earlier records [2, 4] for the Mexican Pacific.

Abnormal production of *Dictyocha sp.* can clog fish gills and respiratory systems, causing suffocation. High densities of silicoflagellates can also cause hypoxia.

Acknowledgements

To Ph. E. Santamaría del Angel (FCM-UABC) for satellite imagery (MODIS-Aqua).

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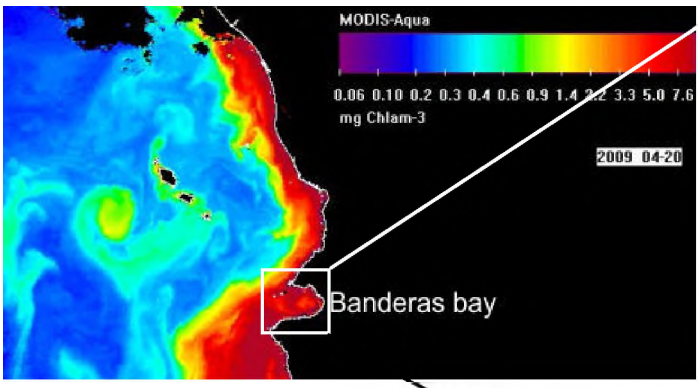

Fig.1. Chlorophyll distribution a concentration, during the Dictyocha californica *bloom in Banderas Bay. Modis-Aqua satellite imagery (April 2009).*

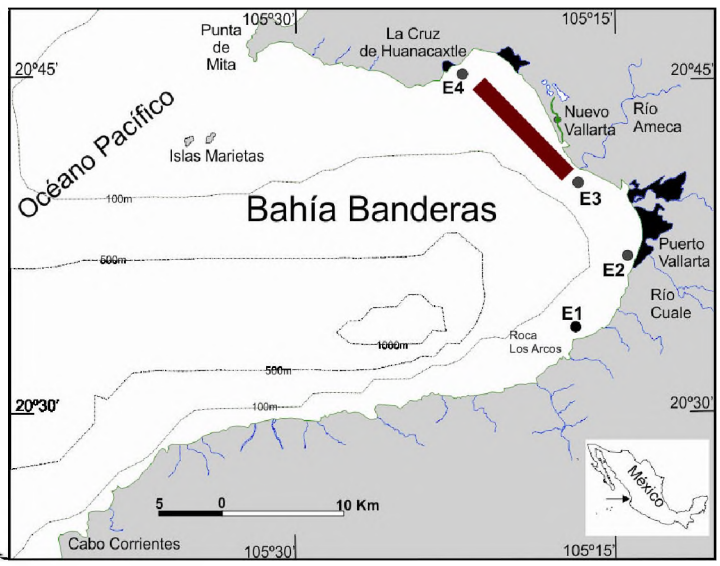

Fig. 2. Location of the HAB event in Banderas Bay , Jalisco, México and sampling stations. Approximate site red bloom.

Heterocapsa triquetra bloom in Golden Horn, Turkey

A red tide of the dinoflagellate *Heterocapsa triquetra* in the shallow uppermost section of the Golden Horn Estuary (GHE) between January and April 2007 was investigated concurrently with physical and chemical variables. The red tide caused orange-brownish surface discoloration. We sampled monthly at selected stations from January to June 2007 (Figure 1). The lower estuary has a depth of 40 m and is strongly mixed. The upper estuary has a maximum depth of 5 m and the circulation is much weaker. Samples were collected using 5 L Niskin bottles. Inorganic nutrients, dissolved oxygen, chlorophyll *a* were measured. Physical data was recorded by a SBE-Sea Logger 25 CTD system. For phytoplankton analysis, 1 L water samples were taken, fixed in buffered formaldehyde, settled for at least 48 h, concentrated to 100 ml, and stored in dark colored glass bottles until examination. The phytoplankton cells were counted with a Sedgewick-Rafter chamber under a light microscope.

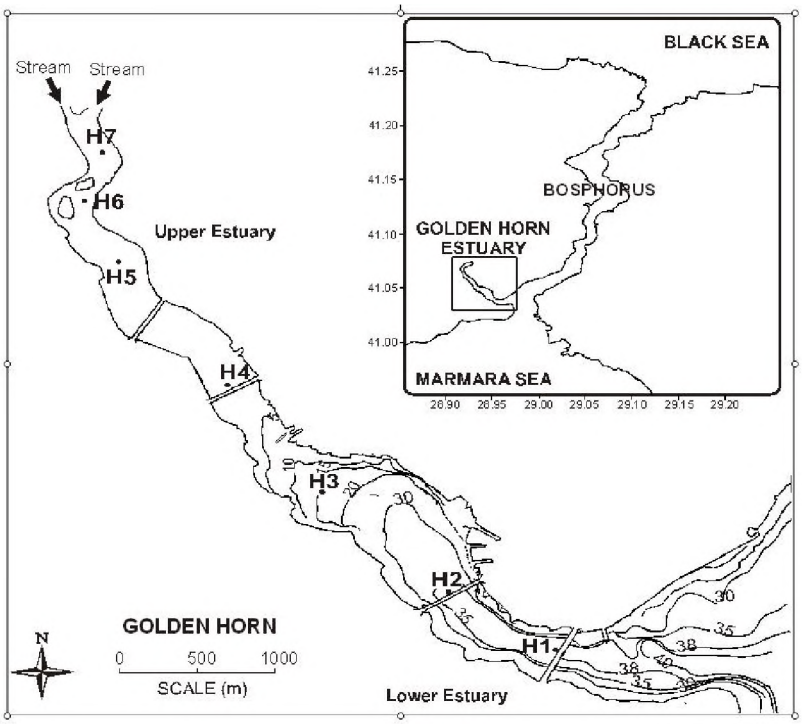

Fig. 1. Study area and sampling stations

During the red tide, temperature ranged from 9.1 ºC to 17.9 ºC and salinity from 18.8 to 19.7 psu in the lower estuary, and from 9.4 ºC to 24 ºC and from 15.2 to 17.2 psu in the shallow upper section. Secchi disc values were from 5 to 9 m in the lower estuary and between 0.2 and 1 m in the upper. *H. triquetra* appeared in the GHE between January and April 2007. Cell densities increased gradually from January, reached a peak in April, and decreased sharply in May; it was rarely detected in June (Fig. 2). *H. triquetra* concentrations were generally low in the lower estuary. Discolored water started in the upper estuary in mid-January 2007 when the water temperature was 9.5 °C and salinity 17.6 psu; at this time cell abundance was 2700 cell ml^{-1} and chlorophyll *a* concentrations exceeded 67 µg L^{-1}. In the upper estuary in February, *H. triquetra* cell densities reached 6000 cell ml^{-1} locally, and chlorophyll *a* 61 µg l^{-1}. In March, counts exceeded 10,000 cell ml^{-1} and chlorophyll *a* was 82 µg L^{-1}.

H. triquetra comprised 100% of the phytoplankton in the upper estuary during the red tide; surface densities there peaked at >19,000 cell ml^{-1} in April (Fig. 2). The highest chlorophyll *a* concentrations (104 µg L^{-1}) and supersaturated DO (20.4 mg L^{-1}) were recorded at this time (Fig. 4). The secchi depth decreased to 0.1 m at Station H7.

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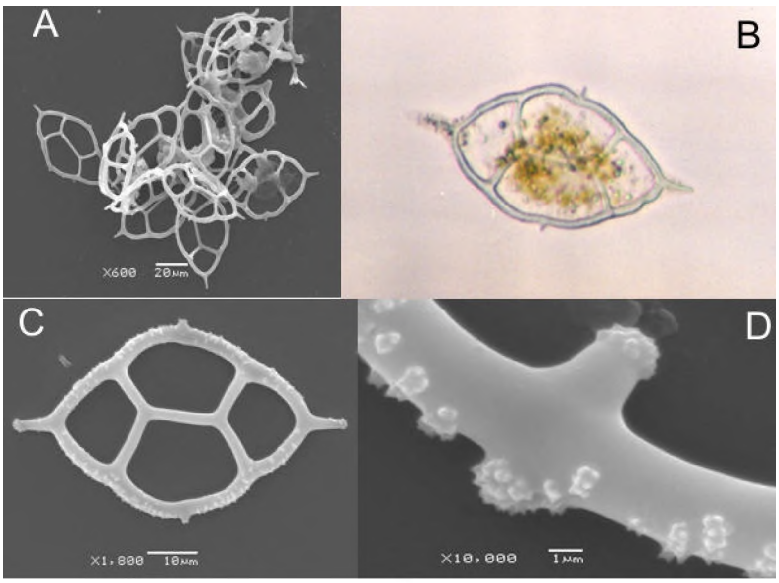

Fig. 3. Dictyocha californica *in Banderas Bay, México. A) Several specimens of the bloom. B) Specimen in vivo. C) Specimen in SEM. D) Detail of small spines.*

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Toxics blooms of *Microcystis aeruginosa* in three Río Negro reservoirs, Uruguay



Fig. 1. Map showing the study area with Bonete, Baygorria and Palmar reservoirs.

The Rio Negro is the most important internal river of Uruguay, it is 810 Km long and drains a basin of 69100 km^2 (Fig. 1). Along the river there is a system of three reservoirs constructed for hydropower generation and actually utilized for multiple uses. The main features of Bonete, Baygorria and Palmar reservoirs are shown in table 1.

The main activities at the Rio Negro basin are cattle breading, agriculture and forestation. These human activities can be considered as the main sources of nutrients to water ecosystems. Previous studies at this system revealed the eutrophic status of the reservoirs [1]. They have a dendritic shape with arms where the retention time is higher than in the main body, favouring the development of algal blooms. The cyanobacteria commonly occur in the phytoplankton of lake and reservoirs and sometime develop as blooms, cause deoxygenation, toxins production and nuisance odours [2]. For Uruguay they are reported since 1982 at various ecosystems such as, Salto Grande reservoir in the Uruguay river and in the La Plata river among others [3–6]. The presence of potentially toxic algae in the three reservoirs of the Rio Negro is frequent, especially in the summer months, though not always bloom events are generated, since they depend on particularly environmental and hydrological conditions.

Table 1. Hydrological caracteristics of the reservoirs

Parameter	Bonete	Baygorria	Palmar
Location	32°49'S	32°53'	33°03'S
	56°25'W	56°48'W	57°27'W
Constructed (year)	1945	1960	1982
Area (km^2)	1070	100	320
Volume (km^3)	8.80	0.57	2.86
Max. Depth (m)	32	20	15
Residence time (d)	150	3	16

Many species are potential producers of cyanotoxins as (microcystin, saxitoxin, anatoxin, cylindrospermopsin), and constitute a threat to human and animal health.

The main species of cyanobacteria potentially toxic reported in the three study reservoirs belong to the genus *Microcystis* and *Dolichospermum* being *Microcystis aeruginosa* the most common species founded.

This specie is distributed widely, and is the main microcystin producer in lakes [7, 8].

This study was conducted in summer, between december 2009 to march 2010 under the water quality monitoring program of the Rio Negro reservoirs carried out by the Limnology Section, Faculty of Sciences in partnership with the national energy agency (UTE). For this study Bonete, Palmar and Baygorria reservoirs were sampled monthly, in both a sampling station near the dam, and in a constrained arm of the main water body. Water samples for chemical analysis were taken at surface with a Ruttner bottle and kept cold until processing. There were recorded *in situ* the water temperature, dissolved oxygen, conductivity, pH, Secchi disc depth and the underwater photosynthetically available radiation (PAR), was measured with a Quantum Radiometer (Licor Instruments). Quantitative phytoplankton samples were collected from the surface and from the end of the euphotic zone (1% of subsurface light intensity) through a Ruttner bottle and integrated into a single sample. Quantitative samples were analyzed using the Uthermöhl method. Also qualitative phytoplankton samples were taken with a plankton net (20 µm mesh size) for taxonomical identification. Samples for quantitative estimation of microcystin-LR were collected from areas of accumulation of cyanobacterial blooms. The microcystin-LR was



Fig. 2. Aereal photography of Microcystis aeruginosa bloom (Palmar reservoir march 2010).

Table 2. Physics and chemicals condition during January 2010 in the three reservoirs.

Parameter	Reservoirs					
	Bonete center	Bonete arm	Baygorria center	Baygorria arm	Palmar center	Palmar arm
Temperature (°C)	27.3	27.2	28.5	28.6	28.1	28.9
pH	8.52	8.7	8.95	8.88	9.3	9.9
Secchi disc (mts.)	0.9	0.8	0.8	1	0.7	0.6
Kd (m^{-1})	0.35	0.31	0.31	0.43	0.7	0.26
OD ($mg.l^{-1}$)	8.1	8	9	8.7	8.8	9.6
Conductivity (μScm^{-1})	67.9	68.1	73.2	73.1	76.1	83
Ammonium ($\mu g.l^{-1}$)	0	0	0	0	0	0
Nitrate ($\mu g.l^{-1}$)	99.8	95.5	95.5	73.9	95.5	95.5
Nitrogen total ($\mu g.l^{-1}$)	446	392.5	481.7	562	695.9	945.8
Phosphate ($\mu g.l^{-1}$)	52.1	47.4	59.9	50.5	81.8	64.6
Phosphorus total ($\mu g.l^{-1}$)	178.11	164.69	198.24	225.06	202.70	263.07

determined by HPLC.

Dense *Microcystis* blooms were observed from January to March in the three reservoirs (Fig. 2).

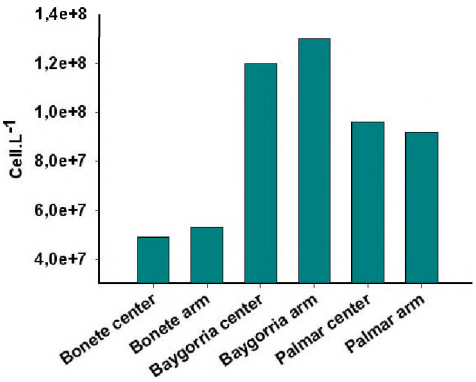

Fig. 3. Abundance (cells L^{-1}) of Microcystis aeruginosa *at six samples station in the Bonete, Baygorria and Palmar reservoirs in January.*

The highest concentration occurred in January simultaneously in the three reservoirs. Baygorria reservoir showed the highest abundance of *M. aeruginosa* at arm station (1.3 x 10^8 cells.L^{-1}), while the minimum concentration was recorded in Bonete reservoir at the station near the dam (4.9 x 10^7 cells.L^{-1}), (Fig. 3). The environmental conditions recorded during January bloom are shown in (Table 2). The three reservoirs were characterized by high values of water temperature, transparency and nutrients concentration. These environmental conditions were maintained throughout the rest of study period.

While the primary function of dams is hydroelectric power generation they are also used for fishing, swimming and as a water source for animals, humans and irrigation. According to HPLC analysis of microcystin-LR, the three reservoirs showed toxicity during the summer months with an increase towards the end.

Table 3. Microcystin-LR concentration for the 3 reservoirs during January- March 2010 (values, $\mu g.L^{-1}$)

Reservoir	Microcystin-LR concentration		
	January	February	March
Bonete	294	197	2109
Baygorria	33	No	551
Palmar	34	2662	1478

Only Baygorria reservoir in February was not toxic. The highest microcystin level was recorded in Palmar reservoir with 2662 mg L^{-1} of extracellular microcystin-LR in February (Table 3).

This study reaffirms the importance of continuous monitoring of water quality, and the importance of studies to determine the causes of *Microcystis* blooms in freshwater ecosystems. In the presence of blooms, extreme care is necessary regarding the uses of water for recreation, for direct consumption by animals or the purification treatment.

Acknowledgements

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Cyanobacteria blooms in freshwater reservoirs of Central Cuba

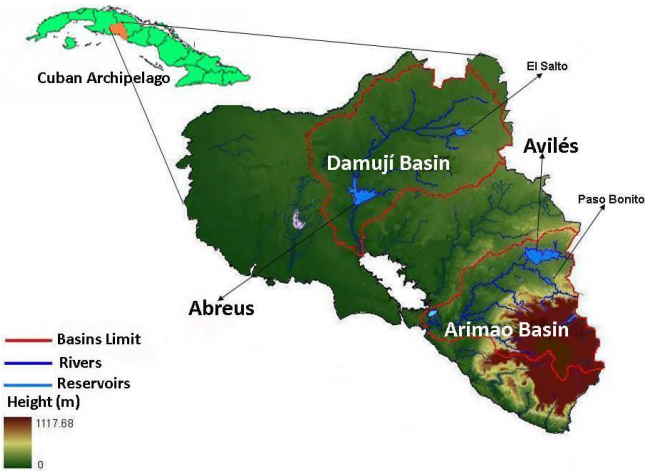


Fig. 1. Map showing Cienfuegos Province and its principal basins and reservoirs.

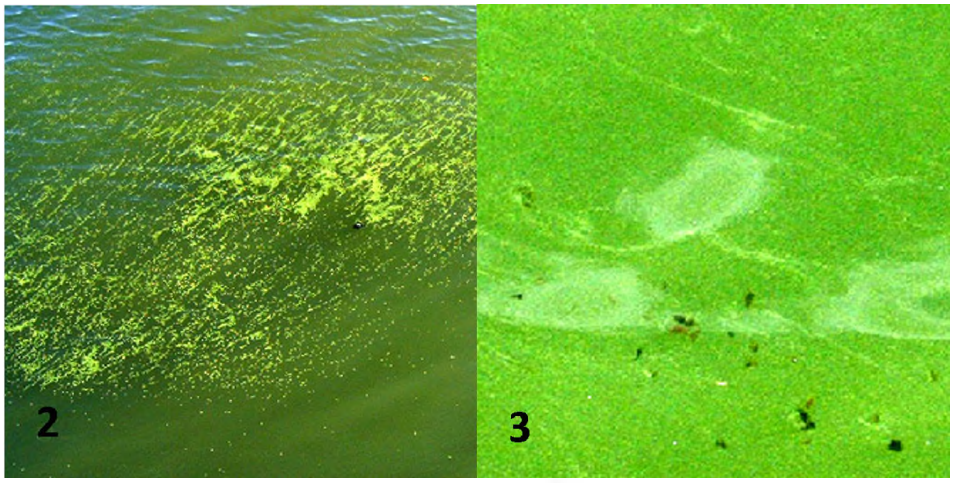
Water blooms were registered in Cuba by Komárek [1] in 16 localities distributed in several provinces, but not in the central part of Cuba. These communities were characterized by cyanobacterial taxa, among them a few *Microcystis* species (*M. aeruginosa* (Kütz.) Kütz, *M. flos-aquae* (Wittr.) Kirchner, *M. comperei* Kom. and *M. wesenbergii* (Kom.) Kom. No toxic events were reported.

Microcystis is morphologically very simple with wide infraspecific variation. Molecular analyses indicate genotype uniformity within this genus, so that species identification is difficult. However, the forms of colonies, cell size, and life cycle are different and characteristic in numerous natural populations and are used for their identification. Their phenotypic taxonomy is even important for investigations of the toxic effect of various *Microcystis* populations and cannot be underestimated.

In March 2009, typical water blooms were detected in two important reservoirs of the Province of Cienfuegos: "Embalse Abreus" and "Embalse Avilés" (Figs. 1-3). Chlorophyll *a* values were 33.52 and 48.92 µg/L respectively. The blooms were composed almost exclusively by two *Microcystis* species: *M. panniformis* Komárek *et al.* 2002 (Abreus), and *M. smithii* Kom. *et* Anagn. (Avilés) (Fig. 4). No toxic consequences were reported in either event.

M. panniformis forms micro- or macroscopic colonies, mainly irregular, rounded or elongated, but not clathrate, with cells densely aggregated in all the mucilage surface. Hyaline and inconspicuous mucilage does not overlap the cells agglomerations. Cells are 3-5 µm in diameter; cell content brownish green, with aerotopes. The species is common in plankton communities of eutrophic ponds, lakes and reservoirs, frequently forming blooms together with *M. aeruginosa* [2]. *M. panniformis* is frequently mentioned in the literature, especially in tropical freshwater bodies, but probably confused with *M. aeruginosa*, or also called *M. flos-aquae* (*M. aeruginosa* f. *flos-aquae* cited by Komárek [1] could be identical with *M. panniformis*). This species, which was involved in the Caruaru tragedy in Brazil in 1996 [3, 4], produces mycrocystins [5, 6].

The typical *M. smithii* forms colonies which are more or less spherical or slightly irregular, never clathrate, with scattered, rarely densely arranged cells. Cells 3-5 µm in diameter, blue-green, bright blue-green or olive-green, facultatively with one or several brownish aerotopes in each cell. According to Komárek & Anagnostidis [7] the taxon occurs in plankton of freshwater clear lakes. There are no available data about toxicity of this species. The Cuban population differs from the typical species by more irregular colonies, more densely arranged cells in the colonies, and also by its ecology.



Figs. 2-3. Microcystis *blooms in the Abreus (2) and Avilés (3) reservoirs.*

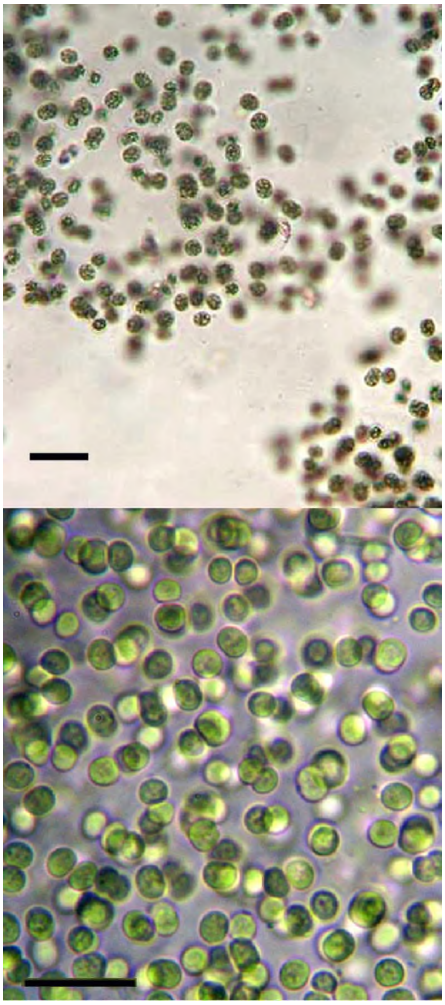


Fig. 4. Microcystis panniformis *above and* M. *cf.* smithii below. *Scales = 10 µm*

(continued from p. 15)

High nitrate + nitrite and low phosphate concentrations, and very high TN:TP ratios were found in the lower estuary. Nutrient levels and the TN:TP ratio decreased gradually from the middle to upper estuary. Trophic index (TRIX) values ranged from 6.01 at H1 to 8.17 at H6 when *H. triquetra* cell densities were highest. *H. triquetra* was most abundant at 13.4 °C and 16.6 psu for phytoplankton growth. A mesohaline frontal zone in the upper estuary with moderate salinity and the weak circulation created conditions for the *H. triquetra* bloom. Nutrient levels decreased sharply in the upper estuary during the bloom. Despite low inorganic nutrient levels, high TN and TP indicate that organic substances from two the streams influence particularly the upper estuary. *H. triquetra* is capable of oxygen depletion in the bottom waters, increasing pH, and low light transparancy can have negative effects on water quality.

Acknowledgements

Thank to the scientists, technicians and captain of R/V ARAR-I. Study supported by the General Directorate of Istanbul Water and Sewerage Administration.

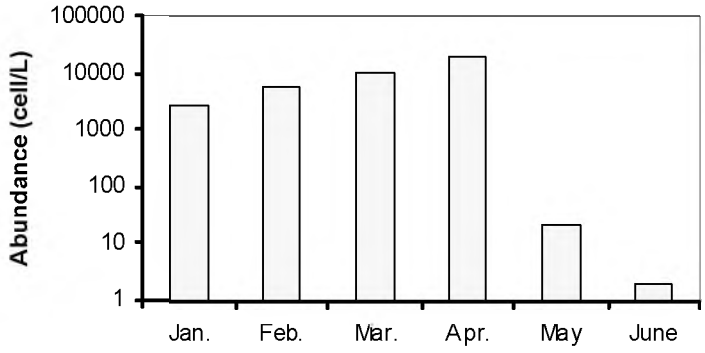


Fig. 2. Monthly variations of H. triquetra *abundance during the red tide.*

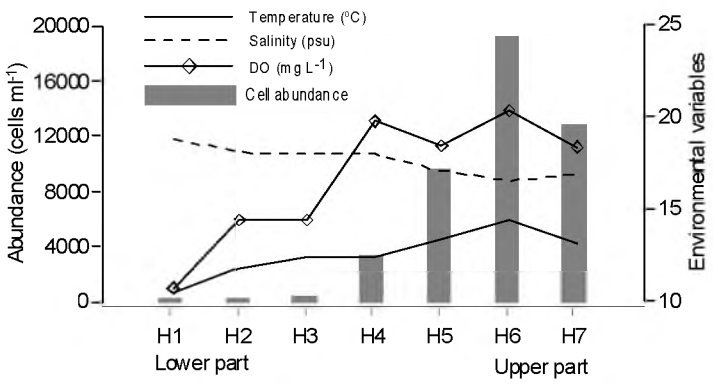


Fig. 3. Abundance of H. triquetra *and some environmental variables in April 2007.*

(Fig. 3). The increase in DO concentrations from the lower to upper estuary indicated the magnitude of the *H. triquetra* red tide. DO values reached more than 2 x SDO (saturated dissolved oxygen) values (DO=20.4 mg L^{-1}, SDO=9.4 mg L^{-1}, 217% saturation) in April 2007; surface pH was high (8.5) in the upper estuary at the time of bloom.

Two streams feed the GHE and cause natural eutrophication [1]. Runoff following rainfall in winter supplies inorganic and organic nutrients critical phagotrophy in nitrogen- and phosphorus-depleted media [2]. Thus, *H. triquetra* may feed mixotrophically on organic particles when inorganic nutrients are insufficient for growth. The TRIX values in April, between 6 and 8, indicate a eutrophic state but low water quality. The *H. triquetra* bloom may be a response to increasing eutrophication. Some *Heterocapsa* species may be harmful, but *H.triquetra* is non-toxic [3]. No toxic effects were associated with the *H.triquetra*bloom, but rapid

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Acknowledgements

To Water Quality Laboratory (CEAC) staff for their important contribution to sampling and analytical assays.

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Harmful Algae News

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A dinoflagellate producer of pinnatoxins

A scrippsielloid dinoflagellate has been shown to be the producer of pinnatoxins (Fig. 1), members of the family of toxic cyclic imines [1]. Cells, mainly in the non-motile form (Fig. 2), have been cultured and tested for toxin production by LC-MS and, up to the present time, pinnatoxin-producing cells have been obtained from New Zealand, Australia and Japan. All are maintained in the Cawthron Institute Culture Collection of Micro-algae (CICCM).

The history of pinnatoxin research has been well documented [2-6], including the discovery of pinnatoxins E, F and G in New Zealand and Australia [7]. However, the dinoflagellate that produced these compounds remained unknown until 2008, when it was found as pitted but otherwise unornamented dark brown cysts (30 µm diam.) in surface sediment samples from the far north of New Zealand [1]. A full description of the new genus and species is in review [8].

Samples obtained from South Australia and positive for pinnatoxin (LC-MS) also contained scrippsielloid-like cells. Four cysts were successfully cultured and the toxicity of cell extracts determined [9]. A similar approach was employed with Japanese (Okinawa) samples resulting in two cysts being cultured. The cyst form is the most common in culture and it is relatively easy to produce mass cultures in a 10 litre plastic bag system. Motile cells (24-32 µm x 20-30 µm; Fig. 2) occur both in culture and in the 'wild', but the excystment trigger has not yet been determined and may involve circadian rhythms.

Eleven New Zealand isolates all produced pinnatoxin E and F, although at different concentrations, whereas

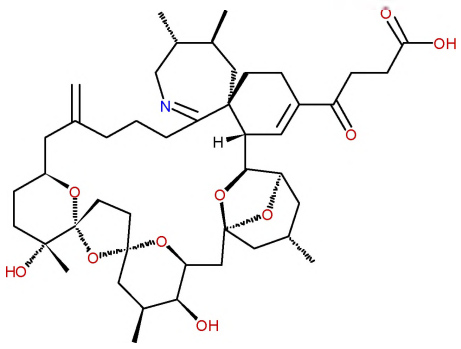

Fig. 1. The chemical structure of pinnatoxin D.

South Australian isolates produced predominantly pinnatoxin G, with lower concentrations of D and E. One isolate also produced pinnatoxin F. Japanese isolates produced pinnatoxin G only.

Based on LSU rDNA and ITS sequence data, the New Zealand/South Australian isolates were found to be identical, but appear to differ from the two Japanese isolates. Further investigations are being conducted in order to determine whether the Japanese isolates belong to a different species.

The toxicity of extracts of the dinoflagellates to mice is of interest, since the LD_{50} by oral administration is very similar to that achieved by intraperitoneal injection. With other cyclic imines, such as gymnodimine and spirolides, toxicity by oral administration is 10 to 1000-fold lower than that by intraperitoneal injection.

Bioassays to test the effect of the dinoflagellate to invertebrate larvae resulted in the death of sea urchin and abalone larvae, and caused sea slug larvae to become moribund. Pacific oyster larvae were mainly impacted when the dinoflagellate cells were disrupted and Greenshell™ mussel larvae were unaffected.

The pinnatoxins are not regulated and only became a topic of interest in New Zealand and Australia when shellfish extracts killed mice in routine bioassays. However, now that the mouse bioassay has been dropped as a requirement by the European biotoxin regulators the closures enforced on the basis of the mouse bioassay no longer occur. No illnesses have ever been reported in Northland related to the consumption of Pacific oysters from areas regularly associated with pinnatoxin contamination, despite the consumption of shellfish gathered from such areas by locals throughout the last fifteen years.

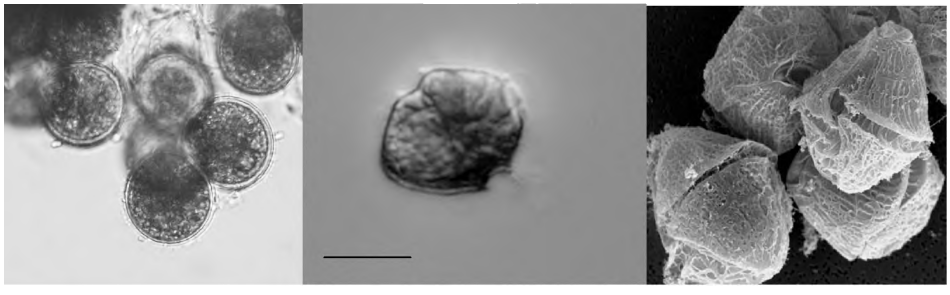

Fig. 2. Light micrographs of cultured non-motile cells (L; 30 µm diam.) and motile cell (centre; bar = 16 µm) of the pinnatoxin E and F-producing dinoflagellate from Rangaunu Harbour, Northland, New Zealand. Scanning electron micrographs (right) of motile cells (approx. 32 x 29 µm).

Acknowledgements

J. Adamson, S. Molenaar and K. Ponikla (Cawthron Institute) for technical assistance; C. Wilkinson (PIRSA, Australia) for samples; NZ Foundation for RST for funding.

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A *Microcystis* bloom under the ice

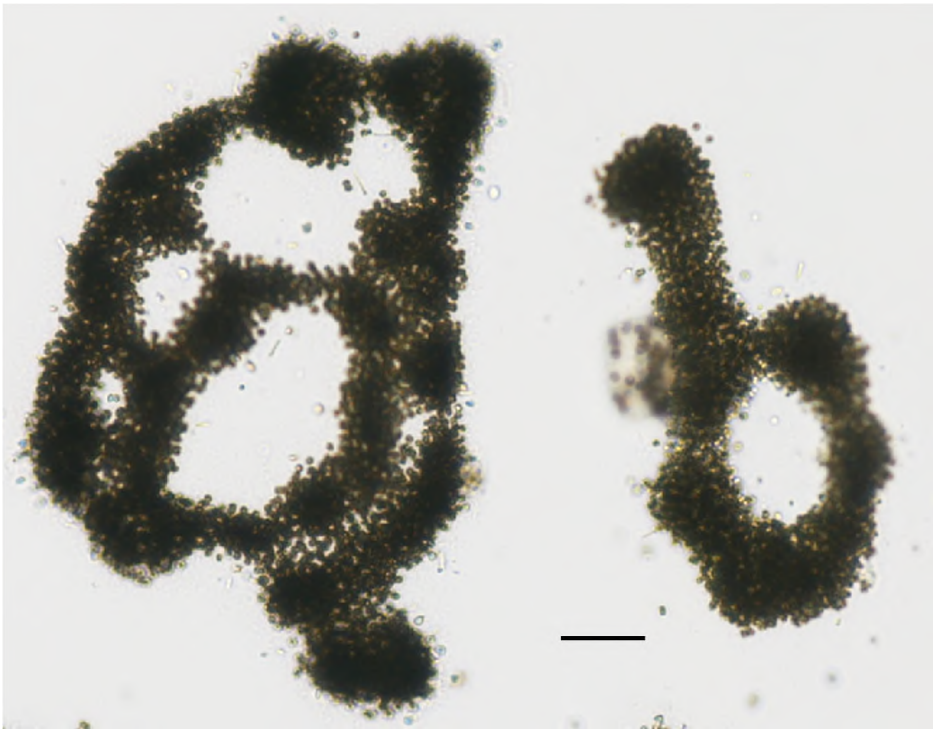

Fig. 1. Light micrograph of Microcystis aeruginosa *colonies collected from the water column of Lake Kastoria. Scale Bar: 50 ìm.*

Microcystis is one of the most common genera dominating the phytoplankton of eutrophic lakes worldwide while *M. aeruginosa* is the best known bloom forming species and a major cause of hepatotoxic blooms [1]. Cyanobacterial blooms are common in eutrophic freshwaters in Greece and cyanobacterial toxin-producing species have been found in all freshwaters studied [2].

Microcystis is known to overwinter on the sediment surface of lakes and re-inoculate the water column in spring from which the epilimnetic population develops in the following summer, as a life strategy [3]. However, it has been also reported that small populations of this cyanobacterium can overwinter as pelagic ones [4] but relevant data are limited [5].

Lake Kastoria is known to host toxic cyanobacterial blooms characterized by high microcystin (MC-LR) concentrations [6]. *Microcystis* morphospecies dominate in the late succesional stage of phytoplankton while during the winter a few colonies were observed on the sediment surface [7]. These morphospecies belong to phylotypes related to the toxin-producing *M. aeruginosa* [8].

A bloom of *Microcystis* morphospecies was recorded under the ice when the air temperature was below zero on 2 January 2009 in the eutrophic Lake Kastoria. During this winter bloom, very large viable colonies of *M. aeruginosa* (Fig. 1) were observed to be attached under the ice (Fig. 2) as well as in the water column. *M. aeruginosa* population density in the water column was high with 585 x 10^6 cells L^{-1} (or 11.7 mg L^{-1}w/w). At the same time, *M. wesenbergii* co-dominated the lake's phytoplankton with a population density of 34 x 10^6 cells L^{-1} (or 1.7 mg L^{-1}w/w). A large number of *Microcystis* single cells were also observed (25 x 10^6 cells L^{-1} or 0.43 mg L^{-1}w/w). Apart from cyanobacteria, cryptophytes were also present in the lake's phytoplankton in lower numbers (2.7 x 10^6 cells L^{-1} or 0.43 mg L^{-1}w/w).

Vasas *et al* [5] reported a large pelagic *Microcystis* (*M. viridis*) population frozen in the ice in a shallow pond near Debrecen, Hungary. Its cells were viable and toxin-producing providing inoculum for a strong toxic water bloom in the following summer. In Lake Kastoria, heavy blooms of *Microcystis* morphospecies were observed both in the summer of 2009 and 2010 (unpublished data).

These results may highlight the importance of the winter growing colonies besides the apparent role of the large sediment populations.

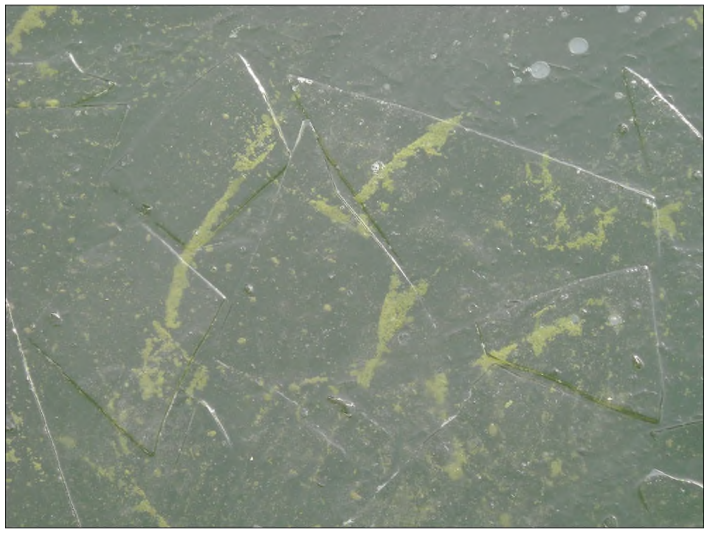

Fig. 2. Microcystis *colonies under the ice of Lake Kastoria.*

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ISSHA's Corner

The International Society for the Study of Harmful Algae (ISSHA) convened the 14th International Conference on Harmful Algae (ICHA14), held in Crete (Greece) (1-5 November 2010) and beautifully organized by the *Hellenic Centre for Marine Research* under the coordination of Dr Kalliopi Pagou. At the inauguration ceremony, a few minutes were dedicated to the memory of Patrick Gentien, who passed away last May.

On Thursday 4 November, the Seventh General Assembly of the Society was held. Beatriz Reguera (President) opened the meeting. The minutes from the previous assembly were approved and council members summarized the activities and achievements of ISSHA in the previous two years: Karin Rengefors (Secretary) presented the new ISSHA website and invited ISSHA members to provide input and criticism; Nina Lundholm (Treasurer) presented the financial statement, covering the period September 2008 to October 2010, and detailed expenditures and funds raised from member's fees, book sales, and the auction; Clarisse Odebrecht (Travel Awards Committee) thanked the Society and the generous sponsors of the ICHA 14 (FAO, NOAA, IAEA, SCOR, UNEP-MAP, Black Sea Commission) for their contributions that allowed financial support to 56 predoctoral students and experts from developing countries and economies in transition to attend the conference. Jennifer Martin (Achievement Awards) summarized the procedure for the elections of Lifetime achievement awards, young scientist award and the Maureen Keller student presentations awards. She announced the decision of the Council to rename the Young Scientist Awards the *Patrick Gentien Young Scientist Awards*.

Gustaaf Hallegraeff (Committee on Publications and Dissemination) presented new material uploaded to the webpage and plans for the future. The publication of the proceedings from the previous conference (ICHA13, Hong Kong 2008) was announced by Prof. Zhou (coeditor) on behalf of the main editor, Prof. K.C. Ho.

Representatives from Mexico, New Zealand and Turkey presented the bids from their countries to host the ICHA 16 Conference in 2016. The three venues were voted by ISSHA members in the following hours, and for the first time, members who were not able to attend the conference were able to vote online. New Zealand was elected as the venue for the ICHA16. ISSHA acknowledges the three countries which bid for their willingness to undertake the laborious task of organizing ICHA conferences. Prof. H.G. Kim, acoompanied by local authorities from his home town, enthusiastically presented ongoing activities for the organization of ICHA 15, to be held in Korea in autumn 2010.

The General Assembly was followed by the ISSHA Auction (which finished at midnight!). The compilation of donations was organized by Anke Kremp, and once more, the auction was a success thanks to the invaluable help of Barrie Dale. Over 11000 $ were collected, to be used mainly for student travel awards for the next conference. Some very valuable donations, such as the hand-



2010 Yasumoto Awards: Yasuwo Fukuyo receives a Takayama carving (a pair of Dinophysis fortii*) with the smiling approval of Prof. Yasumoto (Photo Y. Fukuyo)*



Don Anderson with the Korean jewellery box won at the ISSHA Auction raffle. (Photo D. Anderson)



2010 Yasumoto Awards. Barrie Dale receives a Takayama carving of an Alexandrium tamarense *motile cell and its cyst form. (Photo M. Lion)*

made marine tapestry by Esther Garces' mother, and the beautiful mother of pearl and lacquer jewelery boxes donated by the Korean delegates were raffled. The ISSHA gods blessed Barrie by letting him win the tapestry. Don Anderson was the lucky winner of an exquisite Korean jewelery box.

Achievement awards were announced at the conference banquet.

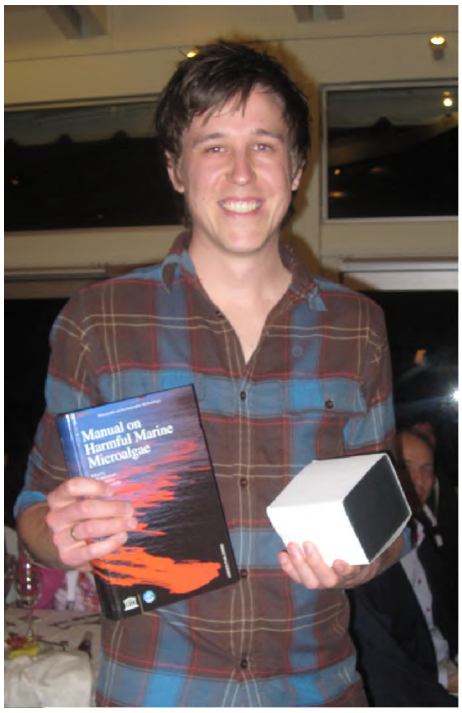
The ***Yasumoto Lifetime Achievement Award*** **2010** was given to Profs. **Yasuwo Fukuyo** and **Barrie Dale**. Short biographies of these two inspiring scientists and great communicators can be found at the *HAB Trail Blazers* section on the ISSHA website (www.issha.org).

Yasuwo Fukuyo was the taxonomist behind the breakthrough discoveries of the causative agents of Diarrhetic Shellfish Poisoning and Ciguatera in the late 1970's. He was a driving force in the promotion of HAB Studies in the WESTPAC region, and probably the taxonomist that has best contributed to the popularization of HABs worldwide with his beautiful plates of microalgae taxonomy and the impacts of HAB events. He felt extremely pleased to receive this award, and a massive carving of a dividing pair of *Dinophysis fortii* (from Prof.Takayama), in front of a smiling Prof. Yasumoto, and said he considered himself such a lucky man because he had the privilege of working with him.

Barrie Dale is "The Geologist" of our HAB community, always putting us back into the right perspective. He made key contributions in the field of dinoflagellate cyst biology and ecology, and in the applications of this research to fossil records. His communication skills excelled in his hilarious performances at the 2006, 2008 and 2010 ISSHA auctions. The Society is deeply indebted to him for them. When Barrie received his prize, and a beautiful carving of an *Alexandrium tamarense* vegetative cell and cyst, he expressed with emotion his view that the greatest thing a scientist can receive is the recognition of his colleagues.

Over 90 PhD students participated in the contest for student presentation awards. Awards for the best oral presentations were given to **Jillian Johnson** (*Global analysis of growth phase-associated transcriptomes in the toxic dinoflagellate, Karenia brevis)* from NOAA (South Carolina, USA) and **Lasse Tor Nielsen** (*Coastal plankton communities appear resilient to year 2100 CO2 and pH changes: evidence from microcosm studies*) from the Marine Biological Laboratory in Helsingor (Denmark). Awards for best posters were given to **Victoria Hewlett** (*A comparison of nutrient effect on haemolytic activity produced by two fish killing species: Gymnodinium mikimotoi and Heterosigma akashiwo*) from the University of Western Ontario (Canada) and to **Heidi Hällfors** (*The Baltic Sea phytoplankton community at the beginning and end of the 20th century–a comparison of historical and modern species data*) from the Finnish Environmental Institute.









2010 Maureen Keller student Awarsd: Top left, Jillian Johnson (Best oral); top right, Lasse Tor Nielsen (second best oral); bottom left, Victoria Hewlett (best poster); bottom right, Heidi Hällfors (second best poster). (Photos by M. Lion)

A beautiful carving of *Alexandrium minutum*, made by Prof. Takayama, was given by the Society to Dr Kalliopi Pagou (Popi) in recognition of the tremendous amount of work she invested to make ICHA 14 a success.

B. Reguera, ISSHA President.
Email: beatriz.reguera@vi.ieo.es

Future events

APRIL 2011

I ICOD - I[st] International Conference on *Ostreopsis* Development

6-8 April 2011, Villefranche-sur-Mer, France

ICOD aims to bring together and synthesize knowledge on ecological, chemical and toxicological aspects of *Ostreopsis* species and the different methods of ecological, economic and public health management of the problem. The meeting will promote exchange between scientists, policy makers and managers of Mediterranean and other temperate countries, in order to optimize the knowledge transfer and reduce the risks linked to *Ostreopsis* development.

More info at: www.obs-vlfr.fr/ICOD.

IPHAB-X 10th Session of the IOC Intergovernmental Panel on Harmful Algal Blooms

12-14 April 2011, Paris, France

IPHAB aims to improve the managerial, scientific and financial resources available for implementation of a global HAB Programme. A primary task is still to identify the required resources, so that we, the international community jointly, can continue to implement activities addressing capacity building, international cooperative research, and communication networks.

More info at: ioc-unesco.org/hab/

MAY 2011

Marine and Freshwater Toxins Analysis - 2nd Joint Symposium and AOAC Task Force Meeting

1-5 May 2011, Baiona, Spain

The symposium will address new developments and methods for the analysis of marine and freshwater toxins, as a joint meeting with the AOAC Task Force on Marine and Freshwater Toxins. Presentations and discussions will deal with needs for detecting STXs, DAs, OAs, AZPs and other seafood toxins, as well as cyanobacterial toxins. In spite of recent progress, many methodological needs remain, ranging from emerging toxins to the ongoing replacement of mouse bioassays with modern and fully validated chemical methods.

Deadline for Abstract submission: 15 February.

For further information please contact: biotoxins.meeting@uvigo.es



JUNE 2011

1st International Conference on "The Molecular Life of Diatoms"

5-9 June 2011, Atlanta, USA

This conference aims to bring together a cross-disciplinary research community that is dedicated to studying the molecular foundations of all aspects of diatom biology and technology

Please register on the website as early as possible to take advantage of the reduced "early birds" registration fee. The deadline for abstract submission is February 28th.

More info at: www.lifeofdiatoms.org

8 ICMSS - 8th International Conference on Molluscan Shellfish Safety

12-17 June 2011, Charlottetown, Prince Edward Island, Canada.

Experts from around the world will discuss the latest scientific advances and their impact on molluscan shellfish safety.

Deadline for abstracts submission: February 7. Early Bird Registration: March 31.

More info at: www.gov.pe.ca/icmss2011/

XII Course COI-AECID-IEO: Monitoring programs for biotoxins according to European regulations

June, Vigo, Spain

The course will be held at the Centro Oceanográfico de Vigo (IEO) in June 2011 (Tentative dates: 8-24). It is organised by the "IOC-IEO Science and Communication Centre on Harmful Algae" and has been designed for Latin American and African university graduates having experience in the analysis of marine biotoxins, which are responsible for starting the regional or national monitoring programs or for strengthening the existing ones.

The language of the course is Spanish.

Further info: vigohab@vi.ieo.es

AUGUST 2011

IOC Identification Qualification in Harmful Marine Microalgae

August, Copenhagen, Denmark

The course will be held at the University of Copenhagen organised by the IOC Science and Communication Centre on Harmful Algae.

The course has two components: a short mandatory preparatory theoretical part provided over the Internet in June followed by a practical laboratory course.

Dates:

Mandatory E-learning part: Over 4-5 weeks in the period May-July.

Practical course at Univ.Copenhagen. Tentative dates: 15-25 August 2011. Optional extension on enumeration techniques from 26-27 August.

Course open for applications.

More info at: ioc-unesco.org/hab/

HARMFUL ALGAE NEWS

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HARMFUL ALGAE NEWS

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HAB Mitigation: Sediments, Flocculants, and the 14th International HA Symposium

There is increasing focus on mitigating HABs in many aquatic systems throughout the world, with innovative and progressive practices most practiced in China and Korea. At the 14th International HA Symposium in Crete last fall, **Gang Pan** and **Dan Wang** presented some recent results indicating use of local coarse sediments mixed with polyaluminum chloride and chitosan to remove a large *Microcystis aeruginosa* bloom in Lake Tai in China. Pan has also distributed a video of the mitigation on YouTube (www.youtube.com/watch?v=xubCJyrZ13o), with substantial public interest. Pan's work has been so favorably received that a boat specifically constructed for routine bloom mitigation has been built and deployed. In other efforts, coagulants were quite effective in precipitating harmful algae and reported as an inexpensive method to improve water quality **(Yuk-kam Kai)** with coagulants increasing collisions of clay particles and increased removal efficiencies **(Xihua Cao, Ting Wu)**. Clays were also used to remove microcystins, the common toxins from *M. aeruginosa* and other cyanobacteria taxa in laboratory experiments, as a precursor to the use of clays in drinking water safety **(Erik Prochazka)**.

The symposium yielded results of additional work in China to examine surface charge of various China clays in removing the raphidophyte *Chattonella marina* **(Xiuxian Song, Ting Wu)**. Efforts and commitments elsewhere have also expanded. Korea has a history since 1995 for active clay mitigation of blooms that threaten its mariculture systems and **Joong Ki Choi** continued those studies by detailing China and Mongolia desert 'yellow dust' removals of several HA species including *Cochlodinium polykrikoides* and *Prorocentrum minimum*. Interest is spreading elsewhere, with other studies in the Philippines with *Pyrodinium bahamense* var. *compressum*, *Alexandrium affine*, *Amphidinium carterae*, and *C. marina* **(Iris Orizar, Peter Paolo Rivera)**. In the USA, a national Prevention, Control, and Mitigation HAB program has been set up within the National Oceanographic and Atmospheric Administration's Center for Sponsored

(Cont'd on p. 2)

New Post-column Oxidation LC Method for Saxitoxins Approved by AOAC International – Training Courses

A new post-column oxidation LC fluorescence (PCOX) method for the paralytic shellfish toxins (saxitoxins) is now approved by AOAC International. Jeff Van de Riet of the Canadian Food Inspection Agency (CFIA), Study Director of the method, had previously published a single laboratory validation (SLV) and followed this with a successful large scale inter-laboratory study that had international participation. Following extensive review, the new method has been given "First Action" status as Official Method of Analysis (OMA) 2011.02. Shellfish included in the validation included mussels (*Mytilus edulis*), softshell clams (*Mya arenaria*), sea scallops (*Placopecten magellanicus*) and oysters (*Crassostrea virginica*). Within the scope of the method are the individual toxins saxitoxin, neosaxitoxin, gonyautoxins-1 to -5, decarbamoyl-gonyautoxins-2 and -3, decarbamoyl-saxitoxin, N-sulfocarbamoyl-gonyautoxin-2 and -3. The overall sensitivity of the method for the toxin profiles and shellfish investigated allows detection of total toxicity above 0.10 mg STX dihydrochloride equivalents per kilogram of tissue (mg STX·diHCl/kg) in the edible tissues. This is 8 times lower than the current quarantine level for the saxitoxins and 4 times lower than possible with mouse bioassay. The PCOX method has already been adopted by the Interstate Shellfish Sanitation Conference (ISSC), the shellfish regulatory group in the USA, as a screening (type IV) method, based on the SLV results. OMA status could clear the way to elevation of the method's status in ISSC and suggest consideration for "type II" method status in Codex Alimentarius.

The PCOX method was developed in a joint effort by CFIA and the National Research Council of Canada (CNRC) both located in the Halifax area of Nova Scotia, Canada. Many researchers in the marine toxins community are familiar with post-column oxidation LC methods developed in the 1980s in Japan

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The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen.

(Continued from p. 1)

Coastal Ocean Research (NOAA CSCOR). That program is providing support to explore bloom mitigation, with recently announced projects on clay flocculation for tidal-fresh Chesapeake Bay *Microcystis* blooms [1], bacteria-mediated bloom mitigation in Delaware's coastal lagoons [2], and prevention of *Alexandrium* cyst excystment in Massachusetts coastal waters [3].

The Place *et al.* project [1] builds on Pan's work as well as that of a 3.5 year research program by an honors undergraduate team at the University of Maryland near Washington, DC [4]. The flocculation abilities, impacts [submersed grass growth, benthic nutrient flux, fate of microcystin, and diel dissolved oxygen dynamics), and socio-economic considerations of clay plus flocculant additions to cultured and field *M. aeruginosa* populations have yielded substantial benefits, with high removal efficiencies identified for local sediments [4], minimal detrimental impacts of flocced and settled *M. aeruginosa* populations [5], and inexpensive and publically-acceptable mitigation possibilities for routine use of the technique **(Kevin Sellner)**.

These sediment and flocculant studies are expanding largely due to anticipated field applications where low costs and technical skill requirements, access to local sediments, and high removal efficiencies warrant consideration. Hopefully, permitting issues will allow field demonstrations in sufficient countries where not only removal efficiencies for natural blooms can be determined but their impacts post-bloom can be quantified and used to inform local considerations for intervening responsibly in recurrent blooms to reduce biomass, toxin and toxicity, and open the system to less harmful phytoplankton.

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by Yasukatsu Oshima of Tohoku University, and in the US by John Sullivan at the University of Washington, Seattle, WA. Like these earlier methods, the recently approved method employs ion pairing in the separation of the toxins and a post-column reaction system to produce sensitive detection based on fluorescent purine derivatives. In addition to performance improvements in the present method, the successful collaboration of the new official method was also made possible by production of certified reference standards by CNRC. The inter-laboratory study supporting OMA 2011.02 will be available in the Journal of AOAC, and the official method itself will be posted in OMA Online at www.aoac.org. Those interested in seeing the method prior to its publication can consult the already published SLV manuscript in JAOAC (J. Van de Riet et al., 2009, JAOAC Int., 92: 1690-1704).

The method and inter-laboratory study results were presented by Van de Riet in Baiona, Spain at "Marine and Freshwater Toxins Analysis, Second Joint Symposium and AOAC Task Force Meeting" held May 1-5, 2011. Website for this meeting is www.biotoxinsmeeting.org and email inquiries should be directed to biotoxins.meeting@uvigo.es. Additional information and discussion of validation and use of officially approved methods were provided by roundtable discussions following presentations by those implementing the methods for paralytic shellfish toxins and lipophilic toxins in the United Kingdom and New Zealand.

The Marine and Freshwater Toxins Task Force and Analytical Community www.aoac.org/marine_toxins/task_force.htm is an international group of experts and stakeholders that addresses official methods validations. In addition to addressing validation criteria, validation studies, and organizing symposia in the US and in Spain, in recent years the Task Force has offered laboratory training on both instrumental methods and rapid test kits for seafood toxins. Most recently a well attended training course was given on the PCOX method, with Van de Riet as instructor, in Seattle, WA, USA in June 2010. The PCOX method training was taught jointly with a semi-automated version of another official LC method, a pre-column oxidation LC method for the saxitoxins, AOAC OMA 2005.06. The latter method was first to offer an official alternative to mouse bioassay in 50 years, and is now applied in the United Kingdom monitoring programs, completely replacing animal-based detection of saxitoxins there. A total of three new official methods for marine toxins have been approved since the 2004 establishment of the Task Force including, in addition to the LC methods for saxitoxins OMA 2005.06 and OMA 2011.02, a commercial ELISA method for domoic acid, OMA 2006.02.

These important efforts and recent developments in marine toxin methodology validation and training will have a significant impact on shellfish monitoring approaches for detecting marine biotoxins in the protection of human health, leading to increasing use of modern analytical methods rather than outdated mouse bioassays.

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C-MANs and BreveBusters in Veracruz, Mexico (Gulf of Mexico)

A binational agreement between the USA (Environmental Protection Agency) and Mexico (Health Department, the Aquarium of Veracruz and the Institute of Ecology) for the monitoring of red tides in the Gulf of Mexico was signed on 22 April 2009. During the period from 7 to 16 September 2010, personnel of the National Data Buoy Center (NDB) of the National Oceanic and Atmospheric Administration (NOAA) worked on the installation of the Coastal-Marine Automated Network (C-MAN) at three selected sites along the coast of Veracruz: in front of La Mancha lagoon, in front of the port of Veracruz, and near Sacrificios Island (Fig. 1). In addition, personnel of the Aquarium of Veracruz were informed about the use, functioning and maintenance of BreveBuster (Fig. 2), a device designed by Mote Marine Laboratory in Sarasota, Florida, to detect *Karenia brevis* blooms by comparing light absorption by particles in ambient water with the light absorption fingerprint characteristic of *K. brevis*.

Unfortunately, on 17 September, Hurricane Karl hit the Veracruz coasts with winds of 185 km h^{-1}, reaching category 3, stopping activities and damaging the C-MAN installed near Sacrificios Island (Fig. 3). On 22 February 2011, personnel of NBD began to repair C-MANs at the three sites of Veracruz and installed the BreveBusters (Figs. 4, 5 & 6). The devices finally started transmitting data on 28 February (Fig. 7).



Fig. 1. Location of C-MAN and BreveBuster stations in Veracruz, southwestern Gulf of Mexico.

For the last two centuries, Veracruz has suffered HABs [1], *Karenia brevis* being the main microalga with notorious toxic effects. During the last decade the occurrence of *K. brevis* has diminished, and *Karenia* blooms seem to have been replaced by recurrent blooms of the non-toxic dinoflagellate *Peridinium quinquecorne* Abé [2]. However, in January 2010, *K. brevis* bloomed along with the morphotype preliminarily called *Karenia* "Mexican hat", causing fish mortality north of the port of Veracruz [3]. The Aquarium of Veracruz has had a weekly HAB monitoring programme in waters near the port of Veracruz and the Aquarium since May 2005, that has let us know the sites where HABs may occur. The Institute of Ecology is implementing a monitoring programme in the La Mancha lagoon area. Universidad Veracruzana focuses on taxonomy, ecology and biogeography of phytoplankton in the Mexican coastal waters of the Gulf of Mexico.

The C-MANs and BreveBusters represent valuable devices that provide data for understanding HABs. BreveBusters are supposed to provide an early alert so that local authorities can make optimal decisions.

C-MAN stations transmit data on barometric pressure, wind direction and speed, air temperature and dewpoint temperature, which can be consulted online by the public: Station VERV4: www.ndbc.noaa.gov/station_page.php?station=verv4; Station SACV4: www.ndbc.noaa.gov/



Fig. 2. A BreveBuster in the Acuario de Veracruz.

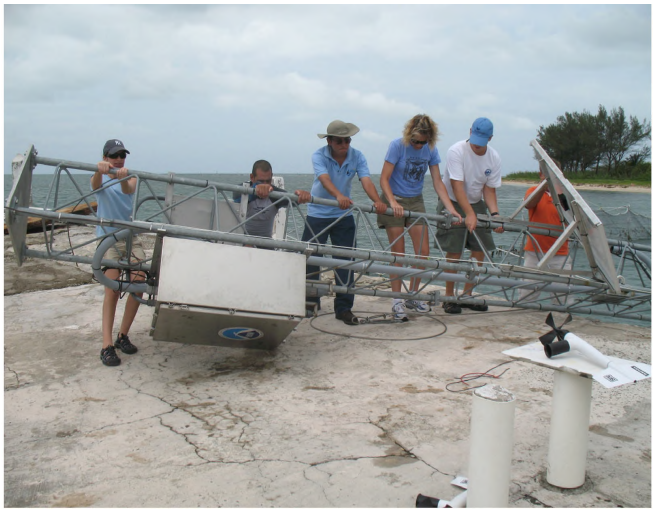

Fig. 3. The damaged C-MAN station near Sacrificios Island,



Fig. 4. Repairing the C-MAN in front of the port of Veracruz.



Fig. 5. C-MAN and BreveBuster at Sacrificios Island.



Fig. 6. Installing BreveBuster near La Mancha lagoon, Veracruz.

station_page.php?station=SACV4; Station LMBV4: www.ndbc.noaa.gov/station_page.php?station=lmbv4&unit=M&tz=STN.

BreveBuster data will be managed by personnel of the Mexican Health Department. Data provided by these devices will undoubtedly be a valuable source of information for joint research *in situ*, not only for the detection of *Karenia brevis* blooms, but also for the understanding of dynamics of other bloom-forming species in the Veracruz region.

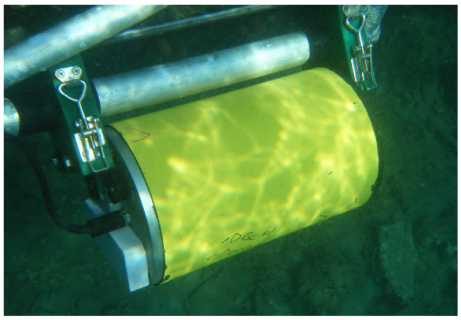

Fig. 7. BreveBuster working at Sacrificios Island.

Acknowledgements

The personnel of Acuario de Veracruz J. Valentín Betanzo-García, L. E. Garduño-Chávez, L. J. Martínez-Hernández, J. C. Albores-Rivero, M. A. Román-Vives, J. Zamudio-Morales, B. Poblete-Carlin and R. J. González-Díaz Mirón helped in the installation of C-MANs and BreveBusters at the three sites, under the supervision of NDB.

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ISSHA's Corner

Bill Silvert

Just when this new issue of HAN was completed we heard the sad news about Bill Silvert. He was a theoretical ecologist who worked at the Bedford Institute of Oceanography in Dartmouth, Nova Scotia, Canada, until he moved to Portugal in 1998. In the last years, Bill lived in the Algarve (Southern Portugal), worked in association with the University of Faro, and even established his own "Centre for Gastronomic Research". Some of us met him when he was still full of energy at the ICES-IOC WG on HAB Dynamics held in Huelva (April 2009). Many HAB/phycotoxin experts will remember Bill as the creator of "Phycotoxins", an e-mail list that since the early 1990's has worked as an excellent network and communication system. Our sincere condolences to his wife Emilia (Mi), researcher at the IPIMAR in the Algarve (micunha@ipimar.pt). More information about Bill is available at http://bill.silvert.org/

ISSHA

International Society for the Study of Harmful Algae

Abstracts 14th ICHA

Abstracts from the 14th International Conference on Harmful Algae (Hersonissos, Crete, 1-5 November 2010) are now available at : www.issha.org.

B. Reguera, ISSHA President. Email: beatriz.reguera@vi.ieo.es

Fish mortality associated with *Alexandrium cf. fundyense* in Lobito Bay, Angola



Fig. 1. Location of the Cais Norte (12º 21'01"S and 13º 34'10"E) and Cais Sul (12º 20'57"S and 13º 34'17"E) in Lobito Bay.

Since the 1950s, harmful algal blooms have become more frequent along the Angolan coast, especially in Luanda, Benguela and Namibe provinces. Between 1953 and 2007, dinoflagellates such as *Prorocentrum balticum*, *Gyrodinium spirale*, *Alexandrium* spp., *Gymnodinium catenatum*, *Gambierdiscus toxicus*, *Pyrodinium bahamense* and *Prorocentrum micans* have been detected, sometimes in association with resource mortalities [1–4]. These blooms and associated mortalities usually occur between July and September, and disappear in October, but are not unknown in the cold season (May-July).

On September 26, 2008 surface samples were collected at 2 stations Cais Norte and Cais Sul in Lobito Bay (Fig. 1), where the water temperature was 22ºC. These samples were fixed with 2% formol. Species identification and counts were made with an Axiovert 200 inverted microscope equipped with phase contrast.

Alexandrium cf fundyense was the dominant species (Fig. 2), with cell concentrations reaching 16 x 10^5 cell L^{-1} at Cais Sul and 14 x10^5 cell L^{-1} at Cais Norte (Fig. 3). Other species identified in low concentrations were *Prorocentrum micans*, *Protoperidinium diabolicus*, *Gonyaulax spinifera*, *Chattonella antiqua*, *Pleurosigma* sp, *Synedra berolenensis* and *Navicula distans*.

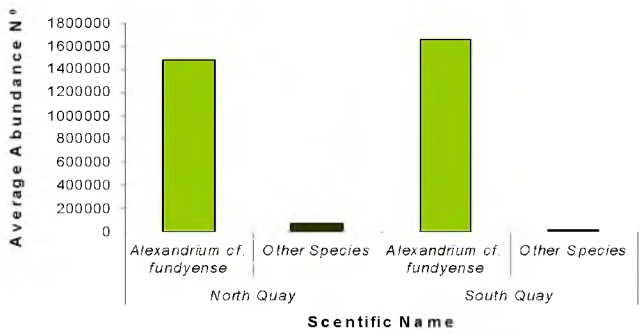

Fig. 3. Abundance of the A. tamarense *and other species in 2 stations of the Lobito Bay.*

The *Alexandrium cf. fundyense* bloom caused mortality of several demersal fish species including *Pomadasy jubelini*, *Albula vulpes*, *Ethamalosa fimbriata*, *Lutjanus agnnes*, *Eucinostomus melanopterus* and *Mugil bananensis*.

In South Africa in 1997, sardines mortalities occurred in St Helena Bay, and *A. catenella* was responsible. Stomach content analysis showed 60 to 80 x 10^3 *Alexandrium* cells in each stomach, and analysis of the viscera revealed toxin concentrations exceeding the harvestable limit of 80 µg toxin 100 g^{-1} of viscera [5]. In the northern Benguela, *Alexandrium tamarense* is a regular red tide species in the Walvis Bay region [5]. *A. tamarense* is recorded in phytoplankton from the central region of the Angola coast [7], a frontal area where the cold Benguela Current and warm Angola Current meet.

An earlier bloom of *Alexandrium* sp. that caused fish and crab mortalities, occurred in Luanda Bay on 24-25 September 2002 with a maximum concentration of 5.1 x 10^6 cell.L^{-1} [8].

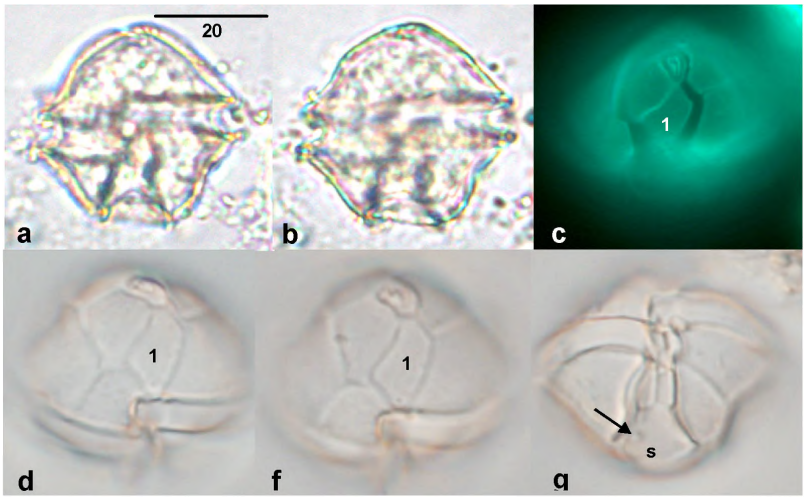

Fig. 2. Alexandrium cf. fundyense*: (a-b) the same cell in different focus, ventral view, (d-f) different cells showing the 1' plate, note the missing ventral pore, (g) Same cell as in (f) showing posterior sulcal plate (sp) and posterior attachment pore (arrow), b-g, scale as in (a). (a-b) bright field microscopy; (c) epifluorescence microscopy and (d-g) DIC microscopy.*

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Harmful algae blooms along the coast of the Baja California Peninsula

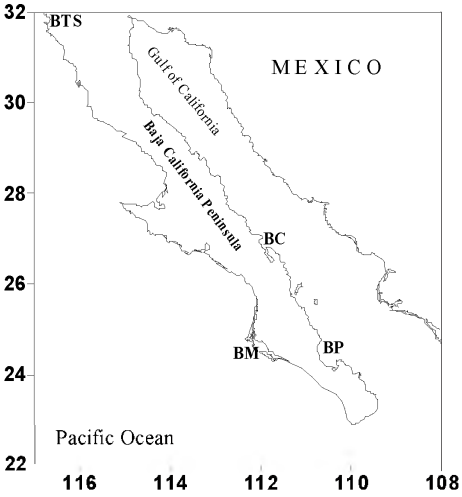
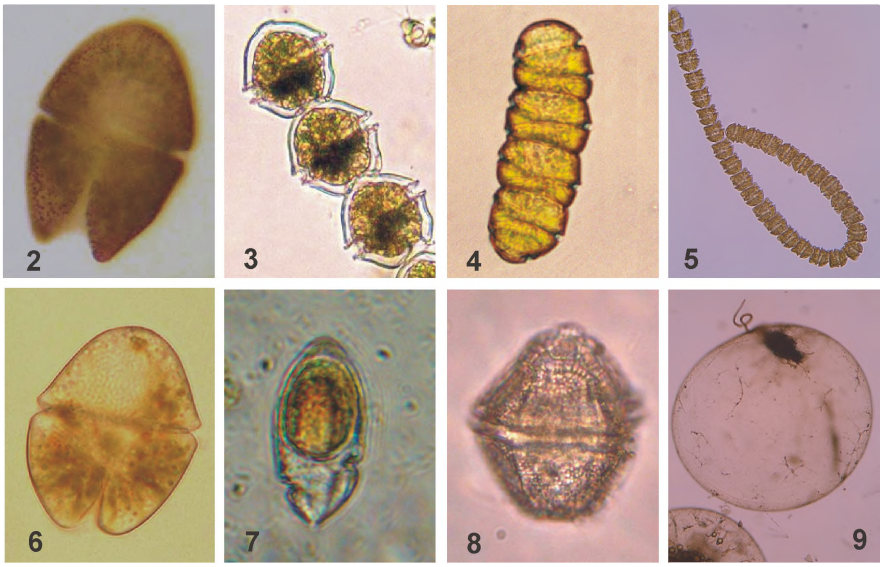


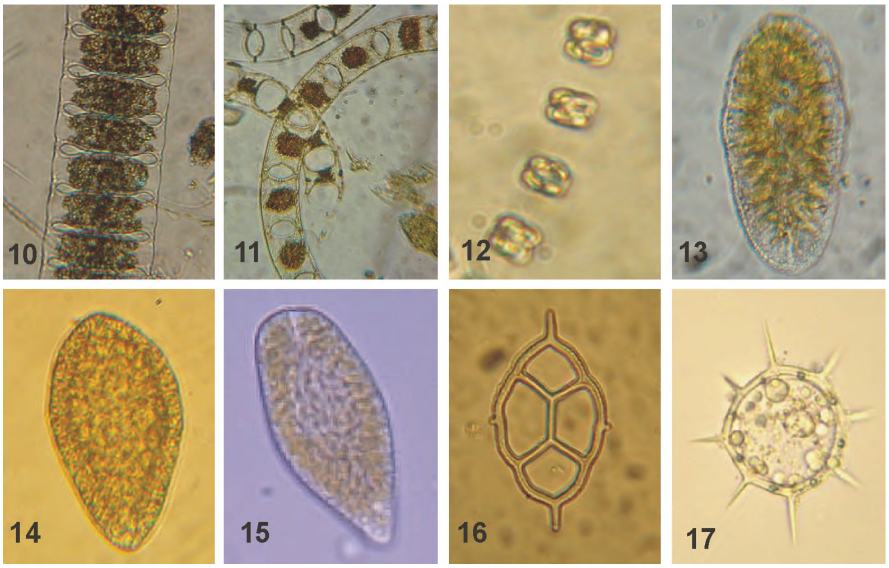
Fig. 1. Baja California Peninsula. Bahía de La Paz (BP), Bahía Concepión (BC), Bahía Magdalena (BM), Bahía de Todos Santos (BTS).

The Baja California Peninsula is located in northwestern Mexico and has a coastal zone of ~3,000 km (Fig. 1). The first studies of the phytoplankton began with sporadic expeditions by foreign investigators on both coasts. They provided the first species lists and noted the areas of high concentrations of phytoplankton. From these antecedents, Mexican researchers studied the phytoplankton communities. Special emphasis was placed on species that produce harmful algae blooms (HABs), which were reported by the first Spanish conquerors and explorers. Officially, the first red tides were recorded in the southern part of the peninsula and in Bahía Concepción in 1878. These blooms were caused by the photosynthetic ciliate *Myrionecta rubra* and the dinoflagellate *Noctiluca scintillans* [1]. An exhaustive review of the literature on red tides and unpublished information indicate that more than 80 events and more than 40 bloom-forming species have been reported. From these investigations, four red tide areas have been studied in the last 25 years. These include: Bahía de La Paz and Bahía Concepción on the eastern coast and Bahía Magdalena, and Bahía de Todos Santos on the western coast (Fig. 1) [2, 3]. Most of the red tides were innocuous. Red tide species of dinoflagellates include: *Akashiwo sanguinea* (Fig. 2), *Alexandrium affine* (Fig. 3), *Ceratium furca*, *C. balechii*, *Cochlodinium polykrikoides* (Fig. 4), *Ensiculifera* spp., *Dinophysis caudata*, *Gonyaulax polygramma*, *Gymnodinium catenatum* (Fig. 5), *Gyrodinium instriatum* (Fig. 6), *Katodinium glaucum* (Fig. 7), *Lingulodinium polyedra* (Fig. 8), *Noctiluca scintillans* (Fig. 9), *Peridinium quinquecorne*, *Prorocentrum gracile*, *P. rhathymum*, *P. micans*, *P. minimum*, *P. triestinium*, *Scrippsiella trochoidea*. Diatoms include: *Bellerochea malleus* (Fig. 10), *Chaetoceros compressus*, *C. debilis*, *C. socialis*, *C. radicans*, *Cylindrotheca closterium*, *Eucampia zodiacus* (Fig. 11), *Hemiaulus membranaceus*, *Nitzschia sigma*, *Proboscia alata*, *Pseudo-nitzschia fraudulenta*, *P. australis*, *Rhizosolenia debyana*, and *Thalassiosira* sp (Fig. 12). Cyanobacteria include *Trichodesmium erythraeum*. Among the raphydophytes, identified species include: *Chattonella marina* var. *ovata* (Fig. 13), and *C. marina* var. *marina* (Fig. 14), *C. subsalsa*, (Fig. 15); prasinophytes include *Pyramimonas grossi* and silicoflagellates include *Dictyocha californica* (Fig. 16), *D. messanensis*, and *D. octonaria* (Fig. 17).

Nevertheless, some microalgae have been harmful, such as blooms of *Akashiwo sanguinea, Ceratium furca, Cochlodinium polykrikoides,* and *Thalassiosira* sp. that caused captive fish mortalities, and deaths of lobsters and cultured oysters [4–6]. Increasing incidents involve *Gymnodinium catenatum* which produces paralytic toxins [7–9], but this has not caused public health problems. Other paralytic toxin producers are *Alexandrium catenella* and *A. tamiyanavichii*. Recently, two varieties of *Pyrodinium bahamense* were detected along the southern coast of the peninsula [10]. The presence of *P. bahamense* var. *compressum* along the coast of the State of Guerrero (central western Mexico) and in the peninsular littoral during 2010 has been linked to El Niño conditions [10, 11]. Several potentially toxin-producing species of *Dinophysis* have been detected (*Dinophysis acuminata*, *D. caudata*, *D. cuneus*, *D. fortii*, *D. norvegica*, *D. mitra*, and *D. rapa*). Among the diatoms, *Pseudo-nitzschia australis* and *P. fraudulenta* have proliferated on both coasts of the peninsula. These species are producers of domoic acid, which was detected in field samples and in bivalves during a June 2006 bloom in Bahía de La Paz [12].



Figs. 2-9. Bloom forming dinoflagellate species from Baja California Peninsula. 2) Akashiwo sanguinea, *3)* Alexandrium affine, *4)* Cochlodinium polykrikoides, *5)* Gymnodinium catenatum, *6)* Gyrodinium instriatum, *7)* Katodinium glaucum, *8)* Lingulodinium polyedrum, *and 9)* Noctiluca scintillans.



Figs. 10-17. Bloom forming microalgae species from Baja California Peninsula. 10) Bellerochea malleus, *11)* Eucampia zodiacus, *12)* Thalassiosira *sp., 13)* Chattonella marina *var.* ovata, *14)* Chattonella marina *var.* marina, *15)* Chattonella subsalsa, *16)* Dictyocha californica, *and 17)* Dictyocha octonaria.

Environmental conditions that cause microalgal blooms along the peninsula are partly understood, known to be associated with some combination of nutrient availability, nutrient ratios, and water temperature. Human activities that increase nutrient concentrations (eutrophication) have minor influence on the increase of HABs in some areas. Microalgal blooms have been examined in experimental and commercial shrimp ponds. Main blooming species include the diatom *Nitzschia sigma* and dinoflagellates *Cochlodinium polykrikoides*, *Ensiculifera* sp., *Peridinium quinquecorne*, *Prorocentrum triestinium*, *P. minimum*, *Scrippsiella trochoidea*, *Protoperidinium pacificum* and *Protoperidinium* spp. A recurrent cause that provokes HABs along the peninsula coast is upwelling [2, 3, 7], which can be sporadic or throughout the year [13]. Upwelling intensity is very variable, and depends on wind strength and direction and currents. Blooms also occur after breakdown of the summer thermocline.

Many microalgae form cysts that can easily be transported to the surface, where they germinate and initiate a bloom. Dinoflagellate cysts are common along the coasts of the peninsula [2, 3, 7, 14]. At least 25 different dinoflagellate cyst types were found in surface net phytoplankton (20 μm) samples in Bahía de La Paz. Some dinoflagellate cysts not previously recorded in the bay are shown in Figs. 18–25. Cysts found were related to their vegetative stages in fixed phytoplankton samples. Temporary cysts of *Gyrodinium*, *Cochlodinium* and *Warnowia* were also found. In many cases during diatoms blooms, especially of *Chaetoceros* spp., resting spores have been observed.

This study was presented for the commemoration of the 25-year celebration of the Planctología Mexicana at the XVI Reunión de la Sociedad Mexicana de Planctología. Funding came from two IPN projects (SIP-20100192, SIP-20110281). The author received support as a COFAA and EDI fellow of IPN.

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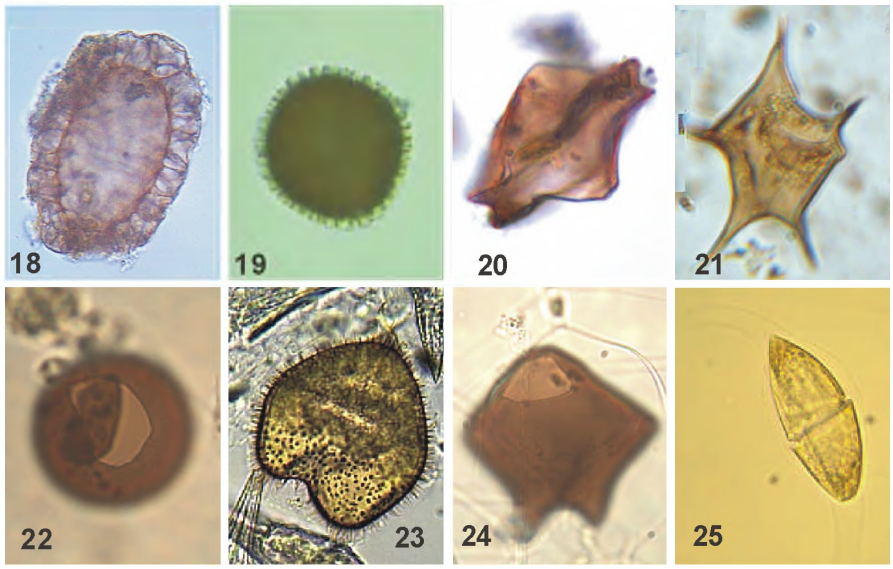

Figs. 18-25. Dinoflagellates cysts found in Bahía de La Paz. 18) Polykrikos schwartzii, *19)* Scrippsiella trochoidea, *20)* Protoperidinium subinerme, *21)* Protoperidinium compressum, *22) empty cyst of* Protoperidinium avellanum, *23)* Protoperidinium claudicans, *24) empty cyst of* Protoperidinium leonis , *and 25) temporary cyst of* Gyrodinium falcatum.

Mortality of marine fauna in Luanda Bay (Angola)

Records of harmful algae blooms (HABs) have been frequent in Luanda Bay [1–4]. These events are more frequent between August and October, during the transition from the cold season to the warm season [1]. This bay is one of the most economically important in Angola and supports artesanal shellfish extraction, coastal fisheries, and recreational activities, and a large commercial and fishery harbour.

Several dinoflagellates (*Prorocentrum*, *Alexandrium*, *Gyrodinium*, *Gymnodinium*, *Pyrodinium*, *Prorocentrum micans*) and diatoms like *Pseudo-nitzschia* (1–5) are the main organisms that have caused blooms in Luanda Bay. On 10 September 2010, a great mortality of demersal and pelagic fish and lobsters was recorded on the beach around the Panorama Hotel (8º47'62" S; 13º13'54" E) (Fig. 1).



Fig. 1. Dead fish on the Panorama Hotel beach

and quantitative phytoplankton analyses were performed using Utermöhl methodology, an inverted microscope with phase contrast "Axiovert 200" and dissolved oxygen determination were done using the Winkler procedure.

The phytoplankton analyses of 30 and 31 August found a multispecies bloom with mainly *Alexandrium tamarense*, *Protoceratium reticulatum*, *Prorocentrum micans*, *Gyrodinium spirale* and *Ceratium furca*. On 10 September, a similar bloom with *Prorocentrum micans* 57 x 10^5 cell L^{-1} representing 61% of the total, followed by the flagellate *Euglena acusformis* with 27 x 10^5 representing 28% (Fig. 2).

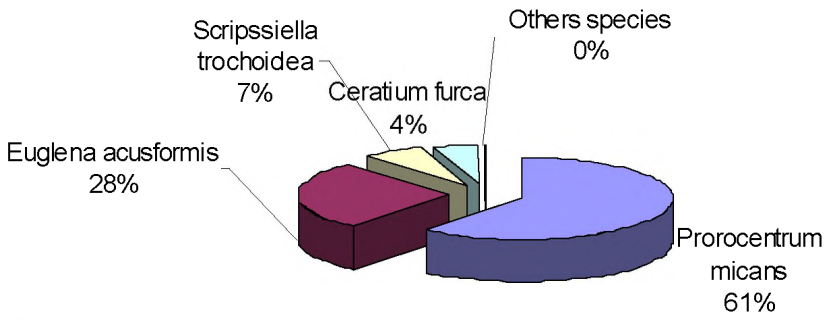

Fig. 2. Percentages of more abundant species on 10 September 2010

In the morning of 30 August 2010, a brown colour was seen in the water off Ponte Cais do Carvão (8º45'80"S; 13º15'75"E) in the bay. In the afternoon of the same day and on 31 August, dead fish, mainly demersal species, were observed.

The dead marine species were: Pelagic fish—*Liza falcipinis*; demersal fish—*Pomadasys incisus*, *Pomadasys peroteti*, *Dentex bernardi*, *Diplodis sarguis campesis*, *Boops boops*, *Pagellus belotti*, *Euconostomis melanopterus*, *Parakuhlia macrophtalmus*; crustaceans such as *Panulirus regius*, and Chondrichthyes *Dasyastis margarita* and *Torpedo torpedo*.

Surface samples for phytoplankton and dissolved oxygen analyses were collected on these dates. The qualitative

Table 1. Qualitative and quantitative analyses of the phytoplankton bloom

Phytoplankton species	Density 10^5 cell L^{-1}
Prorocentrum micans	59.7
Euglena acusformis	27.2
Scripssiella trochoidea	6.5
Ceratium furca	4.4
Peridinium bipes	1.6
Gyrodinium spirale	1.1
Leptocylindrus minimus	0.4
Gonyaulax turbynei	0.2
Peridinium divergens	0.2
Gonyaulax spinifera	0.2
Ceratium fusus	0.1
Dinophysis acuminata	0.1
Peridinium sp	0.1
Scripssiella spinifera	0.1

Other phytoplankton species were present in lower numbers (Table 1).

The dissolved oxygen concentrations (DO) in the water in the August and September events were 2.0 and 2.3 mg ml^{-1} respectively, showing anoxia events caused by multispecific blooms in the Ponte Cais do Carvão and Panorama Hotel areas. In Luanda Bay, high concentrations of *Prorocentrum micans* causing fish mortality [4] are starting to be frequent, although this species has already caused fish mortalities elsewhere in Angola [5]. Trainer et al [6] describe low-oxygen events that suffocate fish and benthic organisms, often linked to the high respiratory demand of the settling organic matter following bloom decay, and perhaps the most common negative effect of high biomass blooms.

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Pseudo-nitzschia pungens (Grunow ex. Cleve) Hasle from Pakistan coastal waters

Some species of the diatom *Pseudo-nitzschia* produce domoic acid, a toxin responsible for Amnesic Shellfish Poisoning (ASP). *Pseudo-nitzschia* species are cosmopolitan and widely distributed in the Atlantic, Pacific and Indian Oceans, with approximately two dozen species reported from various regions of the world [1]: *P. pungens* has not previously been identified in Pakistan coastal waters bordering the northern Arabian Sea. Samples were collected in July, 2007 from inshore waters of Manora Channel, Karachi (24°58'N, 65°59'E), (Fig. 1). Manora Channel is influenced by coastal pollution and sewage discharges from Layari River.

Samples were collected with net tows (55 µm mesh), and preserved in 1% acid Lugol's solution. Specimens were air-dried for scanning electron microscopy (SEM), and mounted on the specimen stub and gold coated in the usual manner. A JSM6380A analytical scanning electron microscope was used, and identification mainly based on keys [2–3]. Light microscopy reveals that *Pseudo-nitzschia pungens* forms stepped colonies that are united by the overlapping of valve ends. The cells are strongly elongated in girdle view, margins are parallel through the valve length, gradually narrowing towards the apics (Fig. 2A-B). Valve tips are attenuated and sharply rounded (Fig. 2A-B). The apical axis ranges from 77.5 to 105 µm and transapical axis ranges from 4.2 µm to 4.6 µm. (Fig. 2B). Fibulae and interstriae are 15 in 10 µm. (Fig. 2B). Striae are as dense as fibulae with 15 in 10 µm. Striae end below the apical ending of the raphe (Fig. 2C-D). Central interspace is absent (Fig.2D). Raphe apical ends are located close to the margin of the valve face. Striae are composed of two rows of large poroids and close to the interstriae (Fig. 2D). There are 3 or 4 poroids in 1 µm (Fig. 2D).

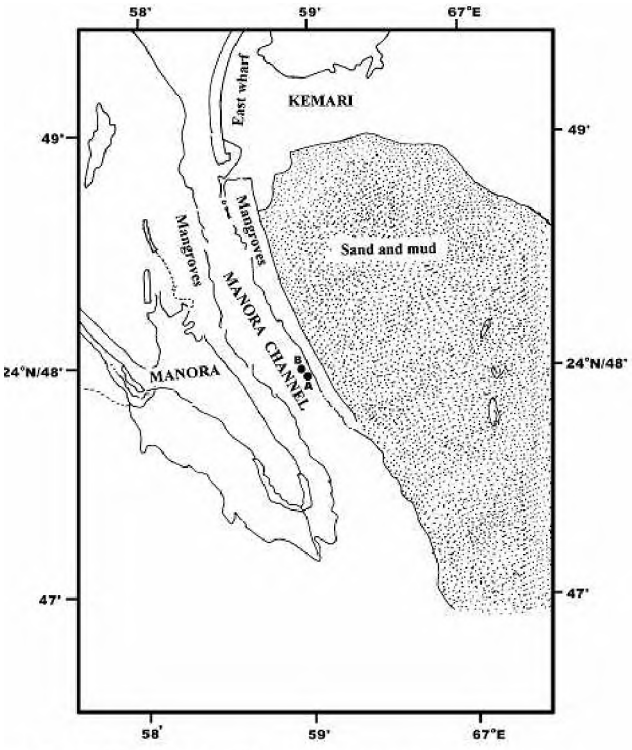

Fig. 1. Map showing the sampling area.

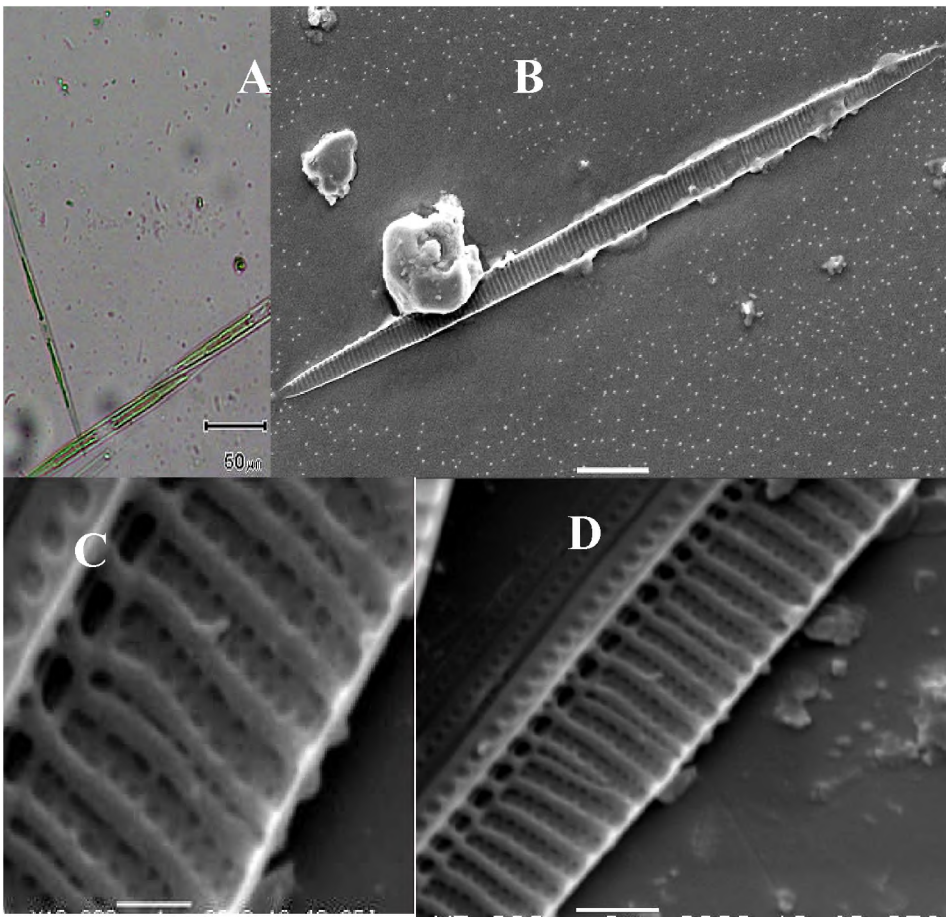

Fig. 2. (A) Light micrograph of Pseudo-nitzschia pungens. *(B) Scanning electron micrographs of* Pseudo-nitzschia pungens*: whole valve shows valve tip, striae, fibulae and poroids: (C, D) Closer views showing two rows of large poroids and 3-4 poroids in 1 µm.*

Acknowledgement

Special thanks to Dr. P. E. Miller (University of California Santa Cruz, CA, USA) for guidance.

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Algal blooms in the Caspian Sea

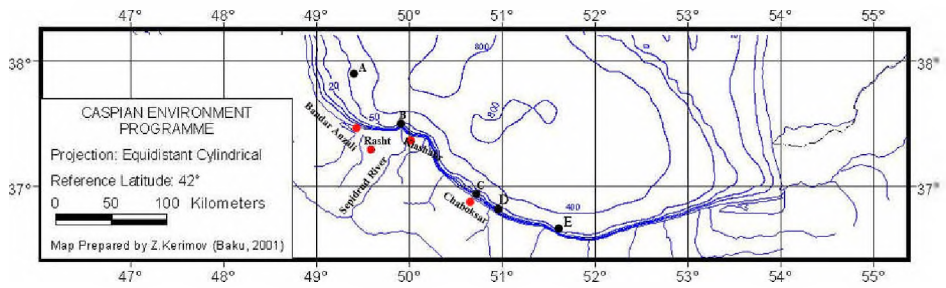


Fig. 1. Map of the study area, Station A: Bandar Anzali coasts near the harbor, Station B: Sepidrud River estuary, Kiashshr Region, Station C: Chaboksar Region, Station D: Tonekabon Region, Station E: Nowshahr Region.

The Caspian Sea is the world's largest inland body of water, lying to the east of the Caucasus Mountains and to the west of the Central Asian steppe, and bordered in the south by Iran. The southern Caspian shores are formed by the Gilan-Golestan lowlands, with the high peaks of the Talish and Elburz Mountains beyond. The region is temperate with high annual average precipitation (Fig. 1). Algal blooms in the south Caspian Sea were first reported in 2005 (station A) when a bloom of *Nodularia spumigena* extended over an area of 2 km^2. In 2006, a bloom of *Hetrocapsa* sp. was observed in Bandar Anzali, then in 2009 and 2010 blooms of *Nodularia spumigena* were reported in the vicinity of Tonekabon (Station D) and Nowshahr (station E) where water depth is 15–40 m.

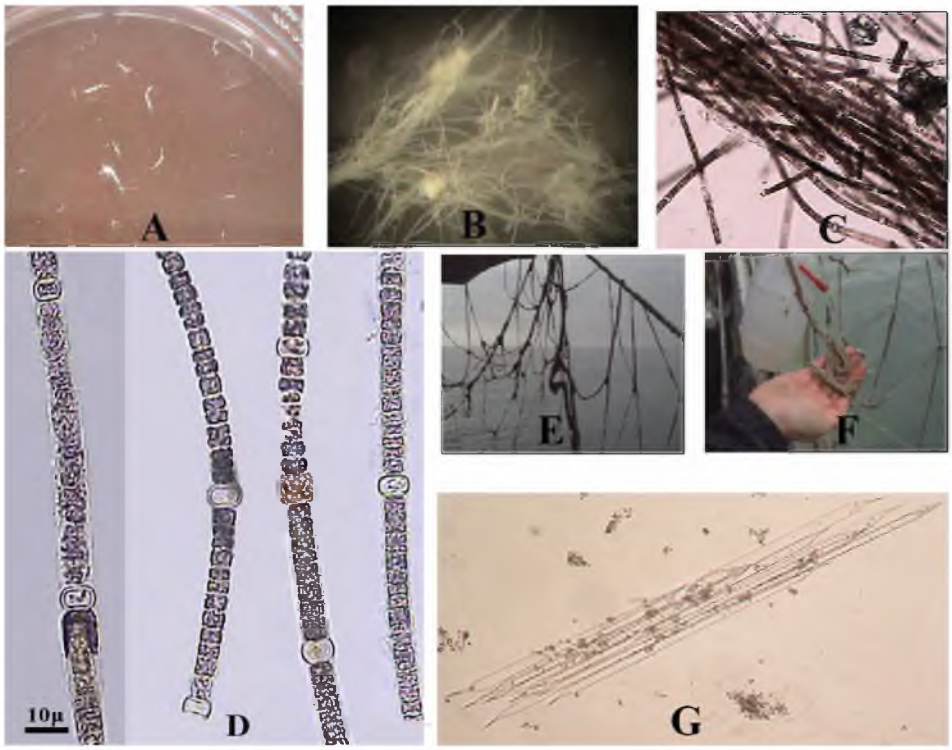


Fig. 2. Nodularia spumigena *filaments collected at the time of bloom in the south Caspian Sea coastline, A:see on water surface with the naked eye. B:* N. *spumigena filaments (20x) C:* N. spumigena *filaments (100 x) D:* N. spumigena *filaments (400x). E and F: The Mass seen in the form of a sluggish layer covering gill nets G:* Pseudosolenia calcar-avis

Bloom of *Nodularia spumigena* appeared as masses of cotton like opaque filamentous algae easily visible to the naked eye (Fig. 2) with 90% of the algae being *N. spumigena*. The surface water temperature at the time of the bloom in September 2005 was 27°C and pH and salinity were 8.4 and 9 PSU respectively. Nutrient concentrations are shown in figure 6.

Other blooms were observed off Chaboksar (station C) in March 2006 and April 2009, some 80 km from station A. The main component of these blooms was *Pseudosolenia calcar-avis* (*Rhizosolenia calcar-avis*) contributed 95% of algal numbers. The algal mass formed a sluggish layer covering gill nets set out for sturgeon fishing, and was traced to the depth of 50m (Fig. 2); it exerted adverse effects on the sturgeon catch. The algal mass was also characterized by colloidal detritus particles in the water column. Blooms of *P. calcar avis* were first observed in the Gorgan Gulf in 1934 and 1935 [1], and throughout the southern Caspian Sea, and in the drainage canal of the Anzali Lagoon in spring and summer

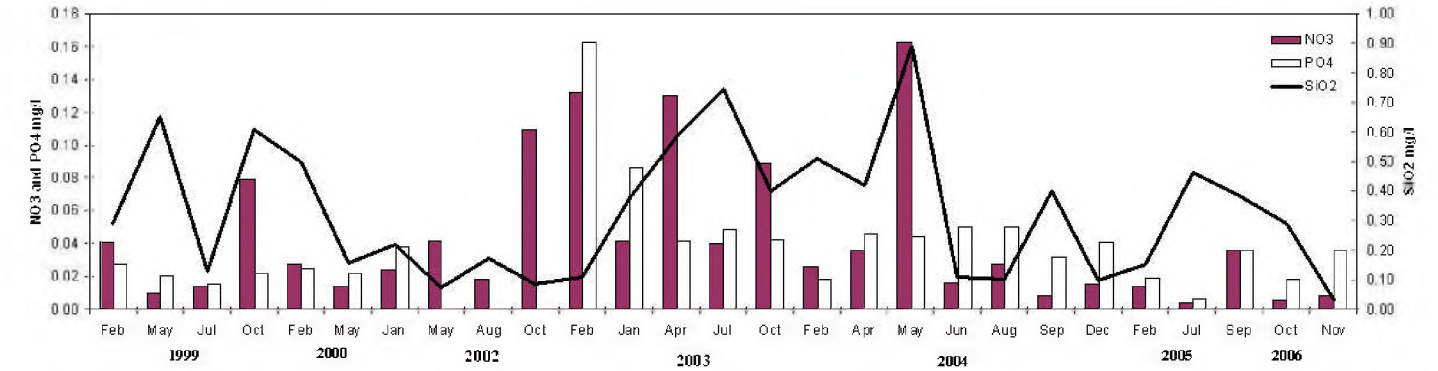
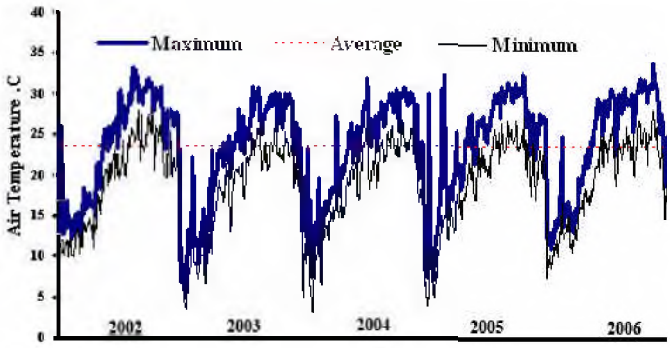
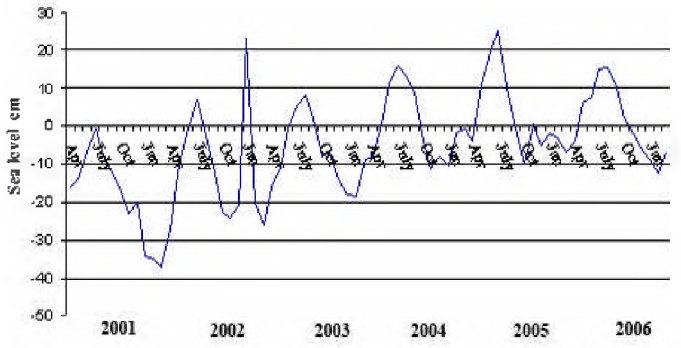


Fig. 3. Average concentrations of N-NO_3^-, P-PO_4^{3+} & SiO_2 at 2-100 m depths in the south Caspian Sea coastline adjacent to Bandar Anzali region (1999- 2006).



Maximum and minimum daily temperature during spring and summer of 2002-2006.



Monthly average of Caspian Sea level (zero on the Y-axis corresponds to - 26.36 m) during spring and summer of 2001-2006.

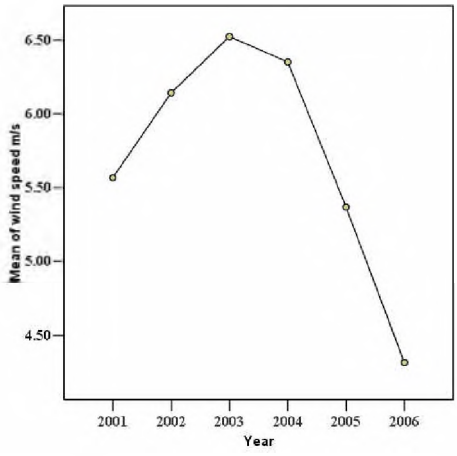
1992-3 [2]. Upwelling of nutrient rich deep water of the Caspian Sea may be a main reason for the observed mass of *P. calcar-avis* although further studies are required [3].

Meteorological records for the five years 2002 to 2006 show that air temperature varied between 2.8 and 33.8°C. Temperature variations during 2005-2006 were significantly higher than 2002-2003 in the study area. Temperature variations in the Caspian Sea are considerable with various trends in different regions. A study conducted by the Caspian Environment Program (CEP; [4]) in 2006 reports a thermocline with a temperature gradient of 13°C at depths between 40-45 m in the Anzali and 30-40m in the Kiashahr regions. The presence of a thermocline and its depth below the surface water influence the biological activities of algae and nutrient cycling, particularly in autumn following the breakdown of the thermocline.

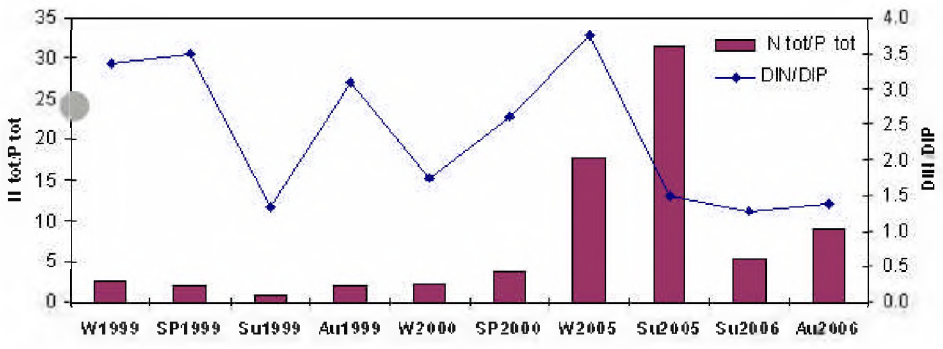
Salinity varied from 9 to 12 PSU from near shore to offshore and pH from 8 to 8.4. Fluctuations in salinity are linked to heavy rainfall and flooding events of the rivers draining into the Anzali Lagoon and the Caspian Sea. Increase in freshwater input probably provided ideal conditions for aggregation of cyanophyte cells.

Caspian Sea level also varied significantly, and the difference was evident in 2005 coinciding with the bloom event. Significant changes in wind velocity in 2005 and 2006 might also have promoted the bloom.

The Sepidrud River discharges heavy sewage loads into the Caspian Sea [5], and the main pollutants are agricultural, food processing, pulp and paper, industrial and urban wastes [6]. Discharges from fish farms and hatcheries are important source of phosphate, especially in shallow coastal regions.



Average daily wind speed during spring and summer of 2001-2006.



Average N_{total}/ P_{total} & DIP/DIN in the south Caspian Sea coastline adjacent to Bandar Anzali region from1999 to 2006, "W., Sp., Su, Au" mean respectively Winter, Spring, Summer and Autumn.

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Continued international commitment to enhance capacity and advance HAB research

In 1991 the IOC established an intergovernmental Panel on Harmful algal Blooms (IPHAB) to foster the effective management of, and scientific research on, harmful algal blooms in order to understand their causes, predict their occurrences, and mitigate their effects. From 12-14 April 2011 the Panel met for the tenth time to identify and decide on priorities for international cooperation on HABs and to recommend to the IOC a work plan for the IOC HAB Programme for the period 2012-2013. Following a presentation by the IPHAB Chair to the full Assembly on 29 June that covered the functions, accomplishments and priorities of the Panel and the IOC HAB Programme, the IOC Assembly endorsed the Work Plan with strong support from many Member States. Additional Member States were encouraged to participate in IPHAB, its task teams and workshops.

Through its working groups, task teams and regional groups the IOC HAB Programme directly engages close to 400 people. A community of 2000 people receives the printed version of 'Harmful Algae News'.

An important task of IPHAB is to review achievements. A brief summary of achievements 2010-2011 includes implementation of ten training courses to identify harmful HAB species and/ on their toxins for protection of public health and in support of critical research; the development and launch by GEOHAB of three new international research plans addressing HABs in fjords and coastal embayments, GEOHAB implementation in Asia, and benthic HABs; the continued strengthening of activities in IOC regional HAB groups - ANCA, FANSA, HANA, and WESTPAC-HAB; provision of the primary communication platform for the international HAB research and management community via the IOC newsletter 'Harmful Algae News' ; scientific synthesis and identification of emerging issues from the ICES-IOC WGHABD, and the continued development of the integrated IPHAB-IODE Harmful Algae Information System (HAIS) with links to OBIS. Additional detail on important accomplishments over the past two decades is summarized in Oceanography Vol. 23, No. 3.

IPHAB-X decided on priorities for the coming biennium.

Regarding regional HAB Programme development it was decided to assess the feasibility of regional networks or groups in the Red Sea-Gulf-Arabian Sea-Northern Indian Ocean region, Southern Africa, and Pacific Islands Countries and Territories.

In relation to biotoxin monitoring, management and regulations a Task Team was established to maintain regular contact with FAO, WHO and other regulatory bodies and recommend priorities for research, capacity development and engagement with regulatory bodies to address the most pressing issues and threats posed by HAB toxins.

The GEOHAB Research Programme, within the joint framework of IOC and SCOR, is expected to conclude its activities by the end of 2013. IPHAB recognized in a resolution that to fully realize the benefits of the accumulated investments in GEOHAB, and to address any new priorities identified by the IOC in collaboration with SCOR, it would be desirable to extend specific CRPs and framework activities within the GEOHAB Terms of Reference beyond the end 2013. The GEOHAB Scientific Steering Committee was requested to present to IPHAB-XI for consideration a revised Science Plan and outline of an Implementation Plan for GEOHAB beyond 2013.

IPHAB noted that more than 150 countries worldwide operate desalination plants to produce drinking water from seawater and recognised that desalination capacity is forecast to grow rapidly in the coming years as demand for fresh water grows. Furthermore, in recent years, HABs have caused serious impacts at desalination plants. It was decided to organise a meeting on "Impacts and management of toxic and harmful algal blooms at desalination plants and related seawater facilities" before the end of 2012.

Acknowledging the progress made by the IPHAB Task Team on Algal Taxonomy in publishing and updating the IOC Taxonomic Reference List of Harmful Marine Microalgae as an integrated element of the World Register of Marine Organisms and the IOC/ HABP-IODE HAIS it was decided to continue the Task Team.

IPHAB also noted that warming of surface layers of the ocean may lead to the geographical spreading of HAB species and an increase in the seasonal occurrence of some HAB species and therefore decided to identify central unresolved issues that limit advances in understanding how projected climate change may influence HAB events by convening an ICES/IOC/PICES workshop of HAB experts.

Finally based on the IOC co-sponsored 'International Workshop on Fish-killing Marine Algae held in Oslo, Norway, April 2011; and recognising that there is increasing concern about the impact of icthyotoxic algae on society and aquaculture in particular, IPHAB decided to establish a Task Team on Harmful Algae and Fish Kills to prepare an overview of the scale of the issue and priorities and to support the organization of a joint ICES/IOC/PICES meeting to better define global understanding of the broad issues.

The Panel elected its new Chair, Dr. Robert Magnien (USA), and Vice-Chair, Dr. Gires Usup (Malaysia). The outgoing Chairs Dr. Leonardo Guzman (Chile) and Phil Busby (New Zealand) were thanked for their commitment and contribution to international cooperation on HABs.

All reports on IOC HAB activities can be found at www.ioc-unesco.org/ hab/ and any extra information about IPHAB, can be provided by contacting the Chairs (rob.magnien@noaa.gov, giresusup@gmail.com) or the IOC Secretariat (h.enevoldsen@unesco.org).

Johanna Fehling *in memoriam*

25 October 1974 - 17 February 2011

Johanna Fehling died after a long fight against recurrent brain tumours on the 17 of February, at home in Germany with her family, at the tragically young age of thirty six. At her request, Johanna's parents released her ashes into the North Sea on Tuesday the 8th of March. She was known to many in the harmful algal community. An *in memoriam* of Johanna including recollections from colleagues and friends is already displayed on the ISSHA website.

Her parents have requested that anyone who wishes to honour her memory can donate to the German brain cancer support group. They have set up an account in her name that is reached from this link (www.hirntumorhilfe.de/en/helfen-spenden/):
Konto 1010 036 900
BLZ 860 502 00 Sparkasse Muldental
keyword: Johanna Fehling
(Unfortunately credit cards are not accepted online)

Career History

2007 – 2010: Post-doctoral Research Fellow, Department of Systematic Botany, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden. Continuing the same project: Rooting the Eukaryote Tree of Life

2005 – 2007: Post-doctoral Research Fellow, Department of Biology, University of York, York, UK. Rooting the Eukaryote Tree of Life



2004: Research assistant, Culture Collection of Algae and Protoza, Dunstaffnage Marine Laboratory, Oban, Scotland.

2000 – 2004: PhD student, Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Oban, Scotland. Thesis: "Diversity, ecology and domoic acid production of *Pseudo-nitzschia* spp. in Scottish waters". The Open University, Milton Keynes, UK. 309 p. Supervisors: Dr. Keith Davidson, Dr. Christopher J. Bolch, Prof. Paul Tett.

October 1996 – August 2000 M.Sc. student: ("Diplom") Institute for Polar Ecology, Christian-Albrechts University of Kiel, Germany. Thesis: "Sympagische Protistengemeinschaften im Arktischen Packeis der Framstrassenregion" ("Sympagic organisms in the Arctic pack ice of the Fram Strait Region)". Supervisor: Prof. Dr. M. Spindler

March 1996 – September 2000: Short-term contracts as a support scientist at the Institute for Marine Research, Kiel, Germany, and the Institute for Polar Ecology, Christian-Albrechts University of Kiel, Germany. Microscopy of marine protists in sea-ice and water samples from the Baltic Sea, Arctic and Antarctic Ocean

October 1994 – October 1996: BSc. student ("Vordiplom") Christian-Albrechts University of Kiel, Germany.



Johanna with Dave Green at the Falls of Lora near Oban

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The Research on Harmful Algae present at the last ICES Annual Science Meeting in Nantes (France)

Last September 2010, the scientific HAB community had the opportunity to showcase its latest advances to a wide research audience at the ICES Annual Science Meeting 2010 that was held in Nantes (France). An entire session was devoted to the Oceanography and Ecology of HABs and covered a range of issues, focusing on physical/biological interactions and climate change but also including other topics. The session was convened by D. M. Anderson, G. Lacroix and Patrick Gentien. Patrick had been working hard to ensure the success of the session until his untimely demise last May 2010. Below, D.M. Anderson offers a nice summary of the communications presented in the event, which commenced with a dedication in Patrick's memory. The presentations covered highly diverse processes occurring at wide spatio-temporal scales, but it was heartening to see the level of GEOHAB connected research which was presented. Talks relating to the GEOHAB modelling workshop and HABs in Stratified Systems Core Research Programme activities were presented during the session.

All participants at the meeting had an opportunity to watch videos, including one resulting from the GEOHAB endorsed project FINAL (Forecasting the Initiation of HABs) which was carried out in France, Scotland and Ireland. Several of the GEOHAB documents were available to participants, including our brochure and the new "HABs in Fjords and Coastal Embayments" report. The new GEOHAB banner was also prominently on display (see photo). In the picture are Robin Raine (chair of GEOHAB, 2006-2009) and Elisa Berdalet (current vice-chair).



Oceanography and ecology of HABs: physical/biological interactions, climate change, and other current issues

Theme Session - ICES Annual Science Meeting

Conveners: D.M. Anderson (USA), G. Lacroix (Belgium), and P. Gentien† (France)

A theme session focusing on HABs was held at the ICES Annual Science Meeting in Nantes, France in September, 2010. This concept was developed during joint meetings of two ICES working groups – the Working Group on Harmful Algal Bloom Dynamics, and the Working Group on Physical Biological Interactions.

The session began with a dedication in memory of our friend and colleague Patrick Gentien, co-convenor of the session who sadly passed away prior to the meeting.

The session covered a wide range of model formulations and HAB species. Some of the models were empirical, including approaches such as utilizing sustained wind from specific directions to generate a "wind index" that had a predictive value for *Dinophysis* and *Karenia* blooms in southwest Ireland (R. Raine). Other models focused on small-scale behavior and physics such as the scales of turbulence that affect HABs (E. Berdalet), and a population health model of the distribution of *Alexandrium* cells infected by the parasite *Amoebophrya* (M. Sourisseau). This study demonstrated that it is realistic to have spatial separation of infected cells from healthy cells with the appropriate behavior. Larger regional-scale models were also presented including a complex ecosystem model that simulated cyanobacterial blooms in the Baltic given different nutritional initial conditions (U. Daewel). Another large-scale model was a coupled physical-biological model of *Alexandrium* bloom dynamics in the Gulf of Maine (D. Anderson). That model captured the regional dynamics of this species with some skill and is now being used in short-term (days) to seasonal (months) forecasts. In this instance the abundance of resting cysts is a strong determinant of the magnitude of the resulting bloom, though results in 2010 demonstrated how large cyst germination can still fail to provide a significant bloom if growth conditions are not favorable in the water column. The session included regional reports on the monitoring of toxic phytoplankton from three Icelandic fjords (H. Gudfinnsson), bio-geochemistry of cyanobacterial blooms in the Baltic Sea (O. Savchuk), population dynamics of *Dinophysis acuminata* in the Ría de Pontevedra in Northwest Spain (L. Velo-Suárez) and transport of *Dinophysis* blooms along

the south coast of Ireland (R. Raine). Looking to the future, a new project entitled "ASIMUTH" was introduced (J. Silke); this will integrate Earth Observation data, models and in situ data to provide regular HAB bulletins in six locations along the western European Atlantic coast. A series of poster presentations complemented the oral session. Topics included climate change and the impact of storms on HABs (S. Aleksandrov), the rate of domoic acid production in cultures with different forms of nitrate (G. Calu), and the detection of domoic acid by using Solid Phase Adsorption (G. Hermann). Regional presentations included the summer phytoplankton in the Baltic (E. Lange) and monitoring programme in the southern Caspian Sea (M. Monshizadeh). A model of the life cycle of dinoflagellates demonstrated the role of life cycle transitions in regulating bloom dynamics (A. Kroll). Overall the breadth of model types presented for different HABs and different management needs were impressive. It is encouraging that this aspect of the HAB field is progressing at a productive pace. At the entrance of the session, the assistants had the opportunity to receive several documents regarding the IOC/SCOR program GEOHAB. These included brochures, the reports of the GEOHAB Core Research Projects "HABs in Stratified Systems" and "HABs in Fjords and Coastal Embayments", and "Harmful Algal Blooms in Asia. A comparative approach".

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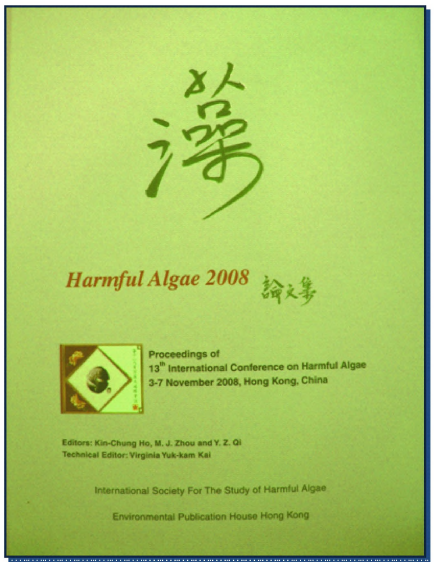
Harmful Algae 2008

Proceedings of the 13th International Conference on Harmful Algae, held at Disneyland Hotel, Hong Kong

Editors:
Kin-Chung Ho, M. J. Zhou and Y. Z. Qi

Technical editor: Virginia Yum-kam Kai



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Future events

AUGUST 2011

Cyanobacteria and Human Health: Merging Ecology, Epidemiology and Neurologic Disorders

4-6 August 2011 Bowdoin College, Brunswick, Maine, USA

Bowdoin College is sponsoring a three-day workshop, preceded by a one-day short course, focused on the linkages between marine and freshwater cyanobacterial blooms, toxicity, and human health impacts. The goal is to bring together specialists in medicine, neurology, toxicology, epidemiology, ecology, oceanography and limnology from medical, academic, research, federal, state and tribal institutions discuss the current state of understanding at the crossroads of these diverse fields as they relate to Cyanobacterial Blooms and Human Health. The workshop will consist of invited plenary lectures, short invited talks and contributed posters in all relevant areas. Participants are encouraged to submit a poster abstract. Further info: /www.bowdoin.edu/earth-oceanographic-science/workshops/index.shtml



DINO 9 – 9th International Conference on Modern and Fossil Dinoflagellates

28 August-2 September 2011, Liverpool, UK

The aim of the conference is to bring together researchers working on present day dinoflagellates with those working on dinoflagellates in the fossil record to foster interdisciplinary understanding and collaboration. Over the years the conference has attracted dinoflagellate workers from across the world who have come together to deepen their understanding of this fascinating group of organisms and gone away with new perspectives and new research avenues to explore. It is in this spirit we offer the 9th conference in the series and cordially invite you to Liverpool to participate in the dynamic social spirit of the city alongside a stimulating intellectual endeavor.
Further info: pcwww.liv.ac.uk/~dino9/index.htm

OCTOBER 2011

Comparative genomics of eukaryotic microorganisms: understanding the complexity of diversity

15-22 October 2011, San Feliu de Guixols, Spain

The EMBO conference on Comparative Genomics of Eukaryotic Microorganism will address the big evolutionary questions of what makes a eukaryote, how multicellularity arose, the evolution of reproduction, pathogenesis, and so on. Lineages of the Eukaryotic Tree of Life (TOL) that until now received only moderate attention, such as the Excavates, Archaeoplastids, Amoebozoa and Choanozoa, will be discussed, together with the better studied Chromalveolates and Fungi.

There will be opportunities for participants to present both short talks and posters, which will be conducted interactively with plenty of time reserved for questions and discussions. In addition there will be interactive workshops on 'Do It Yourself' genomics, databases and bioinformatics.
Contact: heather.barnett@nottingham.ac.uk





OCTOBER 2012

15th International Conference on Harmful Algae

29 October - 2 November 2012, Changwon Gyeongnam, Korea

HAB2012 aims to address all issues related to the causes and effects of marine and freshwater harmful micro- and macro-algae and to serve as a forum for exchanging research outcomes and relevant ideas between researchers, industries, governments and interested parties.
You can find more information at: www.hab2012.kr

Harmful Algae News

Previous issues of HAN and newsletters of the IOC HAB Programme can be downloaded at http://ioc.unesco.org/hab/news.htm

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Write the Editor NOW with news on your work, HAB events in your country or region, or any other matter you wish to share with HAB scientists and managers worldwide. Harmful Algae News has more than 2000 subscribers.

HARMFUL ALGAE NEWS

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Harmful Algae News

AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

No. 45 - January 2012 · www.ioc-unesco.org/hab

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission



This issue of Harmful Algae News marks its 20th anniversary. It also marks the introduction of a new layout and the introduction of a new on-line format. We hope you will like the new formats.

A newsletter for the HAB community was first broached in Takamatsu, Japan, in 1987 and fully formulated at an IOC-SCOR Workshop in Newport, Rhode Island (USA) in November 1991. The first HAN issues were published as a supplement to UNESCO's International Marine Science newsletter, which ceased to exist many years ago. Very few other newsletters have survived as long as HAN in IOC and UNESCO.

A unique aspect about HAN is the dedication of our Editor Tim Wyatt. Tim has from day one taken his job very seriously and has only received very symbolic benefits from the IOC. The IOC wish to express its deep appreciation of Tim Wyatt's unique effort.

Another remarkable aspect is that HAN has survived as a printed newsletter despite the informatics revolution. However, every year the pressure to go e-publishing increases due to the rising cost of postage and with this issue we will introduce the option of subscribing to HAN electronically only. This will save resources. For those that prefer a printed copy and for libraries it will still be possible to subscribe to the printed HAN. You will have a chance to try the new on-line format and if it suits you let us know to which e-mail address you wish to receive HAN in the future. On the back page of this issue you will find further information on how to modify your subscription.

HAN has around 2000 subscribers for the printed version and this has been very stable over the 20 years. At the biennial reviews of HAN by the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB), the Panel has taken this as an indication that the newsletter continues to fill an important role for the HAB research and management community. A role that is complementary to, and in between, those of the peer reviewed journals and the list servers such as phycotoxins, ALGAL-L, and ISSHA.

IOC is pleased that the International Society for the Study of Harmful Algae (ISSHA) uses HAN as its newsletter as it is both practical to the reader and meaningful from a resource point of view.

We hope HAN will continue to be useful to its readers and remind you that you are always welcome to send us ideas, proposals, and constructive criticism. Technology today offers new opportunities to stay connected across the globe and we welcome your thoughts on how we can make the most of them to share and benefit from one another's experiences.

On behalf of Editor Tim Wyatt, the teams at the IOC Science and Communication Centres in Vigo and Copenhagen, the IOC and UNESCO staff in Paris that handle mailing list and printing, and the leadership of IPHAB, thanks to you all for all your contributions over the past 20 years.

Henrik Enevoldsen, IOC of UNESCO

Toxicity and paralytic toxin profile in *Pyrodinium bahamense* var *compressum* and violet oyster in Bahía de Acapulco, Guerrero, Mexico

Harmful algae blooms are common events along the coasts of Guerrero. Since 1991, the local public health laboratory, "Dr. Galo Soberón y Parra", has monitored for red tide-forming species and PSP toxin-producing species along the southwestern coast of Mexico, including toxicity in several marine bivalves. The most common toxic dinoflagellates responsible for red tides in this area are the fish-toxic dinoflagellate *Cochlodinium polykrikoides* and the paralytic shellfish toxin-producers *Gymnodinium catenatum* and *Pyrodinium bahamense* var. *compressum* [1,2]. Sometimes, these three species occur simultaneously along this coast [3].

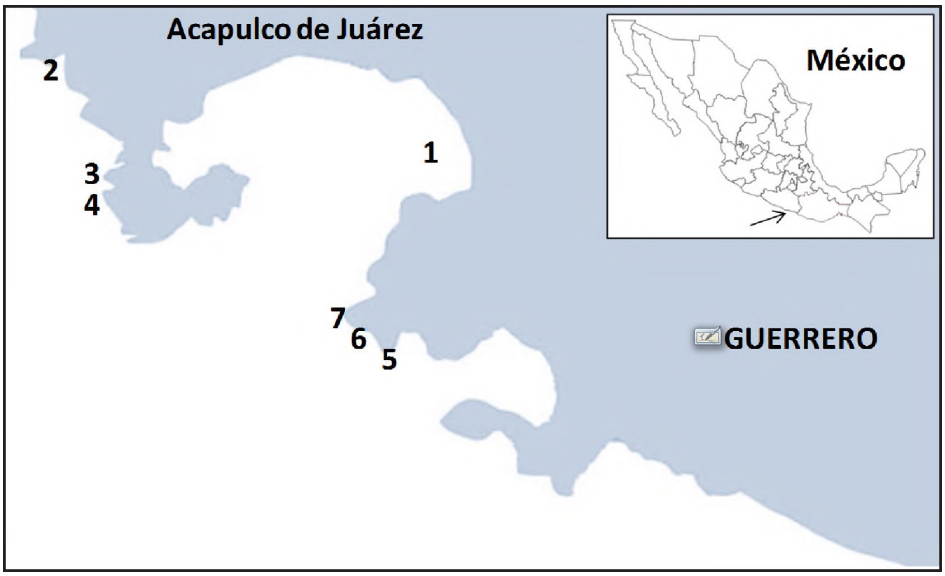

Fig. 1. Sampling stations: (1) Bahía de Acapulco, (2) La Prietilla, (3) La Palmita, (4) Punta Pilares, (5) La Rana, (6) Caja del Muerto, and (7) Punta Bruja.

The first bloom of *Gymnodinium catenatum* was observed in 1999 [4]; its incidence has been increasing since then [2]. *Pyrodinium bahamense* var. *compressum* is broadly distributed along the Pacific coast of Mexico [1,2,3,5]. *Gymnodinium catenatum* and *P. bahamense* var. *compressum* are the main species causing paralytic shellfish poisoning (PSP) along the coasts of the States of Guerrero and Oaxaca.

Two varieties of *Pyrodinium* (*bahamense* and *compressum*) have been recognized [5]. The first extensive outbreak of the var. *compressum* occurred between Zihuatanejo, Guerrero and El Bejuco, Michoacán in November 1996; toxicity ranged from 520 to 6337 µg STXeq 100 g^{-1} in bivalves, and there were six human fatalities and numerous sick people [6]. Eight blooms of var. compressum have since occurred in Bahía de Acapulco [1,3], with toxins in marine bivalves ranging from 21 to 7309 µg STXeq 100 g^{-1}; the highest toxicity occurred in the violet oyster in November 2001 at Las Palmitas, Acapulco.

As part of a continuing toxic microalgae monitoring program, a red tide of *P. bahamense* var. *compressum* was detected in July 2010 in the Bahía de Acapulco (Fig. 1). Two surface samples were collected on 24 July 2010 (Sampling station 1). About 3 liters of seawater were filtered (Whatman GF/F) to determine the presence and the profile of paralytic toxins. Six more seawater samples were collected on 26 July 2010 off Bahía de Acapulco (Sampling stations 2–7) (Fig. 1). Aliquots of 1 mL of these samples were placed in a Sedgewick-Rafter chamber under an inverted microscope (Olympus Axiovert 40 C). Images were recorded at 200× with a digital camera (Cannon Power Shot A64Q) and with a scanning electron microscope (SEM). Six samples of the violet oyster (*Chama mexicana*; Fig. 2) were collected outside the bay to measure shellfish toxicity. Shellfish toxicity was determined by the standard mouse bioassay method (MBM) and by high performance liquid chromatography (HPLC-FLD) [3,7,8].

Pyrodinium bahamense var. *compressum* was the species responsible for this bloom (Figs. 3-7). Cell counts are summarized in Table 1. Abundance ranged from 1000 to 606,000 cells L^{-1}; however, densities of 1.462 × 106 cells

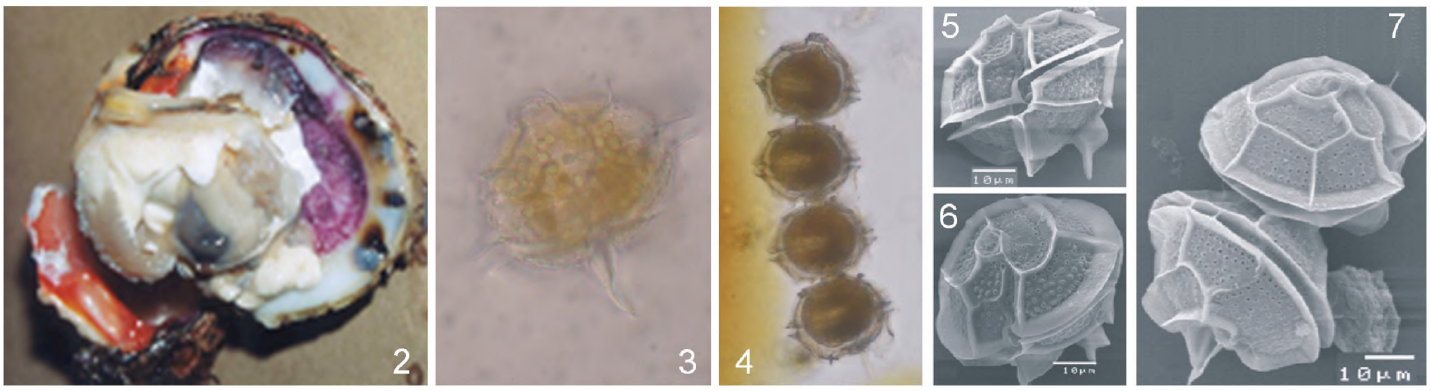

Fig. 2. Violet oyster, a sentinel species used to determine shellfish toxicity in Bahía de Acapulco.
Figs. 3–7. Photomicrographs of Pyrodinium bahamense *var.* compressum *observed in Bahía de Acapulco, Guerrero, Mexico. Cells observed under light microscope (Figs. 3–4) and scanning electron microscope (Figs. 5–7).*

L^{-1} were found in Bahía de Acapulco [7]. This variety occurred as individual cells and two- and six-cell chains. Cell length ranged from 36 to 49 µm. Shellfish toxicity of violet oyster samples analyzed by mouse bioassay ranged from 893.4–1387.53 µg STXeq 100 g^{-1} (Table 1).

Analysis of toxicity in oyster samples using HPLC-FLD varied from 579.03–894.56 µgSTXeq 100 g^{-1} (Table 1). These values are 8–17 times greater than the permitted limit for human consumption. No human poisonings occurred during this event because of the rapid response of the local public health authorities. The toxin profile in the oysters included STX, NeoSTX, GTX3, B1, B2, and C. The toxin profile in phytoplankton included STX, GTX3, B1, B2, and C. STX and NeoSTX were the most important analogue toxins in the oyster samples. Toxin profiles in different marine mollusks included STX, NeoSTX, GTX2, dcSTX, dcGTX2, dcGTX3, and B1 [9]. Variety compressum is highly toxic because its profile is mainly composed of STX, NeoSTX, dcSTX, and B1 [10,11,12] with STX representing up to 85–98 mol% toxin $cell^{-1}$ [12].

On the Guerrero coast, the first PSP incident during 2010 was observed at Playa el Palmar, Zihuatanejo on 20-21 November. Two people exhibited typical symptoms of PSP toxicity after consuming raw clams and they required hospitalization [13]. On 22 December 2010, there was a new bloom of *P. bahamense* var. *compressum*. After eating raw and cooked clams, 12 people in Zihuatanejo had symptoms of paralytic shellfish poisoning; six required hospitalization. The clam Donax punctatostriatus contained 2541µg STXeq 100 g^{-1} [3]. This level is ~30× above the permitted limit for human consumption. Toxicity of these mollusks was linked to the bloom of *P. bahamense* var. *compressum*. Results from the mouse bioassay and HPLC-FLD methods shown significant differences in total toxicity. Toxicity values calculated by HPLC-FLD seem to be underestimates. Studies to validate the use of methods for analyzing paralytic poisons must be performed. Monitoring of species producing these toxins and toxicity levels in marine mollusks along the coasts of Guerrero is ongoing.

This study was funded by the Programa de Vigilancia Sanitaria Permanente y Sistemático de la Marea Roja en el Litoral del Estado de Guerrero, and the Laboratorio Estatal de Salud Pública "Dr. Galo Soberón y Parra" at Acapulco, Guerrero, and by IPN-CICIMAR projects (SIP-20110281, 20110139 and SIP-20110590). I.G.L. received support as a COFAA and EDI fellow of IPN. We are thankful to Ira Fogel (CIBNOR, La Paz) for improving the English text. Yolanda Hornelas-Orozco (UNAM) provided invaluable help with scanning electron microscopy.

Table 1. Abundance of Pyrodinium bahamense *var.* compressum *and shellfish toxicity in the violet oyster in Bahía de Acapulco.*

Sampling stations	*P. bahamense* var. *compressum*	Shellfish toxicity (µg STX / 100 g tissue)	
	Cells L^{-1}	MBM	HPLC-FLD
1 (Bahía de Acapulco)	65000-101000	-	-
2 (La Prietilla)	82000	1159.11	781.68
3 (La Palmita)	16000	1236.30	894.57
4 (Punta Pilares)	606000	1387.53	774.22
5 (La Rana)	1000	1179.36	776.76
6 (Caja del Muerto)	83000	820.35	579.03
7 (Punta Bruja)	1000	893.40	683.38

Table 2. Toxin profile in wild Pyrodinium bahamense *var.* compressum *samples (ng/filter) and in violet oyster (ng/g) collected in Bahía de Acapulco.*

Sampling stations	STX	NeoSTX	GTX 3	B1	B2	C1
Filter 1 - (1)	0,53	-	0,30	0,66	0,24	2,10
Filter 2 - (1)	0,53	-	0,30	2,32	-	0,39
Oyster - (2)	4909,4	94,5	2683,7	10807,0	106,3	28,3
Oyster - (3)	5621,2	108,5	3226,6	10754,2	-	1657,0
Oyster - (4)	4570,0	73,7	2764,9	13133,2	1006,8	40,7
Oyster - (5)	4351,5	146,1	3310,3	10565,3	-	2517,8
Oyster - (6)	2709,2	76,6	2855,0	11461,3	354,4	251,3
Oyster - (7)	3590,5	44,6	3063,2	12106,9	268,4	215,2

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First report of *Pseudo-nitzschia australis* on Moroccan Atlantic Coast and toxicity in Moroccan shellfish

The diatom *Pseudo-nitzschia* has been recorded along the Mediterranean and Atlantic coasts of Morocco. In the monitoring programme, species identification of *Pseudo-nitzschia* is not possible since it requires examination of the fine structure of the cell wall with electron microscopy. The detection and counts of *Pseudo-nitzschia* are reported at generic level [1].

The present work identifies potentially toxic species and confirms the toxicity of the species involved.

Weekly sampling was done on the Atlantic coast: seawater was collected from Oualidia lagoon (32°59'N-08°43'W; 32°58'N-08°45'W), Jemaa Ouled Ghanem area (open sea) (32°51'N-08°53'W; 32°47'N-08°57'W: open sea) and Cap Beddouza (open sea).

Qualitative and quantitative analysis of *Pseudonitzschia* was done from January 2008-December 2009 during the routine HAB monitoring programme. On the Atlantic coast, *Pseudo-nitzschia* spp are frequent components of the phytoplankton (detected in 80%-90% of samples) [2]. *Pseudo-nitzschia* was found in all seasons, in a wide range of temperatures. Highest cell numbers were observed during spring and summer. The maximum count was 5.4 104 cell L^{-1}, off Cap Beddouza in July 2008, at a temperature of 19°C. In Oualidia lagoon, the Pseudo-nitzschia population was composed of large and short chain formers, the former dominant. In the Jemaa Ouled Ghanem area, the large and the short forms were most abundant from April to July 2009.

SEM examination showed one species with large asymmetrical valves, no central interspace (Fig 1.), 7 µm in width; large simple poroids arranged in 2 rows (4 pores in 1µm), 18 interstries in 10µm, and 17 fibules. These characteristics identify it as *P. australis*, a domoic acid producer.

Since 2004, domoic acid was noted in clams and mussels from different shellfish areas located on Atlantic shores, but concentrations rarely reached the legal threshold (20 µg/g of meat) [3]. But in the second week of May 2009, domoic acid in mussels (*Mytilus galloprovincialis*) from Jemaa Ouled Ghanem shores reached 28.58 µg/g meat, and 43.87 µg/g meat one week later. The shores were closed for harvesting. The phytoplankton analyses revealed high *Pseudo-nitzschia* counts in this period (103 to 104 cell L^{-1}).

This is the first ASP event in Moroccan waters with subsequent closure of the areas for harvesting. *Pseudo-nitzschia* is commonly observed in Moroccan Atlantic and Mediteranean waters, and frequently forms blooms in spring and summer. As a DA producer, *Pseudo-nitzschia australis* is implicated in DA accumulation in Moroccan shellfish.

Acknowledgements

We thank Steve Morton (NOAA, Charleston, SC, USA) for his collaboration.

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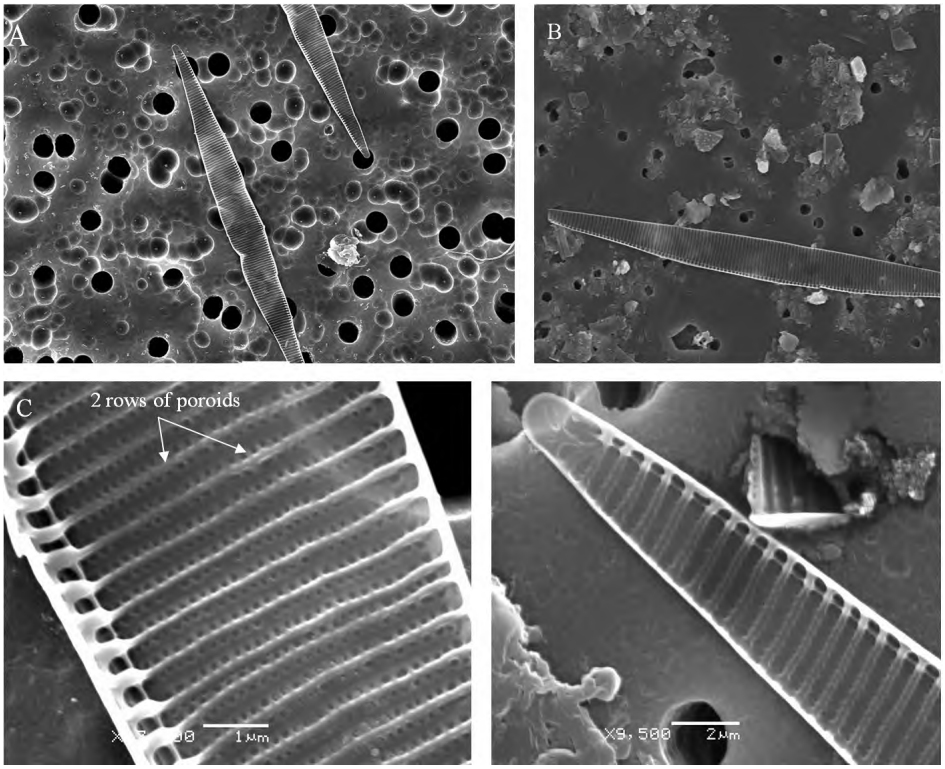

Fig. 1 . Scanning electrons micrographs of Pseudo-nitzschia australis*. A, B) Valve illustrating asymmetrical valve and absence of central interspace. C) Higher magnification of central part of valves showing the two row of poroids / striae, D)*

First report of *Pyrodinium bahamense* in the Straits of Malacca

Pyrodinium bahamense is the main causative organism responsible for paralytic shellfish poisoning (PSP) events in Malaysia. The species was first found on the Sabah Bornean coast in 1970 [1]. No record of *P. bahamense* was reported from other parts of Malaysian waters thus far. The distribution of this dinoflagellate is generally presumed to be confined only to Sabah waters. In our recent plankton survey at Port Dickson, the Strait of Malacca (Fig. 1), we found cells that resembled *Pyrodinium* in the plankton samples. Cells appeared as two-cell chains in very low densities in net samples. Based on the morphological characteristics of the cells, the species was confirmed as *P. bahamense* var. *compressum* (Fig. 2). This is the first record of *P. bahamense* from the Strait of Malacca. The question arose as to whether the species has been overlooked in previous plankton studies or has been introduced by human activity, such as ballast water discharge. This could only be answered with more studies and information obtained on the natural and cultured specimens from Port Dickson.



Fig. 1. Malaysian map showing the sampling sites.

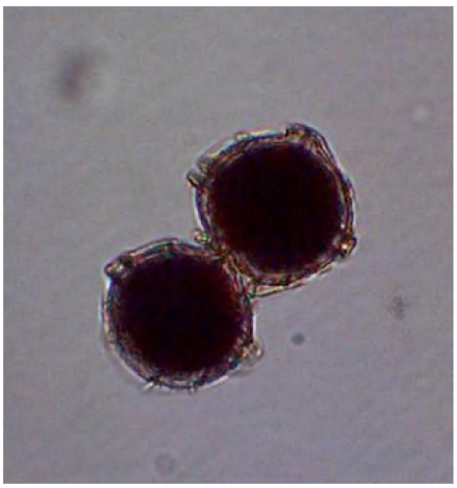

Fig. 2. Pyrodinium bahamense *var.* compressum *from Port Dickson, Malaysia. LM showing 2 cells in chain.*

Alexandrium tamiyavanichii is another highly toxic dinoflagellate responsible for PSP in Malaysia [2]. In Malaysia, the distribution of this species has been confirmed in Sebatu, Melacca (Lim, 1999) [3] and Kota Kinabalu, Sabah [4]. Most recently, the species was encountered in the waters of Sarawak. A clonal culture of *A. tamiyavanichii* was established from the estuary of Samariang, Sarawak. Thecal plate arrangement of cultured cells stained with Calcoflour White was observed by fluorescence microscopy. Based on features of apical pore complex (APC), anterior sulcal plate (s.a.) and posterior sulcal plate (s.p.), the strain was identified as *A. tamiyavanichii.* Toxicity of this strain was further confirmed by ELISA (Skit, Shin Nihon Kentei Kyokai, Japan) and HPLC [5].

The findings of *P. bahamense* from Port Dickson and *A. tamiyavanichii* from Samariang have shown the need to expand monitoring of these species in our waters. Plankton monitoring and toxicity tests on edible molluscs from the areas should be carried out to ensure they are safe for local consumption.

Acknowledgements

We are grateful to Y. Takata for his assistance in the toxicity analysis.

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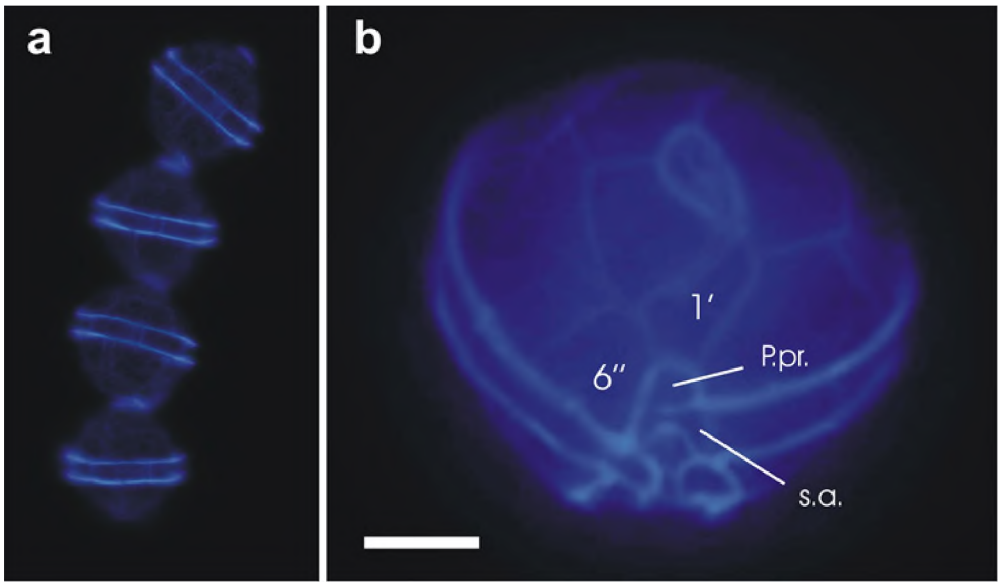

Fig. 3. Alexandrium tamiyavanichii *from Samariang, Sarawak, Malaysia. Epi-fluorescence micrograpsh with four cells in chain (a) and the apical view (b).*

Monitoring potentially ichthyotoxic phytoflagellates in southern fjords of Chile

Autotrophic phytoflagellates have been poorly studied in Chilean coastal waters [1]. These cells are extremely fragile, and are not usually identifiable in preserved samples [2]. *Heterosigma akashiwo* and *Pseudochattonella* cf. *verruculosa* are two species of ichthyotoxic flagellates that have generated significant losses to the salmon industry in southern Chilean fjords. *P.* cf *verruculosa* was initially classified as a rhaphidophyte (as *Chattonella verruculosa*), but DNA sequencing places the species in the Dictyochophyceae [3, 4]. These independent studies renamed it *Pseudochattonella verruculosa* and *Verrucophora verruculosa* respectively. The first of these names currently has priority.

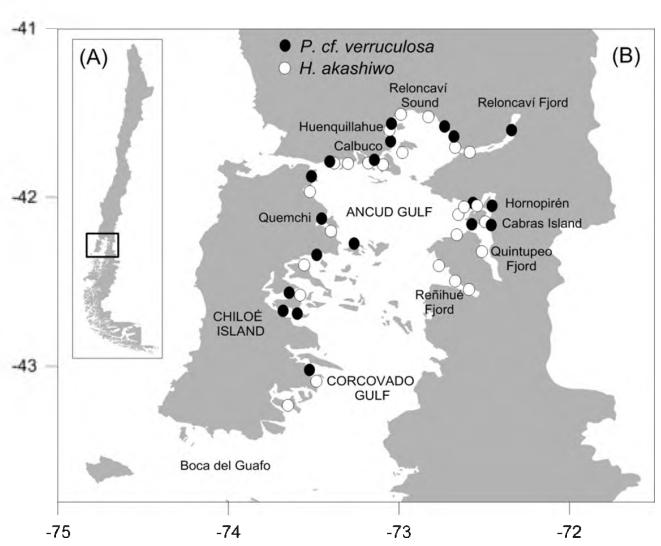

Fig. 1. A) Study area in southern Chile. B) Detection zones of P. cf. verruculosa *and* H. akashiwo.

Pseudochattonella cf. *verruculosa* was first detected by the monitoring program in the Cholgo area (Hornopirén) during summer 2004. In that year, it occurred in Reloncaví Fjord and Sound, Calbuco, North and Central-South Chiloé Island and Quintupeu fjord (Fig. 1B). In this last place, the greatest abundance was 22 cells ml^{-1}, a concentration sufficient to produce farmed salmon mortalities that ranged between 2 and 5%, with a diagnosis that reported neurological and digestive problems. This event coincided with a high sea surface temperature and an intense outbreak of *Vibrio parahaemolyticus*. Subsequently, the presence of this dictyophyte has recurred in the summer months of January and February in the years 2005, 2009 and 2011 (Fig. 2A). These last two blooms have been the most important in terms of cell abundance, reaching concentrations of 394 and 42 cells ml^{-1} in the areas of Huenquillahue and Cabras Island respectively, and comprising up to 75% of the total phytoplankton community (Fig. 2C).

In the spring of 1988, particularly in September, the salmon industry experienced a major crisis due to an intense outbreak of the ichtyotoxic raphidophyte *H. akashiwo*, which was colloquially called "brown tide". Because of high concentrations of this species, greater than 100,000 cells ml^{-1}, there was high mortality of farmed salmon in the inner sea of Los Lagos region (Reloncavi sound, Hornopiren and Chiloé Island). Associated mortalities were higher than 5000 tons and economic losses about US$11 M. Due to this event, the phytoplankton monitoring program was set up, covering a great part of the northern Patagonian inner sea. This program began to detect the presence of *H. akashiwo* again in 1993 and 1994 (Fig. 2B). In these years (from September to December and January to March, respectively), the maximum concentration was 18 cells ml^{-1} and its distribu-

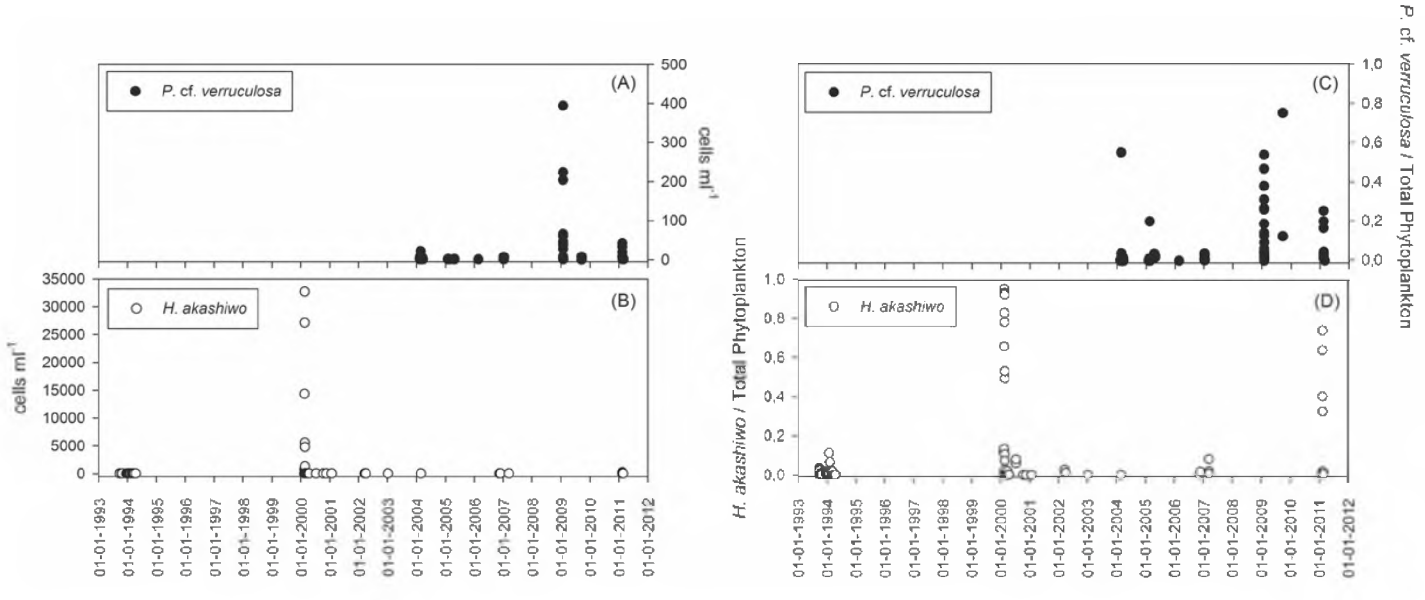

Fig. 2. A-B) Temporal variation in abundance of P. cf. verruculosa *and* H. akashiwo *(cells ml-1). C-D) Temporal variation in ichthyotoxic phytoflagellates/total phytoplankton (cells ml-1) ratio.*

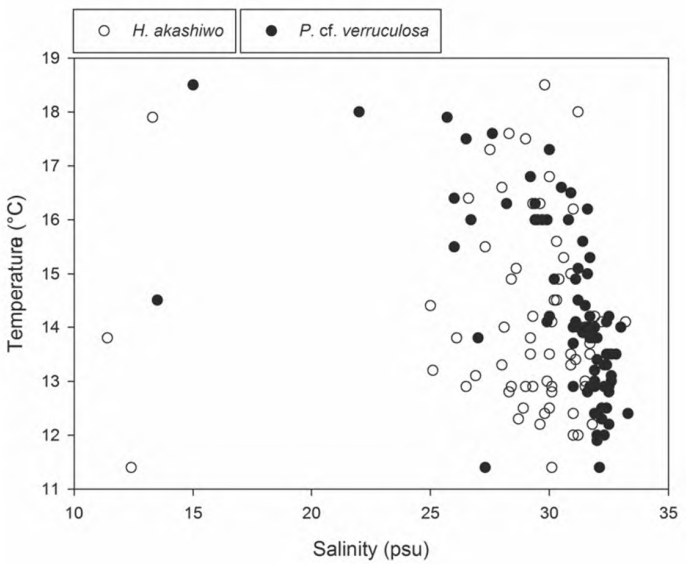

Fig. 3. T-S diagram showing water masses in which ichtyotoxic phytoflagellates were detected.

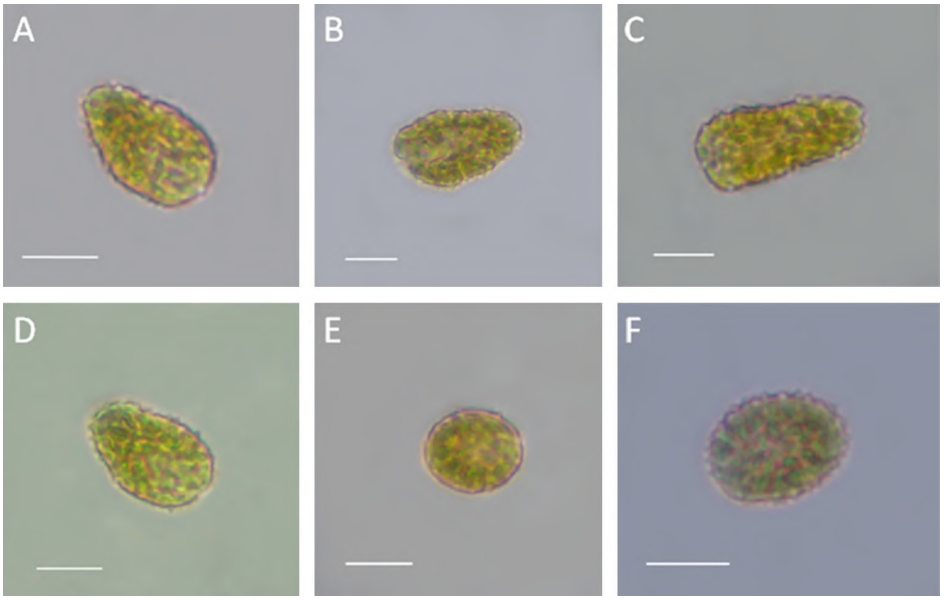

Fig. 4. A-F) Morphological variations of Pseudochattonella *cf.* verruculosa *in southern fjords of Chile. Scale bar: 10µm.*

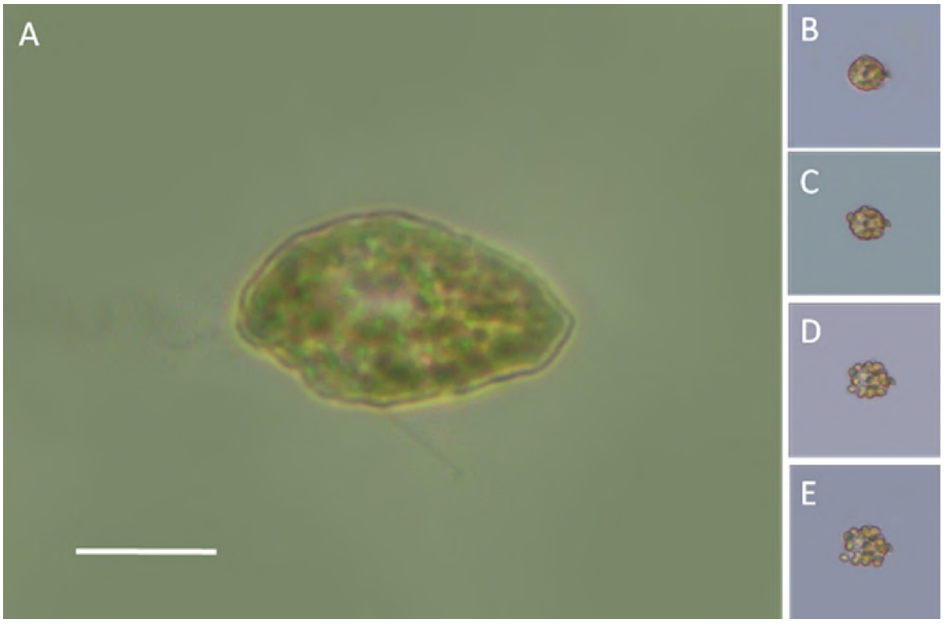

Fig. 5. A. Heterosigma akashiwo *vegetative cell. B-E.* H. akashiwo. *Cell destruction sequence due to Lugol fixation. Scale bar: 10µm.*

tion was restricted to areas of Hornopirén, Reloncaví Sound and Calbuco in 1993, and in 1994 reached the coast of Chiloé. In 2000 an event of great magnitude was recorded again, especially in the Reñihue fjord, associated with mortalities of up to 40% of caged salmon with low abundance of 40 cells ml^{-1}, which corroborates the high toxicity of this species for these fish. *H. akashiwo* impacted farmed salmon during March 2002 on the coast of Quemchi, and more recently in 2011 in association with *P.* cf. *verruculosa*.

Both species, *H. akashiwo* and *P.* cf. *verruculosa*, have been detected in similar ecological niches, primarily in estuarine waters. TS diagrams show these phytoflagellates associated with water temperatures between 11.2 and 17.3 °C (*H. akashiwo*) and between 10 and 18.5 °C (*P.* cf. *verruculosa*), which describes their appearance in the fjord during spring and summer (Fig. 3). Blooms of these species have been observed in stratified waters with salinities between 11.4 and 33.3 psu, but their greatest abundance has been near 25 psu. Both *P.* cf. *verruculosa* and *H. akashiwo* are difficult to identify with light microscopy, mainly due to their high morphological variability (Fig. 4) and their lability in fixing solutions (Fig. 5). There is no information to show these phytoflagellates have been introduced to Chilean waters through ballast water, but their presence has only been recorded since the beginning salmon farming in southern Chilean fjords.

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First record of the ciguatera causing genus *Gambierdiscus* in Brazil

Gambierdiscus was recorded for the first time in Brazil in March 2006 associated with macroalgae *Tricleocarpa cylindrica, Ulva lactuca* and *Caulerpa sertularioides* collected from Maracaípe, Pernambuco state (8° 32' S, 35° 00'W), northeast Brazil. *Gambierdiscus* sp. was present in low numbers (respectively 29, 10 and 17 cells g fresh weigh macrolgae-1) and was accompanied by *Prorocentrum lima* (the most abundant species in the epi-benthic dinoflagellate community of those samples), *Ostreopsis* cf. *ovata, Prorocentrum rhathymum, Prorocentrum emarginatum, Amphidinium* sp. and *Coolia* sp. [1]. The area is a shallow reef close to a small river discharge that drains a mangrove. Mean annual seawater temperature is 26 °C and varies between 25 °C and 28 °C. Studies on the *Gambierdiscus* specimens from northeastern Brazil are being conducted.

More recently, in 2007, Gambierdiscus was also found in macroalgae samples from Rio de Janeiro, at Armação dos Búzios (22° 45' S, 41° 53' W) and Arraial do Cabo (22° 59' S, 42° 00' W, Fig. 1) [2]. In Rio de Janeiro, epi-benthic dinoflagellates were monitored from June 2006 to November 2007. *Gambierdiscus* sp. was recorded for the first time in this region in March 2007. A total of 276 macroalgae samples were collected and analysed during that study from two stations: Forno embayment (Arraial do Cabo) and Tartaruga (Armação dos Búzios) and the genus *Gambierdiscus* was present in 20% of samples. The genus was found associated with macroalgae *Sargassum vulgare, Padina gymnospora, Canistrocarpus cervicornis, Caulerpa racemosa, Amphiroa fragilissima, A. braziliana, Codium intertextum* and *Hypnea musciformis.* However, only in *Sargassum vulgare* and *Hypnea musciformis* samples from Tartaruga, did *Gambierdiscus* exceed an abundance of 10 cells g FW^{-1}. It was more frequently found associated with *Sargassum vulgare*, and was present in 40% of the 52 *Sargassum vulgare* samples analysed from Tartaruga station.

The highest *Gambierdiscus* cell density found in the area was 74 cells g *Sargassum vulgare* FW^{-1} in April 2007, when *Ostreopsis* cf. *ovata* was the most abundant species (238 *O.* cf. *ovata* cells.g FW^{-1}). In May 2007, low total epi-benthic dinoflagellate densities (71 dinoflagellate cells g *S. vulgare* FW^{-1}) were observed, and *Gambierdiscus* was the more abundant epi-benthic dinoflagellate, with mean abundance of 37 cells g *S. vulgare* FW-1. Surface water temperature at Tartaruga varies between 20 and 25 °C while at Arraial do Cabo temperature ranges from 16 to 24 °C, due to coastal upwelling.

The study area fits well the permissive habitats reported for the genus, including annual water temperature between 21 and 31 °C, abundant macroalgae or other substrata for the cells to attach to, low to moderate turbulence, high, stable salinities and attenuated light levels [3]. It is likely that the increased cell numbers recorded at Tartaruga, relative to Forno, may be related to consistently higher temperatures at the former station, due to the influence of coastal upwelling at Forno. However, this assumption requires further studies.

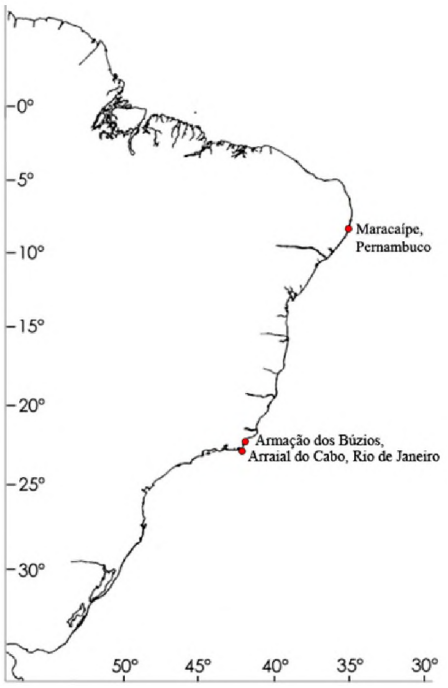

Fig. 1. Map of Brazil showing the three locations where the genus Gambierdiscus *was reported.*

Gambierdiscus specimens from Rio de Janeiro are in agreement with the morphological description of *G. excentricus* recently described in samples from the Canary Islands [4]. The cells are lenticular, anteroposteriorly compressed, round to ellipsoid in apical and antapical view with numerous golden-brown chloroplasts disposed radially (Fig. 2a). The average depth (dorsoventral diameter) of cells is 98 ± 6 (88 -107) µm and width 80 ± 5 (72 - 88) µm (n=27). The thecal surface has nu-

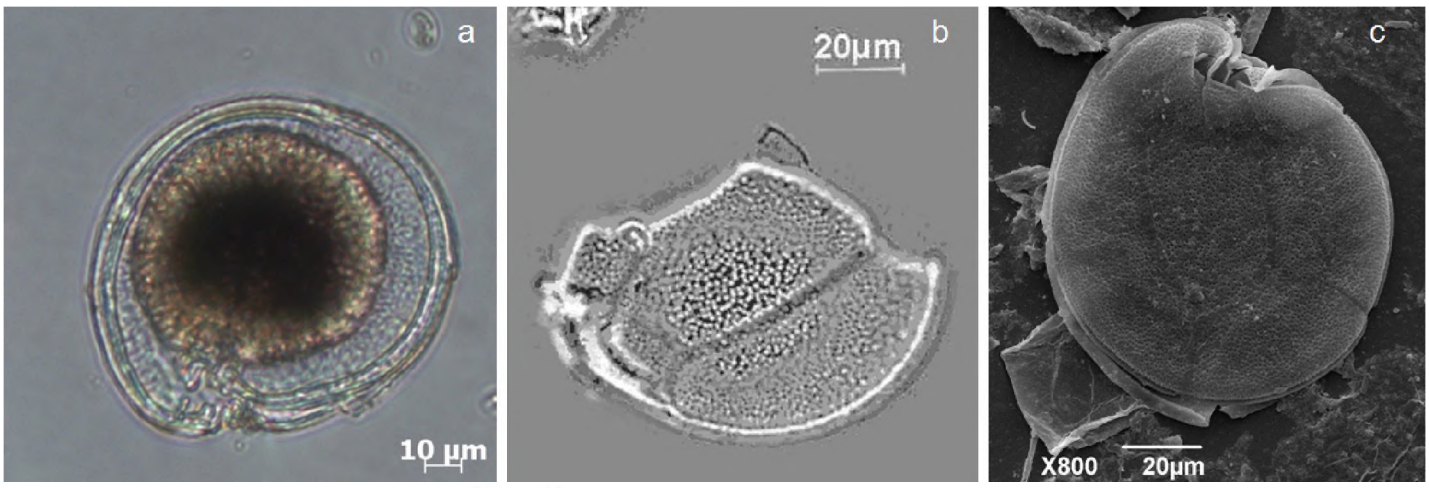

Fig. 2. Gambierdiscus excentricus *from Armação dos Búzios, Rio de Janeiro, Brazil. a) Light microscopy of cell in apical view. b) Part of the epitheca showing the diagnostic 2' plate in light microscopy. c) SEM image of the hypotheca.*

merous irregularly distributed round pores (Fig. 2a,b). The apical pore plate (Po) is oval and ventrally displaced and the second apical plate 2' has the suture 2'/3' about twice as long as the suture 2'/4' (Fig. 2b); plate 6" was not observed. Plate 2"'' is about twice as long as wide, being wider towards the ventral side (Fig 2c).

Although in low abundance, the presence of the genus raises concerns regarding the occurrence of ciguatera. Brazil has an extensive coast and experiences prolonged elevated water temperatures. This condition is favorable for increased *Gambierdiscus* growth, and often correlates with increased incidence of ciguatera [3]. Therefore, it is necessary to establish a monitoring programme for the genus and broaden our knowledge of the geographical distribution of *Gambierdiscus*. There are no official reports of ciguatera in Brazil. However, in some areas of the northeast coast, local communities avoid the consumption of top predator fish of the Lutjanidae during a few months each year, and there have been anecdotal reports of domestic animals intoxicated after eating fish viscera. The possible relations between these observations and ciguatera toxins need further investigation.

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Dear HABs friends and colleagues all over the world!

It is my great pleasure to invite you to Korea to join the 15th International Conference on Harmful Algae(ICHA) from 29 October - 2 November 2012 at the Changwon Exhibition Conference Center(CECO), Gyeongnam, Republic of Korea, under the theme of "Man and Harmful Algal Blooms(HABs)".

The purpose of this meeting is to share new international findings on HABs and to provide an exciting opportunity to celebrate the power of our collaborative research. We will do our best to make the 15th ICHA meeting one of the most informative and interesting international HABs conferences.

I kindly ask you, our HABs friends and colleagues, to join this meeting and also to enjoy the excitement and dynamics of Korean life. In the fall of 2012, the colorful mountains and productive coastlines will be ready to welcome your visit to "Korea XV ICHA2012."



Kim Du Kwan
Governor of Gyeongsangnam-do



Kim Young Man
President of NFRDI



Han Myung Soo
Chair of KORHAB



Kim Hak Gyoon
Chair of Local Organizing Committee, 15th ICHA

The Conference will address the following topics of interest:

- Population dynamics of Harmful Algal Blooms
- Time series of HAB events: Climatic and anthropogenic induced impacts
- Impact of HABs on marine food webs and ecosystem structure and function
- Biological interactions: allelopathy, mixotrophy, parasitism, symbiosis, bacteria and viruses
- New regional HAB events
- Inland Seas and HABs
- Introduction of allien species and HABs
- Cyanobacterial ecology, physiology and bioactive compounds
- Genomics and genetic diversity of HABs
- Toxins: chemical structure and synthesis, detection and analytical methods
- Novel sensor technologies for bio-sensing applications in HAB research and monitoring.
- Harmful Algae culture collections
- Management, mitigation and public outreach for HABs

Two new localities for *Gambierdiscus* toxicus in the Mexican Pacific

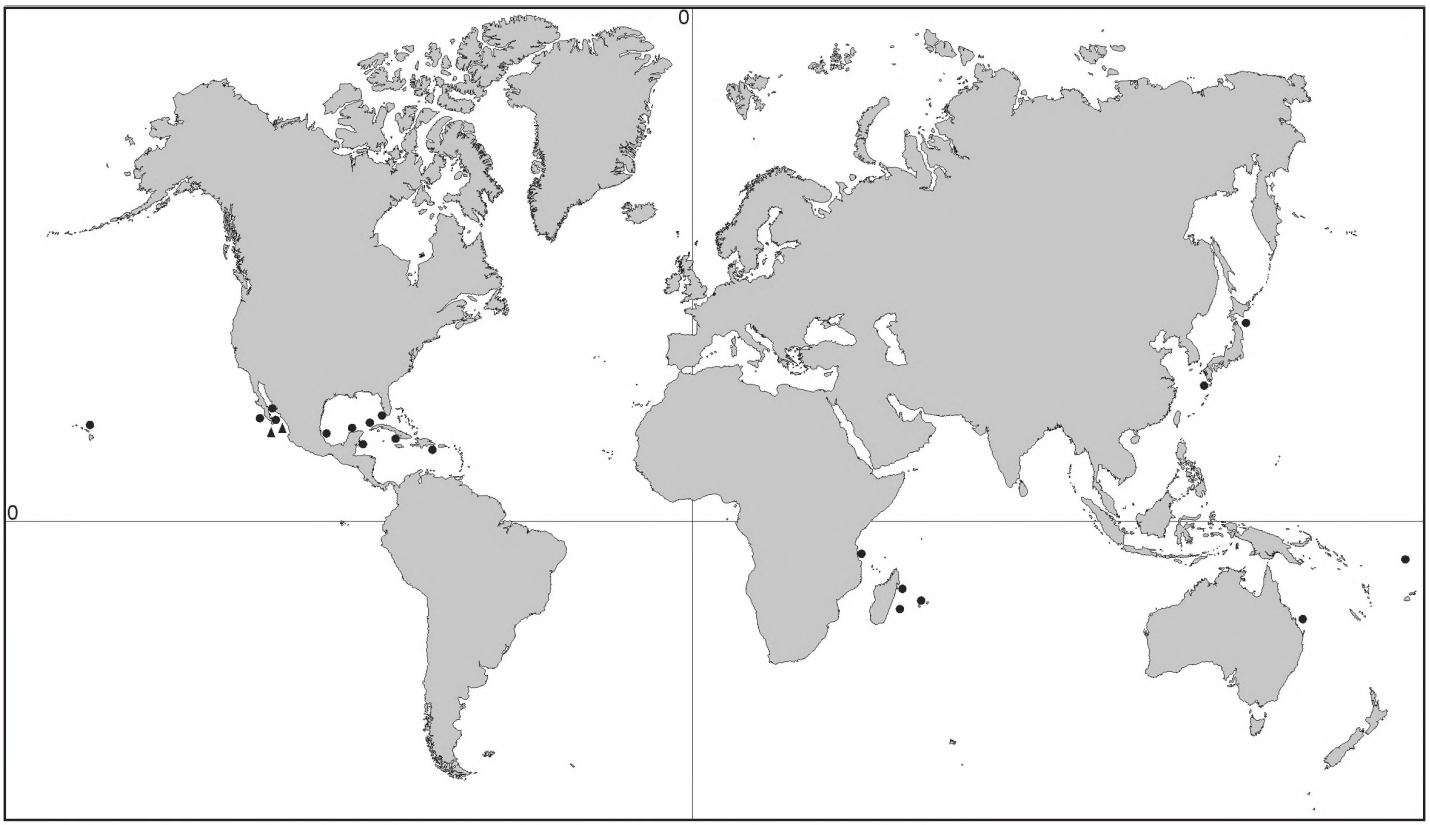

Fig. 1. Global distribution of Gambierdiscus toxicus. *The black triangles are the new locations.*

Gambierdiscus toxicus was described 32 years ago and since then its overall distribution has always been referred to as epiphytic and benthic in circumtropical reefs. It was first described in the Tuamotu Archipelago and the Gambier Islands [1], hence its name in French Polynesia. It was discovered almost simultaneously in Hawaii [2], then in the Indian Ocean [3] , in the Virgin Islands and Florida [4], Belize, Caribbean [5], mostly in Cuba where it represents just over 50% [6], and on the Great Barrier Reef [7]. In the Gulf of Mexico it has been recorded only off the coasts of Quintana Roo [8], Yucatan [9] and in Veracruz [10]. In the Gulf of California it is known from La Paz and Magdalena bays [11] (Fig. 1).

On 25 February 2011, as part of a project "Reproductive biology of coral-destroying sponges" (SEP-CONACYT: 102 239), samples were collected with a 60 micron mesh horizontal plankton sampler [12] around Isabel Island (30 km off the coast of Nayarit) and the largest island of the Revillagigedo Archipelago, Socorro Island (Fig. 2). Large amounts of "smallest discus" were tak-

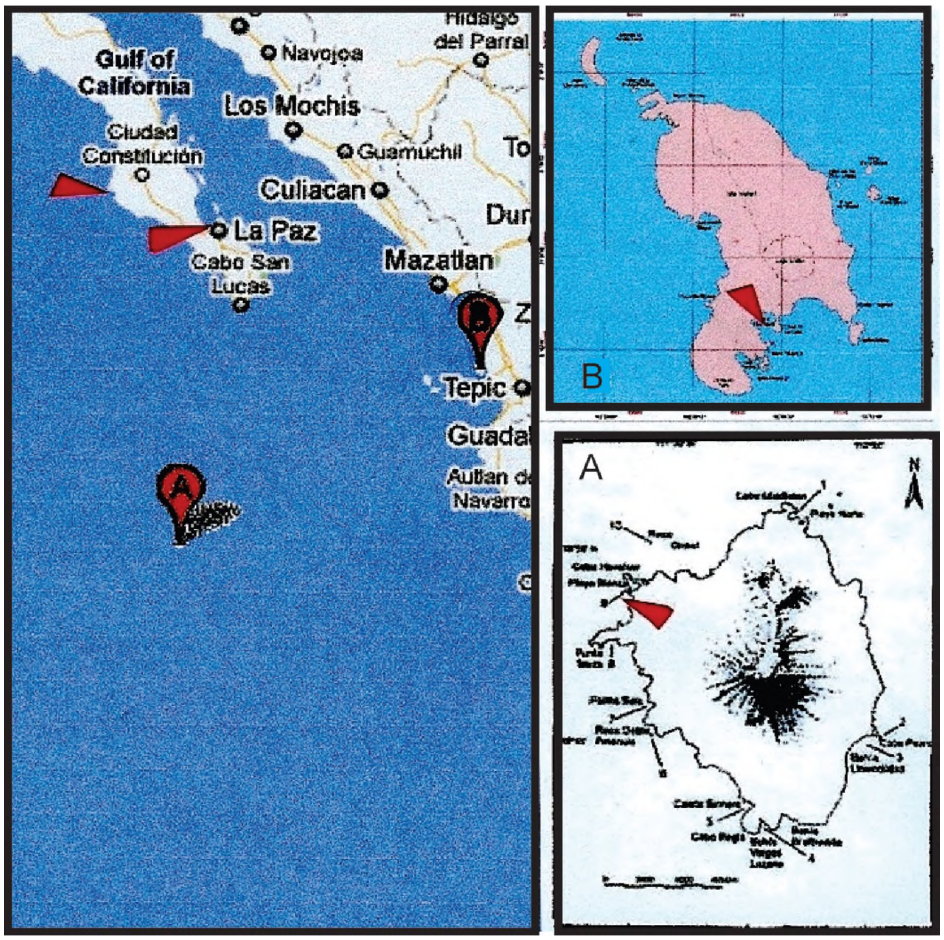

Fig. 2. Location of Isabel and Socorro Islands, Mexican Pacific. Red triangles are sampling sites.

en, later identified as *Gambierdiscus toxicus* Adachi et Fukuyo, 1979 [1]. Close examination of selected specimens with a scanning electron microscope indicate an almost exact coincidence of this material with the morphological characteristics previously described by Okolodkov (Table 1). Only the length of the apical pore complex (APC) was greater [8], and the hook-shaped opening of the specimens from Socorro Island was occluded by a membrane (Fig. 3B). In the Mexican Pacific, there is evidence of ciguatera in La Paz and Magdalena bays and in Alijos Rock and Pardito Island and it has been suspected that this ciguatera is due to *G. toxicus* and *Ostropsis ovata* [13]. However, until now, there were no firm identifications. This study confirms the presence of *Gambierdiscus toxicus*. There is still no evidence of *O. ovata*, but *Ostreopsis siamensis* has been identified on Isabel Island [14].

Table 1. Morphology of Gambierdiscus toxicus *in Yucatan and the Mexican Pacific.*

Caracter (µm)	Okolodkov *et al* 2009	I. Isabel	I. Socorro
Form	lenticulate	=	=
Transdiameter	65-95	76-91.4	97
Long D-V	52.5-70	72-83	94
Long A-Antapical	63-87.5	41.6-50	?
Long. APC	6.23-8.6	8-9	8.06
Wide APC	4.56-5.38	5.23-6.25	4.35
Form APC	ellipsoidal_fishhook	=	to cover
Pore APC	48	45-54	45
Cingulum	Ascends	=	=
List	Smooth	=	=
No.plates	8	=	=
Pore plates Ø	0.4	0.2-0.3	0.4

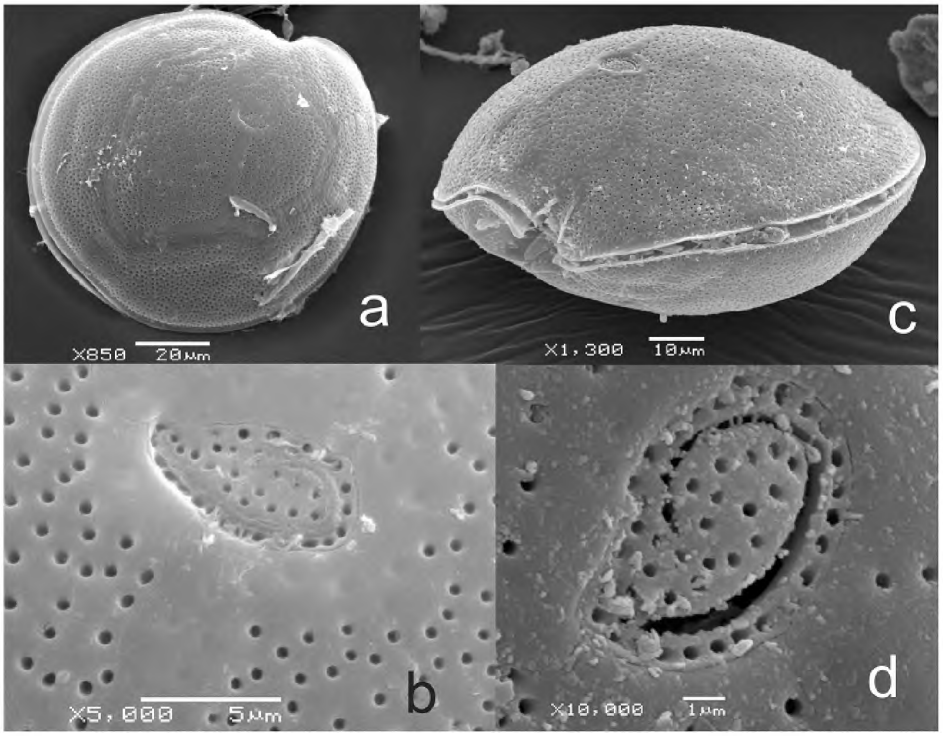

Fig. 3. Gambierdiscus toxicus*: a-b Socorro Island. c-d Isabel Island. a) apical view. b) APC occluded. c) side view. d) APC open.*

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First bloom of *Ostreopsis* cf. *ovata* in the continental Portuguese coast



Fig. 1. Sampling site on Lagos beach

Blooms of *Ostreopsis* in coastal areas are a topic of increasing interest, mainly in the Mediterranean area, due to the potential hazard that species of this genus might cause to human health and the consequent negative effect on the tourism economy. In contrast to typical blooms of other planktonic dinoflagellates, *Ostreopsis*, as an epibenthic genus, proliferates to form a thin pellicle that covers the substrate. Cell aggregates are normally released into the water column after events of increasing hydrodynamism (waves, tides). These aggregates are detectable by sight as mucilaginous flocs in the water column and at surface in shallow waters.

During the last decade, records of *Ostreopsis* events have been increasing in the Mediterranean. Following outbreaks in the W Mediterranean, in the Balearic Islands in 2005 and on the Murcia coast of Spain in 2006, *Ostreopsis* spp were detected in the NE Atlantic, in macroalgae samples from Madeira and the Canary Islands [1]. The species was later identified as *O.* cf. *ovata* [2]. On the Moroccan Atlantic coast off Cape Ghir, *O.* cf. *siamensis* was observed for the first time in 2004 and blooms have been increasing in intensity and frequency since then [3]. In 2008, several fishermen from the Portuguese Selvagens Islands (located at the same latitude as Cape Ghir) became sick after eating a fish from the genus *Seriola*. The phytoplankton community present in seawater samples from those islands and provided to the IPIMAR monitoring programme revealed the presence of *Ostreopsis* spp., although the relationship with the syndrome was not established. On the Portuguese mainland, during 2008, *Ostreopsis* cf. *siamensis* was identified for the first time in the SW upwelling coast of Sines [4]. In the same year, this species was detected in the Portuguese mid-Atlantic Azores Archipelago together with *O. heptagona* and *O.* cf. *ovata* [5].

In September 2011, as part of a sampling program started in 2010 [6], a bloom of *Ostreopsis* was seen on the beach of D. Ana (Lagos coast, south Portugal, Fig. 1) due to the presence of mucilaginous filaments. This information was communicated to IPIMAR, the national phytoplankton monitoring laboratory, which intensified sampling in time and in adjacent areas. The bloom reached densities as high as 5420 cells L^{-1} although concentrations were lower (40 to 320 cells L^{-1}) in adjacent areas. A forecast of bloom transport and aggregation/dispersion was made with the MOHID operational model for the Portuguese coast (http://forecast.maretec.org/), under the framework of FP7-ASIMUTH project (IST-IPIMAR). Local authorities closed several beaches for bathing once informed about the bloom occurrence and model predictions for its transport.

Identification of *Ostreopsis* solely on morphological criteria is difficult, even when details of the thecal plates are taken into acount. As in most *Ostreopsis* blooms, there was great morphological and size variability in cells from the Lagos coast. Cell dimensions fell within the range of *O.* cf. *siamensis* and *O.* cf. *ovata* and were smaller than the other described *Ostreopsis* species (Figs. 2 and 3). Due to the difficulty of finding a conclusive morphological identification, a genetic analysis was performed to both field and cultured strains based on the ITS1-5.8S-ITS2. Genetic analyses revealed the presence of *Ostreopsis* cf. *ovata*. Our sequences, when compared

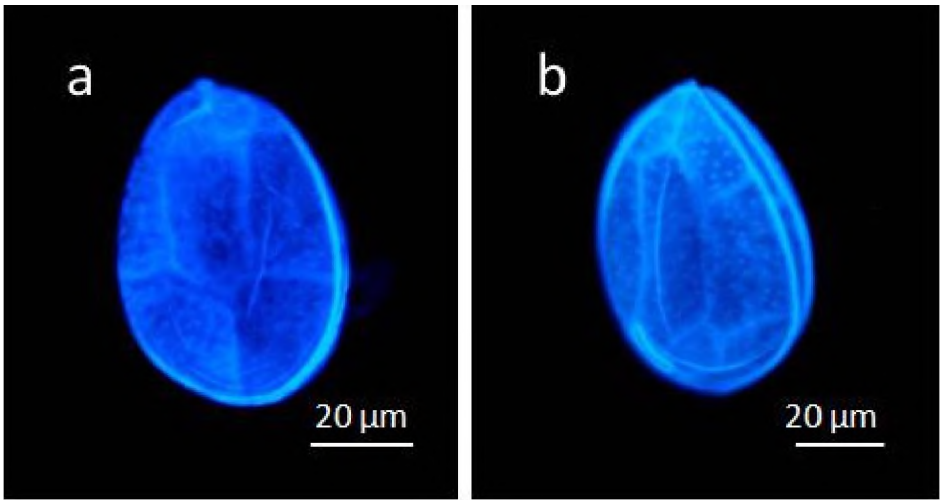

Fig. 2. Calcofluor stained field cells of Ostreopsis. *Hypotheca (a) and epitheca (b) views*

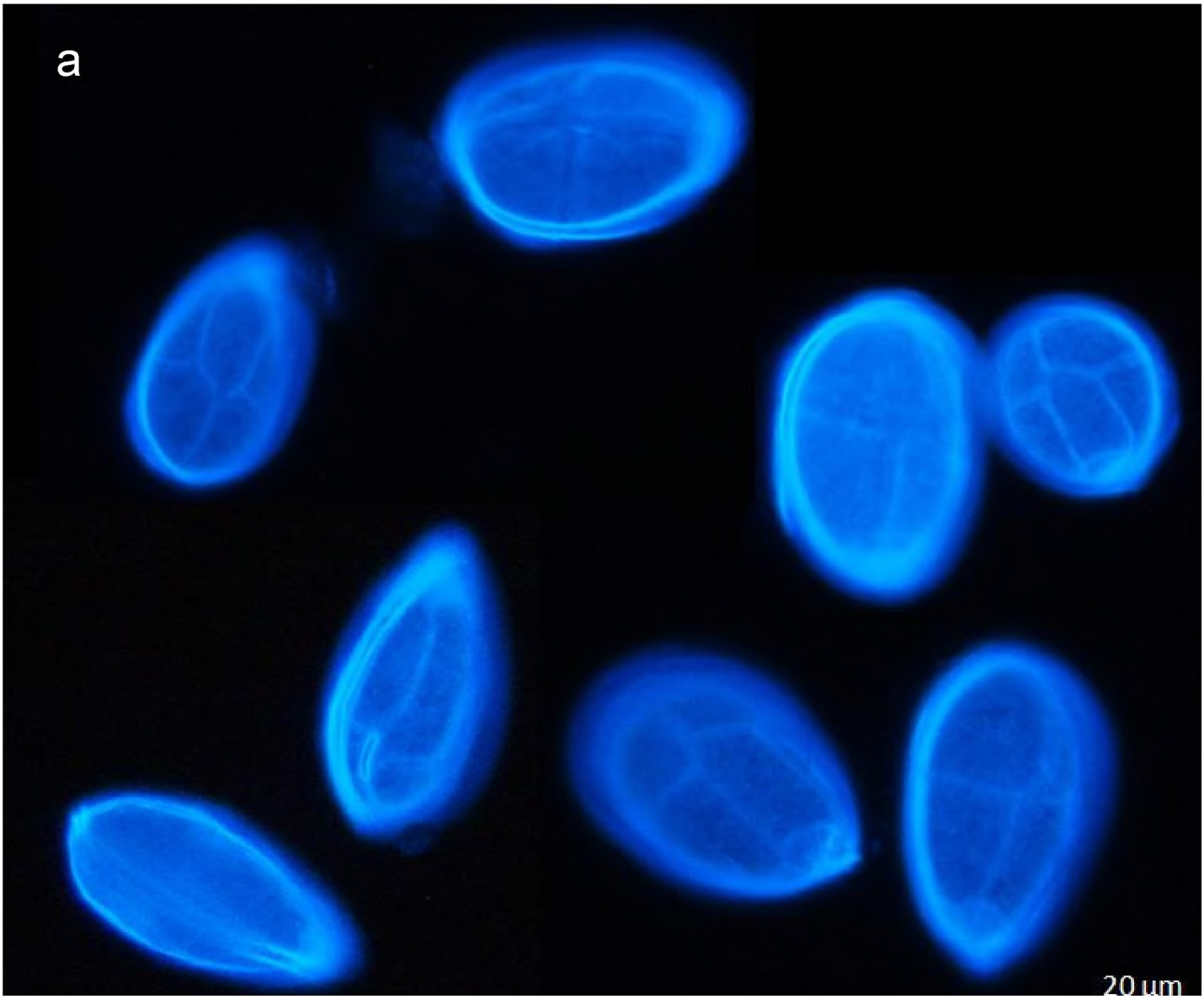

Fig. 3. Field sample of Ostreopsis*: (a) Light microscopy; (b) Calcofluor stained specimens showing morphological variability.*

to the ones of GenBank showed high similarity (99%) with strains from the Mediterranean Sea (e. g. Aegean Sea, Catalan Coast, and Tyrrhenian Sea) [2].

Acknowledgements

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IOC WESTPAC Training course on Taxonomy and Ecology of the diatom *Pseudo-nitzschia*

A UNESCO IOC/WESTPAC training course with the theme: 'Taxonomy and Ecology of the Diatom *Pseudo-nitzschia*' was held 20-24 March 2011 at the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan, Malaysia. The event was jointly organized by UNESCO Intergovernmental Oceanographic Commission sub-commission for the Western Pacific (IOC WESTPAC) and the Asian Natural Environmental Science Center, University of Tokyo, with distinguished speakers from Japan, Denmark and Vietnam.

The training course drew participants from seven countries (China, Indonesia, Japan, Malaysia, Philippines, Thailand and Vietnam), including representatives from government agencies and research institutions.

During the course, participants were given hands-on training in basic and advanced techniques in *Pseudo-nitzschia* identification. These included scanning and transmission electron microscopy, and rapid molecular detection techniques using fluorescence in situ hybridization. A series of lectures was also delivered in the four-day course by Dr Nina Lundholm.

A Harmful Algal Blooms (HABs) Seminar was conducted in conjunction with the course. Nearly 90 participants attended the seminar. The talks were given by Drs Yasuwo Fukuyo, Nina Lundholm and Ha Dao Viet.

Dr Yasuwo Fukuyo, the first vice chair of the IOC/WESTPAC, and the leader of WESTPAC HAB Project have emphasized the importance of HAB research in the region. HAB events have been increasingly reported globally, and cause poisoning and fatalities due to consumption of toxic shellfish, including Malaysia. Besides causing harm to the environment, plants, and animals, these blooms can be detrimental to aquaculture, fisheries, and coastal tourism, as well as the health of local communities. According to the organising chairperson, Dr Po-Teen Lim, such a training course is timely and vital to strengthen the regional network and collaboration in HABs research. It is crucial in standardizing scientific approaches used in identification of the diatom *Pseudo-nitzschia*, and most importantly to enhance human capital to deal with HABs issues, especially in the national and international agencies.



Fig 1. More than 90 participants during the Harmful Algal Bloom Seminar.



Fig. 2. Dr Lim explained on SEM during the hand-on session.



Fig. 3. Dr Ha from Institute of Oceanography, Vietnam talks during the Harmful Algal Bloom Seminar

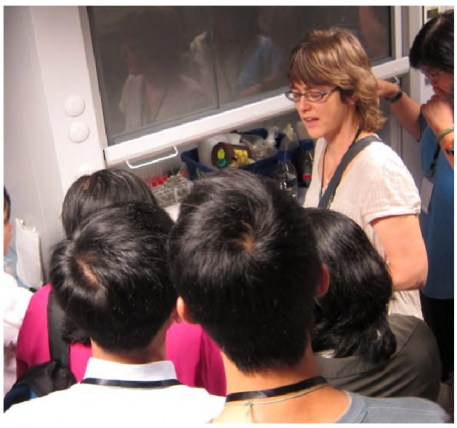

Fig. 4. Dr Lundholm demonstrates acid wash preparation to participants

Application of a Receptor Binding Assay to the analyses of PSP toxins in four species of shellfish in El Salvador

Harmful algal blooms of paralytic shellfish poisoning (PSP) toxin producers have a serious impact on public health and cause millionaire losses to the shellfish industry on the Pacific coast of El Salvador [1]. In 2010 and 2011, high levels of PSP toxins associated with a bloom of *Pyrodinium bahamense* var. *compressum* were detected in several mollusc species and led to shellfish harvesting closures. Recently, the Marine Toxins Laboratory, University of El Salvador (LABTOX-UES), has implemented a receptor-binding assay (RBA) method [2] for the detection of PSP toxins in shellfish. The RBA method is currently used in laboratories around the world as a fast and sensitive alternative to the mouse bioassay. This receptor binding assay for PSP toxins has been evaluated in an AOAC Single Laboratory Validation [3] and has just been approved as an official analytical procedure following AOAC interlaboratory trials. LABTOX-UES, in cooperation with NOAA, had previously used the RBA to determine PSP toxins in sea turtles. The RBA has allowed early warning of the presence of PSP toxins in shellfish in El Salvador, and probably contributed to save human lives.

Material and Methods

Samples of oysters (*Crassostrea iridiscens*), snails (*Plicopurpura columellaris*), crabs (*Carcinus maenas*) and mussels (*Modiolus capax*) were prepared by homogenizing 1-2 g of flesh that was extracted with an equivalent volume of HCl 0.1N. The pH of the extract was measured and corrected (if needed), to get a value between 3.0 and 4.0. This extract was boiled for 5 min, cooled to room temperature, centrifuged (3400 x g) and the supernatant collected an filtered with a syringe (0.45 µm).

The RBA Method: The acidic shellfish extracts were analyzed according to the STX receptor-binding assay. The method is based on the specific interaction between the toxins and their pharmacological target, i.e. the voltage-gated sodium channel, site 1 for PSP toxins. In this assay, a radio-labelled toxin ([^{3}H] saxitoxin) competes with unlabeled molecules for the sodium channel sites in a rat-brain crude membrane preparation. When the binding equilibrium is reached, free [^{3}H]toxins are removed by filtration and collected receptor-bound [^{3}H]toxins are quantified by liquid scintillation counting. The reduction in [^{3}H] toxin binding is directly proportional to the amount of unlabelled toxin present. A standard curve is generated using increasing concentrations of unlabeled toxin standard; the concentration of toxin in extracts of samples is determined with reference to the standard curve. Thus, the RBA is a functional assay that estimates the toxic potential (toxicity) of the extract, in a manner comparable to the mouse bioassay.

Results

Saxitoxin-like activity was detected in all samples analyzed. Detection limits of the assay were about 7.6 µg equiv. STX/ 100 g meat. Samples were diluted, as needed, whenever their concentration of PSP toxins exceeded the linear range of the calibration curve. Similar results were obtained from samples where different dilution factors were used. Concentrations above 2500 µg quiv. STX/100 g meat were detected in oysters and crabs in September 2011 (Fig. 1). Thus, the RBA can analyze samples with toxin levels providing an adequate dilution factor is selected.

Table 1. Concentrations of PSP toxins (µg STX equiv./ 100 g meat) detected with the RBA. A regulatory level of 80 µg equiv. STX/100 g meat (400 mouse units) is applied in El Salvador.

LABTOX Reference	Sampling site	Sample	Shellfish	Date of analysis (dd/mm/yy)	STX (µg equiv/100g)	Mouse Units
MIZ21-24	Mizata Beach	whole tissue	oysters	12/09/11	145.3	727
MIZ26-28	Mizata Beach	whole tissue	oysters	29/09/11	731.4	3657
TAQ01-03	Taquillo Beach	whole tissue	oysters	25/08/11	1872.4	9362
CUC01-03	El Cuco Beach	whole tissue	oysters	31/08/11	138.7	694
MIZ31-32	Mizata Beach	whole tissue	snails	11/10/11	1622	8110
MIZ33-34	Mizata Beach	whole tissue	oysters	11/10/11	2809	14040
MIZ37-38	Mizata Beach	whole tissue	mussels	11/10/11	771.2	3856
ZON01-03	El Zonte Beach	whole tissue	snails	01/10/11	463.2	2316
ZON04-06	El Zonte Beach	whole tissue	crabs	01/10/11	2604	13020

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To IAEA, project *Detection of Marine Toxins through the Use of the Radioassay Method in El Salvador* for its valuable support to set up the LABTOX-UES laboratory and to the National Oceanic and Atmospheric Administration (NOAA) for their cooperation and technical assistance.

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First report of *Dinophysis* species causing Diarrhetic Shellfish Poisoning in British Columbia, Canada

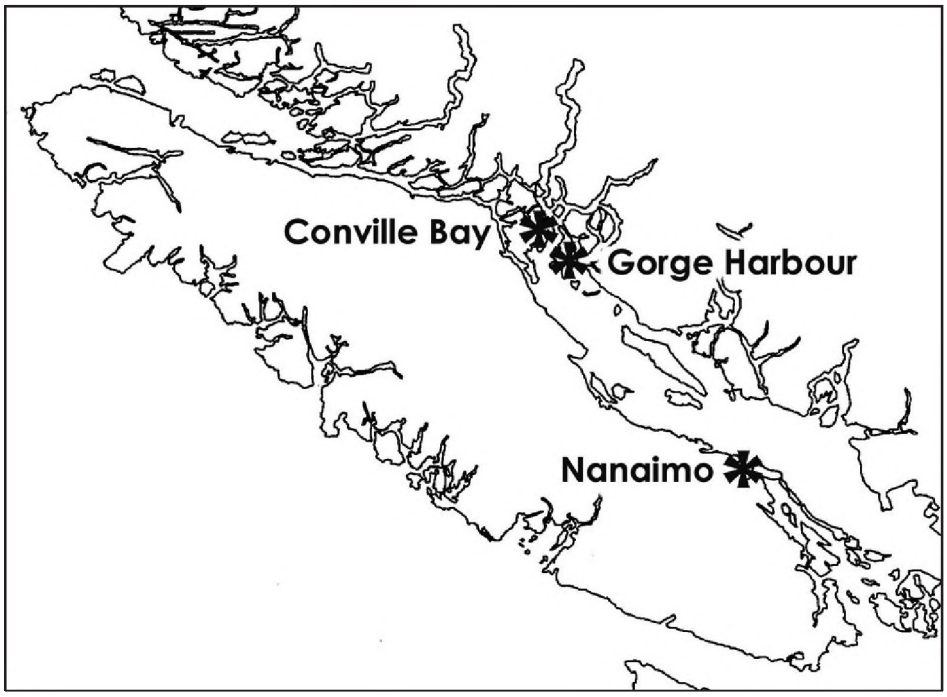

Fig. 1. Map of Vancouver Island and south coast of BC, showing HAMP sampling site (Conville Bay) and DSP-affected mussel harvest site (Gorge Harbour).

The western province of Canada, British Columbia (BC), has a temperate climate and naturally nutrient rich coastal water with high primary productivity. Historically, BC has been one of the areas most affected by harmful algal blooms (HABs), including those of algae species that cause shellfish poisoning syndromes. Routine monitoring of shellfish for the presence of Paralytic Shellfish Poisoning (PSP) toxins in BC began in 1942, following a severe outbreak of PSP with six deaths recorded [1]. Cases of Amnesic Shellfish Poisoning (ASP) have not been recorded in BC; however the ASP toxin, domoic acid (DA), has been found in shellfish [2]. Currently, BC shellfish are tested by the Canadian Food Inspection Agency (CFIA) for the presence of PSP toxins weekly in summer and bi-weekly in winter. Occasionally these samples are also tested for DA.

The first case of Diarrhetic Shellfish Poisoning (DSP) on the west coast of Canada was announced by the CFIA in August 2011. Approximately 60 people became sick from consuming mussels harvested in Gorge Harbour, Cortes Island, between July 19 and August 2, 2011 [3]. After the poisoning outbreak, the CFIA recalled the affected product and issued an official warning to the public not to consume these mussels.

DSP has caused significant problems in other parts of the world, especially in Europe, and presently many countries rely on a combination of phytoplankton sampling and shellfish toxicity monitoring. The early warning level of DSP-causing *Dinophysis* species in water samples that triggers further testing of shellfish flesh for DSP toxins depends on the region, but in most countries is in the range of 100-500 cells L-1.

Species of *Dinophysis* (especially *D. acuminata, D. norvegica,* and *D. fortii*) are very common in BC coastal waters. A two-year study in the 1990s [4] revealed the presence of *Dinophysis* species with concentrations above known DSP-causing levels throughout the sampling area [4]. Another study pointed out that the absence of diagnosed DSP in humans in BC at the time might be due to the fact that diarrhoea, the major DSP symptom, resembles bacterial contamination, so DSP could not be detected without testing specifically for toxins [5]. The absence of early warning due to insufficient testing for DSP toxins and lack of monitoring of shellfish HAB species eventually led to this year's outbreak. Currently, there is no phytoplankton monitoring in BC by government agencies: neither the federal departments of the CFIA and Fisheries and Oceans Canada (DFO), nor provincial or municipal organizations.

As well as threatening human health by causing shellfish toxicity, HABs are a major economic threat to the BC salmon aquaculture industry. Algal blooms in BC are not known to cause a health risk to fish consumers, but HABs are the primary health issue of farmed salmon in BC due to frequent fish kills at farm sites. Striving for efficient HAB management and mitigation, a consortium of BC fish farmers, in association with DFO, initiated the Harmful Algae Monitoring Program (HAMP) in 1999. Since that time HAMP personnel have analysed weekly samples from 1, 5, and 10 m depths at sampling sites on or near fish farms for the presence of harmful algae, which has resulted in warnings of potentially harmful events.

Dinophysis species are not harmful o fish and thus their concentrations re not usually enumerated during IAMP sample analysis. However, in une 2011 the concentrations of *Dino-hysis* at a few sampling sites appeared nuch higher than usual, so HAMP staff tarted counting *Dinophysis* in these areas. This included one HAMP sampling

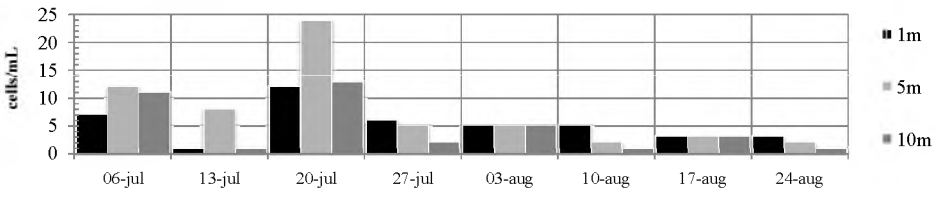

Fig. 2. Dinophysis *species count, Conville Bay, BC, 2011.*

site – Conville Bay – which was about 25 km from the area where mussels were later affected by DSP biotoxin (Fig. 1). The highest concentration of *Dinophysis* species recorded at this site was 24000 cell L^{-1} on July 20 at 5 m depth (Fig. 2). The most common species observed in the samples at this site were: *D. acuminata, D. acuta, D. fortii* and *D. rotundata* (Fig. 3). The most abundant species was D. acuminata; from July 13 to August 17 it comprised 82% of the total *Dinophysis* count (Fig. 4). The highest concentrations of *Dinophysis*, in terms of depth, were usually recorded at 5 m (Fig. 5); for the period of July 13 to August 20 the count for *D. acuminata* on average was 6800 cells L^{-1} at 5 m, and 4000 and 3300 cells L^{-1} at 1 m and 10 m respectively.

The unusually high concentrations of *Dinophysis* in Conville Bay coincided with the Gorge Harbour DSP event. Based on available phytoplankton data and results of CFIA DSP toxin analysis, it appears that DSP toxin exceeded the regulatory limit on July 19th, around the time of highest *Dinophysis* concentrations, throughout the monitored water column in Conville Bay (July 20th: 12000 cells L^{-1} at 1 m, 24000 cells L^{-1} at 5 m, and 13000 cells L^{-1} at 10 m). About two weeks after the highest *Dinophysis* concentrations, the level of DSP toxins returned to acceptable levels in Gorge Harbour shellfish samples.

This DSP outbreak in BC highlights the necessity of monitoring and studying algal species known to produce shellfish toxins. The prevention of shellfish borne illnesses requires not only monitoring of shellfish flesh for the presence of toxins but also analysis of water samples for the presence of harmful algae. Data on the occurrence, type and concentrations of toxic algae provides an early warning of toxic events: which toxin may be expected to be found in shellfish, and when to monitor product for toxins. Knowledge of the local phytoplankton population, ecology of the toxic species, and detecting harmful algal blooms helps to prevent poisoning cases and protect shellfish consumers.

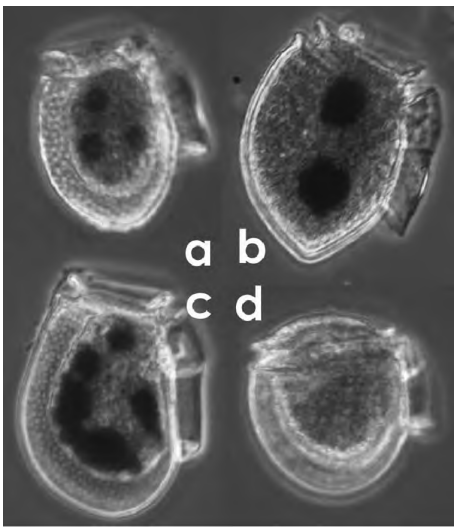

Fig. 3. Most common Dinophysis *species in Conville Bay July 13 - August 17, 2011. a) D. acuminata, b) D. acuta, c) D. fortii, and d) D. rotundata*

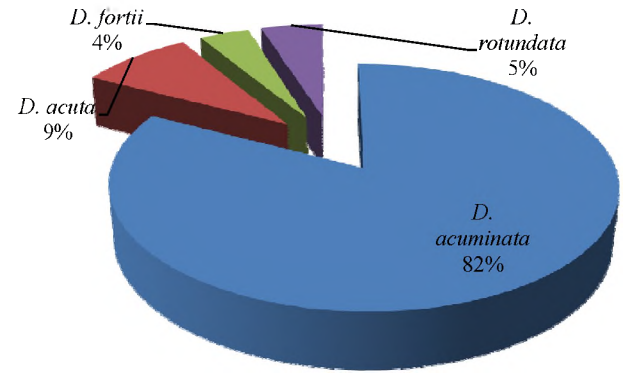

Fig. 4. Percentages of D. acuminata, D. acuta, D. fortii, *and* D. rotundata *in Conville Bay samples from July 13 to August 17, 2011.*

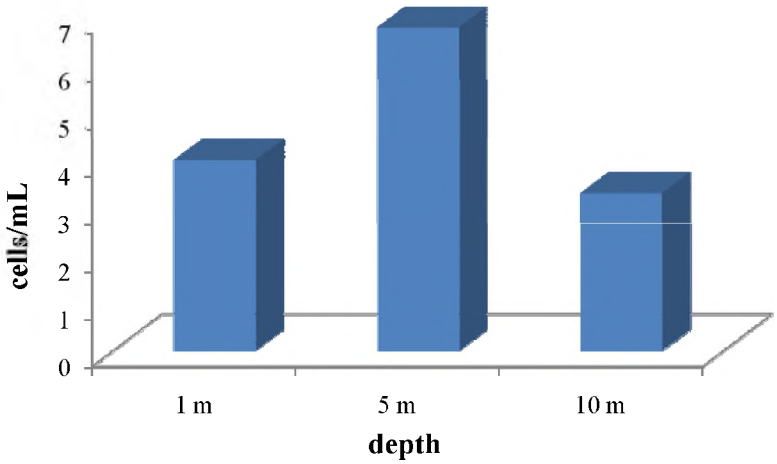

Fig. 5. Average concentrations of D. acuminata *at 1, 5, and 10 m depths from July 13 to August 17, 2011 at Conville Bay.*

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Harmful Algal Bloom Operational Forecast System in the US

A Need Realized

It was October 31, 1987—Halloween Day in the U.S. It seemed to be an otherwise ordinary day, but people on the beaches near Beaufort, North Carolina, were experiencing out of the ordinary respiratory distress. A bloom of "Florida red tide", the toxic algae *Karenia brevis,* had unexpectedly appeared in North Carolina coastal waters for the first time on record. It stayed for almost half of a year.

This persistent and unforeseen harmful algal bloom (HAB) was economically disastrous for shellfisheries, seafood, and tourism, costing an estimated $25 million to North Carolina coastal communities (about $47 million today). A need to better monitor, predict, and plan for events like this—integrating all available data from satellites, field monitoring, and ultimately models— was clear.

The bloom had been transported from Florida via the warm waters of the Gulf Stream. During the event, the National Oceanic and Atmospheric Administration's (NOAA's) polar orbiting environmental satellites were able to collect data that allowed scientists to detect ocean thermal features associated with the bloom and provided a means for tracking it. Before that time, high resolution images of sea surface temperature were not routinely available to coastal managers. This red tide event illustrated the need for near real-time satellite data and gave birth to NOAA's CoastWatch Program. By 1989, NOAA was processing the full resolution satellite imagery on an operational basis and distributing it within a day to the coastal management community.

HAB Forecasting Today

The world of HAB monitoring and forecasting in the U.S. has come a long way since 1987. Since 2004, NOAA, in collaboration with many entities, has been developing and successfully transitioning operational forecasts for HABs that integrate data from satellites and field monitoring with models. (http://www.tidesandcurrents.noaa.gov/hab).

The first operational HAB forecasts in the US were introduced for the eastern Gulf of Mexico (Florida). To this day, operational HAB forecast bulletins, providing *K. brevis* detection and forecast information, are distributed twice weekly to managers during blooms and once weekly during non-bloom periods, with daily beach conditions available to the public via the Web and phone hotlines. The nowcasts and forecasts in the eastern Gulf of Mexico are based on a combination of 1) biological, oceanographic, and meteorological field data from federal, state, and local agencies, 2) satellite imagery, 3) public and animal health reports from state and local agencies, and 4) transport models. The bulletins allow for early and more efficient mitigation efforts by managers and help prevent unnecessary public exposure to aerosolized toxins.

In fall 2010, NOAA similarly transitioned a "demonstration," or proof of concept, HAB forecast for the western Gulf of Mexico (Texas) to operational capability. Texas forecasts focus primarily on early warnings for *K. brevis,* but detecting toxic *Dinophysis acuminata* and modeling potential transport of blooms along the coast is also supported. Texas forecasts also incorporate data from a continuous automated imaging sensor designed by researchers at Woods Hole Oceanographic Institution and operated by Texas A&M University scientists, called Imaging FlowCytobot, that captures images of phytoplankton for validation and enhanced early warning capabilities.

Demonstrations have been underway in several regions within the US for some time: 1) for the past three years in Lake Erie, targeting toxic *Microcystis* blooms (by NOAA), 2) for the past five years in the Gulf of Maine targeting toxic *Alexandrium blooms* (by the Woods Hole Oceanographic Institution and North Carolina State University), and 3) for the past four years in the Pacific Northwest targeting toxic *Pseudo-nitzschia* blooms (by the University of Washington and NOAA). Several hundred managers representing dozens of federal, state, and local agencies receive and use information from these experimental systems to better manage resources and to mitigate public health and economic impacts. Regional HAB forecasts for the Chesapeake Bay and the California coast are also under development. Plans are already being developed to transition the Great Lakes, Gulf of Maine, and Pacific Northwest regional forecasts to operations in NOAA's Center for Operational Oceanographic Products and Services (CO-OPS), in collaboration with state and academic partners, http://www.tidesandcurrents.noaa.gov/hab/development.html.

The Importance of Research

The development of successful, integrated ecological forecasting systems begins with research that includes an understanding of the regional HAB species ecology and oceanography. For example, the harmful algal bloom forecast system for the Gulf of Maine uses a coupled biological/physical model based on such understanding along with *Alexandrium cyst* maps to generate a seasonal forecast of bloom severity—comparable to seasonal climate outlooks. During the bloom season, weekly forecasts of bloom development, transport, and decline are issued. This success has developed over fifteen years, starting with a major regional project to understand the ecology of *Alexandrium* blooms, funded first by NOAA and the National Science Foundation (NSF) through the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) Program. The engagement of multiple funding sources has advanced the research to the present day forecast model for *Alexandrium*.

Research has played a similar role in the development of forecast systems in other regions of the U.S. and will continue into the future, both to improve existing forecasts and develop new ones.

Future of HAB Forecasting in the U.S.

NOAA is taking an integrated approach to managing data synthesis, analysis, and dissemination to serve multiple operational forecasts for impacted regions, recognizing that HAB issues, the state of knowledge, and the components of HAB forecasts are different for each region.

For systems that are operational, both evaluations of the forecasts and



new research discoveries continually identify necessary improvements and valuable enhancements. HAB-specific sensors, deployed on remotely operated vehicles, ships and moorings as part of coastal observing systems, will provide crucial data for more accurate predictions. Some next generation forecasts will incorporate food-web based models that predict fate and effects of HAB toxins. Long-term risk assessments to ecosystems will be important for resource management as well. These are a few examples of the exciting work that lies ahead.

Support for HAB modeling, detection, and forecasting is provided by many federal agencies, including NOAA, the National Aeronautics and Space Administration (NASA), the Centers for Disease Control and Prevention (CDC), NSF, and the National Institute for Environmental Health and Safety (NIEHS). Continuing collaborations between Federal agencies (including funding programs, researchers, modelers, and operational offices), academia, state agencies, nonprofits, industry, etc. will be essential for continued success.

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Gambierdiscus Wiki

A *Gambierdiscus* Wiki has been developed on Wiki Spaces http://gambierdiscuswiki.wikispaces.com/ as a GEOHAB-BHAB product. The taxonomy of *Gambierdiscus* will be evolving for quite some time and this is a forum for helping the community interact.

The aim is to get the information out so it can be used and other information added as the community contributes. Comments are welcome to Dr. Pat Tester at Pat.Tester@noaa.gov

Phycotoxins

You can also get news on HAB research and events at the Phycotoxins-list at the internet.

The address of this list is: phycotoxins@www.agr.ca

To join the list, send "subscribe phycotoxins" to: listserv@www.agr.ca

Archives are located at www. agr.ca/archives/phycotoxins.html

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

The "GEOHAB Core Research Project on HABs in Benthic Systems" presented at ICOD

The "International Conference of Ostreopsis Development" (ICOD) was held in Villefranche-sur-mer, last April 2011 (http://www.obs-vlfr.fr/ICOD/). During three days, scientists, policy makers and managers from the Mediterranean area and other countries exchanged their expertise in order to optimize the knowledge transfer and to reduce the risks linked to the *Ostreopsis* development. At the conference, communications covered many aspects of the ecology, chemistry and toxicology of *Ostreopsis* species. Fruitful discussions took place on how to approach the ecologic, economic and health management aspects of the problems caused by blooms of *Ostreopsis* species in the Mediterranean and around the world's seas.

The ICOD deserved a special interest for the GEOHAB program, given its tight link with the new Core Research Project (CRP) on "Harmful Algal Blooms in Benthic Systems" (BHABs) currently under development. At the ICOD, Adriana Zingone (GEOHAB-BHAB Committee) and Elisa Berdalet (vice-chair of GEOHAB) presented the GEOHAB program to the audience. Furthermore, they summarized the main outcomes of the open science meeting (OSM) held in June 2010 in Honolulu, which represented the start for the GEOHAB-BHABs CRP. At that meeting scientists working towards the understanding of how particular benthic phytoplankton species (e.g. *Gambierdiscus, Ostreopsis, Prorocentrum*) proliferate and cause harm, intensively exchanged their knowledge. During that OSM, research priorities and infrastructural gaps were identified regarding the taxonomy, distribution and ecology of benthic HABs. The outcomes of the open science meeting, including the state of the art in the field of BHABs and a research implementation plan, are part of a report currently in preparation. The ICOD was an excellent opportunity for establishing bridges of collaboration among scientists and managers involved on BHABs and to foster research in this field.

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From left to right: Adriana Zingone, Rodolphe Lemée (one of the ICOD organisers) and Elisa Berdalet.

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Comparative methods for the study of harmful algae: the GEOHAB programme within an Iberian perspective

The Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) programme was present at the "XI Reunión Ibérica sobre Microalgas Tóxicas y Ficotoxinas" ("XI Iberian Meeting on Toxic Microalgae and Phycotoxins") held in Bilbao, from May 31st to June 2nd 2011 (http://www.ehu.es/reunioniberica/).

The GEOHAB reports were made available to participants and a specific presentation of the programme (Berdalet et al.) was offered to the audience. The general characteristics of GEOHAB were reviewed: interdisciplinary and multidisciplinary research with an international scope using a comparative strategy, in order to advance the understanding of HAB dynamics. Implementation of GEOHAB goals is achieved through establishment of Core Research Projects (CRPs) and Regional Programmes, that start with open science meetings, whose published Reports (also freely available at http://www.geohab.info) provide a synthesis of knowledge and needs, as well as a roadmap for the international community.

The presentation emphasized some processes related to HAB events occurring in waters surrounding the Iberian Peninsula (including the Canary Islands), that have contributed to the development and implementation of GEOHAB. The scenarios included, for instance, the Iberian upwelling system, thin layers in the Galician Rías, the Ebro Delta embayments and benthic HABs. The aspects considered in the different CRPs included biogeography, biodiversity and adaptive strategies of target species, eutrophication, physical forcings, modeling and observation at different scales. Important open questions within each ongoing CRP were highlighted to attract further contributions from the scientists attending the XI Reunión Ibérica. Special emphasis was also made to encourage endorsement of ongoing HAB related projects to GEOHAB. The participants acknowledged the important role of GEOHAB for the advance of HAB research and expressed their desire for it to continue this role beyond 2013. In the picture, some participants at the meeting.

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From left to right: Monica Lion, Santiago Fraga, Elisa Berdalet, Teresa Moita, Beatriz Reguera, Emma Orive and Jorge Diogène

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

GEOHAB Updates and News



The GEOHAB Science Steering Committee met recently to coordinate ongoing activities and make plans for the next few years. The SSC added Dr. Patricia Tester (NOAA, USA) to help represent the new Core Research Programme on Benthic HABs, and endorsed several new projects and activities including two workshops (see also the article about the "International Conference on Ostreopsis Development").

Fig. 1. The GEOHAB Scientific Steering Committee meeting in October 2011 at Dartington Hall, UK

Special Issue in the journal Oceanology and Limnology

Harmful algal blooms have been expanding globally, but it has been particularly pronounced in the coastal waters of Asia. One of the major reasons proposed for this expansion in both Asia and throughout the world is the increase of nutrient loading to our coastal waters. To address some of the scientific questions associated with this apparent trend, the GEOHAB Core Research Project on HABs and Eutrophication held its second open science meeting in Beijing, China (October 2009). Results from that meeting were recently published in a special issue of the Chinese journal Oceanology and Limnology (Volume 29, Number 4), comprised of 21 contributions ranging from nutrient utlization to physiology, modeling, and comparisons within and between systems.

GEOHAB Meetings—Save the Date!

There are several planned activities on the GEOHAB calendar—more information and updates are available at the GEOHAB website, www.GEOHAB.info.

29-31 May 2012 Victoria, Canada	GEOHAB Open Science Meeting: Progress in Interpreting Life History and Growth Dynamics of Harmful Algal Blooms in Fjords and Coastal Embayments
22-24 August 2012 Monterey, CA USA	GEOHAB Stratified Systems Intercomparison Work shop: Reconciling Physical and Biological Measurements
25-27 April 2013 UNESCO, Paris	GEOHAB Open Science Meeting: The GEOHAB Legacy and the Future of International Cooperative Research

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Endorsed Projects

As part of the GEOHAB Implementation Plan (2003), the GEOHAB programme solicits and endorses projects that implement a comparative approach to HAB research in Core Research, Targeted Research, Regional/National Research, and Framework Activities. During 2011, multiple projects have been submitted, and are highlighted below. Additional details can be found on the GEOHAB website (www.geohab.info), together with the endorsement request form.

Framework Activities

International Workshop on Fish-killing Marine Algae, 10-11 April 2011, Oslo, Norway. As part of the pilot project "Algefisk" -The effects of algae and algal toxins on fish health and food safety- financed by the Research Council of Norway, and with the co-sponsorship of the IOC UNESCO Harmful Algal Bloom Programme, held a small workshop on marine ichthyotoxic algae. The workshop brought together in an informal environment researchers working on ichthyotoxic algae, to share experiences, ideas and approaches to tackling this under-studied problem. The overall goal is to identify research that will bring better understanding of, and, in turn, improved mitigation options for managing, ichthyotoxic algal problems. Participants include algal biologists, fish pathologists, algal toxin chemists, and aquaculture industry representatives. The lead contact is Dr. Christopher Miles.

International Conference on *Ostreopsis* Development (ICOD), 4th to 8th April 2011 Villefranche-sur-mer (FRANCE). Several species belonging to the genus *Ostreopsis* (benthic dinoflagellates) are common in temperate seas and in particular in the Mediterranean Sea. The ICOD aimed to review the knowledge on 1) ecological, chemical and toxicological aspects of *Ostreopsis* species and 2) on the different methods of ecologic, economic and health management of the problem. The ICOD facilitated the exchange between scientists, policy makers and managers of the Mediterranean and other temperate countries, in order to optimize the knowledge transfer and reduce the risks linked to the *Ostreopsis* development. The event was organised by the French Phycological Society, whose 2011 meeting was held the first two days of the event (4-5 April 2011).

Unveiling the biodiversity of marine benthic dinoflagellates at the beginning of the XXIst century. The project is writing and publishing a (reviewed) book about benthic dinoflagellates. This book will contribute to the benthic GEOHAB Core Research Project because it will summarize all the knowledge about benthic dinoflagellates, present the currently known species diversity and help identifying the taxa. The main part of the book will be taxonomy (species descriptions and illustrations) and thus present the first comprehensive identification help for benthic dinoflagellates. It will be a basic contribution to improve monitoring efforts in this area. The lead editor is Dr. Mona Hoppenrath.

Regional/National Research

Ecology and Oceanography of Harmful Algal Blooms in the Philippines" (PhilHAB's). PHILHABs constitute the HAB research program in the Philippines and proposed to be implemented in three to six years (2009-2014). Results of which can be also useful inputs into the envisional Asian GEOHAB. The main thrusts of the proposal are derived mainly from the scientific program of SCOR-UNESCO's GEOHAB. The general objective of the PHILHABs program is to understand the critical factors and mechanisms underlying the population dynamics of HAB species in various oceanographic regimes particularly the Philippines. Results can be used as basis for monitoring and managing the occurrence, movement, toxicity and environmental effects of various types of HAB's (in the Philippines). The program also intends to develop methods and technologies relevant to the monitoring and management of HABs in the country. The lead contact is Dr. Rhodora Azanza.

Determination of adhesive strength, propagation mechanisms and methods of destruction of different life cycle stages of *Alexandrium catenella*. Is the northwards expansion of *Alexandrium catenella* from southern Chile determined by climate oceanographic features and/or by the increase of aquaculture activities? Blooms of *A. catenella* in Chile are events that have naturally occurred in the southernmost area of the country since 1972. However, these blooms have subsequently been observed in lower latitudes, specifically in areas where there has been a great development of salmon and mussel farming during the last two decades. The reasons for this observed trend are not well known. The present project has as first objective, to study and update the database in the study of the coastal areas of Chile affected by the *A. catenella* blooms in order to investigate the factors suspected as well as the influence of ENSO to affect the spreading of this species towards lower latitudes of Chile. The lead contact is Dr. Gemita Pizarro.

GAMBOS: Estudio de la distribución de dos dinoflagelados bentónicos marinos *Gambierdiscus* y *Ostreopsis*, con especial detalle en la producción de toxinas y su relación con la salud pública (Study of the benthic marine dinoflagellates *Gambierdiscus* and *Ostreopsis*, with a special emphasis on the toxin production and its relationship to public health). The aim of this project is to characterize two recent phenomena in Spanish waters related to *Gambierdiscus* and *Ostreopsis*, which cause an increasing concern in the public health and tourism sectors and the determination of the involved toxins. The main hypothesis that arises is that recent ecological unbalances in the coastal rocky habitats have caused the proliferation of these microalgae which were in the past in low concentrations and did not cause any damage. The lead contact is Dr. José Franco Soler.

Introduced Alien species in the Portuguese estuaries and coastal areas: patterns of distribution, vectors and invading potential (INSPECT). The introduction of non-indigenous or alien species that might become invasive has increased with globalization.

This project aims to study the occurrence of marine alien species in Portuguese estuaries and coastal areas, evaluate the existence of environmental conditions that favor or prevent the establishment of potential invaders and increase public awareness on this threat.

The results are expected to clarify some aspects of the introduction processes, such as the relative importance of vectors of introduction of alien species, the identification of environmental conditions that favor or inhibit invaders, and species with enhanced invasive characteristics. Proposals on the definition of priority areas and species will be one of the outputs of this project, with the aim of supporting managers and decision makers on the allocation of resources to prevent and/or mitigate the impacts of invaders. This project is also expected to increase the cooperation between the scientific community, administration representatives of different sectors and civil society, by having all partners working together to produce information that will be used in raising public awareness for the threats of alien species' introductions. The lead contact is Dr. Ana Amorim.

Regional/National Research

ECOHAB – Modeling favorable habitat areas for *Alexandrium catenella* in Puget Sound and evaluating the effects of climate change. The dinoflagellate *Alexandrium catenella* produces a suite of potent neurotoxins that accumulate in shellfish and cause severe illness or death if contaminated shellfish are consumed by humans. *Alexandrium catenella* form dormant cysts that overwinter on the seafloor and provide the inoculum for toxic blooms the following summer when conditions become favorable again for growth of the motile cell. A 2005 survey of *A. catenella* cyst distribution in Puget Sound, Washington State, identified "seedbeds" with high cyst abundances that correspond to areas where shellfish frequently attain high levels of toxin. However, even at these sites, interannual variability in the magnitude of toxic events is high. The objectives of this project are to (I) determine interannual variations in *A. catenella* cyst distribution in Puget Sound, (II) quantify rates of cyst germination and vegetative growth for a range of temperature, salinity, and light conditions, (III) determine the presence/absence of an endogenous clock that regulates cyst germination, (IV) model favorable habitat areas for cyst germination and vegetative growth, (V) evaluate climate change impacts on favorable habitat areas, and (VI) establish a time series with sufficient depth to provide seasonal forecasts of toxic blooms. The lead contact is Dr. Stephanie Moore.

Study of the genetic diversity and characterization of cultures of dinoflagellates from the genus *Dinophysis* (DIGEDINO). The knowledge about the genetic diversity of dinoflagellates populations is still scarce and at least in the Galician rías it is unknown if there exist homogeneous populations along time and space, or a succession of subpopulations or ecotypes displaying different toxicities. In the present project we propose (1) to study the genetic diversity of Dinophysis in natural samples and cultures during a yearly period, (2) to define hypothetical ecotypes on the basis of the physiological and morphological characterization of clonal cultures in the laboratory, (3) to study the genetic diversity in plastids of *Dinophysis* to confirm if they originate from a single source of they differ geographically, along time or among the studied species and (4) to design specific molecular probes for species/ecotypes of *Dinophysis*, testing their efficiency and reliability to detect these organisms in natural samples.. The lead contact is Dr. Francisco Rodriguez.

Trends in Phytoplankton Diversity and Environmental Changes in Coastal Ecosystems (EDIPHYCE). Over the past decades, marine coastal ecosystems have been exposed to important and rapid environmental changes, mainly caused by human activities. In pelagic coastal systems, modifications of the structure of phytoplankton assemblages in relation with such perturbations have already been identified, thus affecting the whole trophic coastal marine food webs. Long-term phytoplanktonic data sets represent an inestimable source of information to better understand the past and to extrapolate and forecast the future. The objective of this project is to evaluate if and how phytoplankton diversity (including

toxic species) has changed in relation with the main pressures that occurred along the French Atlantic coats and the English Channel, i.e. eutrophication and climatic change. Using a multidisciplinary approach, we propose to carry out retrospective and prospective analyses of decadal resources regarding phytoplanktonic communities, available either in the frame of monitoring activities and hidden in living archives (the sediments) or manageable by computer models. The lead contact is Dr. Annie Chapelle.

Targeted Research

A Regional Cooperative Assessment of the genetic variability of HAB species in relation to their toxicity, population dynamics, and biogeography (ReCoHAB) – a comparative research initiative under GEOHAB Asia. The Project will, as a regional cooperative and comparative research project, focus on two types of HABs (toxin producers and high biomass producers) which are particularly poorly understood in terms distribution, toxicity and management options. Research priorities for these to issues were defined in detail at two expert meetings. The goal is to improve prediction of HABs by determining the ecological and oceanographic mechanisms underlying their population dynamics, integrating biological, chemical, and physical studies supported by enhanced observational and modeling systems. This will be achieved through international regional cooperative research on HABs in ecosystem types sharing common features, comparing the key species involved, and the oceanographic processes that influence their population dynamics.

ECOHAB: A Regional Comparison of Upwelling and Coastal Land Use Patterns on the Development of HAB Hotspots Along the California Coast. California represents approximately 1,000 miles of the Pacific coastline of the U.S. and major fractions of the coastal economy and environmental resources of our nation. Alarmingly, blooms of harmful and toxic algae have increased in frequency and severity along this coast during the past few decades. Although several HAB organisms are present, *Pseudo-nitzschia* (Domoic Acid Poisoning) is rapidly becoming the single greatest threat and problem for human and ecosystem health, potentially eclipsing PSP in severity due to the diverse impacts on the economy through commercial fisheries and tourism, as well as via direct impacts on marine birds and mammals. Our primary objective is to develop a better understanding of the ecophysiological conditions leading to bloom and toxin initiation for *Pseudo-nitzschia,* by simultaneously comparing two "hot spots", Monterey Bay and San Pedro, California. The lead contact is Dr. Raphael Kudela.

ASIMUTH (Applied Simulations and Integrated Modelling for the Understanding of Toxic and Harmful Algal Blooms). A collaborative project entitled "Applied Simulations and Integrated Modelling for the Understanding of Toxic and Harmful Algal Blooms" (ASIMUTH) held its initial partner meeting in Cork Ireland on the 14 and 15 December 2010. This FP7 funded project has 11 research and SME partners from Ireland, France, Spain, UK and Portugal. Through the ASIMUTH project, scientists and industry from these countries along Europe's Atlantic Margin will form a network to produce the first realistic HAB advisory and forecasting capability to the European aquaculture industry. The project will be an applied downstream service of the MyOcean core project of Global Monitoring for Environment and Security (GMES) which is a joint initiative of the European Commission and European Space Agency, which aims at achieving an autonomous and operational Earth observation (EO) capacity. The lead contact is Dr. Julie Maguire.

The effect of algae on fish health and food safety (AlgaFish). The main objective of this pilot project is to establish international partnerships and lay the foundation for an application for the project entitled "The effect of algae and algal toxins for fish health and food safety (AlgaFish)". Marine algae are believed to be the cause of health problems in fish around the world. There is no longer fish farming in large parts of Southern Norway precisely because of toxic algae. The significance of algae and algal toxins for the industry in the rest of the country is very little understood. We will build an interdisciplinary network of Norwegian and international scientists to study the algae problems in connection with fish health, and then secure the knowledge needed to develop potential solutions. A pilot project is testing whether algae can be the cause of the seasonal gill damage in two fish farms in south-west Norway, and whether algal toxins may be the mechanism behind this. In addition, SPATT-disks will be tested to see whether they can be used for monitoring of algal toxins, including ichthyotoxins. The lead contact is Dr. Christopher Miles.

ISSHA

International Society for the Study of Harmful Algae

ISSHA's Corner

Dear ISSHA members:
The ISSHA Council wishes you a very productive 2012!

This year is a busy one and we will need your active participation for an important list of activities:

- Election of new Council Members
- Nominations for the Yasumoto lifetime achievements award
- Nominations for the Patrick Gentien (Young scientist) award
- Bids to host the 17 ICHA Conference (2016)

Our Korean colleagues, coordinated by Dr Hak Gyoon KIM (full time dedication) are doing their best to prepare an impressive 15 ICHA Conference in Changwon (South Korea). Start thinking about the work you want to bring to Korea, ear mark the costs in your budgets, and consult the 15 ICHA website (available through the ISSHA website, www.issha.org). Do not leave everything for the last minute!

Many of you will soon receive a reminder to renew your membership dues.

Do not forget to add a portrait of yourself (login/my profile). It is so nice to be able to recognize faces.



Fig. 1. Participants in the Korean Local Organizing Committee (KLOC), convened by Dr H.G. Kim (KLOC Coordinator) held in Busan, South Korea, 1-2 December 2011.

Future events

Marine and Freshwater Toxins, Third Joint Symposium and AOAC Task Force Meeting and LC-MS/MS lab training on lipophilic toxins

18-22 & 25-26 June 2012, Tacoma, WA, USA.

Topics for the Symposium are the lipophilic toxins, the DSP outbreak in USA and Canada, saxitoxins official methods, domoic acid, emerging toxins, ciguatoxins, cyanotoxins and test kits.

Please reserve the following dates for above meeting June 18-22, then June 25-26, 2012 for LC-MS/MS lab training for diarrheic and other lipophilic toxins.

As many of you know, the US and Canada experienced their first outbreaks of diarrheic shellfish poisoning (DSP) this year right here in the Pacific Northwest. We will have training for detecting these toxins and a full day devoted to them at the above meeting.

Please contact James Hungerford (James.Hungerford@fda.hhs.gov) if you have interest in these sessions.

ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors

12-14 March 2012, Lisbon, Portugal

WGBOSV is open to representatives from ICES and IOC Member States (the non-ICES countries). Indication of interest and further information: Tracy McCollin, tracy.mccollin@scotland.gsi.gov.uk.

ICES-IOC Working Group on Harmful Algal Bloom Dynamics

25-28 April 2012, Oban, United Kingdom

WGHABD is open to representatives from ICES and IOC Member States (the non-ICES countries). Indication of interest and further information: Bengt Karlson, Bengt.Karlson@smhi.se.

GEOHAB Open Science Meeting: Progress in interpreting Life History and Growth Dynamics of Harmful Algal Blooms in Fjords and Coastal Environments

29-31 May 2012, Victoria, Canada

The GEOHAB Core Research Project (CRP) on HABs in Fjords and Coastal Embayments will hold an Open Science Meeting to review and update research on the role of life history and growth dynamics in the control of harmful algal blooms (HABs) in coastal environments and to identify key objectives for future international studies. Organizers: Allan Cembella, Leonardo Guzmán, Marina Montresor, Vera Pospelova and Suzanne Roy. Contact: Dr. S. Roy, Suzanne_Roy@uqar.ca

IOC Qualifications in Identification and Enumeration of Harmful Microalgae

15-25 August 2012, Copenhagen, Denmark

The course includes 90 hours of teaching and is divided into two parts, each consisting of 45 hours of teaching. The first part of the course is an internet teaching programme, while the second part is a practical workshop in species identification. More information: Jacob Larsen, jacobl@bio.ku.dk.

Future HAB Research Priorities Work Shop

28 October 2012, Changwon Gyeongnam, Korea

A GEOHAB meeting-workshop to present ideas and discuss HAB research priorities in the coming decades. For more information: Chair GEOHAB SSC, R. Kudela, kudela@ucsc.edu

15th ICHA 2012 Korea
15차 국제 적조 회의
XV International Conference on Harmful Algae

15th International Conference on Harmful Algae

29 Oct - 2 Nov 2012, Changwon Gyeongnam, Korea

HAB2012 aims to address all issues related to the causes and effects of marine and freshwater harmful micro- and macro-algae and to serve as a forum for exchanging research outcomes and relevant ideas between researchers, industries, governments and interested parties. More information: www.hab2012.kr

10th Advanced Phytoplankton Course: Taxonomy and Systematics

12 November - 1 December 2012, Copenhagen K, Denmark

The 10th Advanced Phytoplankton Course is being organized in cooperation by the Marine Biological Section of the Department of Biology, University of Copenhagen, Denmark, the Stazione Zoologica Anton Dohrn, Naples, Italy, and the IOC Science and Communication Centre on Harmful Algae. The course will consist of training on specific recognition of marine planktonic algae, with emphasis on light microscopy and use of taxonomic literature. The aim of the course is to increase and update the expertise of the students in the identification of diatoms, dinoflagellates, coccolithophores and other phytoflagellates. More detailed information: http://www.bi.ku.dk/apc10.html or e-mail: apc10@bio.ku.dk.



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Intergovernmental Oceanographic Commission

HAB Research and Management in Korea

Overview of HAB research and controls

Outbreaks of harmful algal blooms (HABs) in Korea were sporadic until the 1980s, but became frequent during the 1990s. During the last three decades, HABs in Korean coastal waters have led to deleterious effects such as risks to human health, loss of natural and cultured seafood resources, constraints on tourism and recreational activities, and damage to natural aesthetics and biodiversity. Of major concern are mass fish mortalities and shellfish poisoning syndromes. In August 1981, Korea experienced the first mass mortalities of fish and shellfish caused by *Karenia mikimotoi* blooms in Chinhae Bay. In 1995, a fish killing dinoflagellate, *Cochlodinium polykrikoides*, caused widespread and persistent blooms accompanying the worst economic loss of US$ 95.5million. PSP has also been a troublesome issue for marine food safety since the 1980s.

Since then, Korea has established comprehensive national HAB monitoring including shellfish toxins for early warning and management. Clay dispersal to control fish killing *C. polykrikoides* blooms began in 1996 (Fig. 1). In recent years, HAB research activities are mostly conducted by national institutes, universities, and government supported institutes. The on-going HAB projects are nationwide HAB monitoring, bio-optics and remote sensing, population dynamics, toxin chemistry and toxicity, benthic harmful algae, impacts of HABs, management and mitigation, and awareness and capacity building. Some on-going HAB studies are introduced here.

Hak Gyoon Kim, Pukyong National University, hgkim@nfrdi.go.kr



Fig. 1. Aerial view of merry-go-round clay dispersal off Yokjido Is on south coast of Korea, to control fish-killing Cochlodinium *bloom approaching fish cages, August 21, 2008, photographed by Jong-Suk Jung. Source : Hak Gyoon Kim, Pukyong National University, hgkim@nfrdi.go.kr*

National HAB Monitoring and prediction system

HAB monitoring covering all Korean coasts has been conducted by national institutes since 1996. NFRDI (National Fisheries Research and Development Institute) is primarily in charge of routine red-tide monitoring and conducts monthly HAB surveys at 98 sites in Korean coastal waters, from March to November, to investigate monthly changes of water quality and dominant phytoplankton species. More than 30 fisheries extension service stations under local governments are also responsible for irregular HAB monitoring, whenever water discoloration occurs within or in adjacent waters. In addition, once HABs by fish-killing species are initiated, NMPA (National Maritime Police Agency) also join the red-tide monitoring program and conduct aerial surveillance with helicopter. In addition, NFRDI has conducted a specific monitoring program targeted on *Cochlodinium* blooms since 2000. This monitoring primarily aims at early prediction and warning of *Cochlodinium* blooms based on biological and hydrological data collected in biweekly field surveys from late June to early October. To sustain safe supplies of shellfish products without shellfish poisoning, NFRDI, in collaboration with local fisheries extension services, conducts regular monitoring of oyster, mussel and clam farms. More than 100 sites are monitored monthly from February to November with special emphasis on the eastern part of South Coast where oyster farms are concentrated. When shellfish poisoning is detected, NFRDI increases monitoring frequency from monthly to weekly.

The HAB clearing house in NFRDI collects all available biological and hydrological data from red-tide monitoring institutes and coastal environment observation buoys deployed near aquaculture farms, and meteorological and remote sensing data, to synthesize, analyze and publish a daily red-tide

newsletter. The daily HAB newsletter describes causative organisms, cell densities, and affected areas, and provides warnings and predictions on the development and spread of red-tides: it is disseminated to fisherman and relevant agencies in charge of red-tide monitoring and/or mitigation by ARS (Automated telephone Response System), SMS, Satellite TV, easy fax and internet webpage (www.nfrdi.re.kr).

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National marine biotoxins monitoring and responses

Contamination of bivalves with marine biotoxins such as paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) and other lipophilic shellfish toxins (LST) are controversial issues in Korea. Korea is a major producer of bivalves, and Korean shellfish products are exported to the US, China, Japan, EU and elsewhere (Fig. 2). The occurrence of marine biotoxins in Korean shellfish is considered to be important both in Korea and importing countries. The Marine Biotoxin Monitoring Program in Korea began in 1980, and was initially based on the screening of mussels for the presence of PSP toxins in shellfish growing areas on the south coast. Since the 1980s, 44 cases of PSP, of which 5 were fatal, were reported from the south coast of Korea, and all those who died had consumed toxic wild mussels. On the other hand, neither ASP nor DSP incidents have been officially reported in Korea. Since the 1990s, the PSP monitoring area was expanded to the east and west coasts of Korea, and DSP and ASP monitoring added to the program, which entails the participation of the central government, local governments and fisheries cooperatives.

Marine biotoxins are analysed at the National Fisheries Research and Development Institute, Busan. Local governments act to prevent shellfish poisoning caused by marine biotoxins, for example by closing harvesting areas. At present, PSP is measured by the mouse bioassay, and ASP and lipophilic toxins are analyzed using instruments such as HPLC or LC-MS/MS. Official regulatory limits for biotoxins in shellfish flesh are 800 μg · kg^{-1} for PSP; 160 μg · kg^{-1} for okadaic acid plus dinophysistoxin^{-1}, and 20 mg · kg^{-1} for ASP. When concentrations exceed the regulatory limit, shellfish harvesting areas are temporarily closed; recreational harvesting is also prohibited. PSP has occurred in the spring season, mainly March to May, on the southeast coast of Korea. Jinhae Bay is especially affected, and some areas in the eastern part of the bay exceed regulatory limits every year. Jinhae Bay is a typical semi-enclosed coastal area surrounded by cities and is one of the most eutrophic coastal embayments in Korea. This bay is known for recurrent red-tides including *Alexandrium tamarense* blooms. The eutrophic condition in Jinhae Bay is considered a major factor promoting the growth of toxic phytoplankton such as *Dinophysis* spp. or *Alexandrium* spp. Marine biotoxin occurrences, especially PST, begin in Jinhae Bay and expand to the southeast coast of Korea each year. LSTs in bivalves show remarkable seasonal and annual variations. Some sporadic LST occurrences were observed by mouse bioassay until 2008 when the LC-MS/MS technique was adopted and seasonal variation of LST occurrence could be investigated. Seasonal trends show that high LST values are a spring-summer problem. The major LSTs in Korean bivalves are okadaic acid and dinophysistoxin^{-1}, but occasionally pectenotoxin^{-2} or yessotoxin are detected as a major component. However, the toxicity in whole bivalves tissue has not exceeded the regulatory limit (160-200 μg · kg-1) in Korea, the EU, and the US. ASP is rarely found in Korean shellfish by HPLC analysis, and no poisoning incidents have been recorded.

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Fig. 2. Oyster farms in south Sea of Korea. Oyster production is one of the most important industries. Source : Ji Hoe Kim, Food Safety Research Division, National Fisheries Research and Development Institute, kimjh@nfrdi.go.kr.

Ocean-color remote sensing for HAB monitoring

The National Fisheries Research & Development Institute (NFRDI) has been using satellite remote sensing techniques since the 1990's to provide fisheries information in Korea (Fig. 3). The environmental parameters provided by satellites were initially limited to Sea Surface Temperature (SST) and water turbidity. After the launch of ocean color sensor SeaWiFs in 1997, chlorophyll concentration was considered a key parameter for monitoring red tides and water quality in Korean waters. But the satellite red tide information is corrupted by suspended sediment particles, especially in coastal waters, and remote sensing cannot be used for operational purposes. Moreover, the spatial resolution of SeaWiFS is not adequate to monitor coastal red tide patches. In the early 2000's, Korea Ocean Research & Development Institute (Dr. Y H Ahn, KORDI) carried out the basic research on red tide detection techniques, including the optical properties of phytoplankton, the modeling of ocean color changes by living organisms in the ocean, and inverse techniques of ocean colour. We anticipate that this research will be used in the near future hyper-spectral satellite (like HiCO) to detect and identify the

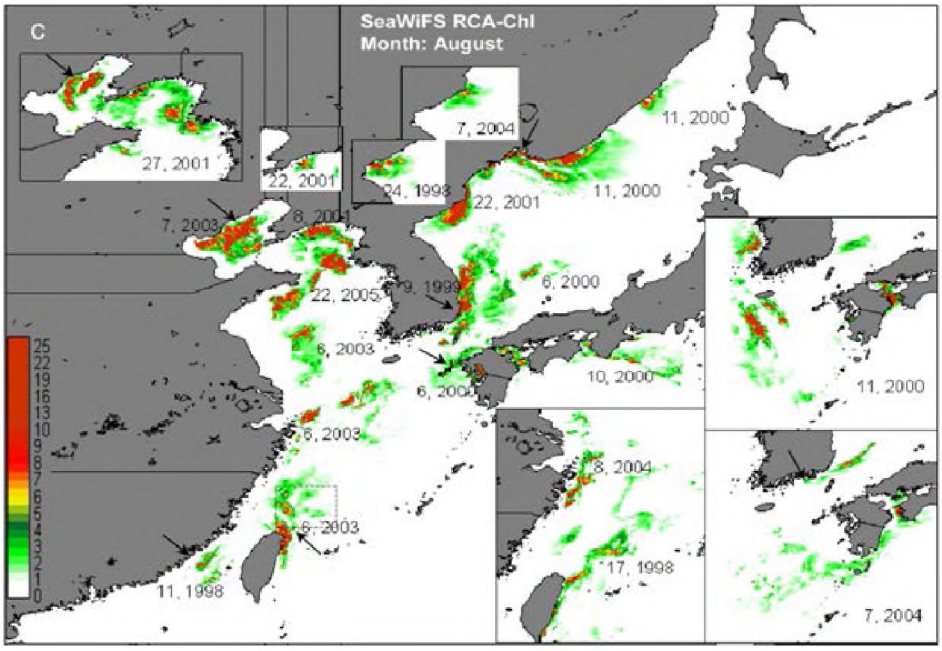

Fig. 3. The spatio-temporal distributions of the SeaWiFS detected red-tide by the Red-tide Chlorophyll Algorithm (RCA) in and around Korean waters during August 1998 – 2006. The bloom dates and years are mentioned in the figure. Source : Yu-Hwan Ahn, Korea Ocean Satellite Center in KORDI, yhahn@kordi.re.kr.

different phytoplankton groups from space. In addition, a new red tide algorithm developed by KORDI for ocean color satellite permits partial separation of chlorophyll from suspended sediment signals. But red tide detection techniques are not yet perfect, and continue to be an ongoing task for ocean colour scientists.

The new technique was applied in northeast Asian waters to the spatial distribution, movement and frequency of red tides. It revealed that most Korean coastal red tides do not develop in situ, but are advected from southern Taiwan. When they enter Korean waters with the current, the causative species meet nutrient rich waters and bloom again. These results indicate that Korean red tides start in the open sea rather than in coastal water. Since 2000, KORDI scientists have studied phytoplankton fluorescence signal (FS) of chlorophyll. The intensity of FS is proportional to chlorophyll concentrations in ocean waters and is not distorted too much by suspended sediment particles. In 2010, the first geostationary ocean colour Imager (GOCI, one of 3 payloads in the COMS satellite), mission developed by KORDI, adopting FS bands, was launched with the support of the Ministry of Land Transportation & Maritime Affairs (MLTM) in Korea. The main mission of GOCI is the monitoring of Korean coastal waters. Red tide monitoring is one of key missions using FS detection. Korea Ocean Satellite Center (KOSC in KORDI) is responsible for the data service and operating of GOCI, and receives images eight times a day. The Korea Aero-Space Research Institute (KARI) controls the COMS satellite. At the 15th ICHA, we will present the activities of satellite red tide monitoring and research results in Korea.

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Study of benthic dinoflagellates and monitoring

Biogeographically, Jeju Island is in the temperate zone based on air temperatures, but some tropical fish and invertebrates have been found here in the last decade. The Tsushima Warm Current, a branch of the Kuroshio Current, with high water temperature and high salinity, has influenced the sea around Jeju Island more strongly than before. We can assume that this island is changing from temperate to sub-tropical. We have found planktonic dinoflagellate evidence of the shift from temperate to sub-tropical, due to strengthening of the Tsushima Warm Current. The ciguatera agent *Gambierdiscus* sp. has been reported recently in Jeju waters. Until now, no toxic events caused by dinoflagellates have been reported; detailed information on the taxonomy, seasonal distribution, diversity and molecular phylogeny of benthic toxic and non-toxic dinoflagellates from this Island is not yet available.

The present study of benthic dinoflagellates has been conducted since March 2011 with the following objectives: to provide an overview of their diversity and systematics, to identify and describe them for environmental monitoring, and to develop monoclonal cultures of strains from Jeju waters. Dinoflagellate samples were collected around Jeju Island and cultures established. Preliminary observations reveal 32 species belonging to 15 genera. Among them, 10 species are reported to be toxic in subtropical and tropical regions and 27 are new to Korean waters. The potentially toxic species include *Amphidinium carterae, A. operculatum, Coolia monotis, Gambierdiscus yasumotoi, G. toxicus, Ostreopsis ovata, O. lenticularis, Prorocentrum emerginatum, P. concavum,* and *P. mexicanum.* Species of *Amphidinium* and *Ostreopsis* are the most abundant, and large numbers of *Ostreopsis* were found in June 2011. *G. yasumotoi* was found in only one location (Hwasun) in June 2011 and *G. toxicus* in Humduk in October and November 2011. *P. concavum* oc-

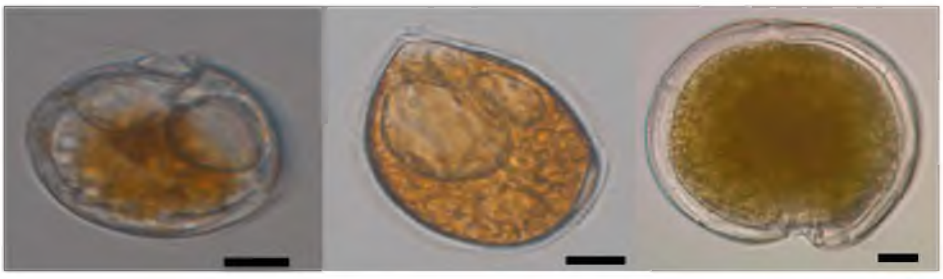

Fig. 4. Coolia monotis *(left),* Ostreopsis ovata *(middle) and* Gambierdiscus toxicus*-like (right) specimens. These subtropical species of harmful algae have been identified around Jeju Island off southwest Korea (scale bar = 10 µm). Source: Joon-Baek Lee (Jeju National University) and Md. Mahfuzur Rahman Shah (Jeju Sea Grant College Program, Korea, jblee@jejunu.ac.kr).*

curred in Shewa in August 2011, and *P. mexicanum* in October 2011 in Hwasun. A total of 150 strains of benthic dinoflagellates were established in the laboratory. Some species like *A. carterae* and *A. operculatum* are being cultured in 300ml, 1 L, 2 L, 3L flasks and 20 L carboys to scale up and optimize culture conditions. Newly recorded species are identified so far based only on LM, and more detailed morphological and genetic study are needed to confirm identifications.

The existence of subtropical/tropical benthic dinoflagellates in Jeju waters may point towards a change from temperate to subtropical. We believe that the occurrence of these species is related to stronger flow of the Tsushima Warm Current. The presence of potentially toxic tropical dinoflagellates (*Coolia monotis, Ostreopsis ovata, Gambierdiscus yasumotoi, G. toxicus*) (Fig. 4) may indicate a risk of potential toxicity in Jeju waters. Studies continue to establish single cell cultures (small scale to mass culture), biochemical (fatty acid, antioxidant, pigment) and toxicity analyses of the cultured strains, taxonomic comparisons using morphometric and molecular phylogenetic analysis, and seasonal abundance and distribution patterns in Jeju waters.

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Studies of planktonic food web and mixotrophy

Our research lines include i) interactions between harmful algae and their grazers or prey (Fig. 5); ii) marine planktonic food webs; iii) dynamics of C, N, P in marine ecosystems; iv) responses by the marine planktonic community to diverse scale environmental changes; v) mixotrophy in marine organisms; vi) horizontal gene transfer among marine protists; vii) invasive species, in particular, subtropical dinoflagellates (*Coolia, Gambierdiscus, Ostreopsis, Prorocentrum,* and *Amphidinium*); viii) *Pfiesteria* and similar species; ix) the taxonomy and eco-physiology of new dinoflagellates and ciliates (we established the dinoflagellates *Stoeckeria algicida, Luciella masanensis, L. atlantis, Paragymnodinium shiwhaense, Gyrodiniellum shiwhaense, Gyrodinium moestrupii* and the ciliates *Strombidinopsis jeokjo, Parastrombidinopsis shimii,* and *Balanion masanensis*). In addition, we are developing methods of controlling harmful algal blooms (i.e. mass-cultured grazers, NaOCl), reducing CO_2 using marine microalgae, and producing biodiesel using marine protists in food webs. For more information:

Hae Jin Jeong, Seoul National University, hjjeong@snu.ac.kr http://plaza.snu.ac.kr/~hjjeong/English.htm

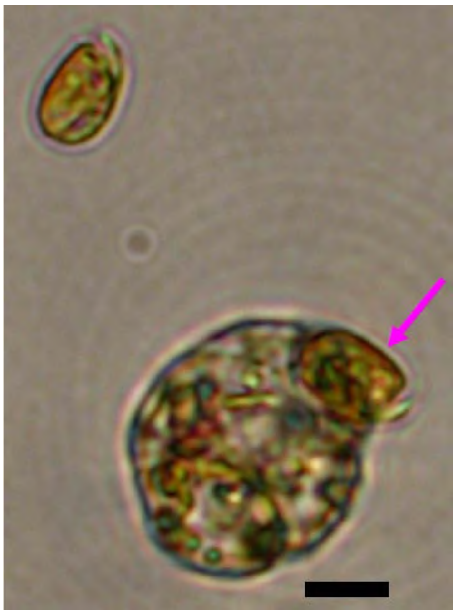

Fig. 5. The mixotrophic dinoflagellate Cochlodinium polykrikoides feeding on the dinoflagellate Amphidinium carterae (arrow). (scale bar = 5 µm). Source: Hae Jin Jeong, Seoul National University, hjjeong@snu.ac.kr; http://plaza.snu.ac.kr/~hjjeong/English.htm

Studies of marine phototrophic microbes (MPMs)

The main research interests of the MPM Lab in Kunsan National University are "strain-based" ecology and biotechnology of MPMs in Korean seas. MPMs include unicellular cyanobacteria, red tide plankton, and symbiotic eukaryotes such as zooxanthellae, parasitic dinoflagellates and epiphytic microalgae. Our study on MPM ecology includes the strain-based ecophysiology of *Amoebophrya* strains, *Mesodinium rubrum,* and *Dinophysis* spp. and the population ecology of bloom-forming MPM species in Korean seas. Successful isolation of *Teleaulax* sp. strains some 10 years ago was extended to setting up Korean *Mesodinium rubrum* strains, which initiated the novel establishment of *Dinophysis acuminata* strain DA-MAL01 in 2006. The availability of the three species in culture opened a new door for our research on the mechanisms for the blooming process, evolution of plastid and cell organelles, and even to multifaceted biotechnological applications of previously "harmful" MPMs (Figs. 6, 7). At present, biotechnological aspects of MPMs strains in our lab are mostly related with *Dinophysis* spp. strains which have proved to be ideal provider organisms of the anti-cancer drug lead, pectenotoxin^{-2}(PTX^{-2}). Nitrogen-fixing unicellular cyanobacterium strains have been isolated and tested for the commercially reasonable production of hydrogen gas within 30 years in Korea.

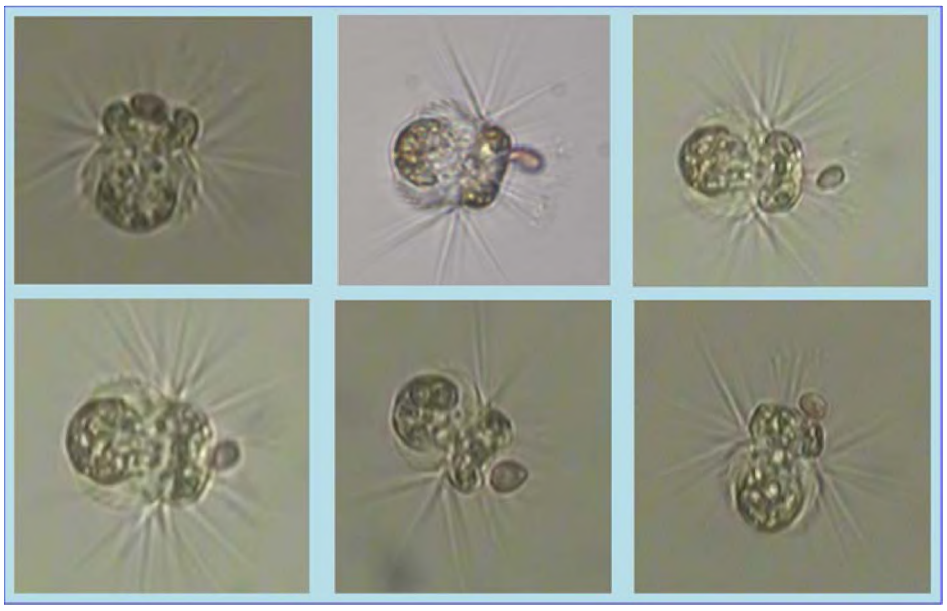

Fig. 6. From the phytoflagellate Teleaulax *to the ciliate* Mesodinium*, the first step of real-time plastid flow through food chain. Source: Wonho Yih, Kunsan National University, ywonho@kunsan.ac.kr*

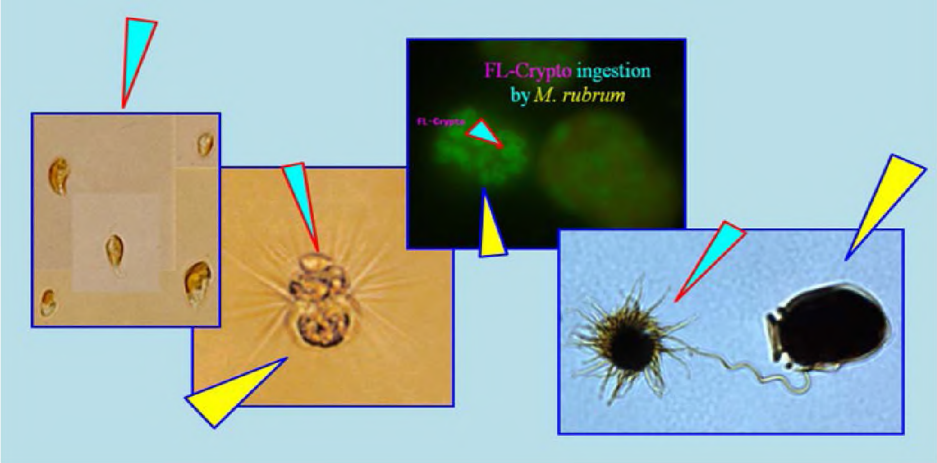

Fig. 7. From Teleaulax *through* Mesodinium *to* Dinophysis. *In a time scale of minutes to days, plastids of the phytoflagellate* Teleaulax *are obtained and reused serially more than two times by the ciliate grazer, dinoflagellate predator, and other even higher level consumers. Source: Wonho Yih, Kunsan National University, ywonho@kunsan.ac.kr*

Single cell (SC) physiology of cyanobacterial strains, such as growth synchrony, recycling of fixed carbon, and oxygen resistance of the hydrogen producing SC system, might be further studied. MPMs with diverse cell dimensions could also be developed as the promising live feed organisms for mariculture of marine animals, as the key organisms for the mobile CO_2 removing units, and also as sources of novel engineered enzymes, toxins, and pigments. In the near future, a kind of TBS (total biotechnology system) using MPM strains as the core could be designed and tested for economically and environmentally sound simultaneous production of multiple bio-products.

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Study of sophorolipid-yellow clay mixture to mitigate HABs

Numerous HAB mitigation methods have been examined in Korea, including yellow clay, marine bacteria, microscreen filtration, sodium hypochloride and microzooplankton. We have recommended the use of sophorolipid to mitigate *Cochlodinium* red tide [1]. Nevertheless, with the exception of yellow clay, these methods have failed to be practically applicable because of high costs, negative side effects, or difficulty of use in the marine environment. It is well known that yellow clay is nontoxic, inexpensive, and easy to use, and has been applied to mitigate HABs in South Korea. To remove above 80% of HABs, dispersion concentration of more than 10 g L^{-1} yellow clay has been recommended. However, this high concentration of yellow clay has been a problem to produce anaerobic bottom environment and negative effects on other plankton and benthic algae. Compared with other surfactants, the sophorolipid showed a good algicidal effect and biodegradation efficiency, but low penetration efficiency into the water column.

To reduce the weak points of yellow clay and sophorolipid, we recommend to use the mixture of 1 g L^{-1} yellow clay and 5 mg L^{-1} sophorolipid. The sophorolipid-yellow clay mixture more efficiently mitigated the HAB (95% removal efficiency) than yellow clay alone (79%) and had a less adverse effect on bacterioplankton, heterotrophic protists and zooplankton than the yellow clay. These results indicate that sophorolipid-yellow clay mixture can mitigate HABs efficiently with environmental and economical effects.

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HABs early warning and mitigation with clay dispersal

To give timely assistance to fishermen and aquaculturists in advance of HAB, NFRDI has adopted a red-tide warning system. It consists of three steps: Red Tide Attention, Red Tide Alert and Warning Lifted. Attention and alert notices are issued when the density of fish-killing species, *C. polykrikoides*, exceeds 300 cells mL^{-1} and 1000 cells mL^{-1}, respectively. Once a red-tide warning is issued, NFRDI and local government recommend fisherman to stop feeding, and supply liquid oxygen and/or disperse yellow clay to minimize impact on resources. NFRDI also has a shellfish poisoning warning system based on the toxin levels of shellfish. When the PST level exceeds the safety limit, 800μg · kg^{-1} of shellfish meat, NFRDI issues a shellfish poisoning warning, and harvesting of shellfish is banned until the warning is lifted.

Mariculture for finfish and shellfish is widespread in coastal Korea. Many pen cages, particularly for finfish culture, are concentrated mostly along the southern coast where there are many islands enabling aquaculture facilities protected from typhoons. However, HABs of *C. polykrikoikes* have occurred since the 1990s in both inshore and offshore waters, and resulted in severe fisheries damage in the area. Yellow clay composed of montmorillonite has been applied since 1996 to minimize damage by *C. polykrikoides* blooms. It is estimated that the application of the clay control method has reduced annual losses from 95 million US dollars in 1995 to 0.2-22.3 million US dollars in 1996-2007. Several devices such as clay dispensers, automatic HAB alarm systems and electrolytic water generators have been developed for fisherman and responsible agencies, to help in early warning for HABs and as tools for effective HAB mitigation. Clay dispensers crumble native yellow clay into a fine size which increases HAB removal rates, and disperse it automatically into HAB affected areas. These have been available to HAB control agencies since 1997. Automatic HAB alarm system equipped with chlorophyll and turbidity sensors has been developed and made available to land-based finfish culture farms since 1999, and provide warning sound or signals when red tide is introduced into aquaculture sites. Another special device, the Electrolytic Clay Dispenser (ECD), an electrolytic water generator and clay dispenser combined, has been developed and deployed to local government since 2001 in Korea. The device is designed to minimize the amount of clay treatment applied, to reduce any ecological impact, particu-

larly on the benthos by accumulation of clay on the sea bed, and to enhance HAB removal efficiency compared to earlier designs of clay dispenser. NFRDI has now started to monitor environmental changes including impacts on areas where yellow clay was frequently introduced during *C. polykrikoides* blooms since 1998. The impact on the fauna, particularly on benthos, is thought to be negligible based on present results, but further long term studies is needed.

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The Eco-STAR project to develop technology for aquatic ecosystem restoration and management

As a part of the Eco-STAR Project (part of Core Environmental Technique Development Project for Next Generation of the Korean Ministry of Environment), the Aquatic Ecosystem Restoration Project group (AERP) was established in 2007 to develop eco-friendly environmental techniques, to be promoted as a competitive project in the 21st century (Fig. 8). A sum of 84.1 billion Korean won (Government 58.5 billion + private 25.6 billion) is earmarked for the Eco-STAR budget, to develop technology for aquatic ecosystem restoration and management over 6.5 years (December 2007 to May 2014); the group has a total of 684 researchers from 75 institutes.

To restore and preserve the health of damaged aquatic ecosystems, AERP develops practical technologies in four areas, i.e., i) to restore and manage aquatic ecosystems; ii) to alleviate stagnation and to purify water quality; iii) to develop and to manage riparian margins, and iv) to evaluate aquatic ecosystem health, on the principle of "mutual survival of humanity and nature." The project also emphasizes the significance of the societal and economic value of aquatic ecosystem restoration, and education and public relations.

There are twelve sub-projects. One of these, the "Development of Nature-friendly Technology for Water Quality Purification", deals mainly with efficient control of harmful algal blooms (HABs) in freshwaters, by evaluating the performance of HAB control techniques using algicidal bacteria and algicidal



Fig. 8. The logo of Aquatic Ecosystem Restoration Project Group signifies the unification of humans and nature. Expressing the shape of a human's body with raised hands in calligraphy, it describes free human image and the wave lines in blue and green express water and forest, respectively. The harmony of two colors expresses the image of human and nature that advance toward the future together. As warm colors, those used for the shape of human mean passion, energy and challenging spirit. Source: Myung soo Han, Han Yang University, hanms@hanyang.ac.kr

substance(s) derived from them. Thus, the project involved i) isolation of various algicidal bacteria active against problem species in South Korea; ii) identification of algicidal substances extracted from these bacteria, and iii) improvement of existing algicides into useful chemical agents (hence termed 'MEDI-TIDE') possessing stronger algicidal activity, more species-specific activity and lower toxicity. At present, iv) the possible use of MEDI-TIDE for the efficient termination of natural blooms of problem species with minimal adverse effects on freshwater systems (reservoirs and rivers) is being assessed under field conditions. Finally, further study of the optimization of mass-production of MEDI-TIDE is planned.

Many blooms represent a serious threat to the use and sustainability of our freshwater resources. The commonest problems (toxicity, food web altering, hypoxia generating), are due to *Microcystis, Anabaena, Stephanodiscus,* and *Peridinium*, and affect drinking water supplies from both health and economic perspectives. These are found annually in most lakes, reservoirs, and rivers, including the Pal'tang (37 °N and 127 °E), Juam (34-35 °N and 127 °E), and Daechung Reservoirs (36 °N and 127 °E) and Nakdong River (35-37 °N and 124-131 °E) of South Korea. Thus, if the project is successfully completed as planned, MEDI-TIDE may be a simple and environmentally friendly solution for the provision of safe potable water, and also effectively prevent blooms and curtail excess algal and cyanobacterial growth.

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Use of molecular probes to understand migration, dispersal and population dynamics of harmful algal bloom species

The first outbreak of *Cochlodinium polykrikoides* was observed in the Nak-Dong estuary near Busan, Korea in 1982. Since then, *Cochlodinium* blooms have occurred in Tongyeong, Geoje, Namhae and Yeosu in southern Korean coastal waters [1, 2]. Finally, it became widespread from the West to the East Sea via southern Korean coastal waters [1, 3]. The frequency and intensity of *Cochlodinium* blooms has increased over time [4] and cell density exceeded 48,000 cells ml^{-1} in 2003. *C. polykrikoides*-related fish kills have gradually increased, with huge economic losses, over 100 million US dollars in Korean coastal waters (NFRDI report) (Fig. 9).

Nevertheless, we still do not fully understand the migration, dispersal and bloom mechanisms of *C. polykrikoides*, about which there are many questions (Fig. 10). Previous research reported that *C. polykrikoides*'s bloom begins each year near Narodo in southwest coastal waters of Korea. But, it is unclear whether *C. polykrikoides* blooms were advected by the Tsushima and Yellow Sea warm currents, branches of the Kuroshio Current, or germinated from seed populations near Narodo. Moreover, *C. polykrikoides* blooms also last for longer periods than other red tides [4]. Some studies suggested that assemblages of *Cochlodinium* (Fig. 11) may be composed of two or more strains or species. Recently, a *C. polykrikoides* population was classified into East Asian (Korean/Japanese), Philippines and American/Malaysian ribotypes by 28S ribosomal DNA [5]. However, it is impossible to identify each *C. polykrikoides* ribotype microscopically due to extremely similar morphologies. Nothing is known about the geographical distribution of these *C. polykrikoides*

ribotypes in Korean coastal waters.

Molecular techniques such as FISH (Fluorescence In situ Hybridization), microsatellite markers and quantitative real-time PCR assays are necessary to detect and quantify each *C. polykrikoides* ribotype. Surveys were performed weekly or bi-weekly from June to November in southern coastal waters of Korea for 3 years (2009-2011). Physical (temperature, pH, DO and salinity), chemical (nutrients and chlorophyll a) and biological factors (phytoplankton, bacteria, HNF, ciliate and micro-zooplankton etc.) were investigated. We selected 35 sampling sites from July to August in 2008 to determine the geographical distribution of each *C. polykrikoides* ribotype. In order to understand the migration and dispersion of *C. polykrikoides*, sampling was extended into the East China Sea, Jeju and Tsushima Island. Then distribution of *C. polykrikoides* ribotypes was analyzed with quantitative real-time PCR assay.

As results of this project, we discovered that Philippines and East Asian ribotypes are widely co-distributed all around Korean coastal waters. The Philippines ribotype was detected even in the East China Sea and around Jeju Island. This implies that the Philippines ribotype may flow into Korean coastal waters via the Yellow Sea warm current. In 2009, we found a mixed bloom of *C. polykrikoides* in which Philippines (summer) and East Asian (autumn) ribotypes showed different seasonal fluctuation in southern Korean coastal waters. These results suggest that the long term persistence of *C. polykrikoides* blooms may be caused by a succession of different ribotypes in the same area.

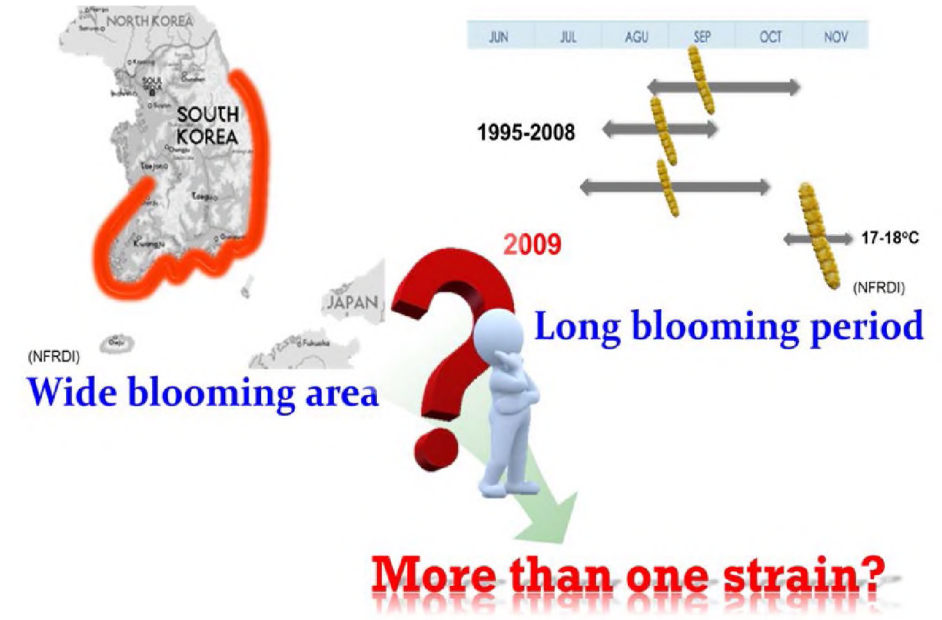

Fig. 9. Huge long-term bloom of Cochlodinium polykrikoides *in Korean coastal waters. Source: Myung soo Han, Han Yang University, hanms@hanyang.ac.kr .*

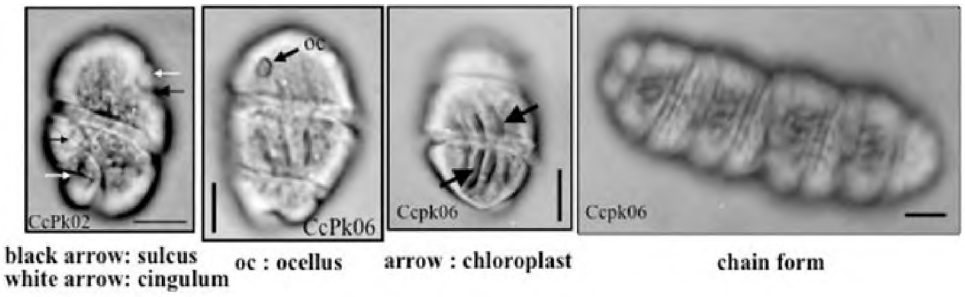

Fig. 10. Micrographs of the red-tide chain-forming dinoflagellate Cochlodinium polykrikoides *(scale bar 10 µm). Source: Prof. MS Han.*



Fig. 11. Harmful algal blooms scum visible in Daechung, South Korea (from http://www.newsis.com/, ©Newsis, 2010).

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Karenia blooms in Gulf of Mexico

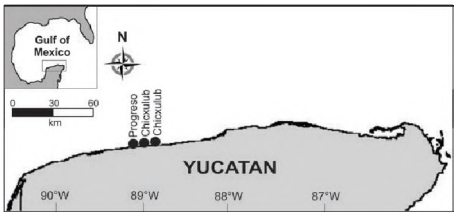

Fig. 1. Sampling sites at the northern coast of the Yucatan Peninsula in August 2011.

species level in water-bottle samples taken in the surface layer, using an inverted Olympus CKX41 microscope in a Sedgwick-Rafter chamber. *Karenia brevis* (Fig. 2 and 3) and *K.* cf. *papilionacea* (Fig. 4 and 5) were rather abundant at two sites, and *K. papilionacea* dominated at the easternmost locality (Fig. 6 and 7). However, most of cells remained unidentifiable under the light microscope due to distortion in cell shape after fixation, and we designated them conventionally as *K.* cf. *brevis and K.* cf. *papilionacea* (Fig. 4 and 5). In all examined cells of *Karenia* species, the nucleus was located in the left part of the hypotheca. In acid Lugol-fixed samples, the details of the sulcus, the cingulum and the apical grove were not seen. The length (L) and the width of cell (W) and the W/L ratio were measured for 199 cells. The morphometrical characteristics for *K. brevis* (including *K.* cf. *brevis*) were as follows: L=17.5-29.5 µm (19.77±1.84), W=19-31.5 µm (24.70±3.08), W/L=0.85-1.53 (1.25±0.12), n=158; for *K. papilionacea* (including *K.* cf. *papilionacea*) L=18-25 µm (21.45±1.42), W=29-37.5 µm (31.49±2.29), W/L=1.33-1.7 (1.47±0.10), n=41.

Table 1. Abundance of Karenia *species in the Progreso-Chicxulub area, SE Gulf of Mexico (cells L^{-1}).*

Sampling site	*K. brevis*	*K.* cf. *papilionacea*	*K. papilionacea*	Total
Progreso	15808	20072	7280	43160
Chicxulub 1	22500	45443	9557	77500
Chicxulub 2	7480	4307	170820	182607

During the last two years, blooms caused by *Karenia* species in the southern Gulf of Mexico have increased in frequency. In the central part of the State of Veracruz, a mixed bloom of *K. brevis* and *Karenia* "Mexican hat" sp. was reported in 2010 [1].

Karenia species appeared in high abundances in the Progreso-Chicxulub area (21°21.421'-21°22.466'N, 89°00.832'-89°07.333'W; Fig. 1; Table 1) at three coastal sites out of 16 visited monthly since 2002. The ranges of the physical-chemical parameters were as follows: temperature 26.7-27.5°C, salinity 36.20-36.32, nitrates 6.75-6.97 µM, nitrites 0.12-0.22 µM, ammonium 0.99-1.80 µM, urea 3.21-4.37 µM, phosphates 0.052 µM (the only measurement), silicates 2.24-4.16 µM, and chlorophyll "a" 0.38-0.46 mg m^{-3}.

We were able to identify two taxa at

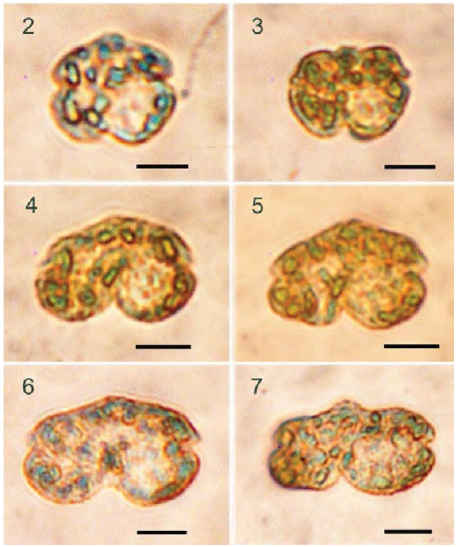

Fig. 2-7. Karenia *species from northern Yucatan: 2-3* K. brevis*; 4-5* K. *cf.* papilionacea*; 6-7* K. papilionacea. *Scale bar: 10 µm.*

To our knowledge, this is the first *Karenia* bloom ever registered along the northern coast of Yucatan Peninsula. Both species mentioned above are known as either producers of brevetoxins or brevetoxin-like compounds [2]. Judging from the recently reported events to the west of the study area, we can expect various *Karenia* species in the SE Gulf of Mexico. According to unpublished data of the state laboratories of public health, *K. brevis* and *K. papilionacea* were also observed on 15 September 2011 in coastal waters of the northern part of the State of Campeche, SW of our study area [3], and on September 22-24 offshore [4]; in August-September 2011, *K. brevis*, *K. mikimotoi* and *K. selliformis* blooms in the coastal waters of the State of Tabasco, further to the west of the State of Campeche [5]. All the species mentioned above have been found in the Gulf of Mexico [2], and we speculate that the latter two and *K. papilionacea* will be found in the SW Gulf of Mexico in the very near future because of physical transport mechanisms. In the northern Gulf, *K. brevis* has been reported from Texas to Florida, and even up the east coast [6]. *Karenia papilionacea* occurs off Texas, Louisiana, and Florida [7, 2, 8], and based on the inferred transport of the 1996 bloom from Florida to Louisiana this species should occur off Alabama and Mississippi.

Acknowledgements

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First report of Dinophysis acuminata bloom in Basque waters, Northern Spain

Phytoplankton has been monitored since 2000 in the Nervión River estuary in the Southern Bay of Biscay (Northern Spain), since 2002 in the coastal waters and estuaries of the Basque Country, and since 2005 along the Cantabria coast. Since then, several toxin-producing harmful species has been identified, including several species of *Pseudo-nitzschia*, dinoflagellates *Dinophysis, Karlodinium, Karenia, Ostreopsis* and *Prorocentrum*, and flagellated forms such as *Prymnesium* spp. and *Heterosigma akashiwo* [1, 2, 3]. The genus *Dinophysis* is one of the most frequently found in the area, but generally appears in very low concentrations (< 100 cells L^{-1}). However, during the spring survey of 2011, high concentrations of *Dinophysis acuminata* complex were observed at two coastal stations, one (L-N20) 2 km from the coast and 4.5 km from the mouth of Nervión River, the other (L-B10) 0.7 km from the coast and 2.7 km from the mouth of the Butron River (Fig. 1). Cells in surface samples were counted by the Utermöhl method. Cell concentrations reached 5 x 10^3 cells L^{-1} at st. L-N20 and 1.8 x 10^4 cells L^{-1} at st. L-B10. Other dominant taxa in the samples were the coccolithophore *Emiliania huxleyi*, with densities around 7 x 10^5 cells L^{-1}, and the haptophyte *Chrysochromulina*, the prasinophyte *Pachysphaera* and the diatom *Pseudo*-nitzschia, all of them with concentrations around 5 x 10^4 cells L^{-1}.

The identification of the genus *Dinophysis* is largely based on the cell contour, the size and shape of the large hypothecal plates, and the shape of the left sulcal lists and their ribs [4]. Taking into account the average measurements of the cells observed (maximum length: 45 µm; dorso-ventral depth: 30 µm) (Figs. 2 and 3), and based on Raho et al. [4], the specimens were identified as *Dinophysis acuminata*, but genetic methods should be applied for a more certain identification.

Dinophysis can produce diarrheic shellfish poisoning (DSP), a major cause of economic losses to shellfish aquaculture, which makes this genus one of the most studied harmful taxa [5]. It is well known that *Dinophysis* species prey on *Mesodinium rubrum*, but on this occasion the ciliate was not observed in the samples; but the highest densities of *Dinophysis* and *Mesodinium rubrum* do not necessarily coincide, and *Dinophysis* peaks may be as much as a month later than the highest densities of *Mesodinium rubrum* [6].

Dinophysis is also reported in very low concentrations in other areas, with values of the order of 10-40 cells L^{-1} [5]. However, with increased the spatial and temporal resolution of sampling, it has been found that the genus can reach bloom proportions more frequently than previously observed. Blooms can take place at different depths, and be concentrated by currents or by vertical migration and subsequent growth. Under favorable conditions of light and prey availability, *Dinophysis* species can attain very high growth rates [5]. The difficulty in finding blooms of *Dinophysis* despite its recurrent presence in coastal waters can to some extent be explained by its aggregation in thin layers of 10 cm to 3 m, which can extend horizontally for many kilometers [6].

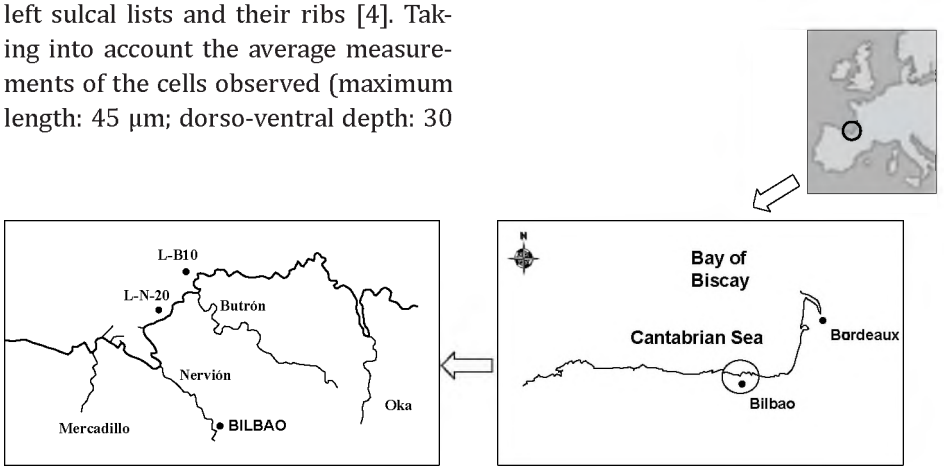

Fig. 1. Location of the two stations





Fig. 2. Micrographs of Dinophysis acuminata *complex*

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Dinoflagellate Bloom Coincides with Marine Invertebrate Mortalities in Northern California

Harmful Algal Bloom (HAB) events that involve non-traditional HAB species are poorly understood but can nonetheless cause extensive marine life mortalities. Documenting these events will be important for increasing our understanding of the ecological impacts of HABs on marine ecosystems. One such algal bloom coinciding with a mortality event impacting marine invertebrates was recently documented in Sonoma County, north of San Francisco, California (USA) in August 2011 (Fig. 1). Reports of this bloom and the marine invertebrate mortalities extended 80km along the nearshore from Bodega Bay: (Lat. 38.33N – Long. 123.02W) north to Anchor Bay, California (Lat. 38.80N – Long. 123.58W). Ocean conditions were calm in the weeks prior to the die-off, and there was an extraordinary display of night-time bioluminescence which persisted through the initial observations of dead invertebrates. Daily samples taken before, during and after the mortality event show the phytoplankton community was dominated by a dinoflagellate in the *Gonyaulax spinifera* (R. Horner pers. comm.) species complex (Fig. 2). SEM images of the bloom samples revealed that two morphotypes of *G. spinifera* were present.

To our knowledge, this is the first confirmed report of an algal bloom which caused invertebrate mortalities in a suite of economically and ecologically important species in northern California. Previously observed die-offs in this region were small in scale and appeared to be associated with low dissolved oxygen conditions following algal blooms or the degradation of drift macroalgae accumulations in protected coves. There have been blooms of the dinoflagellate *Cochlodinium* resulting in invertebrate mortalities along the central and southern Califonia coast, including the loss of over 1 million seed oysters in Morro Bay. The cause of this southern California mortality appeared to be associated with a toxin or something other than oxygen depletion.

The invertebrates that died during the northern California bloom included herbivores and carnivores from multiple phyla including mollusks, echinoderms and crustaceans, but fish deaths were not observed. After dead shellfish and sea stars washed ashore (Fig. 3), quantitative subtidal field surveys in the kelp beds in Sonoma County were conducted. Surveys revealed that at four sites an average of 25% of red abalone, *Haliotis rufescens*, a recreationally fished species and red sea urchins, *Strongylocentrotus franciscanus*, a commercially fished species were dead or dying. Non-fished species were also impacted including gumboot chiton, *Cryptochiton stelleri*, other mollusks, purple sea urchins, *S. purpuratus*, and several species of sea stars including *Pisaster ochraceous*. The spatial patterns of mortality included both coves with reduced water flow and the potential for localized oxygen depletion and exposed headlands where oxygen depletion is highly unlikely. Invertebrates in shallow water (<10m) suffered greater mortality than those in deeper water. Invertebrates within the Bodega Marine Lab (BML) located within the affected region also died during this event, with sea stars exhibiting bizarre arm bending behavior prior to dying, but fish were not affected. Oxygen levels measured in one tank at the lab were normal (97% of full saturation) during the event. Some invertebrates appeared unaffected by the event such as the bat star, *Asterina miniata,* which was observed actively scavenging dead abalone and sea urchins in the field. Using these data, the Fish and Game Commission in California voted in September, 2011 to close the recreational abalone fishery in Sonoma County for the remainder of the 2011 season.

Mussels, *Mytilus californianus,* which bioconcentrate phytoplankton toxins, were collected during the bloom and tested by the California Department of Public Health for Paralytic Shellfish Poisoning (PSP) using the Jellett Rapid Testing Systems lateral flow immunoassay and Domoic Acid toxin using the standard High Performance Liquid Chromatography (HPLC) method [1]. Test results for these toxins were below the minimum detection limits. Two independent labs at the University of California, Santa Cruz (R. Kudela UCSC) and Fish and Game Laboratory at OSPR (D. Crane) detected trace levels of Yessotoxin (YTX) using HPLC-mass spectrometry (UCSC) and tandem mass

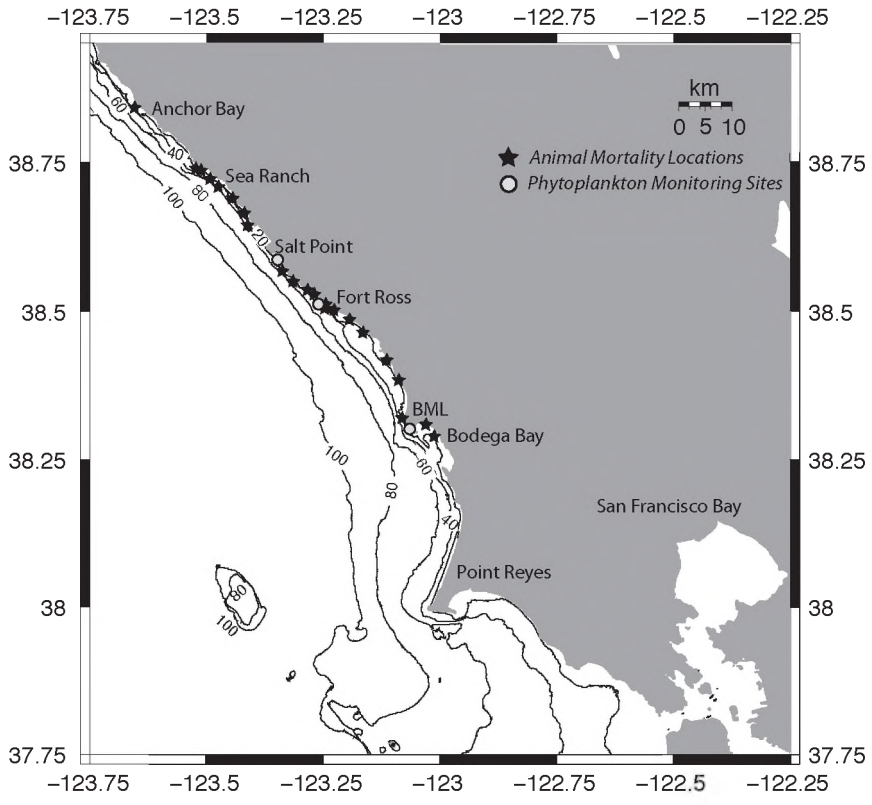

Fig. 1. Map of region in northern California impacted by the harmful algal bloom.

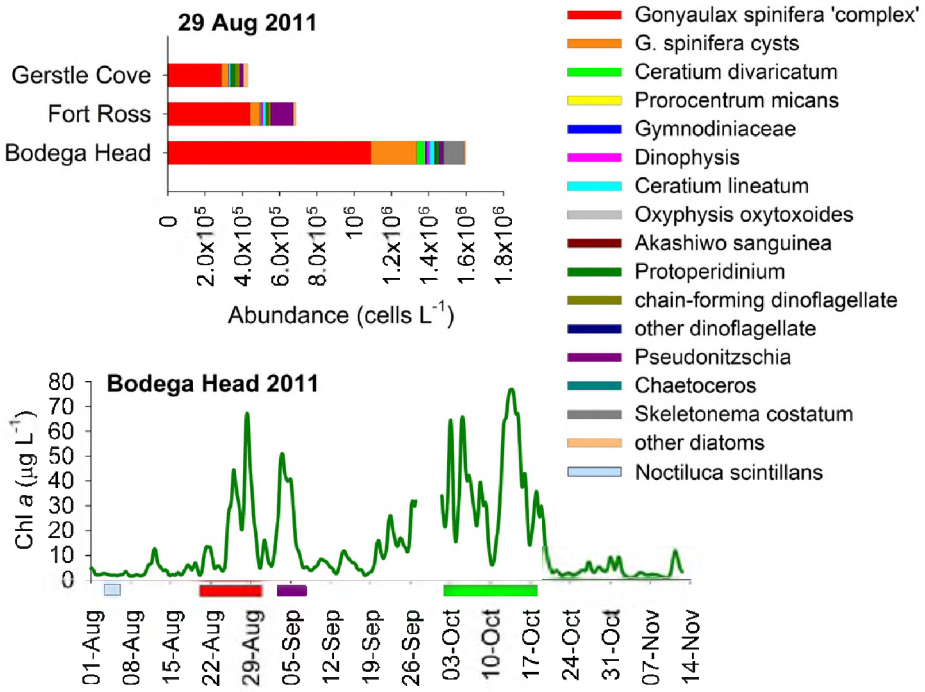

Fig. 2. Chlorophyll a time series showing dominant taxa during visible bloom events at Bodega Head and phytoplankton species abundances from 3 locations in Sonoma County (noted in Fig. 1 with open symbols) where invertebrate die-offs occurred.

spectrometry (OSPR) in both mussels and abalone gut tissue. *Gonyaulax spinifera* is known to produce YTX [2]. The US Food and Drug Administration lab in Dauphin Island, Alabama detected trace levels of YTX, dinophysistoxins and okadaic acid, as well as pectenotoxins, and spirolides. UCSC independently confirmed that other common toxins including domoic acid, okadaic acid, and microcystins were below detection. Histology of the tissues including the gastrointestinal tract, kidney, nervous tissue, gonad, muscle and gills from abalone that died at BML as well as red abalone collected from Fort Ross two weeks after the peak event showed no consistent pathologic changes.

To our knowledge, this is only be the second time YTXs have been detected in California [3]. YTX is a class of disulfate polyether (lipophilic) toxins with at least 90 different analogues identified from phytoplankton and shellfish although the structures of just a few are known [4]. The oral potency of YTX is thought to be exceedingly low, however it is toxic when injected into mice [5]. Abalone and sea urchins are herbivores which feed primarily on drift macroalgae while the sea stars affected here are carnivores that feed on mussels and other invertebrates. The trophic transfer of a toxin from the phytoplankton to non-filter feeding herbivores is unclear as is the oral toxicity of the YTX. Incidental ingestion of the dinoflagellate and/or its cysts on macroalgae is one possible route of exposure as observed with herbivorous manatees dying after consuming sea grass coated with dinoflagellate [6]. Work currently underway is examining the genetics of the *Gonyaulax spinifera* species complex, the mechanism of toxin transfer, as well as any change in the genetic structure of the abalone populations in the region as a consequence of the event. Since *Gonyaulax* forms cysts, the possibility cannot be ruled out that a similar bloom event may occur in the same geographic region in the future.



Fig. 3. Dead red abalone and gumboot chitons wash ashore at Fort Ross Cove, Sonoma County, California August 2011. Photo Credit: Nate Buck.

Acknowledgements

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A Recurring Bloom of Toxic Marine Cyanobacteria above the Arctic Circle

During September of 2008, the waters of Kotzebue Sound, Alaska, turned a fluorescent green color. Given that Kotzebue Sound is above the Arctic Circle, local authorities initially suspected chemical contamination and not a biological origin. Samples were collected and sent to the Alaska Department of Environmental Conservation by the Alaska Department of Fish and Game Kotzebue Office but only tested for the presence of petroleum and petroleum byproducts. A year later (September 10, 2009), another bloom with a "grass seed" appearance occurred in Kotzebue Sound. This bloom was identified by light microscopy as the cyanobacteria, *Aphanizomenon flos-aquae* [1]. This was followed on September 10, 2010, by another bloom that was identified as a mixture of *Aphanizomenon* sp. (3.1 x 10^5 filaments mL^{-1}), *Nodularia* sp. (5 x 10^4 filaments mL^{-1}) and *Anabaena* sp. (1 x 10^4 filaments mL^{-1}; Fig. 1). A cyanobacteria bloom also occurred August 26, 2011, but was very short in duration and was not sampled.

Observational data collected by a Kotzebue resident in a Weather Journal and meteorological data from a nearby weather station (National Weather Service weather station at the Kotzebue Wein Memorial Airport (http://weather.noaa.gov/weather/current/PAOT.html) suggested that in 2008-2010, a combination of ~15.5 °C air temperatures, South to East winds and a rain event followed by clear skies can combine during an overall warm, dry summer to create conditions favorable for the initiation of these cyanobacteria blooms (Fig. 2).

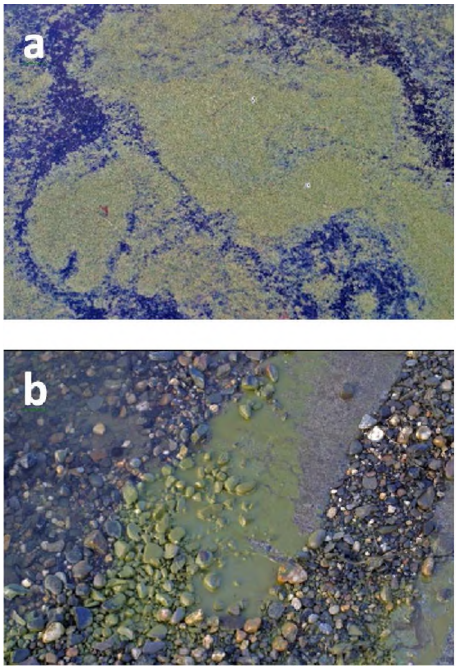

Fig. 1. Photographs of the Aphanizomenon flos-aquae *bloom taken in 2010 A. on the surface of Kotzebue Sound and B. on the shoreline.*

Toxin analyses [2, 3] showed that the blooms were positive for microcystins and/or nodularins in both 2009 and 2010 (Table 1) using the bioactivity-based protein phosphatase inhibition assay. However these two years showed very different toxin profiles when analyzed using a liquid chromatography assay coupled with mass spectrometry. In 2009, only microcystin-LR was detected, though the small sample size (50 mL) would have prevented detection of minor congeners accounting for less than 10% of the total. In contrast, 2010 samples contained no detectable microcystins but consisted solely of the arginine isomer of nodularin (Nod-R, *m/z* MH+=825). Other common cyanobacteria toxins (anatoxin-a, cylindrospermopsin and the PSP toxins) were not observed in these samples and were either not present or below our detection limits (Table 1).

Aphanizomenon species are cosmopolitan and production of the toxins anatoxin-a, cylindrospermospin, and the paralytic shellfish toxins (saxitoxin and its derivatives) has been reported for a number of freshwater members of this genus [4]. Marine species in the order Nostacales produce both microcystins (*Anabaena* species, [5]) and nodularins (*Nodularia spumigena*, [6]), but neither microcystin, as observed in 2009, nor nodularin, as observed in 2010, has been associated with *Aphanizomenon* species in fresh or brackish waters. High values of nodularin production similar to that observed in 2010 have been reported for *Nodularia spumigena* blooms in the Baltic Sea [7].

Cyanobacteria samples collected from the bloom in 2009 and 2010 had their ITS rRNA region extracted, amplified and sequenced [8, 9, 10]. These sequences were compared to others in GenBank. The BLASTn results outlined in Tables 2 and 3 show that the molecular data support the morphological identification of *Aphanizomenon flos-aquae* in the 2009 and 2010 samples. In a 268 base pair region, twenty-two clones spanning the two years had a BLASTn result of 98% and 97% with characterized *A. flos-aquae* strain 83 (Denmark) and strain 326 (Finland), respectively. It is quite possible that generation of more clones would have unveiled other species present. Likewise, using this molecular strategy we uncovered a species (*A. lemmermannii*) belonging to one of the other genera in the 2010 sample identified morphologically (*Anabaena*). There are several *A. lemmermannii* ITS sequences available on GenBank (in addition to the UK sequences present in our BLASTn results) which were derived from characterized cultures [11], but did not reveal them-

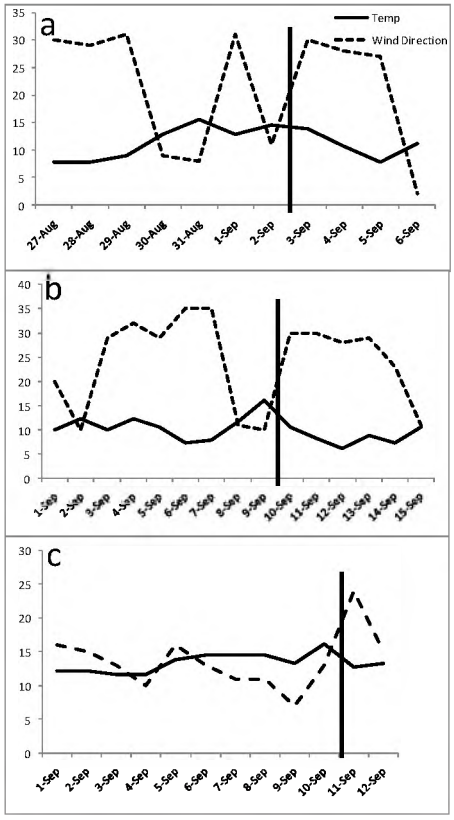

Fig. 2. Air temperature (solid line) and Wind Direction (dotted line) data collected from Wein Memorial Airport over time in 2008 (A), 2009 (B) and 2010 (C). Temperatures are given in degrees Celsius and wind direction is described in tens of degrees clockwise from true north. A value of 36 indicates North, 27 indicates West, 18 indicates South, 9 indicates East and a value of 0 is calm. Note the differences in the x and y axes between the graphs. The vertical solid lines represent the approximate initiation of the bloom.

Table 1. Concentration and detection limit for different cyanobacteria toxins in a sample collected in September 2009 and 2010 from Kotzebue Sound. For comparison purposes, microcystins and nodularins as determined by the protein phosphatase inhibition assay (PPIA) and by liquid chromatography mass spectrometry (LCMS) are expressed in terms of microcystin LR equivalents.

2009

Toxin	Method	Detection limit	Results
Total Microcystins/Nodularins	PPIA	1.2 µg L^{-1}	4.7 µg L^{-1}
Micro./Nod. congeners	LCMS	1.3 µg L^{-1}	3.5 µg L^{-1}
Anatoxin-a	LCMS	1.0 µg L^{-1}	Below detection
Cylindrospermopsin	LCMS	0.6 µg L^{-1}	Below detection
PSP Toxins	ELISA	13.6 µg L^{-1}	Below detection

2010

Toxin	Method	Detection limit	Results
Total Microcystins/Nodularins	PPIA	1.2 µg L^{-1}	790 µg L^{-1}
Micro./Nod. congeners	LCMS	7.0 µg L^{-1}	600 µg L^{-1}*
Anatoxin-a	LCMS	0.2 µg L^{-1}	Below detection
Cylindrospermopsin	LCMS	0.2 µg L^{-1}	Below detection
PSP Toxins	ELISA	13.6 µg L^{-1}	Below detection

*equivalent to 495 µg nodularin-R L^{-1}

Table 2. Top BLASTn results for the consensus sequence from ITS clones from the 2009 (n=12) and 2010 Kotzebue samples (n=10). Six additional clones from the 2009 sample and five from the 2010 sample shared >99% similarity but are not included in the groupings because they contained one polymorphism (one additional from 2010 showed 96 - 98% similarity) with the BLASTn results (data not shown). The query sequence was 362 nucleotides. Only BLASTn results with sequence similarity of 96% or greater are included.

Clone (*n*)	BLASTn result [9]	Sequence designation	location	Ref	GenBank Accession number	Nucleotide overlap	% similarity
2009a (12) JQ433686 2010a (10) JQ433693	Uncultured cyanobacterium	DGGE band #26	Netherlands	14	AY827756	362	99
	Uncultured cyanobacterium	DGGE band #14	Netherlands	14	AY827744	321	99
	Aphanizomenon flos-aquae	DGGE band HV25b	Baltic Sea	15	AF431745	268	99
	Aphanizomenon flos-aquae	DGGE band LJ38	Baltic Sea	15	AF431746	268	99
	Uncultured cyanobacterium	DGGE band #44 and #52	Netherlands	14	AY827782	362	98
	Uncultured cyanobacterium	DGGE band e19	Netherlands	16	AJ619651	322	98
	Uncultured cyanobacterium	DGGE band #9	Netherlands	14	AY827739	322	98
	Uncultured cyanobacterium	DGGE band #19	Netherlands	14	AY827749	319	98
	Aphanizomenon flos-aquae	DGGE band PB98	Baltic Sea	15	AF431747	268	98
	Aphanizomenon flos-aquae var. *klebahnii*	strain 83	Denmark	11	AJ293201	268	98
	Aphanizomenon flos-aquae	DGGE band LJ31	Baltic Sea	15	AF431754	268	97
	Aphanizomenon flos-aquae	strain 326	Finland	11	AJ293200	268	97
	Aphanizomenon flos-aquae	DGGE band BSW9	Baltic Sea	15	AF431755	237	97
	Uncultured cyanobacterium	DGGE band #15	Netherlands	14	AY827745	335	96
	Uncultured cyanobacterium	DGGE band #18	Netherlands	14	AY827748	333	96

Table 3. BLASTn results for six unique ITS clones derived from the 2010 Kotzebue sample. Only BLASTn results with sequence similarity of 96% or greater are included.

Clone (*n*; length)	BLASTn result [9]	Sequence designation	location	Ref	GenBank Accession number	Nucleotide overlap	% similarity
2010a (2; 366) JQ433700	*Anabaena lemmermannii*	BC Ana 0004	United Kingdom[1]	Unpubl.	AY886938	273	99
	Anabaena lemmermannii	BC Ana 0018	United Kingdom[1]	Unpubl.	AY886942	273	98
	Anabaena lemmermannii	BC Ana 0017	United Kingdom[1]	Unpubl.	AY886941	273	98
2010b (1; 362) JQ433701	Uncultured cyanobacterium	DGGE band #9	Netherlands	14	AY827739	322	97
	Uncultured cyanobacterium	DGGE band #19	Netherlands	14	AY827749	319	97
	Anabaena lemmermannii	BC Ana 0004	United Kingdom[1]	Unpubl.	AY886938	257	97
	Uncultured cyanobacterium	DGGE band #15	Netherlands	14	AY827745	335	96
	Anabaena lemmermannii	BC Ana 0018	United Kingdom[1]	Unpubl.	AY886942	273	96
	Anabaena lemmermannii	BC Ana 0017	United Kingdom[1]	Unpubl.	AY886941	273	96
2010c (1; 366) JQ433702	Uncultured cyanobacterium	DGGE band #14	Netherlands	14	AY827744	305	97
	Anabaena lemmermannii	BC Ana 0017	United Kingdom[1]	Unpubl.	AY886941	273	96
2010c (1; 366) JQ433702	Uncultured cyanobacterium	DGGE band #14	Netherlands	14	AY827744	305	97
2010d (1; 367) JQ433703	Uncultured cyanobacterium	DGGE band #14	Netherlands	14	AY827744	306	96
	Uncultured cyanobacterium	DGGE band #19	Netherlands	14	AY827749	306	96
	Uncultured cyanobacterium	DGGE band #9	Netherlands	14	AY827739	306	96
	Anabaena aphanizomenoides	DGGE band K47-m3	Netherlands	14	AY827806	287	96
	Aphanizomenon flos-aquae	DGGE band LJ38	Baltic Sea	15	AF431746	257	96
	Aphanizomenon flos-aquae	DGGE band LJ31	Baltic Sea	15	AF431754	256	96
	Aphanizomenon flos-aquae	DGGE band BSW9	Baltic Sea	15	AF431755	238	96
2010e (1; 362) JQ433704	*Anabaena lemmermannii*	BC Ana 0004	United Kingdom[1]	Unpubl.	AY886938	257	97
	Uncultured cyanobacterium	DGGE band #9	Netherlands	14	AY827739	322	96
	Uncultured cyanobacterium	DGGE band #19	Netherlands	14	AY827749	319	96
	Anabaena lemmermannii	BC Ana 0018	United Kingdom[1]	Unpubl.	AY886942	257	96
	Anabaena lemmermannii	BC Ana 0017	United Kingdom[1]	Unpubl.	AY886941	257	96

[1]Cotswold Water Park

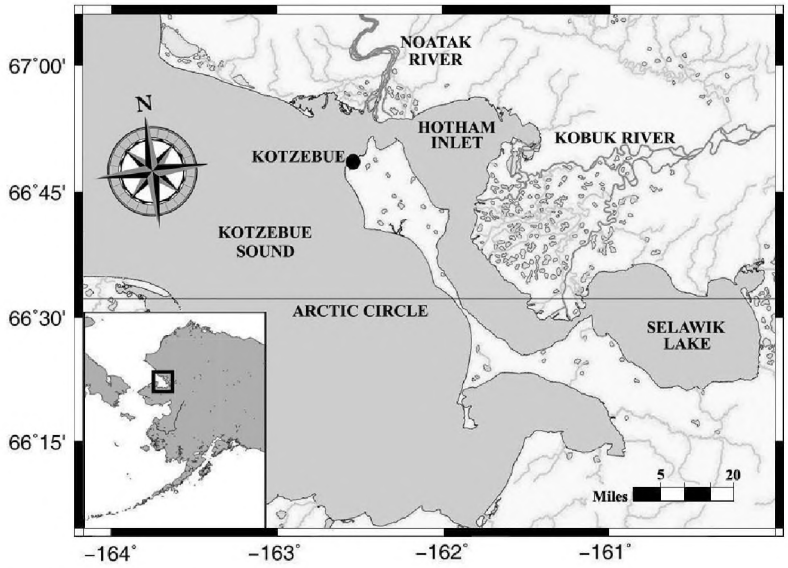

Fig 3 Map of Kotzebue Sound

selves as close matches. Upon further alignments between these sequences, the UK *A. lemmermannii* and our clones 2010a, 2010b and 2010e, it is apparent that these sequences are quite different (data not shown), calling into question the species designation of the sequences available on GenBank. The UK sequence data are unpublished, however the information on GenBank implies that these sequences were derived from isolates. As with many genera and species, more data are needed from characterized cultures/isolates to resolve these gaps in understanding.

Although ITS sequences for *Nodularia* (the third genus identified morphologically in the 2010 sample) are available on GenBank, this genus did not appear in any of our BLASTn analyses. It is quite possible that a more comprehensive clone library (i.e. a greater number of clones) would contain sequences from *Nodularia*. In addition, further analysis of these samples with species/genera-specific PCR would aid in further resolution of the cyanobacterial communities present.

Kotzebue Sound in northwestern Alaska is a partially enclosed embayment adjacent to the Chukchi Sea (Fig. 3). It is fed by three major rivers: the Noatak, Selawik and Kobuk. The Kobuk River is the largest of the three, draining an area of approximately 36,000 square miles and bordered by tundra with some evergreen forest. Salinity in the Sound is highly variable (3-28 during August-September; [12]) and dependent on river input, wind direction during the open water period, ice formation and ice melt during the ice-covered period.

The area is sparsely settled with the town of Kotzebue (3,100 residents) located on the Baldwin Peninsula on the shores of the Sound (Fig. 3). One additional small permanent settlement (Deering; <125 residents) and a few collections of seasonal campsites are also present along the Sound. Many of the residents are native Alaskans who harvest caribou, seal, fish and berries to meet their nutritional needs, with Kotzebue Sound providing 70% by weight of the annual harvest [13]. The Kotzebue Sound watershed has been used in this manner for at least the last few thousand years. Here we combine recent observations and modern molecular and analytical techniques to describe a recurring bloom of toxin-producing cyanobacteria in Kotzebue Sound. The toxicity of this bloom and the reliance of local residents on the Sound for food make this region high priority for harmful algal bloom (HAB) monitoring.

Acknowledgments

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Project MIDTAL (MIcroarrays for the Detection of Toxic ALgae): final workshop report

Detection of harmful algae in phytoplankton samples using light microscopy is difficult because it requires considerable taxonomic expertise and time to screen samples. Moreover, electron microscopy is required for the correct identification of several harmful species. Accurate identification is crucial because mistakes, in the case of false-negatives, can have serious consequences ranging from serious health problems for people and collapsing regional seafood markets as a result of the ensuing panic, and, in the case of false-positives, fishermen are upset if they are forced to stop their activities unnecessarily. New technology is urgently needed to render detection of large numbers of HAB-causing species more accurately, more cheaply and more rapidly.

One promising possibility is the adoption of microarray technology for detection of HAB species using taxon specific probes. DNA-microarrays are aminosilane-coated glass slides onto which a large number of fluorescently labelled DNA-probes can be spotted. Each of these probes can be designed in such a way that it is specific against the ribosomal RNA of a particular HAB-taxon. If that taxon is present in an environmental sample, then the probe will hybridize with the ribosomal RNA of the taxon and the hybridised RNA/probe complex will become fluorescent when excited by UV-blue-light. Probes can be designed in a hierarchical way, i.e. nested sets of probes against nested sets of natural clades. This improves accuracy and greatly reduces the chances of false positive or false negative assignments.

The European 7th Framework Program project MIDTAL (*MIcroarrays for the Detection of Toxic Algae*) developed a microarray for the detection of toxic species and another one for the detection of their toxins. The main objectives of the project were to demonstrate proof of concept of 'microarray' technology for HAB species detection and to develop this concept into a product for commercialization. The MIDTAL team, coordinated by Prof. Linda Medlin (MBA, Plymouth, UK), included nine partners from seven European countries (Table 1) Each partner focused on a different group of HAB-causing organisms (Table 1), designing DNA probes against these taxa, and testing the probes against monoclonal cultures and environmental samples in which the target species were present. Probes were developed against taxon-specific target regions in the nuclear rRNA encoding gene regions (18S and 28S) because these RNAs are abundant in the ribosomes of all cells. Moreover, the availability of a large database that includes sequences from species across planktonic diversity enables the design of highly specific probes.

Table 1. MIDTAL team

Principal Investigators	Institution	HA species targeted
Linda Medlin (Coordinator)	Marine Biological Association of the UK, Plymouth (UK)	*Alexandrium, Pseudo-nitzschia, Dinophysis*
Wiebe Kooistra, Marina Montresor	Stazione Zoologica Anton Dohrn, Naples (Italy)	*Pseudo-nitzschia*
Edna Graneli	Linnaeus University, Kalmar (Sweden)	*Heterosigma, Karenia, Gymnodinium*
Beatriz Reguera	Spanish Institute of Oceanography, Vigo (Spain)	*Alexandrium, Dinophysis*
Robin Raine	Martin Ryan Institute, Galway (Ireland)	*Chrysochromulina, Prymnesium*
Bente Edvardsen	University of Oslo (Norway)	*Pseudochattonella*
Jane Lewis	University of Westminster, London (UK)	*Alexandrium, Prorocentrum*
Katrina Campbell	Queen's University, Belfast (UK)	Toxin microchip
Yolanda Pazos	INTECMAR (Spain)	*Gymnodinium, Alexandrium,, Dinophysis, Pseudo-nitzschia, Gonyaulax*

After three years work, the MIDTAL partners have achieved their objectives. The resulting MIDTAL microarray target species belonging to the major HAB groups, including toxic dinoflagellates (*Alexandrium, Dinophysis, Azadinium, Gymnodinium, Karenia, Karlodinium, Prorocentrum*), raphidophytes (*Heterosigma*), prymnesiophytes (*Prymnesium, Chrysochromulina*), Dichtyocophyceae (*Pseudochattonella*) and the diatom genus *Pseudo-nitzschia*. In addition, Katrina Campbell (Queen's University, Belfast, UK), developed a microchip based on immuno-detection for the direct and multiplexed identification of saxitoxins, domoic acid and okadaic acid in environmental samples.

To demonstrate their achievement, the MIDTAL partners organized a two-day 'hands on' workshop at the Stazione Zoologica Anton Dohrn in Naples (16-17 April, 2012) at which the microarray was presented to the scientific community. Interested stakeholders from the public and private sector from all over Europe (Italy, Spain, France, Ireland, Croatia, Greece) and beyond (Chile and Tunisia) took the opportunity to familiarize themselves with this sophisticated but simple technique. Participants were grouped in small teams and trained by MIDTAL post-docs and researchers to maximise hands-on engagement. The teachers (Lucia Barra, Eva Pérez Blanco, Simon Dittami, Johannes Hagström, Francisco Hernandez, Jessica Kegel, Gary McCoy, Sara McNamee and Joe Taylor) provided the theoretical background and practical details and tips on bench procedures. All participants were keenly interested in applying the microarray technique to environmental studies if the chip were commercially available and suggested that further developments should include the design and incorporation of



Fig 1. Members of the MIDTAL consortium and participants in the MIDTAL Workshop at the Stazione Zoologica Anton Dohrn, Naples, 16-17 May 2012.

probes against toxic species not yet included on the microarray (e.g., palitoxin-producing *Ostreopsis* and ciguatoxin-producing *Gambierdiscus*). The application of the toxin microchip was also presented to the participants during the workshop.

A manual "*MIDTAL: A protocol for a successful Microarray Hybridisation and Analysis*" will be available soon from Koeltz Publishing Co. The microarray produced as a final outcome will be patented and commercialized; negotiations are in progress and the commercial kit will be distributed through the new start-up company MICROBIA in Banyuls sur Mer, France. Further details will be made available through the Midtal website www.midtal.com.

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NEW: "Assessment and management of biotoxin risks in bivalve molluscs"

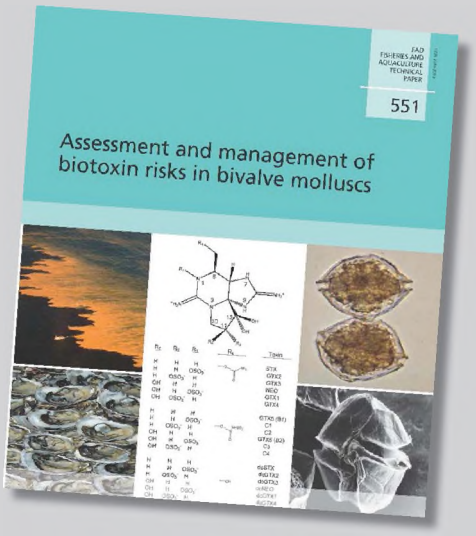
At its 25th session the Codex Committee on Fish and Fishery Products (CCFFP) requested the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to provide scientific advice on biotoxins in conjunction with its work for developing Standards for Live and Processed Bivalve Molluscs.

The CCFFP elaborated further the following specific questions to be covered through this advice:

- Provide scientific advice to the CCFFP to enable the establishment of maximum levels in shellfish for shellfish toxins.
- Provide guidance on methods of analysis for each toxin group.
- Provide guidance on monitoring of biotoxin-forming phytoplankton and bivalve molluscs (including sampling methodology).
- Provide information on geographical distribution of biotoxin-forming marine phytoplankton.

FAO, WHO and the Intergovernmental Oceanographic Commission of UNESCO (IOC) agreed to organize an Expert Consultation to address this request. The group met several times and after some delay the report is finally published:

The following publication/document has been included in the FAO Corporate Document Repository:
http://www.fao.org/docrep/015/i2356e/i2356e.pdf
http://www.fao.org/docrep/015/i2356e/i2356e.zip



ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors (WGBOSV)

The WGBOSW met from 12th to 14th March, 2012 at the European Maritime Safety Agency (EMSA) in Lisbon, Portugal with Brian Elliott (EMSA) as host and Tracy McCollin (United King-dom) as chair. In total there were 39 participants representing 19 countries and EMSA over the course of this meeting and the joint meeting on the morning of the 14th March with the Working Group on Introductions and Transfers of Marine Organisms (WGITMO).

The meeting covered a range of objectives, the group were updated on the status of shipping vector research, discussed methods and procedures used at ballast water treatment test facilities, risk assessment and pathway management methodologies.

Two of the Terms of Reference were completed during a sub-group meeting of WGBOSV in London from 26-27th January, 2012. The sub group met directly before a meeting of the Bulk Liquids and Gases (BLG) subcommittee meeting at the IMO to discuss proposed guidelines on ballast water sampling and analysis. The outcome of the group's discussions was then provided to the BLG meeting the following week by the chair (Tracy McCollin) representing ICES. The approach taken at the meeting was for each country to provide an update on the status of shipping vector research and other interesting information in the form of a National Report. The main outcome of the meeting was a better understanding of the work being carried out in different countries and where there may be opportunities for collaboration, recommendations regarding what information may be needed to carry out risk assessments and how this relates to the current IMO (G7) guidelines and exemptions and a wide ranging discussion regarding the potential improvements that could be made to the IMO guidelines for approval of ballast water management systems (G8).

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Revised Harmful Algal Event Database HAEDAT launched

A longstanding desire to modernise the joint IOC-ICES-PICES database HAEDAT and to improve database functionality has been made possible through a grant provided by the Government of Flanders (Belgium) to the IOC. The obtained results include a shift to using Google Maps which are displayed at the top of every search (fig.1). The maps are automatically zoomed and centred depending on what they have to display, and they can be moved around by clicking and dragging on them. Clicking on one of the markers refines the search to the relevant grid. Maps are also displayed when showing any event with location data (if exact latitude/longitude is available shows this, otherwise shows where the relevant grids are). If a grid code has bounds specified, those bounds are displayed whenever the grid is displayed. All grids associated with a country are displayed by doing a search for that country (fig.2), Users can download the entire database from the 'Browse' page or their 'Options' page. Problems opening the file properly are caused by a bug in Excel, which can be fixed by upgrading to the latest version.

An interface has been added for checking event data. The administrator can check all records, and national editors can check records from their country.

The interface shows 20 records at a time, and only displays the fields that need checking. One can click on the event name to see all the information associated with an event.

An interface has also been added for checking contact data and allows the administrator to check all records, and national editors can check records from their country.

Other Changes includes that dates are checked for consistency when adding/editing an event. An advanced search by grid has been enabled and browsing by region and year has been enabled, and the 'Browse' page now includes the number of events associated with each link. Passwords in the database have been encrypted to make them more secure, and the codebase has been migrated from PHP4 to PHP5.

The future success and usefulness of HAEDAT now entirely depends on that National HAEDAT focal points enter data on a yearly basis and that those countries that are behind in data upload complete the reporting. The IOC data management team and the IODE Project Office in Oostende hope the improved HAEDAT will be well received by data providers and users.

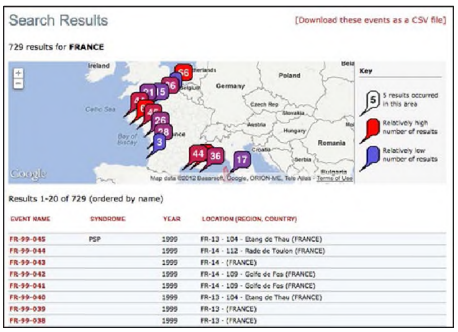

Fig. 1



Fig. 2

Future events

Marine and Freshwater Toxins, Third Joint Symposium and AOAC Task Force Meeting and LC-MS/MS lab training on lipophilic toxins

18-22 & 25-26 June 2012, Tacoma, WA, USA.

Topics for the Symposium are the lipophilic toxins, the DSP outbreak in USA and Canada, saxitoxins official methods, domoic acid, emerging toxins, ciguatoxins, cyanotoxins and test kits.

Please reserve the following dates for above meeting June 18-22, then June 25-26, 2012 for LC-MS/MS lab training for diarrheic and other lipophilic toxins.

As many of you know, the US and Canada experienced their first outbreaks of diarrheic shellfish poisoning (DSP) this year right here in the Pacific Northwest. We will have training for detecting these toxins and a full day devoted to them at the above meeting.

Please contact James Hungerford (James.Hungerford@fda.hhs.gov) if you have interest in these sessions.

IOC Qualifications in Identification and Enumeration of Harmful Microalgae

15-25 August 2012, Copenhagen, Denmark

The course includes 90 hours of teaching and is divided into two parts, each consisting of 45 hours of teaching. The first part of the course is an internet teaching programme, while the second part is a practical workshop in species identification. More information: Jacob Larsen, jacobl@bio.ku.dk.

15th ICHA 2012 Korea
15차 국제 적조 회의
XV International Conference on Harmful Algae

15th International Conference on Harmful Algae

29 Oct-2 Nov 2012, Changwon Gyeongnam, Korea

HAB2012 aims to address all issues related to the causes and effects of marine and freshwater harmful micro- and macro-algae and to serve as a forum for exchanging research outcomes and relevant ideas between researchers, industries, governments and interested parties. More information: www.hab2012.kr

9th International Conference on Molluscan Shellfish Safety

17-22 March 2013, Sidney, Australia

The Call for Abstracts now open. Abstracts should focus around the following themes:Harmful Algal Blooms and Marine Biotoxins, Microbiological and Chemical Risks, Emerging risks from 'Non Bivalve' Molluscs, Risk Assessment and Management, Hazard Inactivation and Elimination Strategies, Production Area Remediation, and Cyanotoxins. ABSTRACT SUBMISSION DEADLINE – THURSDAY 14 JUNE 2012. To submit your abstract please visit www.ICMSS2013.com

ICES-IOC-IMO Working Group on Ballast and Other Ship Vectors (WGBOSV)

18-20 March, 2013, Montreal, Canada

Contact: tracy.mccollin@scotland.gsi.gov.uk

ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

9-12 April 2013 in Vigo, Spain

Contact: Bengt.Karlson@smhi.se

IPHAB-XI: Eleventh Session of the IOC Intergovernmental Panel on Harmful Algal Blooms

22-24 April 2013, Paris, France

Contact: h.enevoldsen@unesco.org

GEOHAB Open Science Conference

25-27 April 2013, Paris, France

Contact: Kudela@ucsc.edu

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Workshop on "Advances and challenges for understanding physical-biological interactions in HABs in Stratified Environments

This workshop will be held at the Monterey Bay Aquarium Research Institute (MBARI, Moss Landing, California, US) on 21-23 August 2012. The activity is part of the Core Research Project (CRP) on Stratified Systems (GEOHAB, 2008). It is meant to be a platform to develop future international research and, as such, it fits GEOHAB's mandate.

The CRP on Stratified Systems concentrates on the fine-scale distribution of microalgae in stratified environments and their relationships to forcing at larger scales. In stratified environments, specific phytoplankton communities containing HABs have been observed to occur in thin layers. In the last 20 years, investigations have been undertaken to further our understanding of the biological and physical mechanisms involved in the formation, maintenance, and dissipation of thin layers. Research has been focused on the relevance of physical microstructure for fundamental life processes of microorganisms (nutrient and light availability, reproduction, life cycle, ecological interactions) and the relevance of thin layers to HABs. Advances in this area have strongly depended on the development of innovative instruments to observe and adequately sample these environments, as well as advanced numerical models.

The goal of the workshop is to review our current understanding of the processes governing the structure and dynamics of HABs in stratified systems. We aim to identify gaps in our knowledge in order to orient future research for their improved modeling and prediction. The workshop is intended for engineers, physicists, biologists, and modellers, who work - or have worked - on the various aspects of phytoplankton dynamics in stratified systems and can thus contribute to provide a multidisciplinary understanding of this phenomenon. We hope to emerge from this meeting with a conceptual model or 'roadmap' of where biological, physical, and chemical measurements of Harmful Algal Blooms in stratified systems should be headed during the next 10 years, as well as a manuscript synthesizing the findings from this meeting. We are hopeful that this setting will also lead to collaborative proposals to conduct multidisciplinary field experiments addressing this subject. These proposals would most likely involve international collaborations. Given that the workshop will be held in August 2012, the outcomes of the meeting will be ready for the final meeting of GEOHAB (April 2013).

The workshop has been structured in six sessions:

Session 1:
Physical measurements at small scale: instruments, measured parameters, obtained data, low-end versus high-end physical/biological measurements. Plenary speaker: Jules Jaffe.

Session 2:
Biological-physical interactions across scales: the formation, maintenance and decay of thin layers. Plenary speaker: Tim J. Cowles.

Session 3:
Living in a thin layer: calculations of biological rates. Plenary speaker: James M. Sullivan.

Session 4:
Integrating fine-scale measurements into a regional modeling approach. Plenary speaker: Hans Burchard.

Session 5:
Discussion about present gaps, future strategy. Leader: Francisco Chávez

Session 6:
Closed meeting of the organizing committee to prepare the report.

In addition, participants are invited to give an oral presentation or poster. Through presentations and group discussion, we will identify the critical remaining questions, and identify new technologies that may be needed to adequately sample HABs in stratified systems in general and in thin layers in particular.

The Registration and Abstract forms as well as other useful information is found at the GEOHAB webpage (www.geohab.info and http://geohab.aqualight.info/). The registration deadline is June 15th, 2012.

Looking forward to meet you at the MBARI

The Scientific Committee

- Margaret McManus, University of Hawaii at Manoa (GEOHAB)
- Francisco Chávez, MBARI
- Hidekatsu Yamazaki, Tokyo University of Marine Science and Technology
- John Ryan, MBARI
- Oliver Ross, Unitat de Tecnologia Marina, CSIC, Barcelona (GEOHAB)
- Elisa Berdalet, Institut de Ciències del Mar, CSIC, Barcelona (GEOHAB-SSC vice-chair)

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Harmful Algae News

AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

In the Land of Bibimbap, Kimchi, and Impeccable Hospitality

Records of red tides and HABs are scattered through Korean archives for nearly 2000 years. **Changkyu Lee** [1] lists 38 between 161 AD and 1820 AD with fish mortalities and human victims. Accounts of what was probably PSP in Jinhae Bay are recorded for the years 1770 and 1820, in the same period as descriptions elsewhere (Vancouver, western Canada in 1793; Combe, eastern Scotland in 1827).

In more modern times, there have been broad shifts in the dominant HAB species in Korean waters. *Karenia mikimotoi* and *Chattonella* spp were more important in the 1970s and 1980s, there was a *Cochlodinium polykrikoides* 'period' from 1993 to 2008, now, since 2000, in decline (**Hak-Gyoon Kim**). In Japanese waters, *K. mikimotoi* and *Heterosigma akashiwo* typically formed red tides in the 1970s, *Chattonella* species in the 1980s, and *C. polykrikoides* in the 1990s. PSP records increased in Japan from 1978 to 1999. There are comparable trends elsewhere, e.g., a period of range expansion of *Pyrodinium bahamense* in the Philippines from 1983 to 1998 (**Rhodora Azanza**). *Gymnodinium catenatum* progressed seasonally from south to north along the Portuguese coast from 1985 to 1995, and again since 2005, but no blooms were recorded between 1996 and 2004 (**Ana Amorim**). Multiannual signals appear in *Heterosigma akashiwo* and *Prorocentrum triestinum* in Narragansett Bay (**Ted Smayda**).

Some of these patterns may be related to climate signals like the Pacific Decadal Oscillation or ENSO, or, as **Patricia Glibert** argues, to the growth of nutrient loading. Both eutrophication and ENSO were invoked to account for changes in a 50 year record of cyst numbers from Changjiang Estuary sediments (**Dou-ding Lu**). But blooms of *Alexandrium* in Japan and *Pyrodinium* in the Philippines do not appear to be related to nutrient loading, and may increase in importance when the latter is reduced (**Yasuwo Fukuyo**). In Japan, *Karenia* blooms have been recorded at least since the 1930s, and *Chattonella* cysts are common in pre-eutrophic sediments. *Alexandrium catenella* appeared in Marlborough Sound, New Zealand in March 2011 for the first time in 18 years of monitoring, and again in 2012 (**Lincoln MacKenzie**). This is a long interval



Photo of Patricio Díaz

to survive without a bloom, but results from Cork Harbour in Ireland suggest that blooms of *Alexandrium minutum* at 7-8 y intervals provide enough cysts to maintain local populations (**Sarah Cosgrove**).

Participants in the Crete conference will recall that the potential impact of anthropogenic acidification on phytoplankton was a theme there. We now learn that natural acidification (pH 6.8 to 8.1) due to fumaroles at a site in the Gulf of Naples has no apparent impact on the local *Ostreopsis* populations (**Davide di Cioccio**).

Older food chain models consisted of diatoms, copepods, and fish; real ones are a great deal more complicated. What was once food (building materials, calories) is now much more, and a consumer must decide what part of its food to digest, and what part to keep for its information value. Serial endosymbiosis is not just something that happened billions of years ago, it is going on all the time (**Veronique Séchet**). The organisms are small, but have 'big bites' [2]. Plastids and genes are passed around like playing cards (**Miran Kim**), trophic chains are really trophic and information networks, the boundaries between autotrophy and mixotrophy dissolve (**Hae Jin Jeong, Per Juel Hansen**), genomes no longer belong to species, and species have multiple genomes. Cryptophytes, for example, have four, the host nuclear genome, mitochondrial and plastid genomes, and a residual nuclear genome (nucleomorph) from the endosymbiont. Bottom-up and top-down regulatory mechanisms blur as more and more 'lateral' processes are identified, and convergence complements divergence.

Graph theory, pioneered by Paul Erdös, is used to explore the architecture and dynamical properties of food webs and other networks. His co-authors are assigned an Erdös number of 1, those who co-authored papers with Erdös' co-authors have an Erdös number of 2, and so on. We might then give *Mesodinium* (and *Karlodinium*) a Teleaulax number (T) of 1 since they acquire it directly, *Dinophysis* a T of 2 since it acquires it from *Mesodinium*; for predators of *Dinophysis* (tintinnids, *Noctiluca*, mussels), T = 3, and for patients with DSP, T = 4. *Dinophysis* derives information from *Teleaulax* via an intermediary which provides sustenance, a more complex linkage pattern than any so far tackled by graph theorists; it may be that such dynamics are beyond the present reach of this approach.

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Information on the distribution, abundance, and physiology of dinoflagellate cysts continues to accumulate. *Alexandrium tamarense* cysts are abundant in the Chukchi and E Bering Seas (**Masafumi Natsuike**), there are significant interannual variations in *Alexandrium catenella* cyst numbers in Puget Sound (**Cheryl Greengrove**) – and, it is claimed, no evidence of an endogenous clock – and *Gymnodinium catenatum* cyst abundance is low in Portuguese shelf sediments (**Teresa Silva**). Other reports dealt with cysts in Manila Bay (**Elsa Furio**), cyst numbers in Alexandria Harbour (**Amany Ismael**), and the effect of cyst age on germination rates of *Alexandrium catenella* and *Scrippsiella* (**Kirsten Feifel**). "Pelagic seed banks" in the Taiwan Warm Current (East China Sea) are proposed to account for blooms of *Prorocentrum donghaiense* (**Xinfeng Dai**). A qPCR count of *Heterosigma akashiwo* cysts compares well with direct counts (**Joo-Hwan Kim**). Dormant stages of diatoms also received attention (**Mineo Yamaguchi**). *Skeletonema marinoi* resting stages are viable for more than 60 years (**Karolina Härnström**).

The rôles of dormant stages in population dynamics continue to challenge us. In Crete, we heard of temporal discrepancies between peak laboratory excystment rates and the appearance of blooms. The mystery continues: field studies in Korean waters now seem to confirm that maximum germination rates of *Alexandrium tamarense*, *Scrippsiella trochoidea*, and *Anabaena flos-aquae* do not coincide with bloom development there (**Myung-Soo Han**). But in Sorsogon Bay, Philippines, model and field studies of *Pyrodinium bahamense* suggest excystment is closely linked to bloom formation (**Aletta Yñiguez**); this may also be so for *Heterosigma akashiwo* (**Elizabeth Tobin**).

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Contributions to this conference series in the past are usually grouped into a few broad categories – organisms, environmental conditions, bloom dynamics, chemistry and pharmacology of toxins, management. The range of organ-



Photo of Patricio Díaz



Photo from www.hab2012.kr

isms has expanded continuously, and the categories have broadened. In his summing up, **Allan Cembella** referred rather negatively to the branching out or dilution of HAB conference topics. This was already a concern at the Rhode Island conference in 1991. Ted Smayda worried then [3] that the community might "splinter into subgroups"; but the splintering is not apparent yet. We certainly belong to various interest groups, but we still attend ICHA conferences and talk to each other.

The HAB community has managed to cope with the addition of all kinds of new species and novel toxins, with brown tides and green tides, with outreach and high tech gadgets and climate concerns, even with the omics revolution (metabolomics, transcriptomics, ecogenomics, see Francisco Rodríguez in this issue). But the focus, to understand and manage HABs, remains intact. It might be that this conference series is one of the few that pioneered cross-disciplinary research, long before it was fashionable. **Øyvind Moestrup** noted that algal toxins have been killing domestic animals for 150 y (in fact much longer), *and we still do not know what they are for.* **Sandra Azevedo**'s review of what cyanobacterial toxins might be for reinforces Moestrup's point.

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The ecology of *Cochlodinium polykrikoides* in Korean waters was reviewed by **Chankyu Lee**. It was reported from Mijo Harbour (**Bum Soo Park**) and Jaran Bay, Korea (**Chang-Hoon Kim**), on the Wenling coast, China (**Douding Lu**), in the Straits of Hormuz (**M.S. Mortazavi**) and Kuwait (**Wafa'a Al Rashed**), in El Salvador and Guatemala waters and in Banderas Bay, Mexico (**Sergio Licea**). Elsewhere in this issue, blooms of this species are described in the Colombian Caribbean, by Andrés Malagón and Laura Perdomo and in El Salvador waters (Jaime Espinoza and others). Temporary resting cysts and planozygotes of this species have been produced in culture (**Ying Zhong Tang**).

Alexandrium records include *tamarense* and *catenella* from southern Korean waters (**Ji- A Park**), *catenella* from Changjiang Estuary, China (**Yunfeng Wang**), and *tamarense, catenella* and *ostenfeldii* in the Beagle Channel, Argentina (**Marcelo Hernando**). An un-named species of *Alexandrium* has bloomed in recent years in the Philippines (**Garry Benico**). Bacteria on *Zostera* may inhibit growth of *Alexandrium tamarense* (**Yuka Onishi**).

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Printing, a prerequisite to widespread literacy, was pioneered in Korea by means of movable metal type in the 13th century, 200 years before its appearance in Germany. More recent Korean enterprise gave the world the first MP3-phone and the first touch screen mobile phone, harbingers of an emergent multimedia neo-literate (post-literate?) era in which images and icons complement or even compete with words, with an accompanying threat of illiteracy. Now humans and some of our machines live in a kind of symbiosis and, we may ask, will science be able to prosper in a world without texts? Shall we tweet our (crowd-sourced?) science to each other with only 140 electronic characters? It would at least ease the information overflow now swamping peer review and editorial filters! Are electronic proceedings a way station en route to intellectual dystopia? And will everything disappear anyway because libraries no longer have funds to archive?

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T. Wyatt

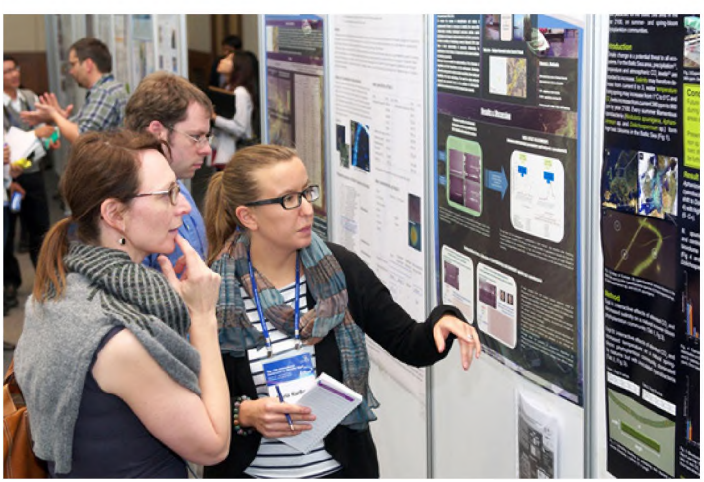


Photo from www.hab2012.kr



Top and bottom, photos from Patricio Diaz. Middle from www.hab2012.kr


15th ICHA 2012 Korea
The 15th International Conference on Harmful Algae




ISSHA
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Top and bottom, photos from Patricio Díaz. Middle from www.hab2012.kr

Some highlights from Gyeongnam

The XVth International Conference on Harmful Algae, ICHA, was held from Oct 29 to Nov 2, 2012 in Gyeongnam (South Korea). It was attended by participants from more than 40 different countries. It was obvious throughout the conference that almost every single detail had been carefully planned in advance by the local organizing committee, chaired by Dr. Hak-Gyoon Kim, and they had taken their responsibilities toward participants very seriously at every moment. Their commitment to timetables and general information about transport and accommodation was excellent. All the "*lost in translation*" problems that might have arisen during our stay in Gyeongnam were averted thanks to the guidelines on the ICHA website. The facilities of the modern Convention Center (CECO) were ideal.

There were 133 oral and 244 poster presentations; only a small selection of them can be mentioned in these few paragraphs. The theme sessions covered all relevant issues concerning the physiology, ecology, life cycles, and modeling of HAB species. These sessions also covered the key issues centred on the relevance of phycotoxins, as illustrated by the fact that 5 out of 31 oral sessions (19 talks) were dedicated to toxin chemistry and toxicity.

In his conference summary, (**Allan Cembella**) noted that toxin research now seems to be a "mature field". He meant that recently no major groups of phycotoxins or human toxin syndromes have been reported. 'Hyphenated methods' (like LC-MS) are now optimized, and toxin specificity and modes of action are well understood. But the toxins of fish killing algae like *Cochlodinium*, raphidophytes and prymnesiophytes, as well as the cyanobacterial toxins, are important exceptions to this view.

New methodological developments and the incorporation of toxin chemistry in multidisciplinary studies (biodiversity, biodistribution, fate of toxins in the food web, grazing interactions, etc) were prominent issues at this meeting. One of these is the 'metabolomics' approach (**Philip Hess**), which couples gradient chromatography and high resolution mass spectrometry to detect metabolites in the cell at a given time and under given conditions. For each species, this procedure yields a large 3D data set, and offers a massive chemotaxonomic tool, the 'metabolome'. Before comparative metabolomics can proceed, the data set is analysed with the Molecular Feature Extraction (MFE) to characterize the metabolome of a given species. This approach identifies new bioactive molecules in microalgae and improves our understanding of the biosynthesis and biogeography of marine compounds.

The search for novel bioactive compounds is also pursued using phenotypic based chemotaxonomy followed by a dereplication step combining solid-phase extraction and ultra high performance liquid chromatography, UHPLC (**Thomas Larsen**). When likely unknown molecules are observed after an initial comparison against a comprehensive compound database, these can be then purified for characterization by nuclear magnetic resonance (NMR).

Molecular tools have been integrated in multidisciplinary HAB studies, not only as complementary taxonomic aids, but also in transcriptomics and ecogenomics. For example, the available saxitoxin (STX) genes are now applied in studies of toxin physiology, metabolic intermediates and regulation of toxins production in paralytic shellfish poisoning (PSP) producers like *Alexandrium catenella* (**Maria Wiese, Shauna Murray**)

Other genomic and transcriptomic studies are related to the use of molecular probes and markers in Amplified Fragment Length Polymorphism (AFLP), qPCR, microarrays and *in situ* hybridisation techniques; biogeographical studies at the population level (**Karin Rengefors, Anke Kremp**) and the assessment of intraspecific diversity associated with toxin production in *Alexandrium tamarense* (**Chen Jiang**) provide examples.

In parallel with the metabolomic approach, metagenomic analyses look likely to play an important role in the future, e.g.in plankton monitoring using Next Generation Sequencing (NGS) methods like Roche 454 (**Satoshi Nagai**).

Although no new toxins or syndromes were announced here, some organisms attracted special attention, like the macroalgal "green tides" in China, and the dinoflagellate *Cochlodinium polykrikoides,* due to the economical damage caused by their blooms; the impact of the latter has been minimized by clay mitigation in South Korean waters, to protect shellfish and aquaculture farms.

Since the isolation of the azaspiracid producing *Azadinium spinosum* in 2008 in the North Sea (east coast of Scotland), its global distribution, toxicity and phylogenetic position have been intensively studied. Some of these advances were summarized by **Urban Tillman** and **Bernd Krock**, and included the recent discoveries of *A. poporum* and *Amphidoma languida*, as well as new azaspiracid analogues in *A. spinosum*.

There is growing interest in the role of parasites in HAB population dynamics, as reflected in the increasing number of studies of their specificity, infectivity and genomics (**Marc Arancio; Lourdes Velo-Suárez; Yameng Lu**). The strangest report at this conference was the mixotrophic behavior of *Alexandrium pseudogonyaulax* and its "toxic mucus trap" (**Hannah Blossom**).

Myung Gil Park and co-workers have made major contributions in the last decade to our knowledge of the dinoflagellate *Dinophysis*, so not surprisingly this organism received special attention at the ICHA meeting. In the theme session on population dynamics of HABs, it was not only *Dinophysis* but necessarily also its ciliate prey *Mesodinium* who shared the "lights on the stage".

Talks on this topic included an update on *Mesodinium* taxonomy, physiology and cellular organization by **Per Juel Hansen**, a presentation by **Miran Kim** with the suggestive title "Is the ciliate *Mesodinium rubrum* a sole prey of the marine dinoflagellates *Dinophysis* spp?"– he provided evidence of *Mesodinium* eating *Chroomonas*, a "green" cryptophyte, whose plastids were not kept as kleptoplastids in *Dinophysis* but only included in digestive vacuoles – and an account by **Meng Meng Tong** of the growth and toxins in *Dinophysis acuminata* under different nutrient regimes. The plot showing that *D. acuminata* does not apparently take up inorganic nitrogen (once *Mesodinium* was removed from the medium, consumption stopped), is one of the most intriguing results from this conference.

Benthic harmful algae, mostly *Ostreopsis* and *Gambierdiscus*, pose a major threat to human and environmental health in warm areas, and have apparently spread to temperate regions too, as shown by **Adriana Zingone**. These genera and others like *Coolia* and *Prorocentrum* were the focus of a theme session and several posters, but benthic microalgae attracted less attention than at ICHA XIV in 2010. Among the presentations covering this issue, molecular studies included qPCR for detection of *Gambierdiscus* (**Tomohiro Nishimura**), and physiological and phylogenetical research on *Ostreopsis*, *Gambierdiscus* and *Coolia* in Korean and Japanese waters (**Chui Pin Leaw; Akira Ishikawa**). A new domoic acid producer, the diatom *Nitzschia bizertensis*, was reported in a Tunisian coastal lagoon (**Donia Bouchouicha**).

Other highlights include a digital holographic cinematographic study grazing behavior of copepods exposed to toxic microalgae (**Pat Tester**), the systematics of the pinnatoxins-producing *Vulcanodinium rugosum* species complex (**Kirsty Smith**), the first occurrence of DSP toxins above the FDA level on the East coast of the US (**Theresa K. Hattenrath-Lehmann**), general issues of global significance like ocean climate change and its influence on HABs, fisheries and public health.

This is only a sketch of a diverse and fruitful conference. HAB research is an active and dynamic field of science which blends basic and applied research as few others, from systematic studies of algae, modeling of their natural populations, behavior and physiology in the laboratory, to the continuous development of equipment and analytical methods for toxin detection, HAB forcasting, and *in situ* identification of microalgae. All this supports the evolution of international monitoring programmes, sanitary regulations, and a better understanding and protection of our environment.

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Blooms of *Botryococcus braunii* in Nozha Reservoir, Alexandria (Egypt)

Nozha Reservoir (31.19°N, 29.37°E) south of Alexandria city is an enclosed, nearly circular freshwater body with a surface area of about 5.5 km^2 and an average water depth of 2.1 m. It receives its water supply from the Nile via Mahmoudia Canal. The reservoir suffers from progressive pollution indicated by the ongoing increase in cadmium concentrations in its sediments [1]. It produces 200-250 Tons y^{-1} of popular fishes (Mullet, Tilapia, Bayad, Carp, and Catfish).

Irregular sampling occurred from January to June 2012 during water discoloration periods, and an intensive monitoring of the ambient physical, chemical conditions, abundance, distribution, and the amount of lipids of *Botryococcus braunii* was done at 5 sampling sites in the first month. Blooms of this unicellular photosynthetic cholorophycean that forms colonies resemble large floating mats on the water surface. According to [2], *B. braunii* converts 3% of the solar energy to lipids which can reach 70% of the algal biomass; when *B. braunii* oil is cracked, it yields a distillate containing 67% gasoline, 15% aviation fuel, 15% diesel fuel and 3% residual oil [3]. The lipid production ability of this alga has been investigated at different growth phases under different culture conditions [4].

Water temperature varied from 13°C in January to 28°C in June, salinity 1.4-2.5, pH 7.8-8.9 and DO 7.2-9 mg L^{-1}, affecting fish survival. The effect of thermal stratification was ignored due to the shallowness of the pond and the effect of prevailing winds.

A controlled fertilizer programme is applied to Nozha Reservoir to increase fish production. This also seems to favour the occurrence of recurrent harmful algal blooms, in 1986, 1994, and 2003, mainly of Cyanobacteria (*Anabeana, Microcystis*). However, there was a dramatic change in species composition in 2012 with predominance of *B. braunii*. It formed intermittent dense blooms between January and June. The green-yellow water turned to brown, and covered the entire lake during calm periods. This species was the only constituent of the phytoplankton community in January (maximum 55.3x103 colonies L^{-1}). The high alkalinity changes (300-420 mg L^{-1}) might explain these mono-specific blooms by damaging the phytoplankton diversity. These blooms caused massive fish mortality (losses about 3000 US $ d^{-1}); it is the first reported harmful bloom of *B. braunii* in Nozha Reservoir. The bloom peak in January occurred at 14°C, 2.1 salinity and pH between 8.7 and 9.5, raising dissolved oxygen content to 10.6-11.6 mg L^{-1}. Nitrate fluctuations (0.03-0.9 mg L^{-1}) seems unlimiting while, phosphate (0.04-0.1 mg L^{-1}) and silicate (0.1-4 mg L^{-1}) were of more importance. Up to 30-50% of the dry weight of *B. braunii* were long chain lipids.

Unless major changes in the physicochemical properties (especially pH) of the water take place, red tides pose a serious threat to the Nozha Reservoir ecosystem. The knowledge of how *B. braunii* can gain and maintain dominance is useful to those who intend to grow ponds of it as a hydrocarbon source in Egypt.

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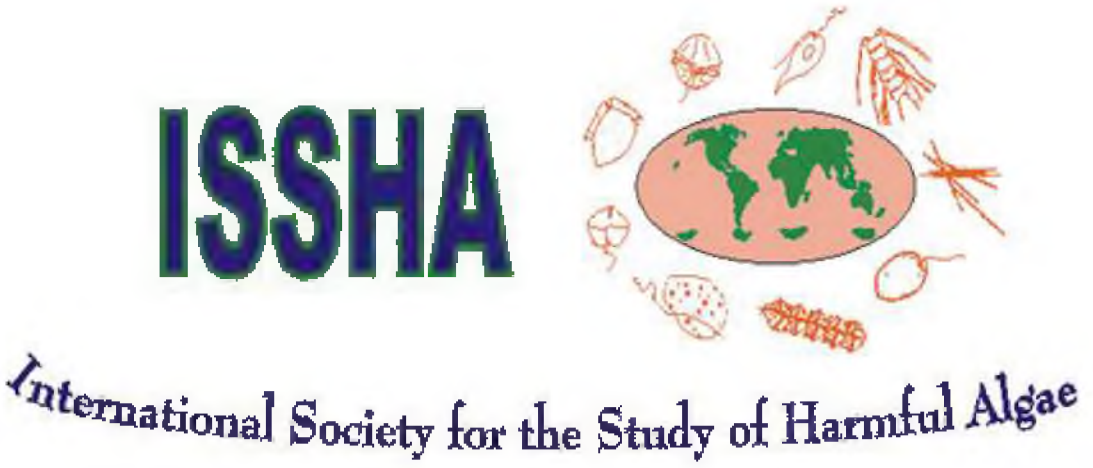


ISSHA President's Corner

The International Society for the Study of Harmful Algae (ISSHA) convened the 15th International Conference on Harmful Algae (15 ICHA), held in Changwon, South Korea (29 October-2 November 2012). A beautiful hand-embroidered ISSHA flag, a gift from our Korean hosts, waved in the wind by outside the Gyeongnam Conference Centre (CECO). During the closing ceremony, a very symbolic act took place when the ISSHA president took this flag from Dr Hak Gyon Kim (Chair of the Korean Organizing Committe) and passed it to Dr Lincoln MacKenzie, chair of the next 16th ICHA local organizing committee.

The ISSHA Council meeting was held on 28 October before the conference. The 8th General Assembly of the Society was celebrated on 1st November. Beatriz Reguera (President) opened the meeting. The minutes from the previous assembly were approved and ISSHA committee chairs summarized activities and achievements in the previous two years. Ian Jenkinson (Statutes and Bylaws) presented proposed changes to update functioning of the Society to modern times: electronic voting instead of ballots, election years coinciding with those of ICHA conferences, etc. All changes were accepted by the Assembly. Nina Lundholm (Treasurer) presented the financial statement, covering the period October 2010 to October 2012, and detailed expenditures and funds raised by member's fees, book sales, and the auction graciously conducted by Barrie Dale at the 14 ICHA (Crete). Clarisse Odebrecht (by correspondence) prepared the Travel Awards Committee report, thanking sponsors of student travel awards (FAO, SCOR, NOAA) and in particular the generosity of the Gyeongnam Provincial Government who provided free accommodation for young scientists at the dormitory of the Gyeongnam Officials Training Institute (GOTI) and daily transport to the Conference Centre. Altogether, 43 young scientists (predoctoral, master and post docs) from 20 countries presenting oral and poster communications at the Conference were given grants.

Vicepresident Gustaaf Hallegraeff (Chair of Publication and Dissemination Committee) announced that for the first time, a free on-line publication of the proceedings from the 14th ICHA (Crete, Greece, October 2010) is available through the ISSHA website. A lively debate followed on the convenience of making on-line proceedings freely available just to the ISSHA members during the first 1-2 years. But this would represent discrimination towards non-ISSHA members attending the conferences. After post-assembly discussions among the Council members, a decision was made that future proceedings will be available to ISSHA members and to all participants registered at that ICHA conference who will be given a complimentary member-like use of the website for 2 years.

Vicepresident Jennifer Martin (Achievement Awards Committee, co-chaired with Rita Horner) reviewed procedures followed by the Committee to examine nominations, by ISSHA members, for the *Yasumoto Lifetime Achievement Award* and the *ISSHA Young Scientist Award*. The same committee had the task of securing double evaluations for the 45 students who competed for the *Maureen Keller Award* for best oral and poster communications.

2012 was election year in ISSHA. Some members had to step down because their period of service for the same office was due, and some others had to do so due to changes in their professional situation. Results of the elections were presented by Karin Rengefors (Secretary and Chair of Elections Committee) and are listed at end of this report.

Lincoln MacKenzie (Cawthron Institute), on behalf of the local organizing committee, presented the venue for the 16th ICHA to be held in Wellington, New Zealand (http://www.icha2014nz.com/) from 27 to 31 October 2014. All ISSHA members attending the Assembly welcomed with enthusiasm the bid by Brazil (Federal Institute of Santa Catarina), presented by Dr Luis A. Proença and supported by well known HAB experts from the South America, to host the 17th ICHA in 2016 in Florianápolis (Santa Catarina State, Brazil).

The ISSHA Auction, now a tradition, followed the ISSHA General Assembly. Due to unexpected throat irritation, Barrie had to decline to act as auctioneer. But the auction took place with great success thanks to Sandy Shumway volunteering, who demonstrated her great skill acquired from years of practice at the National Shellfisheries Association auctions.

All achievement awards were announced during the luxurious farewell dinner buffet that followed the traditional folk dances and music organized at the CECO Convention Hall.

ISSHA wishes to express its gratitude for the hospitality and generosity of the Gyeongnam Provincial Government, for the clockwork efficiency exhibited during the conference at the CECO Convention Centre, and for the enthusiasm and dedication of the Korean Organizing Committee, in particular its chair Dr Hak-Gyoon Kim, from the Republic of Korea National Fisheries Research & Development Institute. A beautiful carving of *Cochlodinium polykrikoides* by Prof. Takayama was given to him as a symbolic recognition for all his efforts.

New ISSHA officers and council members

Officers
President: Beatriz Reguera (Spain)
Vice-Presidents: Robin Raine (Ireland) & Mingjiang Zhou (China)
Secretary: Karin Rengefors (Sweden)
Treasurer: Henrik Enevoldsen (Denmark)
Past-president: Patricia Tester (USA)

Council
Ana Amorin (Portugal)
Anke Kremp (Finland)
Anna Godhe (Sweden)
Aurelia Tubaro (Italy)
Christine Band-Schmidt (Mexico)
Esther Garcés (Spain)
Gustaaf Hallegraeff (Australia)
Ian Jenkinson (China, France)
Katerina Aligizaki (Greece)
Keith Davidson (United Kingdom)
Lincoln McKenzie (New Zealand)
Marta Estrada (Spain)
Philipp Hess (France)
Suzanne Roy (Canada)



Hak-Gyoon Kim receives his ISSHA present: a carving from Prof Takayama reproducing the Cochlodinium Conference logo (Photo from www.hab2012.kr).

ISSHA 2012 Achievement Awards

The *Yasumoto Lifetime Achievement Award* was given to Prof. Øjvind Moestrup (University of Copenhagen, Denmark). The *Maureen Keller Awards* for the best student communications were given to: Bum-Soo Park (South Korea), Hannah Blossom (Denmark), Theresa K. Hattenrath-Lehmann (USA) and Thanh-Luu Pham (Japan)



Prof. Øjvind Moestrup

Prof. Øjvind Moestrup received the *Yasumoto Lifetime Achievement Award* for excellence in his lifetime dedication to research on the biology, taxonomy and ultrastructure of microalgae. In addition, Prof Moestrup has made an outstanding contribution, through the *IOC Science and Communication Centre on Harmful Algae* in Copenhagen and the *IOC Reference List of Harmful Microalgae*, to training and communication on the taxonomy of HAB species.

Øjvind was born on 15 December 1941. He grew up on the island of Funen and in Jutland, Denmark, where his father was a vicar. He started studying biology at the University of Copenhagen in 1962 with particular interest in zoology and initially intended to do his Master's degree on the cytology of flatworms. However, in 1965, he was contacted by Prof Tyge Christensen, who asked him to consider a job as demonstrator at the Institut for Sporeplanter which he accepted. In 1967, Tyge suggested Øjvind accept an invitation from Prof Irene Manton, University of Leeds, England, the leading expert on ultrastructure of algae, to learn electron microscopy. It was there in 1968–1969, where Øjvind learned the techniques necessary for studying ultrastructural details of algae – and he has basically done this ever since. The topic for his Master's thesis was the ultrastructure of *Vaucheria*, and he revealed that the genus belonged to the golden-green algae. In the period 1970–1974, Øjvind was assistant professor at the Institut for Sporeplanter and studied spermatozoids in different groups of algae. In 1972, a new transmission electron microscope was sponsored by the Danish Research Council, and Øjvind moved onto nanoflagellates inspired by a bloom in Kaikoura, New Zealand, including the description of two new haptophytes (*Phaeocystis scrobiculata* and *Chrysochromulina novae-zelandiae*). Øjvind began reconstructing the flagellar apparatus of several different flagellates, such as *Chrysochromulina, Mamiella, Nephroselmis, Pyramimonas, Pseudoscourfieldia* and *Bicosoeca*, which ultimately turned into a whole issue of *Phycologia* and was used as a DSc thesis at the University of Copenhagen in 1983.

See more information on Prof. Øjvind Moestrup's achievements on "Trail Blazers" at the ISSHA website (www.issha.org)



Bum Soo Park

Best Oral to Bum Soo Park (Hanyang University, Seoul, South Korea) for his contribution on "*Distribution and seasonal fluctuation of diverse ribotypes of* Cochlodinium polykrikoides *in Southern Korean coastal waters*" (B.S. Park and M.-S. Han). Bum Soo Park is a PhD

student (supervised by Prof. Myung Soo Han) at the Life Science-Natural Sciences Department in Hanyang University (Seoul, South Korea). He received his bachelor's degree from the same university in 2006 and was a visiting scholar at the University of Washington (Seattle, USA) in 2007-2008. He won best paper awards of The Korean Society of Phycology (2011) and the Korean Society of Environment & Ecology (2011). Park has worked with different microalgal groups (diatoms, dinoflagellates, small flagellates) and the importance of life cycles in their ecology. Currently he is interested in the application of advanced molecular tools in the study of the main HAB species causing socio-economic problems in Korea, in particular of *Cochlodinium polykrikoides.*



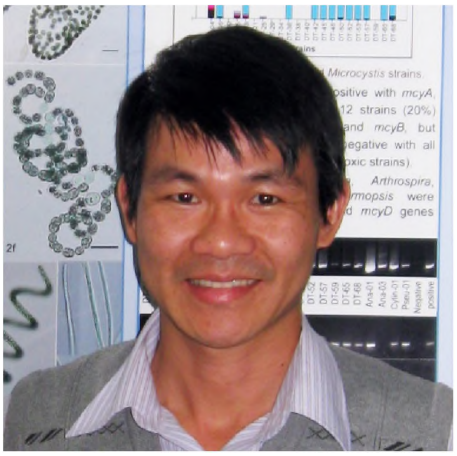
Hannah Blossom

Honorable Mention to Hannah Blossom (University of Copenhagen, Helsingør, Denmark) for her oral presentation "T*oxic mucus traps: a novel mechanism that mediates prey uptake in the mixotrophic dinoflagellate* Alexandrium pseudogonyaulax", coauthored by N. Daugbjerg and P.J. Hansen. This work was done as Hannah's Master degree, completed at the University of Copenhagen, under the supervision of Per Juel Hansen and Niels Daugbjerg. Hannah received her bachelor's degree from Northeastern University, during which she did an internship in Don Anderson's lab - sparking her interest in harmful algae. Hannah is now studying ichthyotoxic algae as part of a Danish strategic research project called HABFISH, led by Per Juel Hansen. She is currently focusing on *Prymnesium parvum* and its effects on fish as well as co-occurring algae. Hannah's research interests include the role of allelochemicals in phytoplankton interactions, particularly in mixotrophic harmful algae species. Originally from New York, Hannah has been living in Denmark for 3 years now, and has enjoyed the adventures of living abroad.



Theresa Hattenrath-Lehmann

Best poster award was given to Theresa K. Hattenrath-Lehmann (Stony Brook University, USA) for "*The Emergence of toxic* Dinophysis acuminata *blooms in a New York estuary*", co-authored by S.L. Morton and C.J. Gobler. Born and raised in Queens (New York), she left the big city to attend University of Maine at Machias (UMM) where she received her B.S. degree in Marine Biology with a focus on mariculture. The university is on the Gulf of Maine, a region well known for large-scale recurrent *Alexandrium* blooms and her professor, Dr. Gayle Kraus, encouraged her to attend a phytoplankton monitoring workshop at Bigelow Laboratory in Boothbay Harbor, Maine. There she became interested in HABs. Her Master's research, initiated in 2007, was on the effects of nitrogen loading on *Alexandrium* densities and toxicity, at Stony Brook University (advisor Christopher Gobler). Her studies concluded that nitrogen loads promote the intensity of PSP events and that nitrogen derived from a waste water source was promoting *Alexandrium* blooms in this system. In addition, she discovered that another harmful algal species, *Dinophysis acuminata,* was blooming in Northport Bay and that shellfish contained DSP toxins above regulatory limits. She is currently a Ph.D. student of Dr. Gobler. Her topic: to assess the causes of the onset, maintenance, and demise of blooms of *Alexandrium* and *Dinophysis*. Theresa would like to continue a career path investigating the root causes of harmful algal blooms, but is also interested in programmes concerning protection of human health against toxic algae. This last topic had been a large part of her Master's work and she found it gratifying to interact with NY state agencies and assist in monitoring programmes designed to prevent shellfish poisonings.



Thanh-Luu Pham

Honorable mention to Thanh-Luu Pham (University of Tsukuba, Japan) for his poster on "*Toxic Cyanobacteria and Microcystin Profile of Numerous Cyanobacterial Strains Isolated from Dau Tieng Reservoir, Vietnam*", co-authored with T.-L. Pham, T.-S. Dao, K. Shimizu, N. Sugiura and M. Utsumi. Thanh-Luu holds a M.Sc. degree from the Ho Chi Minh City University of Technology and is currently working toward his Ph.D. degree at the Aquatic Eco-Engineering Lab, University of Tsukuba, Japan. His Ph.D thesis research, supervised by Professor Motoo Utsumi, focuses on distribution of microcystins in aquatic environments, and covers several aspects including isolation of Cyanobacteria and detection of microcystin production, such as in the present poster. He also has a strong interest in discovering and understanding the mechanisms of distribution and bioaccumulation of microcystins in several aquatic organisms

Clogging up the works: a newly recognized aspect of HAB's

In the minds of most people, Harmful Algal Blooms are associated with phycotoxins and toxicity. This has certainly been the case among water engineers and policy makers in the exponentially growing desalination industry who have only relatively recently become aware of HAB's. Of course, the occurrence of algal blooms and red tides was not unknown to the desalination community but concern for possible residual toxic contamination in desalinated product waters deriving from HAB's in the intake waters of desalination plants has only recently been acknowledged. For example, the title of an invited plenary given by Don Anderson at the European Desalination Society meeting at Barcelona in 2012 was "The global problem of red tides and harmful algal blooms: an emerging threat to desalination plants". Obviously assessing whether current technologies adequately remove toxins in product waters destined for human consumption is of critical importance and has huge economic implications for the desalination industry.

However, there is another impact of HAB's on desalination facilities that has so far scarcely been considered. For years, plant operators have been aware of fouling problems caused by large quantities of biomass from occasional algal blooms entrained in intake water; these can usually be dealt with quite effectively by treating the incoming water with coagulants and/or filtration. Only recently, however, has there been recognition of a more problematic aspect of HAB's occurring in the vicinity of desalination intakes. As HAB's develop, and even more so when they decline, they generate large amounts of unseen organic microgel particles that are now known to be critical drivers of biofilm formation on filtration membranes and other sensitive surfaces [1,2]. Biofilm which leads to clogging of the reverse osmosis (RO) membranes is a major and extremely expensive problem for the desalination industry.

The most extensively studied of these microgels are transparent exopolymer particles (TEP), first described by Alldredge et al. in 1993 [3]. TEP are microscopic, deformable, organic gel-like particles visualized by staining with Alcian Blue, a dye specific for acid mucopolysaccharides. TEP range in size from >0.4 µm to 200-300 µm and appear in many forms; amorphous blobs, clouds, sheets, filaments or clumps (Fig.1). In addition to polysaccharides, many other substances, including proteins, nucleic acids and trace elements can be associated with these microgels. The presence of highly surface active polysaccharides explains the strong tendency of these particles to form metal ion bridges and hydrogen bonds. As a result, TEP are 2 to 4 orders of magnitude more "sticky" than phytoplankton or mineral particles, with a high probability of attachment upon collision. Thus small microgels may aggregate with each other or with other detrital fragments to form marine or lake "snow". In oceans and lakes. TEP are often colonized by bacteria and other microorganisms and may serve as the matrix for "hot spots", sites of intense microbial and chemical activity within the water mass. Extensive research has shown that TEP play extremely important roles in the ecosystem functioning of both marine and freshwater environments, especially in respect to the cycling and transportation of organic carbon [4,5]. Some examples of TEP occurring in Eastern Mediterranean coastal waters are shown in Fig. 1.

These particles may derive from numerous sources. In some aquatic environments, TEP form abiotically from dissolved organic exudation products by processes of coagulation and gela-

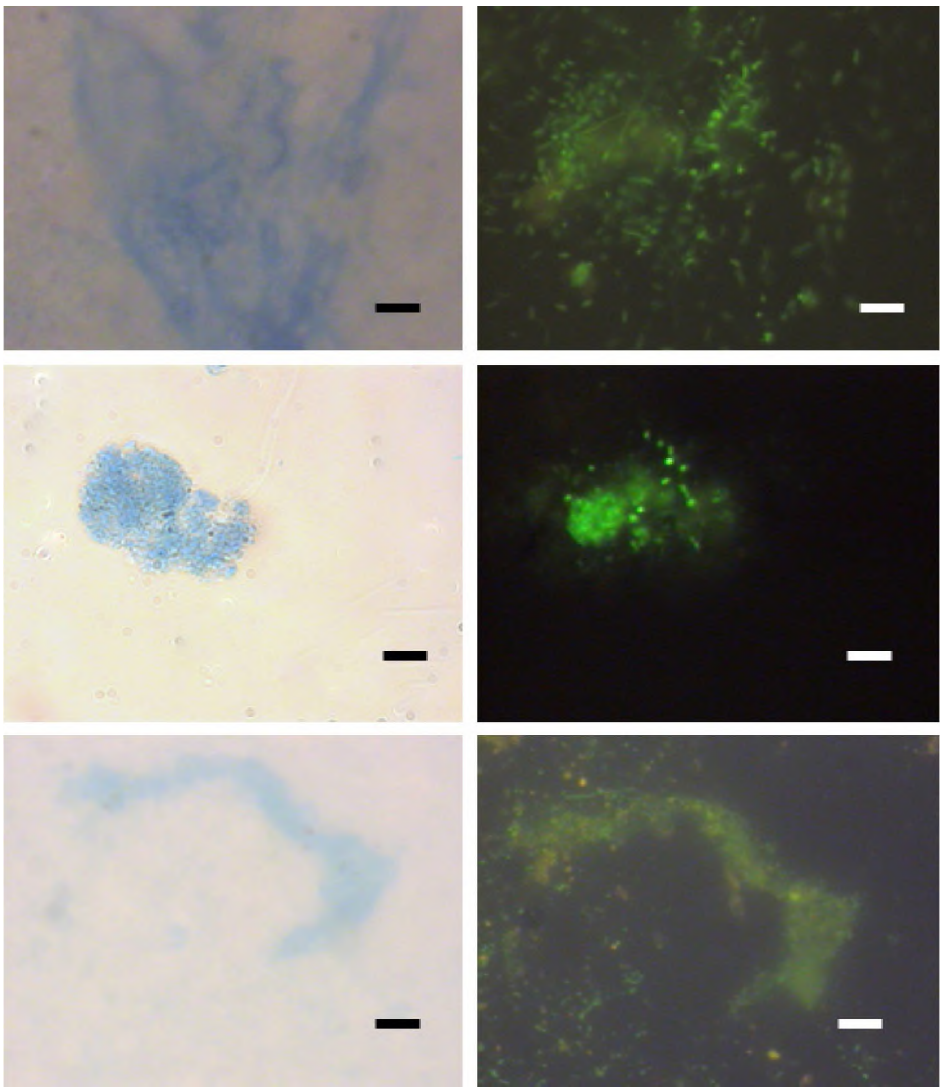


Fig. 1. Transparent Exopolymer Particles (TEP) in eastern Mediterranean seawater. TEP particles when stained with Alcian Blue dye are colored blue under regular microscope illumination (left panels). When the same microscope fields are viewed with ultraviolet epifluorescent illumination (right panels), bacteria staining with SYBR Green appear as bright green spots or rods. The faint green staining of some TEP is probably due to nucleic acids adsorbed to these particles. Scale bars 10 µm.

tion or by bubble adsorption. Considerable amounts of TEP are also produced from the gelatinous envelopes surrounding diatoms and other algae and from bacterial mucous. TEP is also released under stress conditions or at senescence by algae and cyanobacteria [6] or from the breakdown of marine or lake "snow". Therefore, the occurrence and subsequent demise of a HAB in the vicinity of a desalination plant intake would be expected to generate exceptionally high levels of TEP.

Recognition of the involvement of TEP in biofilm formation on filtration membranes and other sensitive surfaces is relatively recent. Based on the known characteristics of TEP, in particular their extreme stickiness and the observation that numerous bacteria were frequently observed on or in many of these particles Berman and Holenberg [7] first suggested that TEP should be involved in the biofouling process. However, it was only in subsequent experimental studies that TEP were actually shown to be actively involved in aquatic biofilm formation and that TEP levels in source water were significantly correlated with the rate at which membranes clogged [8]. Further studies by Bar-zeev et al. [1] led to a revised model for aquatic biofilm formation which included TEP as a significant player in the process. In this work, the term "protobiofilm" was proposed for large TEP heavily colonized by bacteria and other microorganisms on the basis that such microgels possess all the characteristics of biofilm with the exception of attachment to a surface.

Although relatively few data are available, the general impression is that present pretreatment technologies in desalination plants are not particularly effective in dealing with TEP, given the gel-like characteristics of these particles [2]. To date, experimental studies show reduction of only 30 to 50% in the concentrations of TEP reaching the reverse osmosis (RO) membranes that are the critical filtration step in most present day desalination facilitites. If indeed HAB's generate large amounts of these microgels then contamination of the intake waters by TEP may present a much more serious operational problem to the desalination industry than the extremely low levels of toxic residues apparently remaining in the processed waters. In any event, there now appears to be some awareness that HAB's of the biofouling potential of these microgels.

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Tom Berman died this April while hiking in the Galapagos Islands. He was one of the founding fathers of the Kinneret Limnological Laboratory, its first director from 1967 to 1971, and again from 1986 until 1998, when he retired. He made major contributions to our knowledge of algal nutrient uptake and excretion, the microbial loop, organic nitrogen as a resource for algae, and recently transparent exo-polymers (TEP) and their role in desalination technology. A special session to commemorate him is being organised at the upcoming SIL congress in Budapest, 4-9 August.

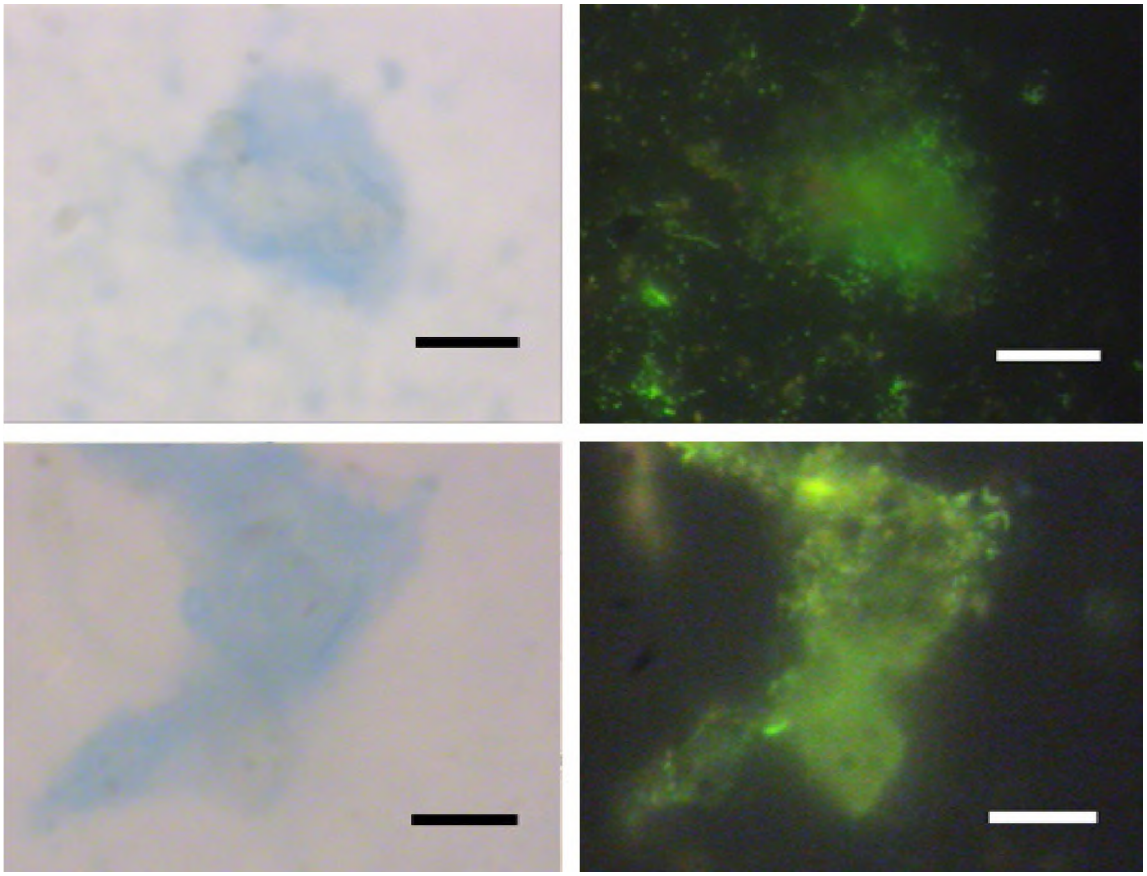

Fig. 1 continued. Transparent Exopolymer Particles (TEP) in eastern Mediterranean seawater. TEP particles when stained with Alcian Blue dye are colored blue under regular microscope illumination (left panels). When the same microscope fields are viewed with ultra-violet epifluorescent illumination (right panels), bacteria staining with SYBR Green appear as bright green spots or rods. The faint green staining of some, but not all, TEP is probably due to nucleic acids adsorbed to these particles. Scale bars 10 µm.

A *Cochlodinium polykrikoides* bloom in El Salvador



Figure 1. Sampling stations in El Salvador coastal waters, from east to west: Gulf of Fonseca, El Cuco, Puerto El Triunfo, Costa del Sol, San Diego, el Muelle, Punta Roca, El Sunzal, El Sonte, Chalpa, Taquillo, La Perla, Los Cóbanos, Garita Palmera. Cochlodinium polykrikoides *present (red), absent (green), (March 2012).*

A bloom of the fish killing dinoflagellate *Cochlodinium polykrikoides* that reached densities of 215 x 106 cell L^{-1} is reported from El Salvador coastal waters. Brown patches 3 km wide and 13 km long, visible by naked eye, developed in March 2012, causing fish mortalities, and social alarm amongst locals and tourists. Environmental conditions associated with this bloom were recorded at monitoring sites (from 13°10´N 87°54´W to 13°40´N 90°02´W) throughout the country.

El Salvador is subject to frequent paralytic shellfish poisoning events associated with blooms of *Pyrodinium bahamense* var. *compressum*. Even moderate cell densities of *Pyrodinium*, not visible to the naked eye, have caused human fatalities and deaths of sea turtles. PSP events in recent years (2010, 2011, 2012) have been detected by the Laboratory of Marine Toxins of the University of El Salvador (LABTOX-UES) and toxins analyzed with a receptor binding assay (1, 2). Blooms of *Cochlodinium polykrikoides* in the Eastern Pacific have been reported before in Costa Rica, Guatemala, Mexico and California (3, 4). During the second week of March 2012, brown patches approaching the surf area were observed by local people from La Libertad. By March 23, overlapping patches formed a 3 km wide band that extended along 13 km of coastline. This event coincided with Easter, when many tourists visited the area, and caused great social alarm. The high densities of *Cochlodinium polykrikoides* caused mortality of benthic fish populations, a putrid smell from decaying organic matter, and severe headaches to visitors and local residents. A member of a group surfing in the affected area who fell and swallowed water in the middle of the patch suffered from diarrhea for three days.

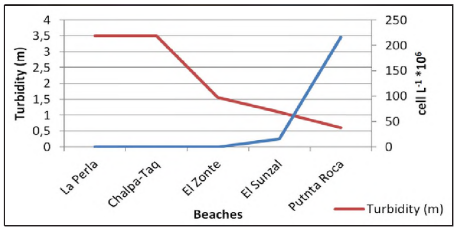

Figure 2. Cell counts and turbidity observed at La Libertad, the area most affected by the Cochlodinium polykrikoides *bloom.*

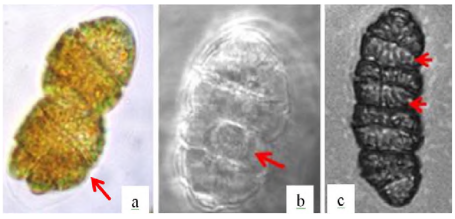

Figure 3. Micrographs of Cochlodinium polykrikoides *collected in El Salvador and criteria used for taxonomic identification. (a) Sulcus very close to cingulum; (b) Large nucleus located in anterior part of epicone; (c) Elongated rod-shaped chloroplasts.*

LABTOX-UES carries out routine monitoring of potentially toxic phytoplankton and phycotoxins along the coasts of El Salvador and had the opportunity to describe this event and warn the relevant health and fisheries authorities. Recreational activities were forbidden in the department of La Libertad to protect the public.

Water samples were taken with 10 L Niskin bottles at 1 and 2 m depth, and phytoplankton by vertical hauls with a 20 µm net. Cells were identified with a Carl Zeiss Axio Imager M1 microscope with phase contrast, and counted in Sedgewick Rafter chambers with an Axiovert 40 CFL inverted microscope. Salinity and temperature were measured with a HACH 56 probe and turbidity with a Secchi disc. Localities sampled are shown in Fig. 1.

During March and April high density patches (up to 215 x 106 cell L^{-1}) of the dinoflagellate *Cochlodinium polykrikoides* were observed (Fig. 1). Samples collected on 23 March revealed that the densest patches were found off Punta Roca and by the pier of La Libertad harbour. Lighter patches were found by the western beaches (Fig. 2). Cell counts decreased from 1.8 x 106 cells L^{-1} on March 29 to 3.3 x 105 cells L^{-1} on May 3. By April 11, patches extended along the entire coast to the Guatemala border. During April and May, a dense patch occurred off Cóbanos, a coral reef area. No *Cochlodinium polykrikoides* were found in samples from May 17 to June 19. Temperatures at 1 m depth were between 26.8 and 28.7°C, and salinity between 34 and 35 psu, characteristic of oceanic waters. Secchi disc depth varied from 0.6 to 3.5 m (Table 1). This is the first record of an ichthyotoxic red tide of in El Salvador waters. The cells were concentrated in the upper 2 m of the water, mainly between Puerto La Libertad and El Zonte beach.

Table 1. Environmental data from beaches affected by Cochlodinium polykrikoides *bloom in March 2012.*

Beaches	T (°C)	Salinity (psu)	Turbidity (m)
La perla	28.3	34.7	3.5
Chalpa	28.4	35.1	3.5
El Zonte	28.3	34.8	1.6
El Sunzal	28.7	35.0	1.1
Punta Roca	26.8	34.8	0.6

Acknowledgements

To CONAMAR, CENDEPESCA, MARN, MSPAS, Alcaldía Puerto La Libertad, the Navy, and students at LABTOX-UES. This work was partially funded by IAEA Technical Cooperation Project ARCAL RLA 7/014 (2009-2013).

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Authors

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Cochlodinium polykrikoides bloom in the Colombian Caribbean



Figure 1

The occurrence of the dinoflagellate *Cochlodinium polykrioides* is reported for the first time in the Colombian Caribbean. Two blooms of this species, with maximal concentrations of $5x10^6$ cell L^{-1}, occurred in the rainy seasons of 2010 and 2011 in Santa Marta Bay. As a result of local currents and winds, the residence times of the blooms were brief, and no adverse effects on local wildlife were observed. The source of these blooms, associated with heavy and persistent rainy seasons and freshwater discharges, is unknown.

In the evening of 25 October 2010, a striking large brown patch of seawater delimited by froth was observed in Santa Marta Bay (Colombian Caribbean coast) (Fig 1). The patch, with a north to south orientation, was driven by the wind into the Bay and adjacent areas, and reached an extension of approximately 6 km^2. The same event recurred on 26 and 29 October and finally on 12 November, 2010. On 31 October and 1 November 2011 patches with similar features were again observed. Phytoplankton samplings were carried out following each patch sighting. Analyses of the samples revealed these patches were constituted by high densities of *Cochlodinium polykrikoides* (Table 1).

These rainy season blooms (October-November) of *P. polykrikoides* occurred with nutrient concentration values within the historic range for the area [1]; nevertheless sea surface temperature was above average and there were sudden declines in salinity. Figure 2 shows the annual variability of these parameters between 4 January 2010 and 23 April 2012. Ahh *et al.* [2] have shown that *C. polykrikoides* blooms in South Korea are associated with increased temperature; they hypothesized that heavy monsoon rains and typhoons, causing heavy floods and input of terrestrial nutrients, are key factors triggering the blooms.

Brown patches of *Cochlodinium* in Santa Marta were sighted in the afternoon and disappeared at night. A local current known as the Panamá-Colombia Countercurrent flows along the Colombian Caribbean coast from SW to NE [3]. This current is more intense in the rainy season, when the northeast Trades are weaker, reaching the coasts of La Guajira in October-November. Data from the meteorological station of the University in Santa Marta (Comarta) showed that when the patches appeared in the Bay, the predominant wind direction was from SSE to SE during the day, reversing to NNW to NW and W at night. All this suggests longshore advection of the blooms from south to north by the Panamá-Colombia Countercurrent as well as cross shelf transport: inshore in the evening, when they were driven into the Bay by local winds, and flushed offshore at night.

Little is known about the origin and causes of the blooms In Santa Marta Bay. Nevertheless the physical conditions associated with them (temperature, salinity, winds, currents, intense and persistent rainy season) suggest that they may have been initiated in the river plumes of the Ciénaga Grande de Santa Marta (a reserve of the biosphere) or of the Magdalena River, and advected to the north by the countercurrent until it reached Santa Marta Bay and the Tayrona Park coast. Persistent high-density blooms of *C. polykrikoides* in semi-enclosed embayments are reported to have a very negative impact

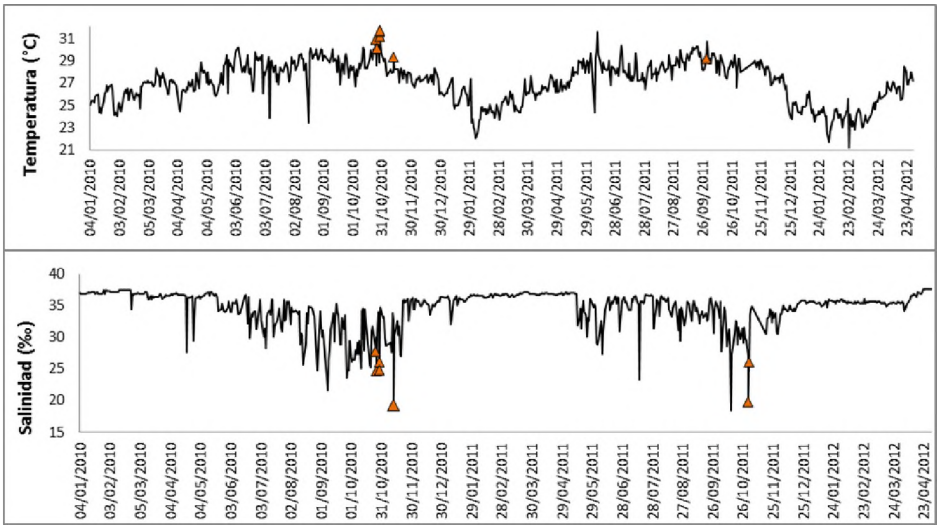

Figure 2. Sea surface salinity and temperature in Santa Marta Bay between 4 January 2010 and 23 April 2012. Orange triangles indicate when brown patches were observed in 2010 and 2011 (http://siam.invemar.org.co/siam/redcam).

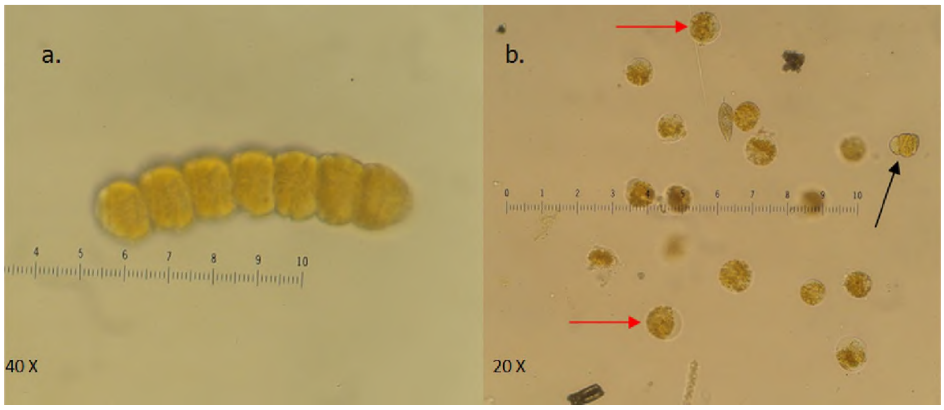

Figure 3. Micrographs of Cochlodinium polykrikoides *(Margalef) from brown patches occurring in Santa Marta Bay (Colombian Caribbean) a) Chain of seven vegetative (naked) cells. Each micrometre unit equals 2 µm. b) Different life cycle stages individual cells of* C. polykrikoides*: red arrows point at thecate and black arrows at naked or vegetative cells showing the cingulum around the cell. Each micrometre unit equals 4 µm.*

on cultivated fish in South Korea [4]. Blooms in Santa Marta were very short-lived and this may explain why harmful effects on the fauna were not reported.

Cochlodinium polykrikoides formed chains of 2-8 cells and sometimes solitary specimens were observed (Fig 3). Cells ranged in size from 30-40 µm long, with a two-lobed hypotheca. Cells were more and more oval as the number of cells per chain increased, but single or paired cells was ellipsoidal. Nucleus in anterior (epicone) position and numerous cylindrical chloroplasts; cingulum descended in distinct left-handed spiral nearly twice around the cell. This description corresponds to that of the naked or vegetative stage in the life cycle of *C. polykrikoides* [5]. Thecate specimens are an intermediate stage leading either to resting cysts or to vegetative cells able to grow in suboptimal conditions [6]. In the Santa Marta blooms, thecate cells became vegetative (naked) cells (Figure 2b), suggesting that the population was actively growing.

Acknowledgements

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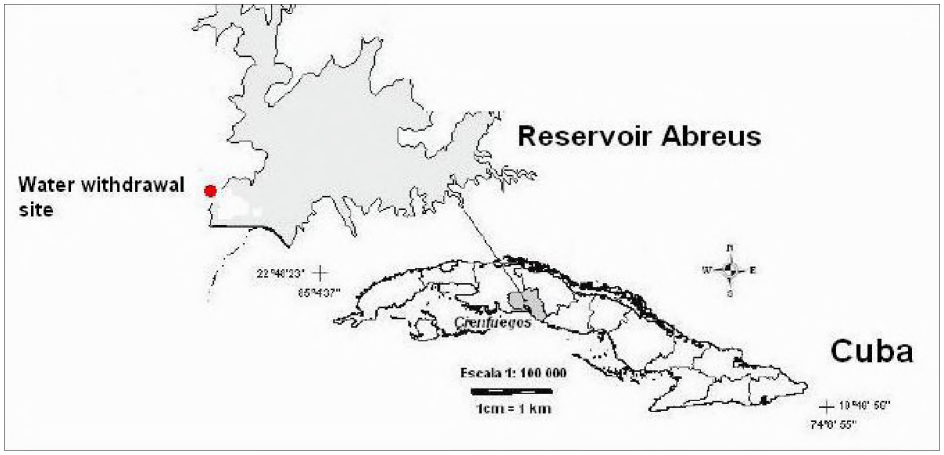
"Red-tide world" Seoul National University, October 26, 2012



A special symposium on red tides "Red-tide world" was held at Seoul National University, Korea on October 26, 2012 just prior to the 15th ICHA held in Changwon, Korea. Eight talks were given by the invited speakers; "Forecasting the New England red tide: Current status, and ongoing efforts to improve accuracy and usefulness" by **Don Anderson** (WHOI), "Mixotrophy in red-tide organisms" by **Hae Jin Jeong** (Seoul National University), "Dense algal blooms, elevated pH, and regulation of estuarine biogeochemical processes" by **Diane Stoecker** (U of Maryland), "Long-term variations of *Heterosigma akashiwo* in Narragansett Bay and its environmental control" by **Ted Smayda** (U of Rhode Island), "The red-tide ciliate *Mesodinium rubrum* in the Korean coastal waters" by **Wonho Yih** (Kunsan National University), "Harmful algal blooms, Shellfish aquaculture and restoration" by **Sandy Shumway**, "New perspective on the relationship between harmful algal blooms and cyst germination in Korean waters" by **Myung-Soo Han** (Hanyang University), and "Taxonomy and phylogeny of the dinoflagellates. A new start based on ultrastructure and molecules" by **Øjvind Moestrup** (U of Copenhagen).

Cyanobacterial bloom in the Abreus Reservoir, Cienfuegos, Cuba



The Abreus Reservoir, constructed in 1985 is located on a very important basin in the Central Province of Cienfuegos. Its main tributaries are Damují, Jabacoa and Limones. The reservoir was originally planned for agricultural and industrial purposes, but in recent years supplies tap water to residential sectors of Cienfuegos.

In March 2009 a typical water bloom formed exclusively by *Microcystis paniformis* was recorded in the reservoir [1]. The event provoked a 'Territorial Investigation Project' to determine the water quality of the reservoir, and included a phytoplankton monitoring programme. As a preliminary result of this investigation, in October 2010 another water bloom with 1.1 x 107 cell L^{-1} was detected at the water withdrawal site of the reservoir (see Table 1), but now composed mainly of filamentous cyanobacteria, species of *Anabaenopsis, Arthrospira, Cylindrospermopsis, Dolichospermum* and *Raphidiopsis*: no toxic events were recorded.

The species found are known mainly from tropical regions; hence some taxonomical comments of these populations may be of interest.

Table 1: Some characteristics of superficial water from water withdrawal site, Abreus reservoir, Cienfuegos, Cuba. October 2010.

Conductivity	381,3 µS/cm
$O_2D.$	8,64 mg/L
pH	7,68
Temperature	27,5°C
$N\text{-}NO_3$	32 mg/L
$N\text{-}NH_4$	0,045 mg/L
$N\text{-}NO_2$	6,1 mg/L
$P\text{-}PO_4$	0,016 mg/L
Chlorophyll a	23,9 mg/L

Anabaenopsis sp. (Figs. 1-2)

Characterized by solitary trichomes, 5.6-7.5 µm wide, short, irregularly coiled, constricted at the cross-walls, without mucilaginous sheaths; vegetative cells ± barrel-shaped to short cylindrical usually with aerotopes; heterocytes terminal, spherical, rare broad oval, 5,6-8,7 µm in diameter; akinetes absent. Abundance in the samples: 1.3 x 105 cell L^{-1}. No akinetes were observed, thus it is not possible identify the species, but some features suggest *A. arnoldii* Aptekar, but the mucilaginous sheath of this species is lacking in our specimens. *A. tanganyikae* (G.S.West) Wolosz. et Mill. has also been found in Cuba [2, 3], but is morphological quite distinct.

Arthrospira cf. *khannae* Drouet et Strickland in Drouet 1942 (Figs. 11-12)

Trichomes with regular loose screw-like coils, solitary, free floating, blue green, 2.8-5.6 µm wide, unconstricted or very slightly constricted at the granulated cross-walls, spirals 22.4-30.8 µm wide, 44.8-58.8 µm length; terminal cells rounded, very slightly capitate; cell content usually with aerotopes. Abundance in the samples: 2.3 x 105 cell L^{-1}. This species is related to *A. khanne*, but the trichomes are not attenuated at the ends and the spiral dimensions are distinct, typically 20-22 µm wide, 20-35 µm long. Our specimens resemble *A. massartii* Kufferath, but this species is benthic, without aerotopes. The typical *A. khannae* has a tropical distribution, from India, Vietnam and Zambia [4].

Cylindrospermopsis curvispora Watanabe 1995 (Figs. 3-4)

Trichomes slightly constricted at cross walls, irregular spiral coils, but circular in outline, 4.2-5 µm wide; cells cylindri-

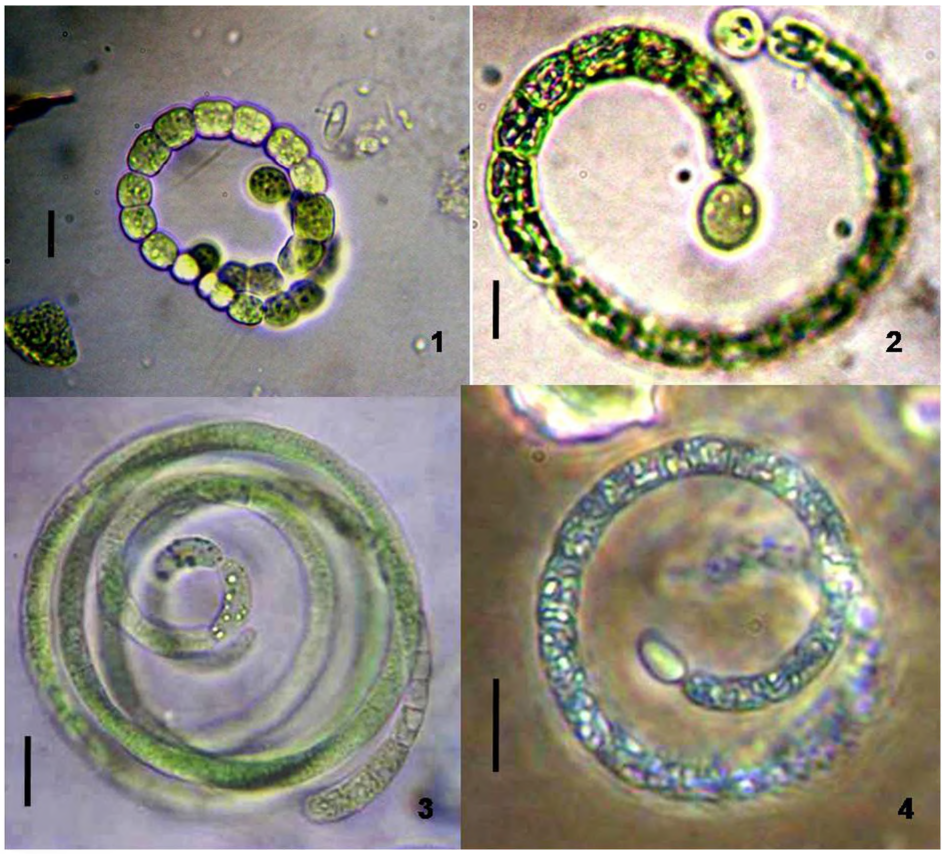


Fig. 1-4: 1-2) Anabaenopsis *sp., 3-4)* Cylindrospermopsis curvispora *Watanabe. Scale = 10 µm.*

cal, 7.5 x 5 µm; terminal cells cylindrical rounded; cell content usually with aerotopes; heterocytes terminal, conical ovoid, rounded at ends, 6.7 x 2.5 µm; akinetes 10 x 2.5 µm, cylindrical-kidney-shaped, sometimes curved, mostly in pairs, distant from heterocytes. Abundance in samples 1.4 x 106 cell L^{-1}. This species, known from central Japan, Sri Lanka and southern Africa [5], was previously recorded in Cuba from Laguna Las Canteras, Cienfuegos [1].

***Dolichospermum* cf. *flos-aquae* (Lyngbye) Wacklin et al. 2009 (Figs. 5-6)**

Trichomes solitary, free floating, constricted at cross walls, irregular spiral coils, but circular in outline, 5.6 µm wide; cells ± spherical, with aerotopes; heterocytes spherical, 7 µm in diameter; akinetes distant from heterocytes, cylindrical oval, 14-19.6 x 8.4 µm. Abundance in samples: 6.0 x 105 cell L^{-1}. This planktonic species is known from eutrophic water bodies around the world [6]. The Cuban specimens have smaller akinetes, not kidney-shaped.

***D.* cf. *solitarium* (Klebahn) Wacklin et al. 2009 (Figs. 7-8)**

Trichomes solitary, free floating, ± straight, constricted at cross walls, 5.6 µm wide; cells ± spherical, with aerotopes; heterocytes spherical, 5.6-8.4 µm in diameter; akinetes distant from heterocytes, cylindrical, 14-19.6 x 5.6-8.4 µm. Abundance in samples: 6.0 x 106 cell L^{-1}. Typical specimens with wider trichomes, vegetative cells citriform and akinetes larger, are known mostly from northern regions of temperate zones [7].

***Raphidiopsis curvata* Fritsch et Rich 1929 (Figs. 9-10)**

Trichomes solitary, free floating, isopolar, 2.8-4.4 µm wide, unconstricted at the cross-walls, irregularly curved to sigmoid, relatively long, gradually tapering to ends; terminal cells elongate pointed; cells cylindrical, one to two times longer than wide; akinetes cylindroids, 8.4 x 2.8 µm. Abundance in samples: 2.5 106 cell L^{-1}. Raphidiopsis Fritsch et Rich is ill-defined and polymorphic, and may be a non-heterocytous stage of other genera such as *Cuspidothrix* or *Cylindrospermopsis*. According to Li et al [8], *Raphidiopsis* and *Cylindrospermopsis* are phylogenetically related, but the identity of these genera is not yet clear [9].

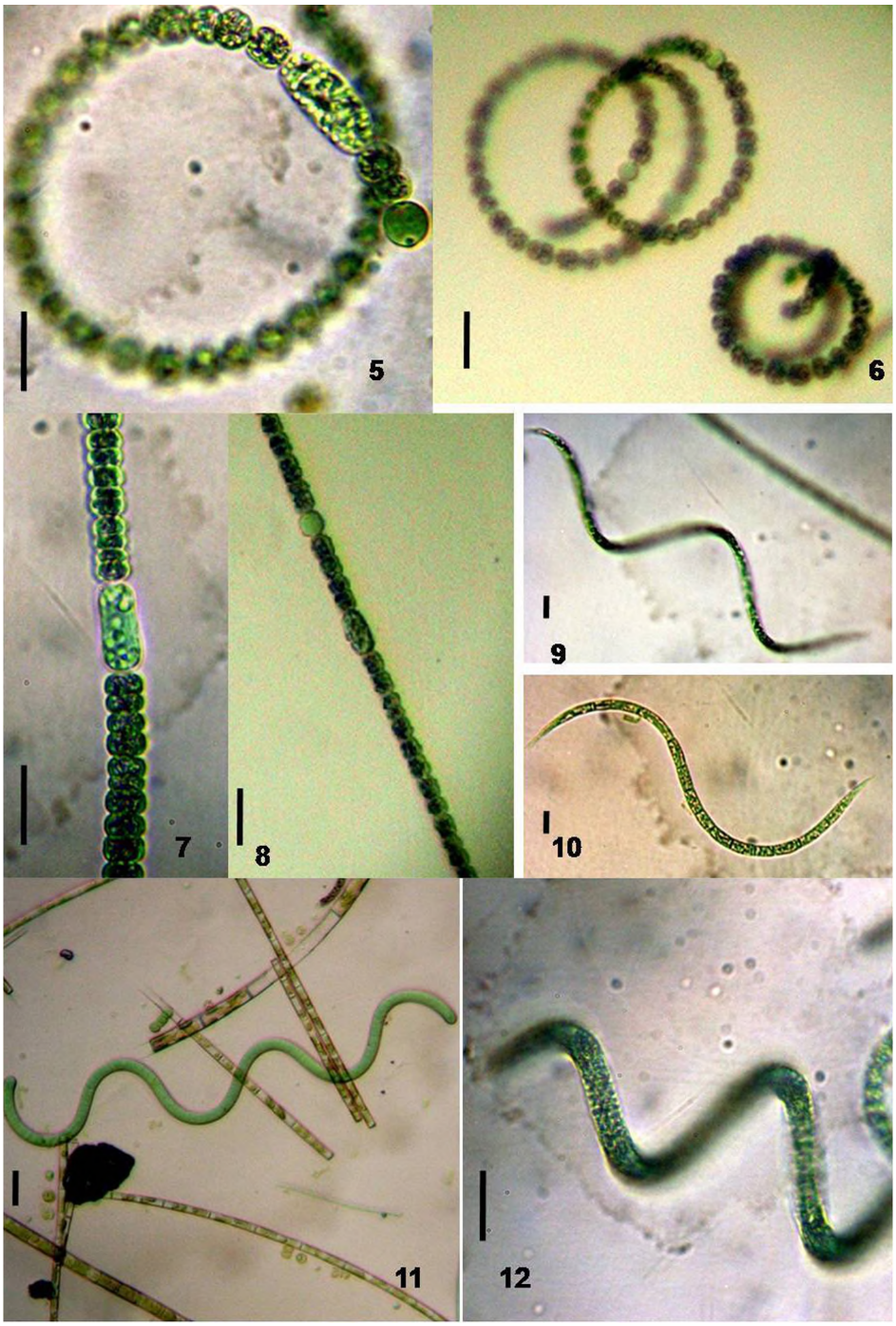


Fig. 5-12: 5-6) Dolichospermum *cf.* flos-aquae *(Lyngbye) Wacklin et al., 7-8)* D. *cf.* solitarium *(Klebahn) Wacklin et al., 9-10)* Raphidiopsis curvata *Fritsch et Rich., 11-12)* Arthrospira *cf.* khannae *Drouet et Strickland in Drouet. Scale = 10 µm.*

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Gambierdiscus and related benthic dinoflagellates from Madeira archipelago (NE Atlantic)

Ciguatera fish poisoning (CFP) has been of increasing concern in recent years since the expansion of this food-borne disease from tropical and subtropical areas of the Pacific and Caribbean to warm temperate areas like Macaronesia (Azores, Madeira, Canary and Cape Verde Islands) and the Mediterranean [1, 2]. Often associated with the CFP-causing dinoflagellates, *Gambierdiscus* spp., are the palitoxin-producing *Ostreopsis* spp., which were also recently recorded from new areas and of concern to human health issues [3].

In 2005, the occurrence of *Ostreopsis* cf. *siamensis* from Madeira (~32.5°N, 17°W) (Fig. 1) was reported for the first time [2]. The sample was collected in 2002 by Fraga (pers. communication) and cultured since then in Vigo, Spain, under strain numbers VGO610, VGO611, VGO613 and VGO614. After molecular studies, they were reassigned to *Ostreopsis* cf. *ovata* [4]. In summer 2007, several wardens from the Natural Park Madeira stationed at the Selvagens Islands, about 300 km to the south of Madeira Island (Fig. 1), suffered light ciguatera-like intoxications after eating locally caught fish (*Seriola* sp., *Sparisoma cretense, Serranus atricauda, Bodianus scrofa, Balistes capriscus* and *Pagrus pagrus*). A more severe CFP incident was reported by fishermen in summer 2008 after eating a *Seriola* sp., also caught around the Selvagens Islands [5]. Immediately after that episode, water samples were collected by the national monitoring laboratory INRB-IPIMAR, and *Ostreopsis* sp., *Amphidinium* sp. and *Prorocentrum* sp., were detected. *Ostreopsis* sp. was reported with cell concentrations as high as 700 cells L^{-1} [6].

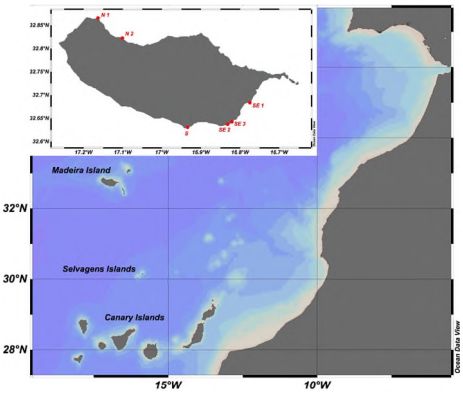

Fig. 1. Madeira archipelago including Selvagens Islands; insert: Madeira Island showing sampling sites.

Since then, CFP-like episodes have been reported almost every year [7, 8]. Several different ciguatoxins were detected in *Seriola* spp. caught in 2009 at the Selvagens Islands (a CTX of the CTX-3C group; a CTX analog, similar to C-CTX-1, C-CTX-2, I-CTX-1, and I-CTX-2; CTX-4A/B or an analogue) [9].

In light of these incidents, we sampled different seaweed around Madeira to enumerate *Ostreopsis* at 2 sites in the north (N 1-2), aad 3 sites in the southeast (SE 1-3), and at 1 site in the south (S) during occasional SCUBA-dives from 2008 to 2012 (Tab. 1, Fig. 1). All samples were preserved immediately with 4-5% formaldehyde in seawater until analysis. In the laboratory the samples were vigorously shaken during one minute to detach epiphytic dinoflagellates, and passed through a cascade of different meshes to retain the larger particles. *Ostreopsis* sp. (Fig. 2) cells were counted by light microscopy using a Sedgewick Rafter S52 counting chamber. The seaweeds were identified, blotted to remove excess water and wet weights determined; they varied from 0.5 to 77.6 g. Sea surface temperatures around Madeira usually range from 17°C in February/March to 24°C in September.

Cell numbers (Table) range from zero to 12639 cells per g wet weight, found in November 2009 at site S. Compared with other studies conducted during bloom phases, where cell numbers reached one to two million cells per g wet weight (e.g., 1.4 x 106 cells per g wet weight in New Zealand [10]), the maximum of present study is low.

Higher cell numbers were found at site S and very low cell numbers at sites N. At sites SE, both zero and high num-

Table 1 Ostreopsis *sp. cell numbers on seaweed substrate (cells per g wet weight ± 95% confidence limit) at sites around Madeira, 2008 to 2012.*

Sample #	Date	Site	Type of substrate	Cells g^{-1} WW
1	22.10.2008	S	*Stypopodium zonale*	158 ± 74
2	22.10.2008	S	turf (cf. *Jania*)	1176 ± 208
3	26.10.2008	SE 1	turf	0
4	26.10.2008	SE 1	Aufwuchs	11752 ± 2850
5	05.11.2008	S	Aufwuchs/*Sargassum vulgare*	1601 ± 380
6	07.12.2008	N 1	cf. *Lophocladia*	0
7	07.12.2008	N 1	cf. *Lophocladia*	0
8	07.12.2008	N 2	*Zonaria tournefortii*	86 ± 86
9	07.12.2008	N 2	*Gracilaria* sp.	5 ± 10
10	02.05.2009	SE 2	*Z. tournefortii*/cf. *Laurencia microcladia*	0
11	02.05.2009	SE 2	turf	0
12	05.06.2009	S	*Padina pavonica*	919 ± 141
13	05.06.2009	S	Aufwuchs/*P. pavonica*	3423 ± 437
14	05.06.2009	S	*P. pavonica*/*Halopteris filicina*/*S. vulgare*	2580 ± 188
15	08.06.2009	SE 3	*Corallina*	108 ± 43
16	08.06.2009	SE 3	turf	0
17	30.07.2009	S	turf	667 ± 162
18	03.11.2009	SE 3	Aufwuchs	2360 ± 357
19	03.11.2009	SE 3	*Dictyota*	2857 ± 415
20	18.11.2009	S	turf	4150 ± 340
21	18.11.2009	S	*Corallina*/turf	12639 ± 1105
22	08.06.2011	S	Aufwuchs	2962 ± 1140
23	12.05.2012	SE 3	*Dictyota*	1706 ± 194
24	12.05.2012	SE 3	*S. zonale*	417 ± 77
25	12.05.2012	SE 3	turf	702 ± 146
26	15.05.2012	S	*Stypocaulon scoparium*	487 ± 103
27	15.05.2012	S	cf. *S. vulgare*	303 ± 83
28	15.05.2012	S	turf/*Corallina*	8504 ± 487
29	14.08.2012	S	turf	11312 ± 1034
30	14.08.2012	S	turf/red algae	2856 ± 501

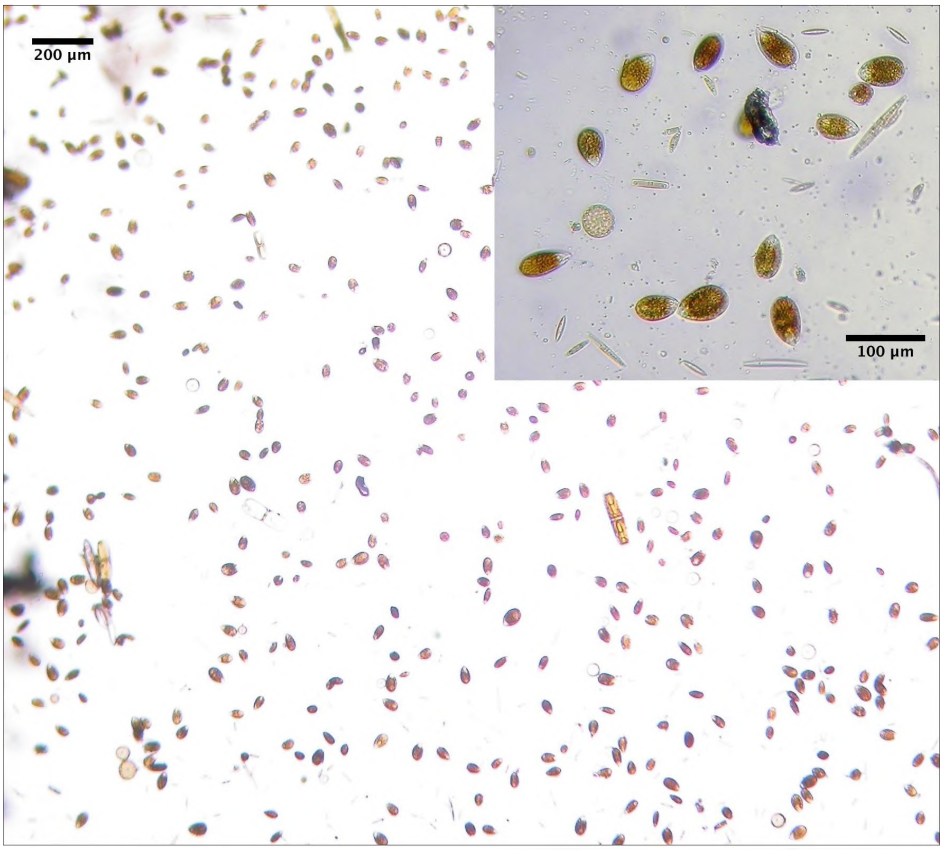

Fig. 2. Field sample of Ostreopsis *sp., LM, scale bar 200 µm, scale bar insert 100 µm*

bers of *Ostreopsis* cells were observed. It is likely that higher water temperature and a higher concentration of nutrients, as well as a less exposed environment favored the proliferation of *Ostreopsis* sp. [10-12] at sampling sites S and parts of SE. In general, the south coast is less exposed to the predominantly north-eastern swell.

Higher numbers of *Ostreopsis* were found mainly on very thin layers or turf substrates and less on erect, frondose seaweeds. The higher surface to volume ratio might be the reason for higher abundance of *Ostreopsis* sp. on thin and turf seaweed layers [13].

During counting and when observing live samples, species of *Prorocentrum* (Fig. 4), *Amphidinium*, *Coolia*, and *Gambierdiscus* were detected. The latter was observed in a fixed sample from October 2008, and recently (August 2012) in a live field sample (Fig. 5). This is the first record of *Gambierdiscus* sp. in the archipelago. Further studies are needed to determine the species. Recently, *G. excentricus* was described as a new species in the Canary Islands [14], so similar assemblages may be present in the nearby Madeira archipelago where oceanographic conditions are similar.

Our findings confirm the recent spread of more tropical HAB-species into warm temperate waters, which may be a response to climate change, introductions by ship traffic, and eutrophication of coastal areas [15, 16].

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Fig. 3. Ostreopsis *sp., LM, scale bar 25 µm*

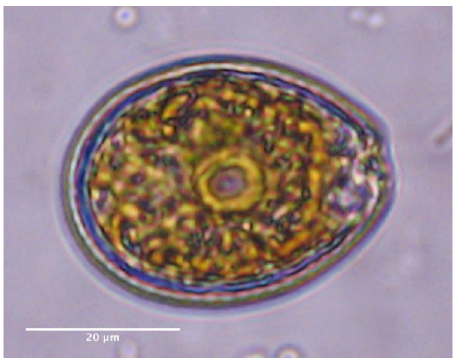

Fig. 4. Prorocentrum *sp., LM, scale bar 20 µm*



Fig. 5. Gambierdiscus *sp., LM, scale bar 50 µm*

A *Phaeocystis* bloom in the Cuban Archipelago



Fig.1. Cayo Largo del Sur, south-western platform of Cuba

The success of *Phaeocystis* and its impacts on marine ecosystems have been linked to their capacity to form large gelatinous colonies [1]. *Phaeocystis* colonies produce hemolytic substances, and are responsible for clogging fishing nets, repelling fish, for oxygen depletion, and for negative impacts on benthic life. These blooms affect tourism and recreational activities due to the water discoloration and deposition of thick layers of odorous foam on beaches [2].They also produce dimethyl sulphide, which produces cloud-condensation nuclei, increasing cloud cover and affecting regional climates [3].

During late March 2012, a bloom of *Phaeocystis* sp. occurred in Cayo Largo del Sur, a small cay in Los Canarreos archipelago, on the south-western Cuban platform (Fig. 1). This site is highly hydrodynamic coast. A red-brown water discoloration persisted for 15 days. The bloom occurred within 200 m of the shore and extended along 22 km of coast. As well as discoloration, abundant foam and mucilage were observed (Figs. 2-5).

Seawater was sampled for phytoplankton and physico-chemical analyses. The ratios Dissolved Inorganic Nitrogen/Dissolved Inorganic Phosphorus and Total Nitrogen/Total Phosphorus were calculated. The algal samples were studied using a light microscope Laborlux, Leica-Leitz with phase contrast equipped with Motic digital camera.

We observed large colonies formed by cells embedded in a firm gelatinous matrix. Cells are slightly kidney-shaped to irregular ellipsoidal to sphaeroidal, 5-6 µm in diameter, with two yellow-green to brownish, parietal plastids (Figs. 6-12). Our alga corresponds well with the generic description of *Phaeocystis*, however the species identification needs further studies.

High concentrations of ammonium and organic nitrogen were recorded along beaches. The high organic nitrogen/total phosphorus ratio indicates the presence of excess nitrogen (Table 1 and 2). *Phaeocystis* blooms are reported in nitrate-rich areas, either naturally or due to anthropogenic inputs [4]. High dissolved oxygen and chemical oxygen demand indicated the presence of a microalgae bloom with high production of organic matter.

Several factors could contribute to the bloom occurrence. Among them, sea surface temperature was slightly warm for this period, with temperature between 26.2 and 27.5 °C [5]. Strong winds (40 km h^{-1} with maximum intervals of 70 km h^{-1}); the velocity, dynamic, and direction of currents to the area (Fig. 13), from east to west could also increase nutrients (organic and inorganic) available in the Jagua depression [6]. But cultural eutrophication due to anthropogenic activities in Cayo Largo del Sur cannot be ruled out.

Sahara dust might be another factor involved in the occurrence of the *Phaeocystis* bloom. Two weeks before the bloom, the southern coast of Cuba



Fig. 2-5. Water discoloration and foam.

Table 1. N, P and Si nutrients at five beaches from Cayo Largo del Sur (DIN = Dissolved Inorganic Nitrogen, DON = Dissolved Organic Nitrogen, TN = Total Nitrogen, TP = Total Phosphorus).

Beaches	NO_2^- + NO_3^- µmol/L	NH_4^+ µmol/L	DIN µmol/L	DON µmol/L	TN µmol/L	PO_4^{3-} µmol/L	TP µmol/L	SiO_4^{4-} µmol/L
Sirena	0,07	0,35	0,42	35,99	36,41	0,04	0,25	1,78
Mal Tiempo	0,60	2,40	3,00	47,88	50,89	0,22	0,68	1,19
Blanca	0,28	0,04	0,32	42,39	42,72	0,07	0,51	1,53
Los Cocos	0,29	0,70	0,99	48,26	49,25	0,16	0,78	1,02
Tortuga	0,10	0,87	0,96	63,46	64,42	0,10	0,15	3,64

Table 2. DIN:PO43- and TN:TP ratios at five beaches from Cayo Largo del Sur.

Beaches	DIN/PO_4^{3-}	TN/TP
Sirena	11	144
Mal Tiempo	13	75
Blanca	5	83
Los Cocos	6	64
Tortuga	10	439

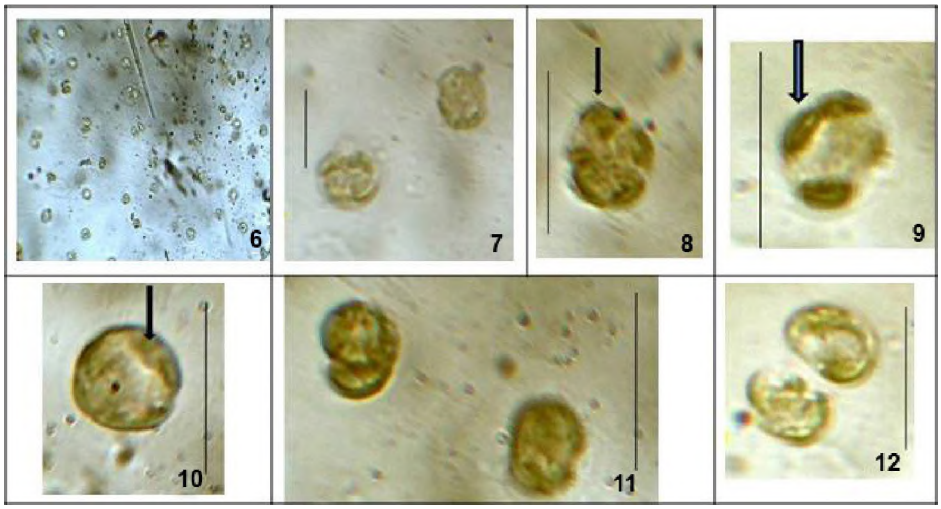

Fig.6. Interior detail of colonies. Fig. 7-12. Different forms of colony cells (arrows=chloroplasts). Scale = 10 µm

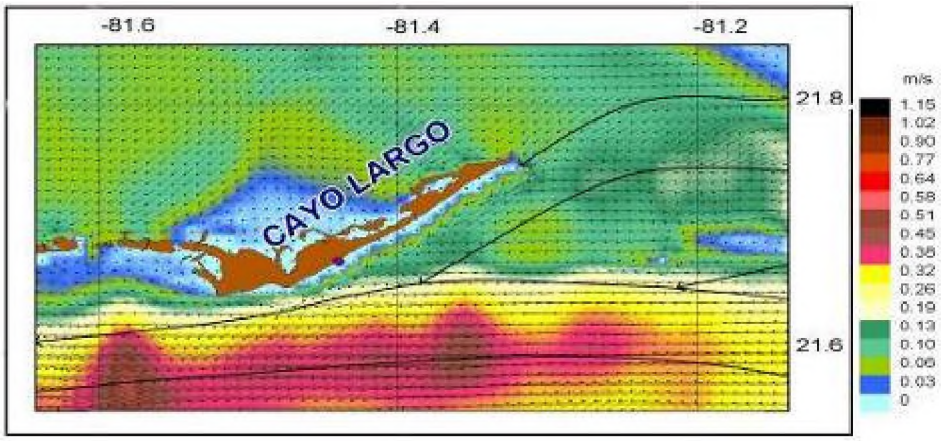

Fig. 13. Offshore currents around Cayo Largo del Sur, south-western platform of Cuba (March 22, 2012).

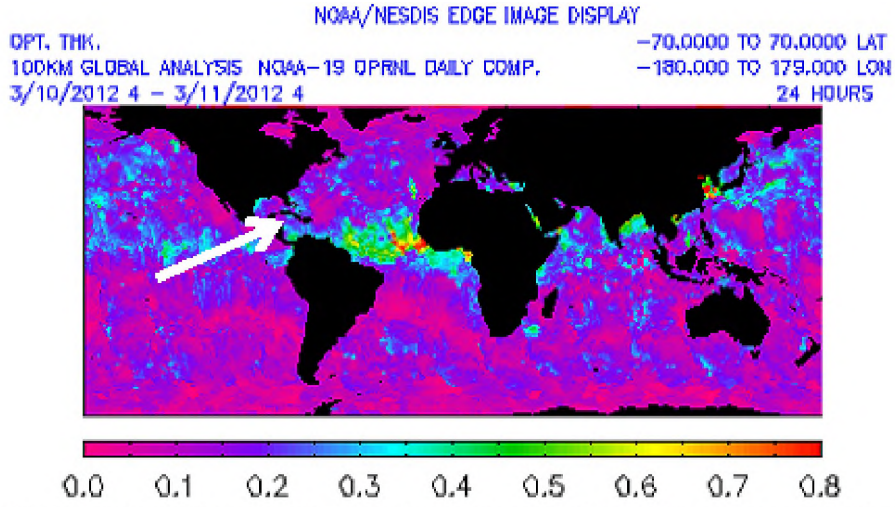

Fig. 14. Inputs of Sahara dust in the south-western coast of Cuba (during two weeks before the Phaeocystis *bloom).*

received inputs of Sahara dust (Fig. 14). It has been argued that desert dust provides iron which contributes to phytoplankton blooms in tropical and subtropical coastal environments [7].

No major mortality of marine organisms was observed in the key; however the bloom affected tourism because of the presence of abundant foam, water discoloration and bad odours. To our knowledge, this is the first record of a *Phaeocystis* bloom in the Caribbean islands.

Acknowledgements

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Gambierdiscus in Kuwait

The diversity of benthic dinoflagellates along Kuwait's shore has been well documented recently [1, 2], but with emphasis on sand-dwelling intertidal species and hyper-saline habitats [3]. During recent surveys of the coastal benthic biota along Kuwait's shore initiated by Kuwait Institute for Scientific Research (KISR) in November 2011 – December 2012, various coastal habitats were examined and numerous samples including intertidal and subtidal sediments, seagrasses, macroalgae, and algal turf were collected.

On 17 November 2012, subtidal sandy sediments were collected within small inshore Qit'at Julai'ah coral reef (28°53'4''N; 48°16'38''E) on the southern Kuwait's coast (Fig.1). Sediments and near bottom water were sampled by snorkeling at 2 m depth. In both sediment and water samples, we found low numbers of globular dinoflagellate cells that resembled *Gambierdiscus*. Similar cells were found epiphytically on floating brown macroalgae from two sampling sites to the south of the Khiran in a small semiclosed lagoon (28°38'6''N; 48°23'28''E), and on the shore (28°37'47''N; 48°23'30''E) on 24 November 2012 (Fig. 1). *Gambierdiscus* cells were present in two of the 16 macroalgae samples analyzed, and were observed in low abundance on *Padina tetrastomatica* (6 cells g^{-1} wet weight) and on *Sargassum oligocystum* (4 cells g^{-1} wet weight). *Gambierdiscus* was found at water temperatures of 23.5-25°C and at salinities of 41.2-42.4. It occurred along with other epiphytic dinoflagellates of the genera *Amphidinium, Coolia, Ostreopsis,* and *Prorocentrum*.

Examination by light and epifluorescent microscopy allowed detailed observation of morphology and plate tabulation (Fig. 2). Cells were photosynthetic with numerous golden-brown chloroplasts, globular in shape in lateral view, oblong in apical/antapical view and slightly laterally flattened, with dome-shaped epitheca and truncate-conical hypotheca. Cells had a depth of 54-73 µm, a width of 46-61 µm, and a length of 49-70 µm, with an average width-to-depth ratio of 0.86 and length-to-width ratio of 1.14. The pre-median cingulum was deeply excavated, descending, and was displaced by one x its width. The apical pore plate (Po) was elongated, characteristically fish-hook shaped and centrally located. The second antapical plate 2'''' was fork-shaped and rather large, with robust and beveled fork edges. Based on the morphological characteristics of the

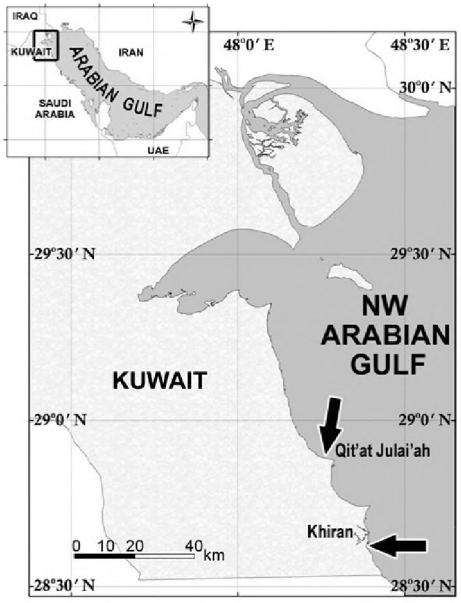

Fig. 1. Map of Kuwait showing where Gambierdiscus yasumotoi *was detected.*

cells, the globular *Gambierdiscus* species from Kuwait was assigned to *G. yasumotoi* Holmes 1998. Following the original description [4] and key to the species of *Gambierdiscus* [5], the cell shape, dimensions, plate tabulation and structure of the 2'''' from Kuwait's material confirmed the identification and allowed to distinguish our finding from the other globular *Gambierdiscus* species, *G. ruetzleri* [5].

Since the original description of *G. yasumotoi* from macroalgae on the coral reef of Pulau Hantu Island (Singapore) [4], its distribution has been referred to as tropical Western Pacific [5-7]. However, recently the globular *Gambierdiscus* species morphologically close to *G. yasumotoi* has been reported from coastal waters of Pakistan, sub-tropical North-West Indian Ocean [8]. The detection of *G. yasumotoi* in Kuwait's coastal area is the first record of the possible ciguatera agent in the Arabian Gulf, and the second report of this species from the Indian Ocean.

G. yasumotoi produces a maitotoxin-like compound lethal to mice [4], and its toxicity has been confirmed by detection of toxic strains in Indonesian waters [7]. The detection of this toxigenic dinoflagellate in Kuwait indicates the potential for future occurrence of Ciguatera Fish Poisoning (CFP).

G. yasumotoi was recorded in Kuwait coastal habitats in low numbers, but its density may be underestimated from our samples: it was found on various substrates, in sediment, and attached to floating macroalgae. While attached

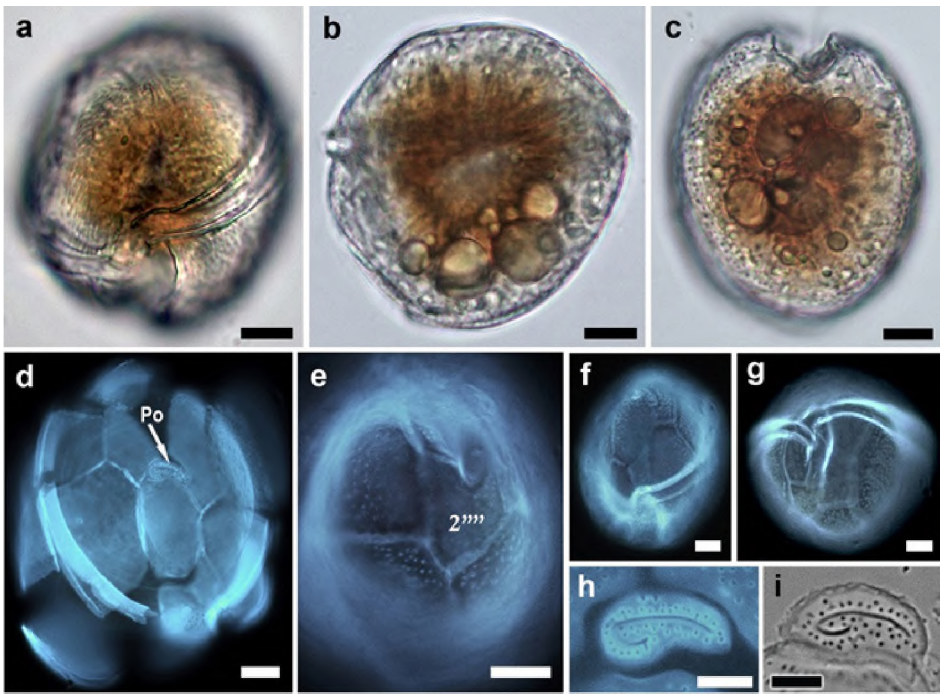

Fig. 2. Gambierdiscus yasumotoi *from Kuwait. a-c - light microscopy of cell in ventral (a), lateral (b) and antapical (c) view; d-g – epifluorescence image of squashed theca in apical view (d), cell in antapical view with the diagnostic 2'''' plate (e), and cell in apical (f) and ventral (g) view; h-i – apical pore plate (Po). Scale bar = 10 µm (a-g), 5 µm (h, i).*

macroalgae in shallow habitats are the prefered hosts of *Gambierdiscus*, their detached floating branches may be exposed to wave action, leading to detachment of the epiphytic dinoflagellates.

The southern part of Kuwait's marine area closely resembles the preferred habitats reported for *Gambierdiscus* [9] in terms of annual water temperature, salinity, hydrological features and available substrates to which cells can attach. Kuwait's coast stretches for about 180 km, occupying the northwestern corner of the Arabian Gulf in latitudes between 30°N and 28°30'N (Fig. 1). Hypersaline, shallow and subtropical environments provide a broad range of habitats, from extensive mudflats in the north to numerous sandy and rocky beaches extending to the Saudi Arabian border. Moreover, some of the world's most northerly coral reefs are located within southern Kuwait waters, and *Sargassum* grows in a discontinuous linear strip parallel to the coast where appropriate substrates exist. The average annual seawater temperature is 23.8°C, varying seasonally from 14°C during winter up to 30.5°C in summer, whereas the southern and offshore waters are generally warmer with an annual average temperature of 25.6°C [10].

Kuwait appears to be free of ciguatera; there are no official reports of CFP in this area to date [11], and *Gambierdiscus* has never been recorded here previously among other potentially ciguatera-related benthic dinoflagellates from the genera *Ostreopsis* and *Prorocentrum* [12, 13]. Our detection of *G. yasumotoi* in Kuwait is the first record of possible agent of CFP in the NW Arabian Gulf, and indicates a need for further studies of benthic dinoflagellate assemblages in the region.

Acknowledgements

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Bloom of *Pyrodinium bahamense* var. *compressum* in Ambon Bay, eastern Indonesia

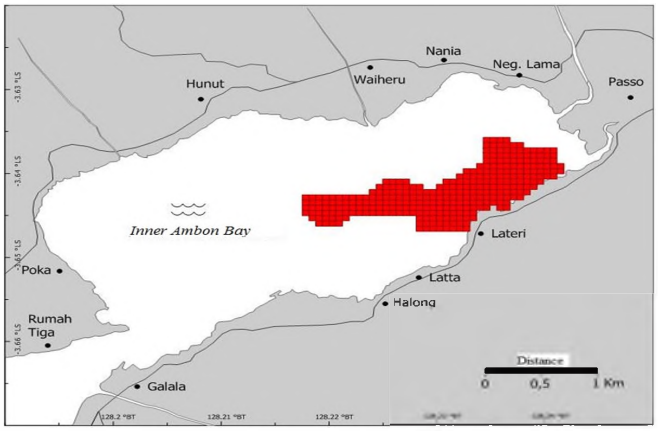

Fig. 1. Bloom area of Pyrodinium bahamense *var.* compressum *in Inner Ambon Bay, July 2012.*

Ambon is a small island located in eastern Indonesia with a population of about 600,000 people. It has a tropical climate influenced by northeast and southeast monsoons. Annual rainfall is approximately 600 mm occurring from May to September, associated with the southeast monsoon season when cooler air from Australia blows to this area. Dry season is associated with the northwest monsoon from October until March, characterized by increased air temperature and less rainfall.

Ambon Bay is an estuary with a sill; the inner bay averages 30 m depth and about 6 km^2 in area. The outer bay, which opens to the Banda Sea, is about 25 km long. The narrow and shallow sill, 15 m deep and 300 m wide, limits water circulation in inner Ambon Bay, making it susceptible to stagnation [1]. Increased development in the Bay and associated pollution inputs may alter the chemical regime of the inner bay.

Pyrodinium bahamense var. *compressum* is responsible for paralytic shellfish poisoning (PSP) in the tropical Pacific. This armored, bioluminescent dinoflagellate causes toxic red tides in coastal waters of several countries in southeast Asia; first event was reported in Papua New Guinea in 1972 [2]. It occurs in the Philippines, Malaysia, Indonesia and other countries in the region. In Indonesia, PSP caused by *P. bahamense* var. *compressum* was first recorded in Kao Bay in 1994 [3]. Subsequently, the first PSP followed in Ambon Bay which led to illness of more than 30 people and the death of 3 children after consuming shellfish collected in the area [4].

Beginning in 2007, monitoring of Ambon Bay water quality and plankton has been conducted to examine water quality alterations due to land development on Ambon Island. It has been reported that the land degradation produces massive sediment pollution which can trigger phytoplankton blooms in this area [5]. High precipita-

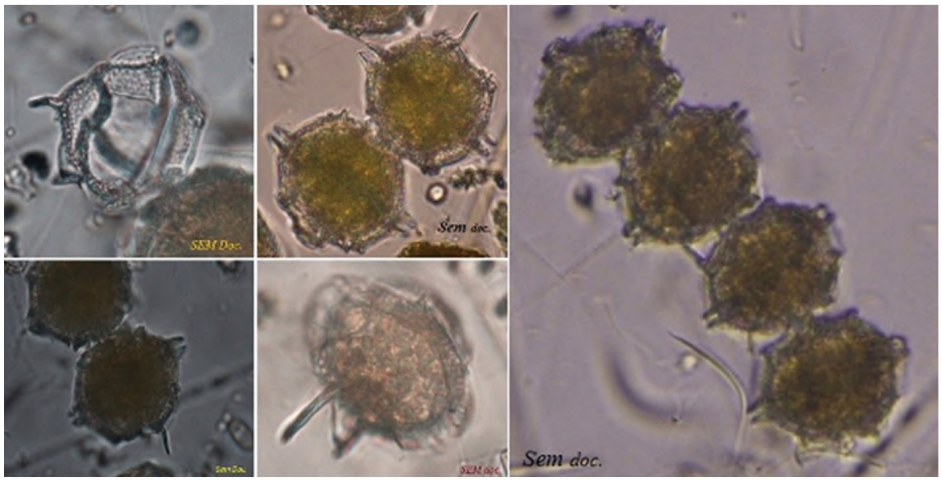

Fig. 2. Pyrodinium bahamense *var.* compressum *found in Ambon Bay, July 2012 (photo : Sem Likumahua, Plankton Lab, Ambon)*

tion in recent years has led to eutrophication in Ambon Bay during the rainy season. Monitoring showed that *P. bahamense* var. *compressum* is normally a minor component of the phytoplankton community between June and July [data unpublished]. The most recent bloom was encountered in July 2012. Two days with heavy rain trigged high runoff, and about 110 hectares of yellowish brown water occurred in the inner bay. The bloom was from 2 meters depth to the surface as shown by the CTD Chl-a profile. The bloom was dominated by *Pyrodinium* and the diatom *Chaetoceros* sp. which reached 2 x 106 and 1.2 x 106 cell L^{-1} respectively. A week later, on 1 July 2012, human illness was reported after consumption of shellfish collected in Ambon Bay. Seven people were nursed in the local hospital for a week before they recovered. The bloom killed hundreds of fish cultured in inner Ambon Bay. The Ambon city government issued warnings against harvesting shellfish in the inner bay. Monitoring of this species abundance and associated hydrological conditions need to be conducted continuously in Ambon Bay.

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Alexandrium tamiyavanichi on the north coast of Perú



Fig. 1. Distribution of Alexandrium tamiyavanichi*. Letters indicate localities where species has been recorded. (*) This report.*

Alexandrium tamiyavanichi Balech is a producer of potent Paralytic Shellfish Poisoning (PSP) toxins. It has been recorded in the Gulf of Thailand, Malaysia, Japan, Vietnam, Hong Kong, Philippines, South Africa, northeastern Brazil and the Mexican Pacific (Figure 1). Harmful algal blooms associated with toxicity in molluscs occur in Peruvian waters, causing human health risks and impacts on the shellfish industry. Therefore, shellfish production zones are monitored weekly under a national program. At least four *Alexandrium* species have been recorded on the Peruvian coast: *A. peruvianum* (originally described as *Gonyaulax peruviana*[1], the non-toxic *A. monilatum*[2] and *A. affine*[3] and recently, the potentially toxic dinoflagellate *A. minutum* (Baylón *et al.*, in prep.). This report describes the occurrence for the first time of *Alexandrium tamiyavanichi* in Peruvian waters and the Tropical East Pacific, and its main morphological characters.

In October (spring) 2011 and February (summer) 2012, phytoplankton samples were taken in Paita Bay (05°04'17"S-81°07'19"W) and Máncora (04°06´29´´S-81°04´06´´W), north Perú (Fig. 2). These areas are not dedicated to commercial mariculture: they are influenced by subtropical equatorial waters and in part by upwelling, small estuaries and urban discharges. Samples for species identification were collected by vertical net hauls (10 µm mesh size) and preserved with 2% formalin. Samples for quantification were taken with a hose (15 m) or 5L Niskin bottle (5 m depth) and were fixed with Lugol solution. Surface temperature was measured with a digital thermometer. Counts were made by the Utermöhl method using a Nikon inverted microscope Eclipse TS100.

Qualitative analysis revealed an *Alexandrium* species forming chains of up to 10 cells, which had the same length and wide (40 µm average) and 35 µm transdiameter (Fig. 3A and 4A). This species was present in very low concentrations (≤ 40 cells L^{-1}). Surface temperature was 20°C in Paita and 25°C in Máncora. Microscopic examination showed an oval apical pore complex (APC) with large central comma-shaped foramen, various small marginal pores along the left margin, and a large anterior attachment pore (aap) near the right margin of the comma head (Fig. 3B). The first apical plate (1') was rhomboid and wide connected directly to the apical pore plate containing a ventral pore (vp) on the posterior part of the right margin (Fig. 3C and 4B). The anterior sulcal plate (S.a.) had a precingular part (p.pr.), almost triangular anteriorly (Fig. 4B) and a large sharp hook shaped

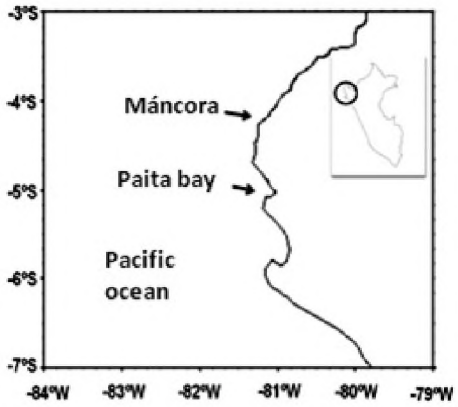

Fig. 2. North coast of Perú showing the two localities where A. tamiyavanichi *was found.*

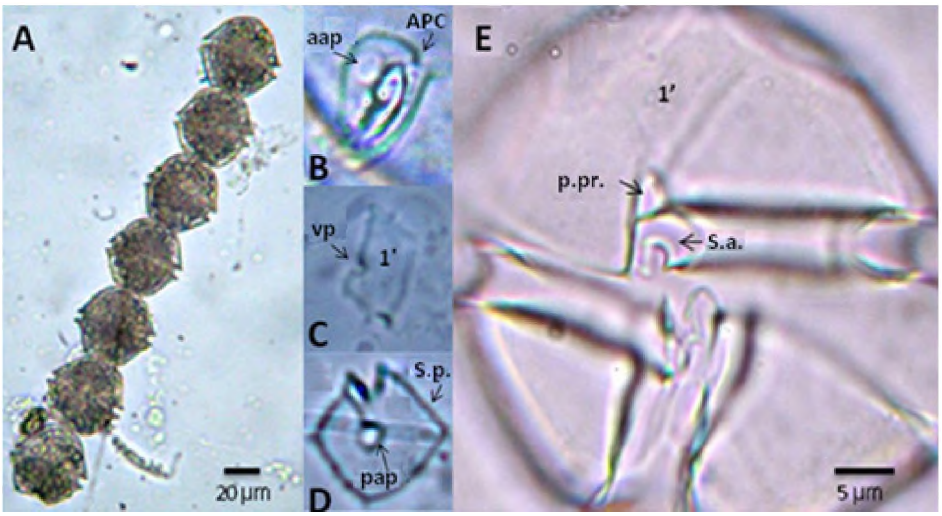

Fig. 3. Light micrographs of A. tamiyavanichi *from Paita Bay, Piura, Peru. A) Cells in chains. B) View of apical pore complex (APC) showing large anterior attachment pore (aap). C) View of 1' plate, wide and rhomboid with its ventral pore (vp). D) Detail of posterior sulcal plate (s.p.) showing posterior attachment pore (pap). E) Central view of intact cell showing 1' plate and anterior sulcal plate (s.a.) and its precingular part (p.pr.).*

apophysis posteriorly. Both regions are divided by a transversal furrow (Fig. 3E). A posterior attachment pore (pap), almost central, connected to the right margin plate through a narrow furrow (Fig. 3D) was observed in the rhomboid and nearly symmetrical posterior sulcal plate (S.p.) of *tamarense* type. Based on these characters and refs (5, 6), the species was identified as *Alexandrium tamiyavanichi*.

Differences between isolates of *A. tamiyavanichi* from Peruvian waters and those originally described by Balech in Southeast Asia were minimal. *A. cohorticula* is morphologically very similar to *A. tamiyavanichi*; Balech (1995) distinguishes the two according to the anterior sulcal plate (S.a.) which is larger and square, 1' plate is narrower and irregular and the posterior edge is right and almost horizontal; besides, it also has a wide sulcal fin. But the two species may be synonymous4.

Off the northern Peruvian coast in 06°S, there is a convergence of warm Tropical Eastern Pacific (TEP) water and cold Humboldt Current water. It is unlikely that *A. tamiyavanichi*, generally found in tropical waters, and stenohaline, extends far from the TEP, except during high environmental variability due to ENSO (El Niño Southern Oscillation). The presence of this dinoflagellate might be caused by current transport, or it may have been introduced by human-assisted dispersal such as ballast water transport. As far as we know, this is the first record of *A. tamiyavanichi* from Peru and in the TEP.

At present, cases of human poisoning due to PSP are unknown in Perú, despite sporadic occurrence of these toxins mainly in scallops from the central coast and associated provisionally with *A. peruvianum*[8]. Elucidation of the toxic nature of this species is still ongoing in Perú. The presence of *A. tamiyavanichi* presents a health risk via potential contamination of molluscs and an economic threat to the local seafood industry. At this time, its follow up should therefore be included in the national monitoring program. Furthermore, countries within the TEP subregion from Ecuador to Costa Rica should also be alert. Likewise, in Peruvian Northern coast we should be alert in north Peruvian coastal waters for the possible presence of other toxic dinoflagellates such as *Pyrodinium bahamense* and *Gymnodinium catenatum*, which occur in areas where *A. tamiyavanichi* is present.

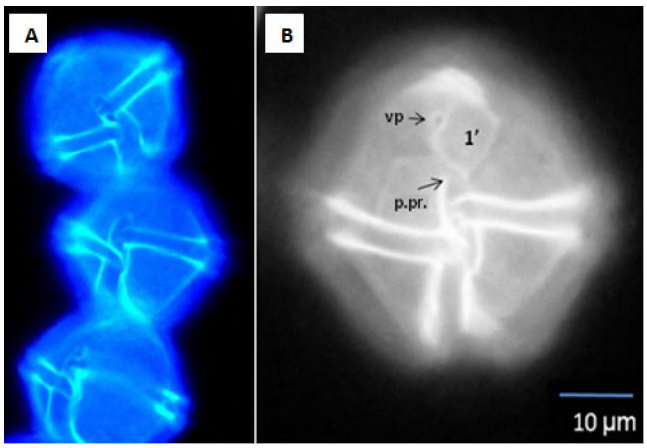

Fig. 4. Epifluorescence microscopy of A. tamiyavanichi. *A) Cells in chain. B) Details of an intact cell showing ventral pore (vp) and precingular part (p.pr.).*

Acknowledgments

We thank Santiago Fraga (IEO, Vigo, Spain) for confirmation of the species, CERPER-Piura for their support, and Carolina Fuentes Rivera-Trucíos for improving the English.

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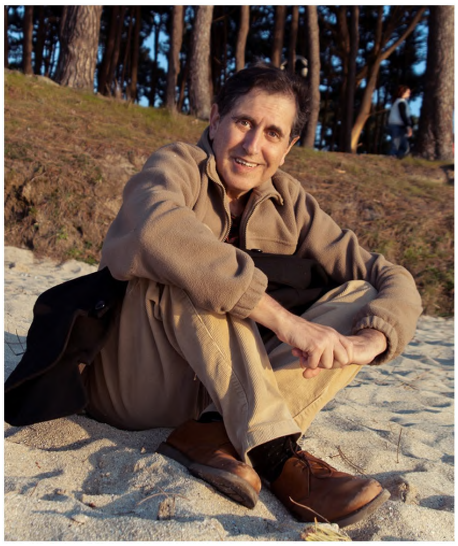
Manuel Zapata (in memoriam)

The changing colours of the sea make up one of the most captivating views of nature. For those who had the privilege of being co-workers of Manuel Zapata, the hues of colour and light at sea surface always bring to our minds the immense variety of plankton pigments, whose knowledge he contributed so much to enlarge. Unfortunately, his death in April 2012 abruptly ended a fruitful scientific career that provided us not only many concrete achievements but also a special mode of contemplating nature.



Manuel's life was always linked to the sea. Born in A Coruña (on the Atlantic edge of continental Europe), he received his BSc and PhD degrees in biology from Santiago de Compostela University. He worked for many years in the oceanographic research section of the Marine Research Centre (Galician Autonomic Government) and had recently (2009) joined the Institute of Marine Research (Spanish Council of Scientific Research). He was passionate about his scientific interests and everyone who listened to him talking about phytoplankton, marine ecology or algal physiology was immediately fascinated with the topic. He made substantial contributions to our knowledge of algal pigments. These are summarized in 50 peer-reviewed publications and book chapters on the characterization of their chemical structures, the development of innovative chromatographic methods for their analysis, their distribution in plankton taxa and their use as signatures for characterizing natural phytoplankton communities.

A few examples associated with HAB species are the pigment studies of haptophytes (Zapata et al. 2001, 2004), dinophytes (Zapata et al. 2012) and the diatom *Pseudo-nitzschia* (Zapata et al. 2011).Many of these publications involved different Spanish and foreign laboratories, as he was always ready to engage new collaborations to widen the significance of his studies and learn from the expertise of other colleagues. They will surely miss his knowledge and irreplaceable contribution in the future. Manuel participated in several international HAB conferences, in the NATO Bermuda workshop celebrated in 1996, and in the more local Iberian meetings on HABs. He was an outstanding member of the HAB "club".

To his friends, Manuel was exceptionally bright, gifted with a brilliant (and rather surrealistic) sense of humour. He was the best colleague and the best friend. We will always remember him.

José L. Garrido, Santiago Fraga and Francisco Rodríguez

Ernesto Fattorusso Obituary 1937-2012

I am profoundly saddened to communicate to the HAB community that Ernesto Fattorusso, professor emeritus of Organic Chemistry at the University of Napoli Federico II, passed away on 7 July 2012. He was a distinguished scientist appreciated not only for his enthusiasm about science, but also for his brilliant personality, extraordinary creativity, communication skills, kindness, thoughtfulness, and great humanity. Ernesto was endowed with an elegant way of approaching people, deep humbleness in expressing his ideas, and great enthusiasm in passing on his knowledge. This is a huge loss not only for the Neapolitan group, but also for the entire marine natural products community.



Ernesto Fattorusso was born in 1937. He got his degree in Chemistry in 1960 and became full professor of Organic Chemistry in 1975. He served two terms as dean of the Faculty of Pharmacy and he has long been Director of the Department of Chemistry of Natural Products at the University of Napoli Federico II. His outstanding research activity, which is documented by more than 320 papers, is impressive for the range and variety of contributions he made, many of them pioneering. He focused his research on the chemistry of bioactive metabolites from marine organisms. Over the past two decades of his career, he played a pivotal role in depicting the phycotoxin scenario across the Mediterranean Sea. With LC-MS detection and NMR-based structural elucidation, he identified many toxins belonging to the group of okadaic acid, yessotoxins, spirolides, and lately of palytoxins, among others.

Ernesto Fattorusso was held in high regard by the whole Marine Natural Products Chemistry community. Due to his scientific achievements in the field, he received a number of prizes, the last of which being the Paul Scheuer Award 2010, awarded for the first time to a European scientist.

Patrizia Ciminiello
Dipartimento di Chimica delle Sostanze Naturali, Napoli, Italy

Tribute to Phil Busby: 1947-2013

Phil Busby, in his capacity as Principal Advisor (Shellfish) at the Ministry of Agriculture and Forestry (MAF) in New Zealand, touched many in his productive career of putting quality assurance of molluscan shellfish on the world map. He retired from this position due to poor health on 31 October 2012 and passed away on 8 March 2013.

Phil initially wanted to become a plumber, but changed his mind to train as an Inspector of Health in the New Zealand Department of Health. He enjoyed many adventures doing field work in Wellington and Invercargill including rare trips to the mutton bird islands off New Zealand's southern coast. He gave up the field work to join the Department's Head Office to run the training course for health inspectors in 1983. Those who went through the course under Phil will never forget his no-nonsense marking style! From 1989 MAF took over running the New Zealand Shellfish Quality Assurance Programme and with it came Phil – initially on secondment from the Department of Health. He became responsible for all public health aspects of the New Zealand Shellfish Quality Assurance Programme, and in 1992 wrote the first New Zealand Shellfish Standard. The 1993 Karenia bloom in Hauraki Gulf and resulting Neurotoxic Shellfish Poisoning outbreak which led to New Zealand wide harvesting closures, catapulted him into the national and international spotlight. He did rise to the challenge, simply was always there, made a difference through persuasion and presence, and maintained his position of authority with grace, charm and flair. He effected major change through (sometimes polarising) debate and arguments, preferably over a few beers in a bar. Debates on human health significance of brevetoxins, gymnodimine, pinnatoxins, Phil has seen them all. He was responsible in 1996 for the requirement that Cawthron Institute acquire accreditation prior to phytoplankton being accepted as part of the New Zealand Marine Biotoxin Management Programme. Phil organised twice yearly Seafood Safety workshops through what is now called Ministry of Primary Industry. The informal proceedings of these meetings capture the significant changes in focus



as research and regulation on harmful algal blooms matured in New Zealand and tried to keep up with each other. In 2001 he was responsible for the validation and approval of LC-MS methods for ASP and DSP marine biotoxins, in 2003 for introducing mandatory hydrolysis of DSP samples, and in 2004 the approval of a LC-MS Screen Test Method for Brevetoxins. Each of these initiatives represented international trend setting decisions and showed the resolve of Phil to provide novel outcome based solutions to improve public health protection. In 2004 Phil was asked to chair the Joint FAO/IOC/WHO Expert Workshop in Dublin on Biotoxins in Molluscan Bivalves and he went on to Chair the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Molluscan Bivalves in Oslo. His role was facilitated through his position as Vice Chair of the UNESCO Intergovernmental Oceanographic Commission Panel on Harmful Algae Blooms and Chair of the Panel Task Team on Biotoxin Monitoring and Management. The respect in which he was held by his national and international peers showed in his re-election in 2009 to both positions. In 2006 Phil drafted and put in place the Bivalve Molluscan Shellfish Regulated Control Scheme (affectionately known as the BMSRCS) comprising new shellfish safety regulations and specifications to take the New Zealand Shellfish Programme through the years ahead and provide access to markets throughout the world.

Thank you for all your contributions, Phil. You will be a hard act to follow!

Gustaaf Hallegraeff
Institute for Marine and Antarctic Studies, University of Tasmania, Australia

Lesley Rhodes, Lincoln McKenzie
Cawthron Institute, New Zealand

Cath McLeod
South Australian Development Research Institute, Australia

Doug McLeod
Glenelg Shellfish, Australia

Dorothy J. McCoubrey
Dorothy-Jean & Associates, New Zealand

Jim Sim
Ministry for Primary Industry, New Zealand

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Charles S. Yentsch, Visionary Ocean Scientist, dies at 85

Charlie was honored as a leader and innovator. Yet he will be remembered as a "beloved maverick" by those who knew him best. Learning about the seasons, cycles, and ever-changing dynamics of the ocean was Charlie's life-long passion. Creating a positive and equitable learning environment shipboard, in the laboratory, and at home was his commitment. Compassion was his hallmark.

Since childhood, Charlie was a student of the seas. As a kid in Kentucky, he was fascinated with fossils of marine organisms in the limestone. As a California teenager, he studied waves from his post as a lifeguard and experienced waves as a surfer. He set up a business to free-dive for and market abalone. The diving triggered his fascination of sunlight interacting with water. As an adult, he turned his father's and uncles' mechanical skills with trucks toward the measurement of light in the sea and the tiny marine organisms dependent on sunlight. He held a reverence for all life and found the simplest forms most compelling. Always non-hierarchical in his belief of human potential, he put every person and every idea on a par to be tested. He cherished skeptically-minded colleagues.

He served in the U.S. Navy during WWII as a fire-fighting instructor in San Diego (1944-1946). The GI Bill and no job prospects encouraged his formal education at the University of Louisville (B.S. 1950), Florida State University in Tallahassee (M.S. 1953), and the University of Washington in Seattle. Employment included the Woods Hole Oceanographic Institution, Woods Hole, MA (1956-1967); Nova University Oceanographic Center in Fort Lauderdale, FL (1967-1970); and the University of Massachusetts Marine Station, Gloucester (1970-1974). In July of 1974, Charlie and wife Clarice founded the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, ME. Within a few years, this institution had become a world-class laboratory attracting innovative scientists from around the world.



Photo: Bob Mitchell

Charlie was regarded as one of the great pioneers of modern oceanography, and one of the first to conceive of and advise NASA on the potential of satellite observations of ocean biology. In 1985 he received a Ph.D. *Honoris causa* from Long Island University, Southampton, NY. The American Association of Limnology and Oceanography presented Charlie with a Lifetime Achievement Award in 1999. In 2010, The Oceanographic Society bestowed the honor of the Nils Gunnar Jerlov Award and also named him a Fellow of The Oceanography Society.

Charlie had a deep sense of time, space, and place. He enjoyed going to sea and world travel, with his family accompanying him when possible (despite a quote on his office door that read "Traveling with children is like traveling third class in Bulgaria"). Yet he was most fond of being home, defined by a wood fire, kids and pets, "tea" time, humor, a well-prepared meal fresh from the sea, and then the evening topped off with a puff on a Partagas and a good read. His descendents are three sons and two granddaughters. Charlie was proud that each one of the five is now an astute naturalist.

If you are so inclined, the next time you set sail, see sunlight reflecting off the ocean, dance the swing, listen to Dixieland, or sing Spirituals – raise a toast to Charlie.

Clarice Yentsch
Fort Lauderdale, Florida, USA

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IOC/WESTPAC Scientists Develop Technical Guidelines for Ciguatera Studies

On 5-6 November 2013, an IOC/WESTPAC Training Workshop on Toxic Marine Organisms was organized at the University of Tokyo, Japan, with one of its major objectives to develop regional technical guidelines on ciguatera studies, drawing on experiences from Japan.

Ciguatera Fish Poisoning (CFP or Ciguatera) is a foodborne illness caused by eating certain reef fishes contaminated with ciguatoxins and its analogues originally produced by toxic dinoflagellates of the genus *Gambierdiscus.* Large reef-dwelling fish in tropical and sub-tropical waters accumulate marine toxins by eating toxic algae growing on coral reefs, and are most likely to cause ciguatera poisoning. CFP is difficult to prevent as ciguatoxic fish do not carry a strange odor or taste, and cooking fish does not inactivate the heat-stable toxin.

CFP is the most problematic algal poisoning syndrome, with reported cases amounting to 20,000 – 30,000 per year, but no effective management system has been developed yet in the IOC/WESTPAC region. Despite the low number of reported cases of CFP in the region, scientists are concerned that there must be many unreported cases in the region, and reports may increase in the near future, given that CFP occurs mainly in tropical and subtropical coral area.

"We must provide scientifically reliable information to prevent and reduce the impact of CFP" said Dr. Dao Viet Ha, WESTPAC Project Leader on Toxic Marine Organisms, emphasizing the need for one regional standard method for CFP studies. The two days workshop, with the contribution of invited Japanese experts on ciguatoxins and other marine toxins, aimed to improve knowledge on the present situation of CFP in Member States, and develop a regional technical guide on the collection of ciguatera poisonings data, including clarification of clinical symptoms, identification of possible causative food, sample collecting for screening for toxicity and toxin identification.

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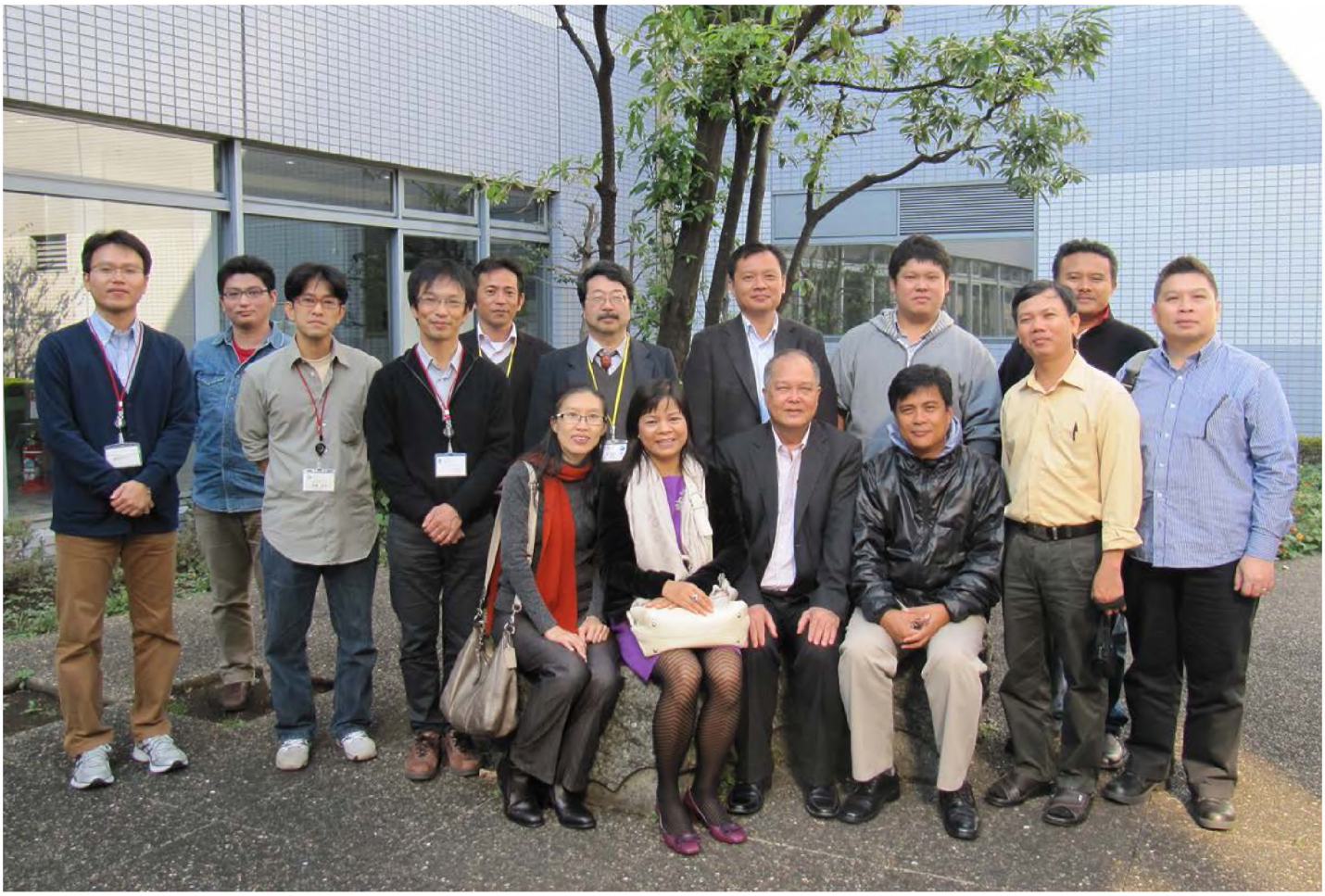

Participants in the 3rd IOC/WESTPAC-TMO training workshop in Japan, 5-6 Nov. 2013.

First report of an *Ostreopsis ovata* bloom on Abruzzo coast (W Adriatic) associated with human respiratory intoxication

At the end of August 2013, the first bloom of the benthic dinoflagellate *Ostreopsis ovata* occurred on the coast of "Trabocchi", Chieti Province, in the Abruzzo Region, Western Adriatic Sea. The bloom formed a brown velvet mat covering natural and artificial rocks, seaweed thalli, and mollusc shells, and persisted for about 1 month. By the end of September, *O. ovata* was only found sporadically. This species was first reported on the Abruzzo coast in summer 2008 [1]. Since then, it has been quite abundant every year, but it had not previously formed a bloom nor raised concerns. Here we describe the first event with human intoxications and mussels/octopus mortality related to an *Ostreopsis* bloom in Abruzzo coastal waters. The distribution of *Ostreopsis* was mostly observed at three rocky beaches, where several cases of human intoxication were recorded, from late August to mid-September 2013.

Ostreopsis species are benthic dinoflagellates generally recorded in tropical and subtropical seas, but the worldwide records of this genus have increased markedly in the last decade and this trend is likely to continue [2]. *Ostreopsis* belongs to the family Ostreopsidaceae: cells are narrow, ovate in dorso-ventral view with biconvex, drop-shaped theca, scattered pores and eight sulcal plates [3]. The life cycle includes a resting stage, probably a hypnozygote [4]. This species is occasionally planktonic but generally benthic on macroalgae, rocks, sediment or detritus aggregates, and forms a mucilaginous matrix within which the solitary cells can move [5].

Ostreopsis ovata produces palytoxin-like (PTX) compounds [6]. Blooms of *O. ovata* can cause hypoxia, anoxia and benthic invertebrate kills by forming mucilaginous layers hosting thousands of cells covering both biotic and abiotic substrates [7]. In addition, formation of toxic aerosols by wave action can produce asthma-like symptoms. In temperate zones, *Ostreopsis* blooms are associated with intoxication by inhalation [8], skin irritation by contact, and mass mortalities of invertebrates. Intoxication by ingestion has not been reported, even when PTX and/or analogs have already been found in seafood by chemical or toxicological analysis [9].

O. ovata has been recorded in the Mediterranean since the 1970s on French coasts. It was first detected on the western coast of Italy (Tyrrhenian Sea) in the 1980s [10]. In the last decade, massive blooms of this species have become more frequent, above all in the Tyrrhenian and Ligurian Seas, causing death of benthic organisms and human health problems such as respiratory difficulties and skin irritation [11]. Over the last decade, people swimming or exposed to marine aerosols along the Tyrrhenian and Adriatic coasts of the Mediterranean Sea have reported a range of symptoms (rhinorrhoea, bronchoconstriction, coughing, fever, dermatitis) associated with high densities of *O. ovata*. In the early 2000s, the most important inhalation intoxications were observed in Italy in 2001 [12] and in 2005 [13], and in Spain in 2004 and 2006 [14], affecting more than 200 persons each time (swimmers, beach goers, local residents). Since then, *Ostreopsis* blooms has been detected in several northern and southern Mediterranean countries [15].

The Trabocchi coast is characterized by shallow rocky coves with pebbly bottoms. The appearance of the *O. ovata* bloom led to intense sampling; it was mostly present off three rocky beaches, Rocca San Giovanni, Fuggitella and Fossacesia (Fig.1). Sampling was carried out from 29th August to 13th September when respiratory problems were reported and poisoning occurred. The water column and different substrates were sampled for phytoplankton and chemical-physical analysis. Samples of macrophytes (15-25 gr fresh weight) were collected from depths between 0.5 and 1.5 m, placed in plastic bottles with filtered seawater and kept in the dark. A surface water sample was immediately fixed with Lugol's solution to estimate the abundance of epiphytic dinoflagellates in the water column (in cells L^{-1}). Macrophyte samples were shaken for more than one minute to detach epiphytic cells. The material was then passed through 250 and 100 µm mesh sieves to remove large particles, and was fixed with Lugol's solution. Epiphytic abundance was expressed in cells gr^{-1} fresh weight of macrophyte (fwm). The fixed material was settled in 2–10 ml chambers for the appropriate time according to Utermöhl's method.

The highest water column concentrations coincided with maximum epiphytic abundance. The highest *Ostre-*

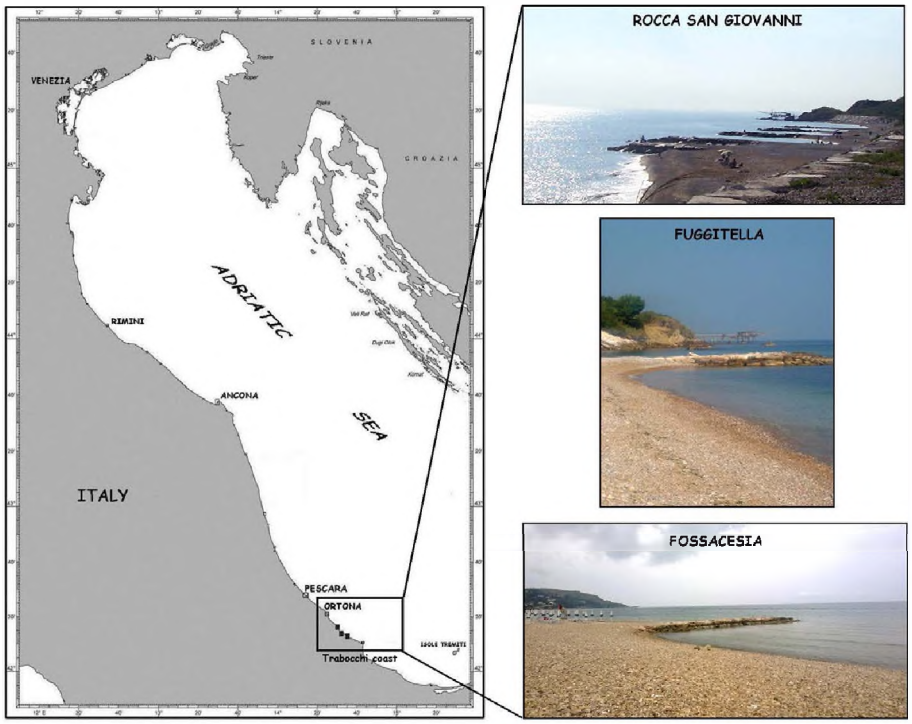

Fig. 1. Trabocchi coast (Adriatic Sea – Abruzzo Region) and location of sampling sites



Fig. 2. Towns affected by O. ovata *bloom where beaches were closed for bathing: (a) Rocca San Giovanni and (b) Fossacesia. The brown biofilm of the epiphytic* O. ovata*: c) on rocks and d) inside flask. Light microscopy photographs of* O. ovata *bloom: e) and f) field samples with high cell counts, 50x magnification, scale bar = 100 µm and 400x magnification, scale bar = 50 µm respectively. g) Scanning electron micrographs of* O. ovata *from isolated strain: epithecal view with pore plate (Po) short and straight (arrows), scale bar = 5 µm. h) LM epifluorescence of* O. ovata*: epithecal view with plates Po and 1', 1000x magnification, scale bar = 10 µm.*

opsis concentration recorded reached 5.28 x 10^5 cells g^{-1} fwm and 9.21 x 10^4 cells L^{-1} in the water column on 6th September 2013 at Fossacesia during the *O. ovata* bloom maximum, with 25 °C water temperature, 34.3 psu salinity and 6.7 mg L^{-1} dissolved oxygen (106% saturation).

During the period of the *O. ovata* bloom, there was strong coloration of the water and a prominent biofilm on rocks. The water had a strange smell like eggs, and dead mussels and octopus were also noticed during the bloom. Thirty two cases of human intoxication occurred, ten of whom required medical assistance. Symptoms included severe pain in the trachea, nausea, breathing problems, vomiting and fever. The mayors of the affected towns subsequently prohibited bathing on beaches from Rocca San Giovanni to Fossacesia. Since it was the first human poisoning event due to a toxic microalgae on the Abruzzo coast, there was much fanfare in local media (newspapers and television).

A week later after a weak gale, sampling on September 13th at Fossacesia revealed the highest concentration of *O. ovata*. There were then 8.35 x 10^4 cells g^{-1} fwm on macrophytes and 7.28 x 10^3 cells L^{-1} in the water column, 24 °C water temperature, 33.3 psu salinity and 7.0 mg L^{-1} dissolved oxygen (108% saturation). Thus *Ostreopsis ovata* concentrations had greatly decreased; but the number of degraded cells increased. The water was still smelly and slightly brownish and nutrient concentrations almost unchanged.

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Germinating cysts of *Heterosigma akashiwo* from marine sediments of British Columbia, Canada

Heterosigma akashiwo (Hada) Hada ex Hara et Chihara is one of the most noxious algae causing harmful algal blooms (HABs) worldwide and has been the principal fish killer of farmed salmon in British Columbia (BC) since the late 1980s; in 1997 alone the cumulative loss to the BC aquaculture industry exceeded CAD 10 million due to extensive blooms of *H. akashiwo* [1]. During the period from 1999 to the present, BC's Harmful Algae Monitoring Program (HAMP) has observed a continuous series of losses of cultured salmon due to *H. akashiwo* and in 2009 – 2012 direct economic losses of BC-farmed salmon due to HABs totaled more than CAD 16 million (HAMP, unpublished data), with *H. akashiwo* causing the majority of these losses.

To improve local knowledge of HABs, in 2012 a 2-year collaborative research project with Fisheries and Oceans Canada (DFO), the aquaculture industry, and the HAMP was initiated; one of the primary goals was the isolation and culture of harmful algae species collected from local finfish and shellfish aquaculture sites. Two sources were examined for species isolation: (1) live bloom samples from farm sites and (2) sediments collected from sites with past observed HABs, as some harmful algae produce cysts that play an important role in initiating subsequent blooms [2]. Here we discuss the results of harmful algal isolation from sediment samples.

In late winter and spring 2013, sediment samples were taken from 16 sites in 3 areas around Vancouver Island, BC (one sample from each of 4 sites in Baynes Sound, 4 sites in Okeover Inlet, and 8 sites in Quatsino Sound) (Fig. 1). Target species for isolation and germination were:

- *Alexandrium* spp. – which cause Paralytic Shellfish Poisoning (PSP). Numerous PSP closures in summer 2012 were recorded in Baynes Sound and Okeover Inlet [3];
- *H. akashiwo* and *Chattonella* sp. – which caused fish mortalities in late summer 2012 in Quatsino Sound [4].

Sampling was done on February 28 (Baynes Sound), April 10 (Quatsino Sound), and April 18 (Okeover Inlet), 2013 using van Veen and Ekman grab samplers (Fig. 2). Sediment samples (3 – 5 L) were taken from depths of 5 – 45 m with sites within areas being located at least 250 m apart. Sites were chosen based on the assumption that cysts accumulate in the same areas as silt and clay [5]. The top 3 cm of sediments in the grab samples were collected, mixed well, placed in re-sealable plastic bags, and immediately stored in a cool, dark place until delivered to the DFO laboratory in Nanaimo. Samples were kept in the dark at 4 °C until germination experiments were conducted. All 16 sediment samples were used for 2 sets of experiments: germination under regular laboratory conditions with (1) culture medium only and (2) culture medium with addition of a chemical to suppress diatom growth.

The first germination experiments were started 1 – 4 days after collection dates (i.e. March 1 for Baynes Sound, April 12 for Quatsino Sound, and April 22 for Okeover Inlet). A small portion of the collected sediments was sieved to produce a 20 – 120 µm fraction, and ~ 1 – 2 mL of the sieved sediments were added to petri dishes (3 replicates for each site) containing modified HESNW medium [6] – i.e. without added silicate and organic phosphates partially substituted with inorganic phosphates. The cultures were incubated at 18 °C under continuous illumination (20 µmol photons m^{-2} s^{-1} provided by full-spectrum fluorescent lamps). Petri dishes were scanned twice a week at 10 – 60X magnification using an inverted microscope. Samples were refreshed with additional media approximately weekly.

One to 2 days after the start of the experiments, diatom cells began to appear in the petri dishes. After 1 week, all dishes had noticeable coloration with various diatom species present, the most abundant being *Skeletonema costatum, Thalassiosira* spp., and *Chaetoceros* spp. In all replicates, diatoms reached several thousand cells mL^{-1} in 2 – 7 days. After a week, in many replicates, *H. akashiwo* cells were seen, sometimes in concentrations up to several hundred cells mL^{-1}. Within 2 weeks, most of the petri dishes had at least several cells of *H. akashiwo* and in some samples dinoflagellate cells also began to appear. After 3 weeks, all replicates had *H. akashiwo* cells, from a few cells mL^{-1} to several thousand cells mL^{-1}. Some samples from Quatsino Sound had thick surface accumulations of *H. akashiwo* – termed "rafts". The sample with the thickest rafts was from Kultus Cove (Quatsino Sound), which is known to be a hot-spot for *H. akashiwo*

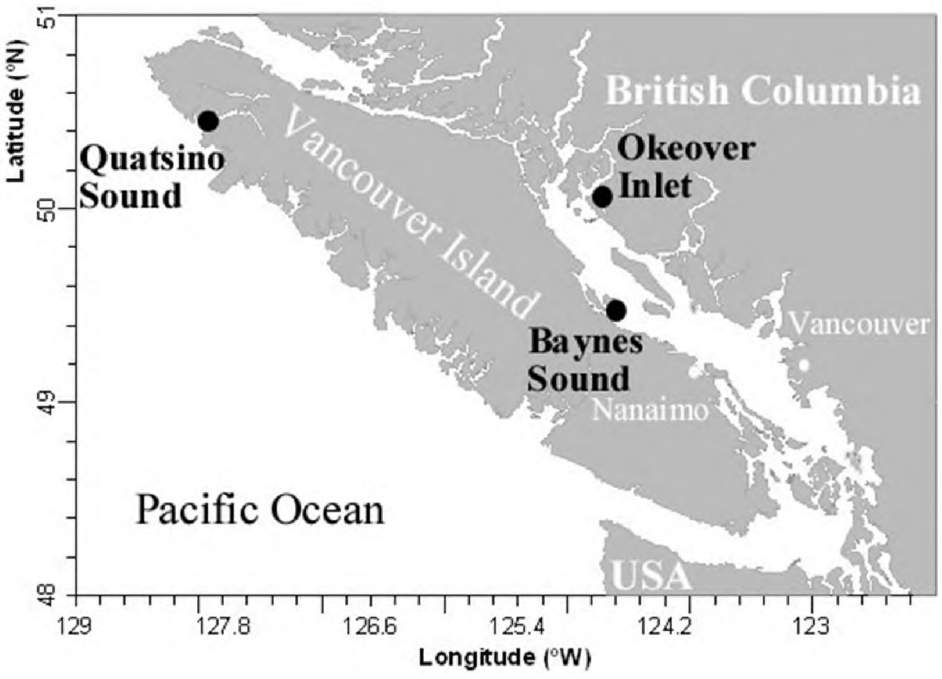

Fig. 1. Map showing sampling areas.



Fig. 2. Sediment sampling at Quatsino Sound on April 10, 2013.

blooms [N. Haigh, unpublished observations]. Some of these rafts were then used for serial dilution attempts to obtain pure cultures [7]. Serial dilution was performed in a laminar flow hood using a micropipette and sterile 24-well plates. This method involves dispensing and subsequent dilution of the sample in media in well plates where, with each transfer, the concentration of the cells in the sample is decreased by a factor of 10. After a further 2 weeks, cells of *Alexandrium* spp. started to appear in Okeover samples, although these species never reached concentrations that would have made isolation practical. After about 6 weeks, a mixture of other small flagellates dominated all petri dish replicates: the experiment ended after ~8 weeks.

The second set of germination experiments was set up at the beginning of June, 3 – 4 months after collection dates. The same germination method (as described above) was used but with the addition of germanium dioxide to the culture medium to suppress diatom growth [8]. Sediments stored in the dark at 4 °C were processed as above and added to 3 replicate petri dishes with media (which included ~ 0.5 – 1 mL (in each petri dish) of prepared stock solution of 2 mg GeO_2 in 100 mL of MilliQ water [9]). After several days, *H. akashiwo* cells were found in some replicates and in about 2 weeks all samples had at least a few cells of *H. akashiwo*. In 2 – 3 weeks, many replicates had thick rafts, providing good material for *H. akashiwo* serial dilution. In 2 weeks, samples from all 4 sites in Okeover Inlet had a few *Alexandrium* cells and other dinoflagellates present. Only a few diatom cells were seen in some replicates (mostly from the Okeover area); diatom growth was greatly inhibited and concentrations never reached 100 cells mL^{-1}. Samples with suppressed diatom growth were better suited for cell isolation and in this way several pure cultures of *H. akashiwo* and *Alexandrium* spp. were successfully established.

Conclusions

All 16 surface sediment samples collected in 3 areas around Vancouver Island produced viable *H. akashiwo* upon incubation. At 18 °C, under continuous illumination, *H. akashiwo* germinated in 1 – 3 weeks. Germanium dioxide stock solution added to media inhibited diatom growth, allowing easy germination and isolation of *H. akashiwo* from sediment samples containing cysts. This technique makes it possible to obtain cultures of *H. akashiwo* from various locations even when vegetative cells are not present in the water column.

Acknowledgments

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Saxitoxin analyses with a receptor binding assay (RBA) suggest PSP intoxication of sea turtles in El Salvador

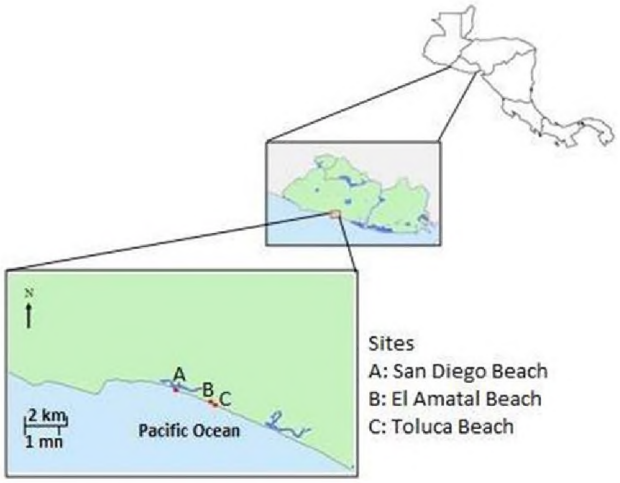


Fig. 1. Locations on El Salvador coasts where stranded sea turtles were found, mainly on San Diego (13°28'16.03"N, 89°15'49.01"W), El Amatal (13°27'37.00"N, 89°14'16.00"W) and Toluca (13°27'47.53"N, 89°14'0.92"W) beaches, October 2013.

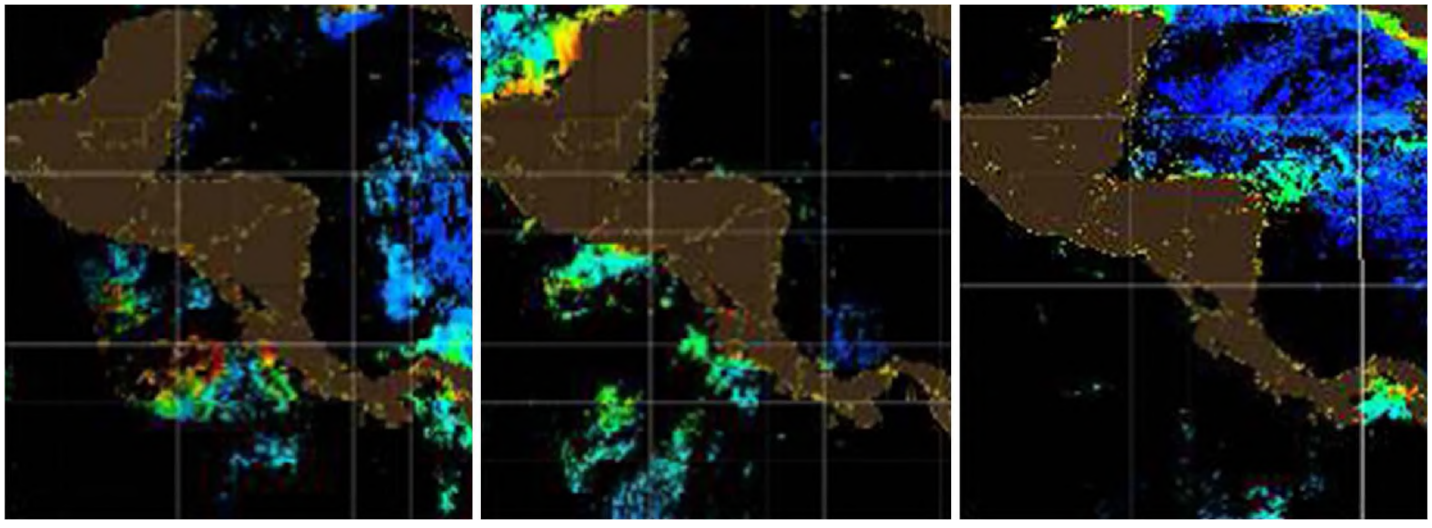
Great efforts are invested on the Eastern Tropical Pacific coast to protect sea turtles, evaluate their populations, and identify threats to their survival. Toxic HABs represent a major threat to turtles, and have been associated with mortalities. The main species found in coastal waters of El Salvador are the green (*Chelonia mydas*), the hawksbill (*Eretmochelys imbricata*), the olive ridley (*Lepidochelys olivacea*) and the leatherback (*Dermochelys coriacea*) sea turtles [1].

There are records of dead sea turtles on El Salvador beaches. During a PSP outbreak in 2005, which included 3 human deaths, 206 dead sea turtles were reported. Liquid chromatography (HPLC) analyses revealed concentrations of saxitoxin of 27.9-627.8 µg STX eq ·100g^{-1} in brain tissue of *Lepidochelis olivacea* and *Chelonia mydas* [2].

PSP events in El Salvador are associated with blooms of *Pyrodinium bahamense* var *compressum* [3]. In November 2009 brownish red patches were observed 46 nm off the coast between Bahía de Jiquilisco and Acajutla and 5 dead turtles were reported in the area. This event was associated with *P. bahamense* cf *compressum* (unpubl. data). In May 2010, intense patches reached the coastline and 17 dead sea turtles were reported from El Salamar and Las Tunas beaches on the eastern end of the country; later in October 2010 a new report of 12 dead turtles was associated with a bloom of *Alexandrium* cf *peruvianum/ostenfeldii* in Los Cóbanos beach. In May 2011, a Receptor Binding Assay (RBA) was applied for the first time to test the presence of STX and its analogues (STXs) in samples from dead specimens but no toxins were detected in that occasion (unpubl. data). Since 2009 the Laboratory of Marine Toxins from the University of El Salvador (LABTOX-UES) carries out a monitoring of potentially toxic phytoplankton. In addition, PSP toxin analyses (by RBA) in oysters (*Crassostrea iridiscens*), sea snails (*Plicopurpura columellaris*), crabs (*Carcinus maenas*) and mussels (*Modiolus capax*) have been performed routinely since 2011 [4].

Between late September and early October 2013 there was a mortality of 201 turtles (information from the Ministry of Environment and Natural Resources). Dead specimens were found mainly on beaches in the department of La Libertad [5] (Fig.1).

Following the sea turtle mortality in 2013, experts from LABTOX-UES in cooperation with officials from the Centre for Development of Fisheries and Aquaculture (CENDEPESCA) collected water samples for qualitative (20-µm plankton hauls) and quantitative (10-L Niskin bottles, Sedgewick Rafter counting chambers) analyses of phytoplankton (Carl Zeiss, Axio Imager M1 and Axiovert 40CFL microscopes



MODIS-Aqua CHLOR_A 2013.09.08 | MODIS-Aqua CHLOR_A 2013.10.27 | MODIS-Aqua CHLOR_A 2013.11.11

Fig. 2. MODIS satellite images from dates before, during and after the report of sea turtle mortality in El Salvador.

Table1. Phytoplankton cell densities in water samples collected from El Salvador beaches during the sea turtle mortality, October 2013.

species		La Libertad (15 Oct) cells L^{-1}	Acajutla (16 Oct) cells L^{-1}
Pyrodinium	*bahamense*	20	-
Cochlodinium	*polykrikoides*	880	520
Gymnodinium	***catenatum***	**480**	**5400**
Gyrodinium	*instriatum*	120	480
Alexandrium	*monilatum*	-	4080

respectively). MODIS satellite images (Chl a fluorescence) from dates before and during the mortality are shown in Fig. 2. Samples of stomach, intestines, liver and kidneys were taken from 16 specimens of *Chelonia mydas* and *Lepydochelis olivacea*, as well as from oysters (*Crassostrea iridiscens*) and kept at – 80 °C until analysis. Analyses of STXs were carried out by RBA, a competitive receptor binding assay in which the radio-labelled [3H]STX competes with unlabelled STXs for the sodium channel sites present in a crude membrane preparation of rat brain. When the binding equilibrium is reached, free [3H] toxins are removed by filtration and collected receptor-bound [3H]toxins are quantified with a liquid scintillation counter with microtiter plate format MicroBeta trilux1450 LC. The reduction in [3H]toxin binding is directly proportional to the amount of unlabelled toxin present. A standard curve is generated using increasing concentrations of unlabeled toxin standard; the concentration of toxin in extracts of samples is determined in reference to the standard curve [6].

The most abundant potential producer of PSP toxins found in the phytoplankton samples was *Gymnodinium catenatum* (5400 cells L^{-1}) (Table 1). Analyses of STXs in liver, intestine and stomach tissue samples from the dead sea turtles showed levels ranging from 116.11 to 478.28 µg STX eq. $100g^{-1}$ (Fig 3). Toxin levels in oysters (58.33 µg STX eq. $100g^{-1}$) were below the regulatory level (80 µg STX eq. $100g^{-1}$).

Stranded vertebrates are not seen very common on El Salvador coasts, so we assume that sea turtles were members of the fauna most affected by the toxic outbreak. The low densities of potentially toxic phytoplankton found in inshore samples and of toxins in oysters (below regulatory levels) would not justify the levels of STX found in the turtles. But we know that medusae, which are carnivorous megazooplankters, are part of the diet of some turtles. We suggest that offshore toxic phytoplankton, eaten by herbivorous zooplankton was transmitted to the sea turtles through medusae acting as vectors of the toxins and caused their death.

Acknowledgements

To IAEA for its valuable support to set up the LABTOX-UES laboratory. To the Ministry of Environment and Natural Resources MARN and the Centre for Development of Fisheries and Aquaculture CENDEPESCA for their support in monitoring harmful algal blooms.

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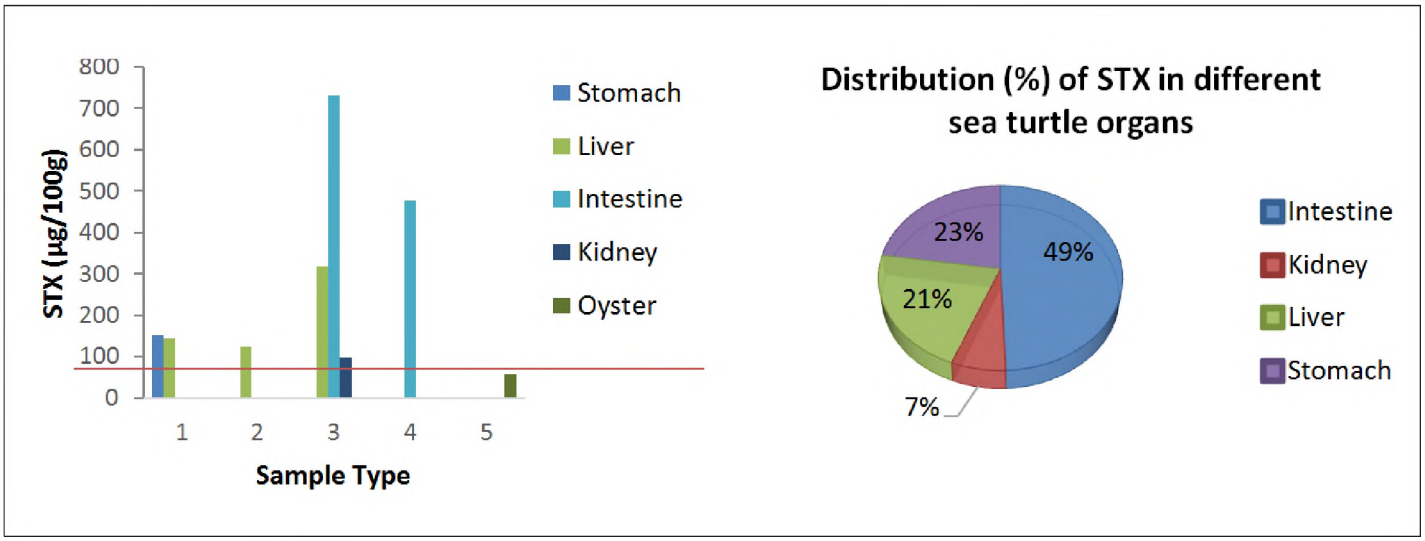

Fig. 3. Left, saxitoxin levels found in sea turtles and oysters from El Salvador applying the RBA (red line indicates the regulatory level); Right, distribution of STXs found in different sea turtle tissues.

Fish die-offs along southern coast of Baja California

Many algal blooms are innocuous to marine fauna or increase productivity of coastal zones. Harmful algal blooms (HABs) can cause negative impacts on other or¬ganisms via production of toxins, by mechanical damage, or by other means [1]. Although HABs are common along the coasts of the Baja California [2], few events have been reported along its southern coast [3, 4, 5]. A bloom of *Pseudonitzschia* sp. occurred from December 1995 to January 1996 at Cabo San Lucas. More than 150 brown pelicans died [4]. In March 1996, a bloom of cyanobacteria and *Chatonella* sp. provoked mortality of benthic fish & fan corals at Cabo San Lucas [4]. A bloom of *Prorocentrum minimum* (2.4–3.0 × 10^6 cells L^{-1}) and *Ostreopsis siamensis* (0.2–0.4 × 10^6 cells L^{-1}) occurred on April 2002 in the Los Cabos area [3]. A small bloom of *Noctiluca scintillans* were observed at Los Barriles on 24 March 2007. Densities of *Noctiluca* at Los Barriles were 250–950 × 10^3 cells L^{-1}. *Gymnodinium catenatum* also occurred at densities of 98 × 10^3 cells L^{-1} (this report). Another smelly bloom occurred on 8 March 2007 at Los Frailes (Fig. 1). No marine faunal die-offs were detected in either event. More recently, two varieties of *Pyrodinium bahamense* were reported at the southern end of Baja California. This region is an important area for fisheries [6]. A fish mortality occurred on 16–21 June 2011 in the Los Cabos region (Fig. 2), first reported by the organization "Red para la Protección de la Tortuga Marina" (Redprotormar, Marine Turtle Protection Network) at El Zacatito, and extending northwards to Playa San Luis. To the south, fish mortality was reported at the Hotel Sheraton. The largest die-offs occurred at La Fortuna (23°09′0.54″N, 109°29′ 39.54″W). Recently, The Procuraduría Federal de Protection al Ambiente (PROFEPA) and the Dirección de Ecología of Los Cabos mentioned died-offs observed at La Playita (23°3′38.98″N, 109°40′7.47″W), and later at Punta Gorda (23°05′00.46″N, 109°34′33.70″W) and La Fortuna (23°09′00.54″N, 109°29′39.54″W). Approximately 20 km of beaches were heavily affected (http://www.youtube.com/watch?v=Yk4o5NRt6ac). The most abundant fish on the beaches were damsel bass (*Hemanthias signifer*; Fig. 3a) and yellowtail surgeonfish (*Acanthurus* sp.; Fig. 3b). Less abundant were azure parrotfish (*Scarus compressus*; Fig. 3c), blue-bronze sea chub (*Kyphosus analogus*; Fig. 3d), and California sheephead (*Semicossyphus pulcher*; Fig. 4). This is the worst fish die-off recorded on this coast. Fish were not eaten by sea birds, crabs, or other marine fauna. Many ctenophores (*Beroe* cf *ovata*) accumulated near the beaches. Ctenophore swarms have not been reported previously in these waters. The die-offs were probably related to the presence of red tide patches reported by fishermen during this event; however, no bloom samples were taken. Die-offs of pelicans occurred shortly before and after the fish die-offs. Phytoplankton samples were collected on 25 June 2011 in the affected area. The most common microalgae were *Chaetoceros* spp., *Thalassionema nitzschioides, Thalassiosira* sp., and at least three species of *Pseudonitzschia*. The ciliate *Myrionecta rubra* was also found. Toxic dinoflagellates in the samples included *Coolia monotis, Dinophysis acuminata, D. caudata, D. fortii, Ostreopsis heptagona, Prorocentrum gracile, Prorocentrum micans,* and *Prorocentrum minium*. Per-



Fig. 1. Smelly bloom in Bahía de Los Frailes.



Fig. 2. Fish die-offs occurred along the shore of Los Cabos Region (red area).



Fig. 3. Fish die-off on 16–21 June 2011 at La Playita and the Hotel Hilton beach on the Los Cabos.

sistent cold winds from the south produced cooler currents, water temperature dropped from 24.5 to 18 °C along much of the Southern Baja coast (Eric Brictson, pers. comm.). Health authorities responded rapidly and no human intoxications were recorded. According to a local authority (Pablo César Támez), ~50 t of fish were removed from beaches over two weeks [7]. Fish markets and seafood restaurants were monitored by the Comisión Estatal para la Protección de Riesgos Sanitarios [8]. The mass die-offs caused by these HABs resulted in economic loss and contributed to pollution of coastal waters. Fish catches of commercial importance were prohibited while the toxicity in fish was detected (Mario García Isais). After this die-off, a training workshop on red tides was organized in Cabo San Lucas. Monitoring of red tide-producing species, particularly toxic species, is ongoing along the coast of the Los Cabos region.

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*Fig. 4. Male sheephead (*Semicossyphus pulcher*) stranded on beach near Los Cabos.*

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Pseudo-nitzschia australis blooms are not always toxic

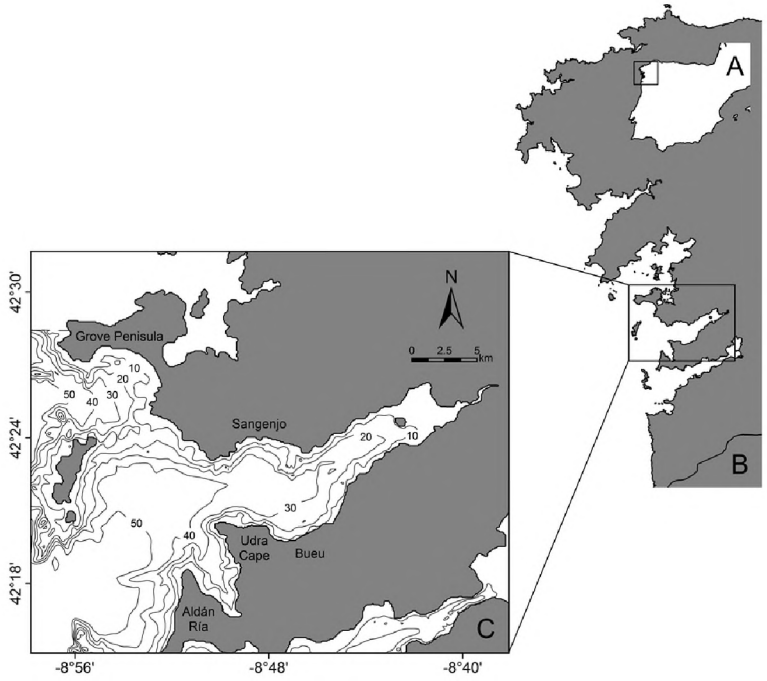

Fig. 1. Maps showing A) Iberian Peninsula; B) Galician Rias Baixas and C) Study area in Ría de Pontevedra.

Diatom species of the genus *Pseudo-nitzschia* H. Peragallo are regular components of the microphytoplankton assemblages in the Galician Rías (NW Spain). Twelve species of this genus have been confirmed as producers of domoic acid (DA) [1, 2], a neurotoxin which causes Amnesic Shellfish Poisoning (ASP). Eight potentially toxic species of *Pseudo-nitzschia* have been reported so far in Galician coastal waters [3]. But the occurrence of domoic acid in shellfish above regulatory levels (ASP outbreaks) in this area has mainly been associated with high concentrations (> 10^5 cell L^{-1}) of *Pseudo-nitzschia australis* Frenguelli [3, 4]. Phytoplankton succession in the Galician Rías [5] is closely related to the seasonal (March to October) upwelling pattern [6]; *Pseudo-nitzschia* species appear at an intermediate stage in the upwelling-downwelling cycles (late spring-summer), following large centric diatoms [5]. Since June 1992, the Technological Institute for the Control of the Marine Environment in Galicia (INTECMAR) carries out a weekly monitoring of potentially toxic phytoplankton (including *Pseudo-nitzschia* spp.) and phycotoxins, according to EU Directives, on Galician coasts (Fig. 1). ASP outbreaks in the region have proven to be short-lived events leading to mussel harvesting bans of one to a few weeks duration. Nevertheless, they represent a serious threat to sustainable exploitation of pectinids (*Pecten maximus, P. jacobeus*). These species have slow depuration rates for DA (0.007 day^{-1} in *P. maximus*) [7] and may need months or even years to eliminate toxins.

Since the first event reported in the Galician Rías in autumn 1994 [4], ASP outbreaks have been reported almost every year in northwest Iberia [8], including the coast of Portugal [9]. The long retention time of DA in pectinids and its impact on the scallop industry in Galicia and Scotland led to implementation of specific regulations (Commission Decision 2002/226/EC) to control DA in pectinids in the European Union [10].

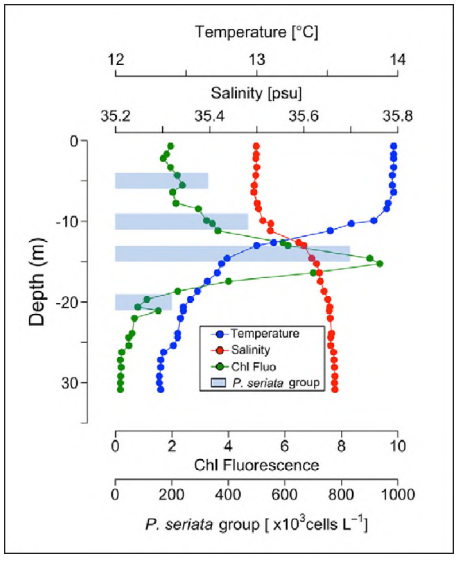

Fig. 2. Vertical distribution of temperature (blue), salinity (red), chl-a fluorescence (green) and Pseudo-nitzschia seriata *group cells density (blue bars) recorded during the survey on 28 May 2007.*

A dense bloom of *Pseudo-nitzschia* spp. was recorded during the *HABIT-Pontevedra 2007* survey carried out in Ría de Pontevedra, from 28 May to 7 June 2007 [11]. *Pseudo-nitzschia* species were identified following Hasle [12]. Particulate DA concentrations were analyzed by liquid chromatography-multiple tandem mass spectrometry (LC-MS) according to Furey et al. [13]. Weekly monitoring results from INTECMAR during the cruise period also reported the occurrence of a *Pseudo-nitzschia* spp. bloom (> 10^6 cells L^{-1}) that reached its annual maxima on 28 May 2007 [14].

Results from the cruise showed that the bloom formed a thin layer of *Pseudo-nitszchia seriata* group spp., dominated by *P. australis* at 14 m depth, associated with marked density gradients in the water column (Fig. 2). Despite the predominance of *P. australis* (Fig. 3), DA was not found (detection level 0.02 µg DA mL^{-1}) in any of the samples with high *Pseudo-nitzschia* densities. Nor were shellfish harvesting bans implemented by the Galician authorities due to the occurrence of ASP toxins in mussels during the cruise period.

It is well known from laboratory cultures of toxigenic *Pseudo-nitzschia* species that large differences in cellular toxin content can be observed during population growth, with the maximum per cell usually found during the late exponential or stationary phase [2]. It could be argued here that the toxin content of *Pseudo-nitzschia* was below detection limits during the cruise and increased later in the season. But there were no mussel harvesting bans in Galicia due to ASP for the whole of 2007 (www.intecmar.org). This can only be explained by the existence of very weakly toxic or non-toxic strains of *P. australis.*

Our results show the occurrence of a non-toxic *Pseudo-nitzschia* bloom, dominated by *P. australis*, the species

commonly associated with ASP on the Atlantic coast of Iberia (Spain and Portugal). Therefore, the detection of high densities of "known" toxigenic species of *Pseudo-nitzschia* is not enough for monitoring purposes, if it is not accompanied by toxin analyses to confirm the presence of domoic acid. In this context, monitoring based on ASP toxin content per volume of water in addition to routine *Pseudo-nitzschia* cell counts, would be a more realistic early warning tool for seafood safety management. This is applied in the monitoring programme of the U.S. west coast [15], where a combination of microscopic monitoring of the algae and assessment of cellular toxicity (expressed as "particulate DA") using test strips (Jellett Rapid Tests) gives an effective early warning of shellfish toxification events, reducing the risk associated with potential toxicity in razor clams.

Acknowledgements

We thank INTECMAR for weekly reports on phytoplankton distributions in the Galician Rías Baixas. S. Fraga helped with identification of *Pseudo-nitzschia* spp. and J.M. Franco with HPLC analysis. This work was funded by ASIMUTH project (*Applied Simulations and Integrated Modelling for the Understanding of Toxic and Harmful algal blooms*) from the 7th Framework Programme (ASIMUTH/SPACE-Grant Agreement 261860) of the European Commission. Patricio A. Díaz is supported by a PhD fellowship from the BECAS–CHILE Programme of the National Commission for Scientific and Technological Research (CONICYT), Chile.

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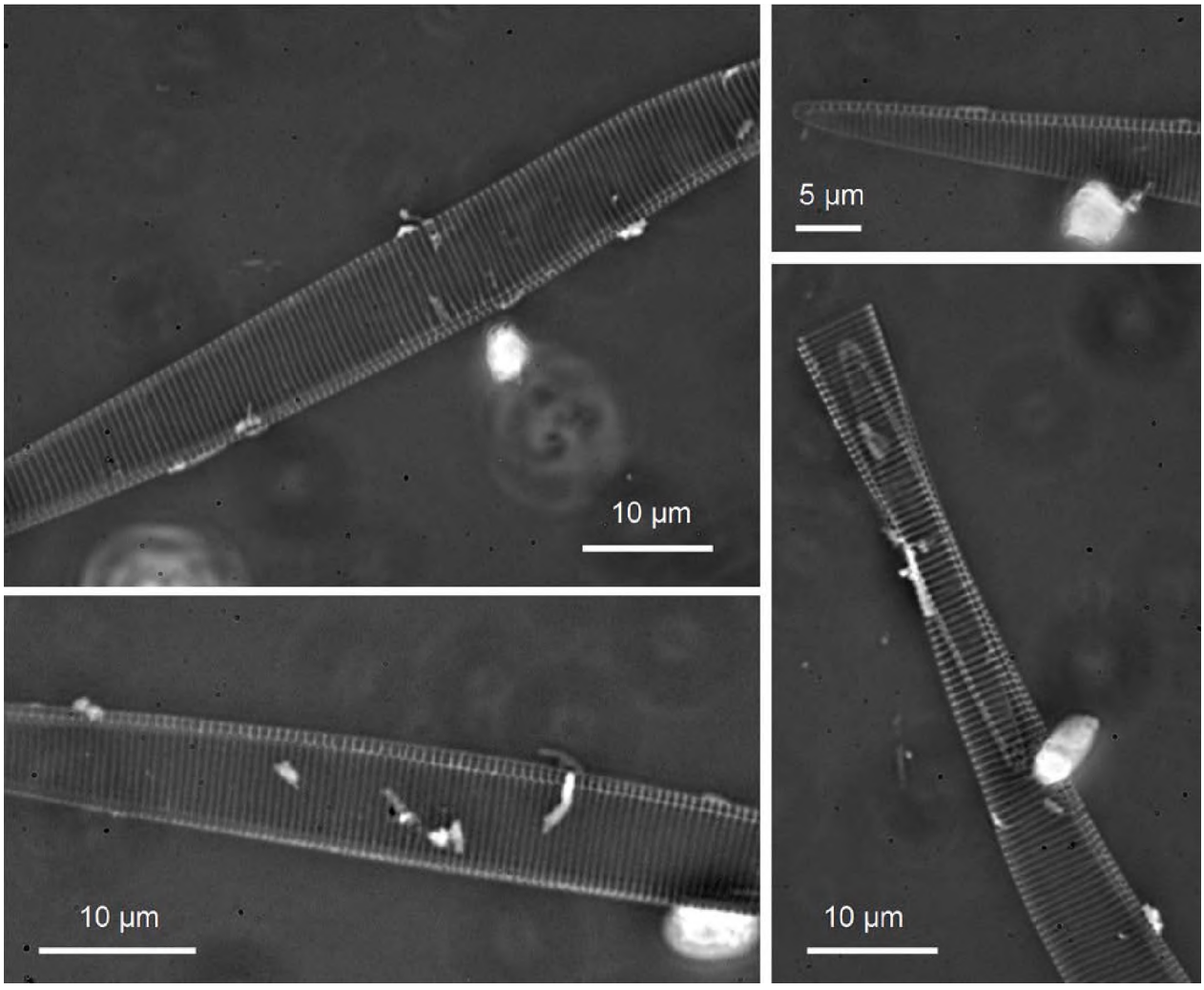


Fig. 3. Phase contrast micrographs of Pseudo-nitzschia australis *found in Ría de Pontevedra (NW Spain), during a bloom in May 2007.*

Blooms of a *Chattonella* species (Raphidohyceae) in La Redonda Lagoon, Northeastern Cuba

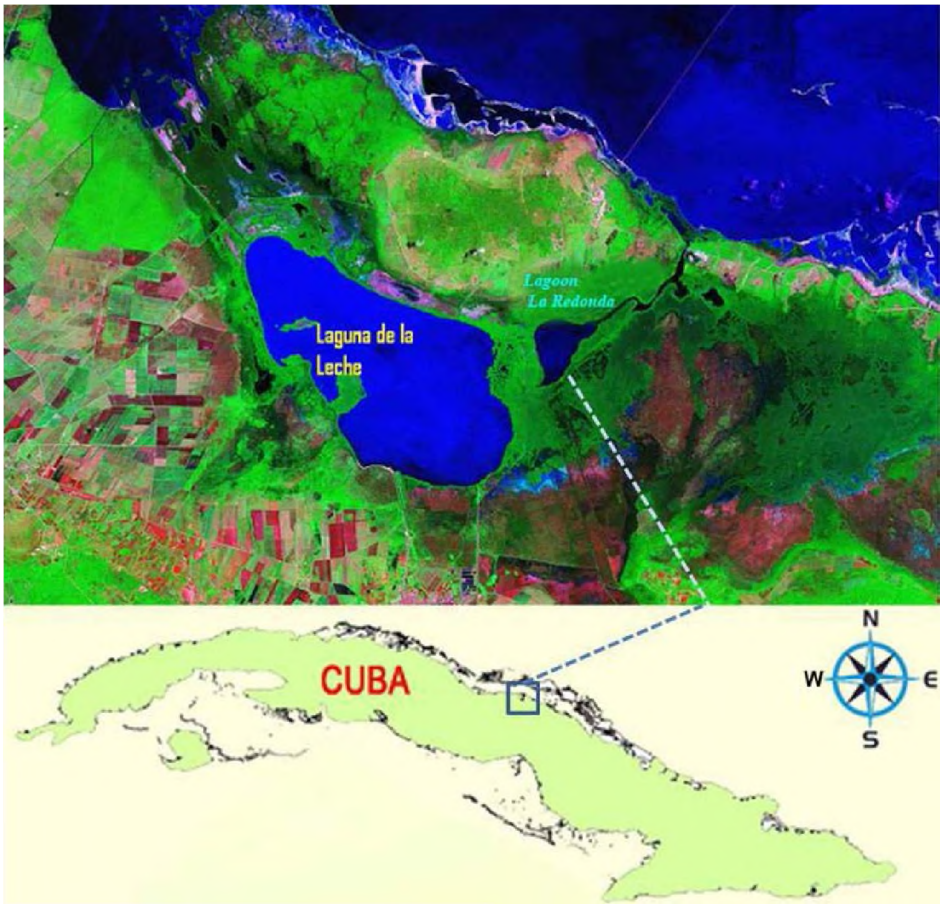


Fig.1. Map showing the study area: Lagoon La Redonda, northeastern coast of Cuba.

Species of the genus *Chattonella* Biecheler (Raphidophyceae) are widely distributed in temperate and subtropical/tropical areas of the world. Blooms of *Chattonella* have been associated with mortality of captive and wild fish populations [1-4]. The genus according to Hara et al. [5] comprises seven species, among which, *C. antiqua* (Hada) Ono 1980 and *C. marina* (Subrahmanyan) Hara et Chihara 1982 are known to be the most harmful fish-killing species. "*Chattonella* red tides" are most frequent in east Asia [6], but species of this genus have been reported from Brazil and The Netherland coasts [7,8].

La Redonda Lagoon together with Laguna de la Leche, belong to a shallow brackish lagoon system located in northeast Cuba (Fig. 1). Table 1 shows the main characteristics of this lagoon (González de Zayas & Lestayo, *in litt.*) which has been used as a reservoir for *Micropterus salmoides* Lacepède 1802 (largemouth black bass). No red tides or fish mortality were previously detected in the area, but the fish population density had decreased in the last years, due apparently to poor larval survival.

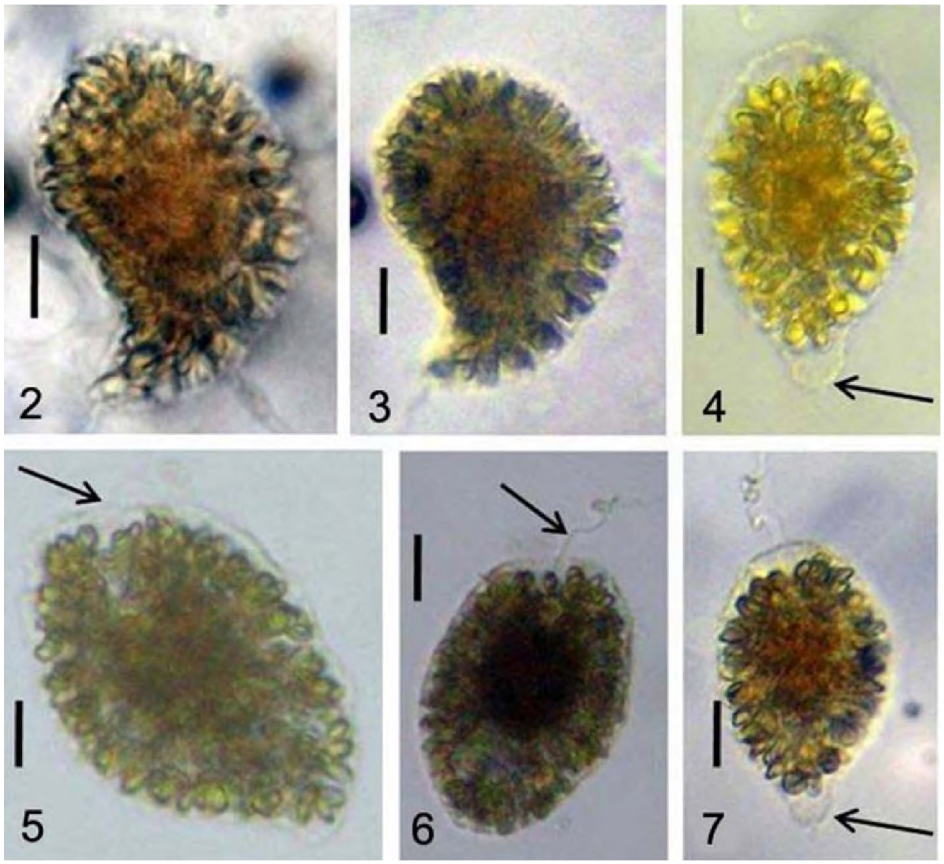
Plankton samples collected in La Redonda Lagoon in November 2013 and observed in a Laborlux Leica-Leitz microscope revealed an abundant (9.66 x 10^4 cells/L) population of a flagellate whose main morphological features corresponded with those of the genus *Chattonella*: i.e. cells with i) two subequal, heterodynamic flagella emerging from the bottom of an apical cell depression and ii) numerous yellowish-green or yellowish-brown chromoplastids radially arranged in the cytoplasm (Figs 2-8). Members of this genus were not found earlier in Cuban waters.

Cells of the Cuban strains were obovoid, oblong to near heart-shaped (probably corresponding with earlier steps of cell division), 19,6-47,6 µm length and 12,6-30,8 µm broad, slightly flattened, lanceolate or somewhat asymmetrical in lateral view, with an anterior end rounded or flat-rounded with a median depression. From the bottom of these depressions emerge two subequal, heterodynamic flagella. Posterior end acutely rounded or very often with a terminal short, colourless, nearly straight, cylindrical or slightly curved tail. Chromoplastids are many, radially arranged, occupying the entire cytoplasm or withdraw from the posterior tail, yellowish-green or yellowish-brown, ellipsoid to cuneiform, each with a hardly visible, naked pyrenoid. Contractile vacuoles, eyespots and ejectisomes (mucocysts) are lacking. Reproduction by cell division occurs along the longitudinal axe (Figs 2-8).

These Cuban specimens could be identified either as *C. marina* (cells are obovoid with the posterior tail acute and evidently curved) or *C. subsalsa* (cells are obovoid or nearly oblong with acutely rounded, straight posterior end); then the diagnostic features of both species overlap, except for the presence of ejectisomes (mucocysts) which should be present in *C. subsalsa* but not *C. marina*. According to traditional taxonomy (based on morphological features), different authors consider the two identical, but taking into account the presence of ejectisomes and no thylakoids penetrating the pyrenoids in *C. subsalsa*, Hallegraeff & Hara [3] maintain them as separated spe-

Table 1. Some characteristics of La Redonda Lagoon, Cuba, after González de Zayas & Lestayo (in litt.); figures are mean values.

Area	4,5 km^2
Depth	3 m
pH	7,98
Temperature	27,37°C
Salinity	1,61 psu
Total N	104,0 µM
Total P	3,1 µM
Ca	1,38µg/L



Figs. 2-7. Chattonella *cf.* subsalsa*: 2-3)* C. marina*-like; 3,7)* C. subsalsa*-like, but with a short cylindrical posterior tail (black arrow); 5) earlier stages of cell division (black arrow); 6) longer flagellum (black arrow).*

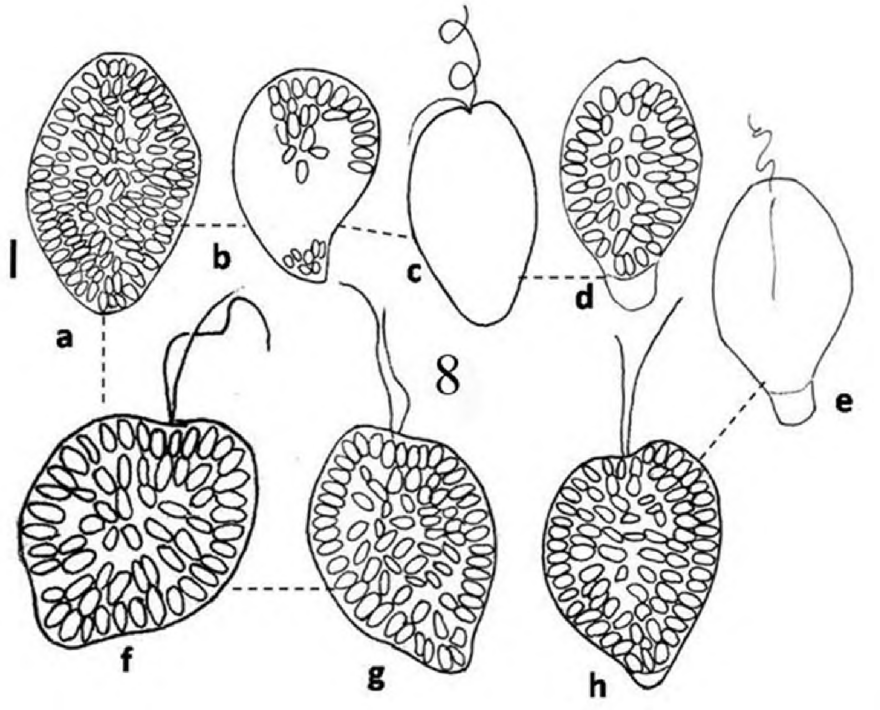


Fig. 8a-h. Chattonella *cf.* subsalsa*; a)* C. subsalsa*-like; b-c)* C. marina*-like; d-e)* C. subsalsa*-like, but with a short cylindrical terminal tail; f-h) earlier steps of the longitudinal cell division.*

cies. Bowers et al. [9], based on gene sequence analysis, accepted them as different species in spite of their weakly supported results. Because our population was found in a low salinity environment we prefer to consider it "*ad interim*" as *C. subsalsa* Biecheler. Further studies are needed for a definitive identification.

This is the first time phytoplankton is studied in this lagoon ecosystem. Attention should be paid to the potential relation of *C.* cf. *subsalsa* and low survival of largemouth black bass larvae. Moderate concentrations of *Chattonella* were found in our sampling, but eventual blooms (>10^6 cells L^{-1}) could cause mass mortalities of fish in the lagoon.

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Foam events due to a *Phaeocystis* bloom along the Catalan Coast (NW Mediterranean)

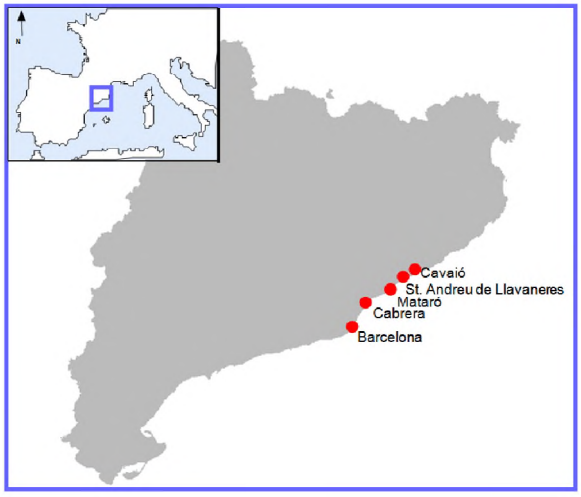

Fig.1. Location of the beaches where foams were observed.

Species of the genus *Phaeocystis* (Prymnesiophyceae) have a world-wide distribution and can produce nearly monospecific blooms. Their life cycle is generally polymorphic with free living non-motile and flagellated cells, and colonies composed of few to thousands of cells embedded in a mucilaginous matrix [1]. Massive blooms of the colonial phase have been observed in nutrient-rich waters such as those of North European coastal zones and polar regions. These blooms may cause problems for local fishing activities (because of clogging fishing nets) and are often associated with an accumulation of foam at the coast, which may have negative consequences for the tourist industry. In addition, some species may produce substances that are toxic to fish [2].

The presence of *Phaeocystis* in the Catalan Sea (NW Mediterranean) has been reported on several occasions. This genus is an important component of the winter-spring phytoplankton community in offshore waters of the NW Mediterranean, outside of the diatom-dominated patches that develop after deep convection in the Liguro-Provençal region [3]. On the other hand, foam events have been recorded throughout the year along the Catalan coast (especially during autumn and spring), but only one observation in March 1996, off the coast of Montroig (Tarragona) had been related to the presence of *Phaeocystis* (M. Delgado, personal communication). In fact, in most cases, the occurrence of foam on the Catalan coast could not be directly associated with any particular phytoplankton species.

In March 2006, foam was observed at various locations along the central coast of Catalonia (NW Mediterranean). A foam event lasting for one day was first detected on March 19 by five ARGUS video cameras focusing on the Barcelona city coast (Fig. 1, 2a). These cameras started to monitor the area in 2001; in addition, since March 2002, water samples are collected regularly (usually once a month) at 8 coastal stations (1.1 to 1.4 and 2.1 to 2.4, Fig. 2a), within the framework of the Coastal Oceanographic Observatory programme (http://coo.icm.csic.es/content/barcelonaelb). Phytoplankton counts by inverted microscopy are routinely carried out for the surface samples of Station 1.4. One week later (March 27), the Harmful Algae Monitoring Programme (focusing on harmful and noxious species along the Catalan coast) detected foam on several beaches along approximately 40 km of shoreline to the north of Barcelona (Cabrera, Mataró, St. Andreu de Llavaneres and Cavaió beaches; Fig. 1, 2b). Phytoplankton samples (fixed with acidic Lugol´s solution) taken four days prior to foam detection (March 15) at station A (Fig. 2a), revealed the presence of high *Phaeocystis* abundance, both in surface waters and at depth (decreasing from 1.64 x 10^6 cells L^{-1} at the surface to 0.80 x 10^6 cells L^{-1} at 30 m depth; Fig. 3a). One day later (March 16), a phytoplankton sample (fixed with formaldehyde-hexamine) taken at Station 1.4 also revealed a very dense *Phaeocystis* population (Table 1). Similarly, water samples (fixed with Lugol´s solution) from the Cabrera, Mataró, St. Andreu de Llavaneres and Cavaió beaches presented more than 5 x 10^6 cells L^{-1} of Phaeocystis (Table 1). High *Phaeocystis* concentrations remained in coastal waters of Barcelona for more than one month, given that high concentrations of this organism were recorded at station A on April 4, (increasing from 0.41 x 10^6 cells L^{-1} at the surface to 1.62 x 10^6 cells L^{-1} at the bottom, Fig. 3b), and at station 1.4 on April 27 (Table 1).

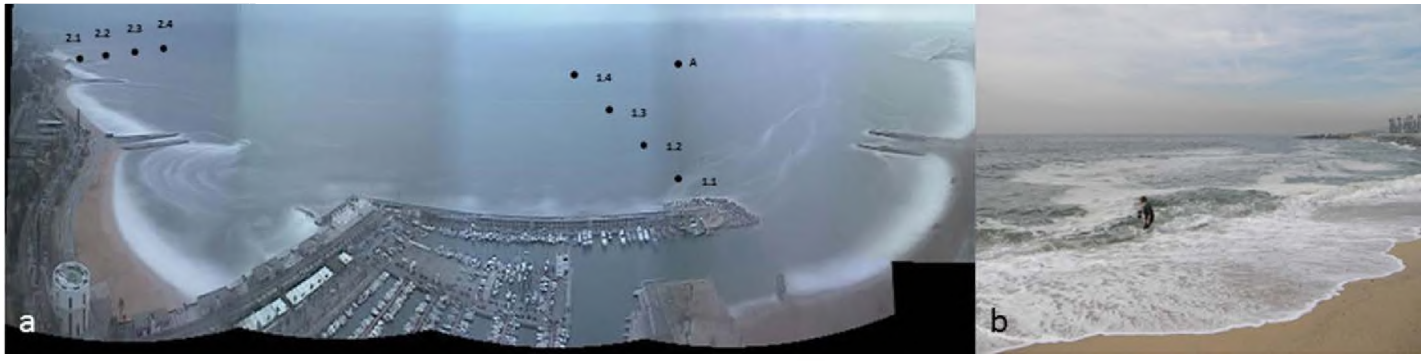

Fig. 2. a) Photography of the Barcelona coast taken with an ARGUS video camera system showing the presence of foams. Location of the sampling stations is indicated by black dots. b) Foams at the Mataró beach (Photo: Catalan Water Agency).

Table 1. Surface concentration of Phaeocystis *cells and Chlorophyll-a (Chl-a) at sites where formation of foam was observed.*

Location	Station	Date (dd/mm/yy)	*Phaeocystis* (10^6cells L^{-1})	Chl-*a* ($\mu g\ L^{-1}$)	Programme/Projects
Barcelona	1.4	16/03/06	0.77	2.61	Coastal Oceanographic Observatory
Barcelona	1.4	27/04/06	0.01	0.76	Coastal Oceanographic Observatory
Barcelona	A	15/03/06	1.64	2.14	MicroRol
Barcelona	A	04/04/06	0.41	1.22	MicroRol
Cabrera	Coastal line	27/03/06	15.4		Harmful Algae Monitoring
Mataró		27/03/06	11.0		
St. Andreu de Llavaneres		27/03/06	8.18		
Cavalò		27/03/06	5.94		

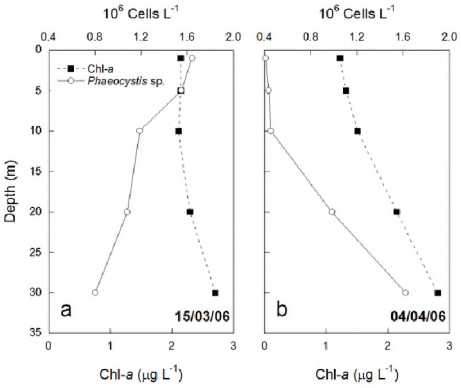

Fig. 3. Vertical profile of Phaeocystis *sp. abundances (cells L-1) and chlorophyll-a (Chl-a) concentrations (µg L-1) at Station A (Barcelona) for a) March 15, 2006 and b) April 4, 2006.*

The bloom of *Phaeocystis* was not monoespecific, since high concentrations of diatoms were also found (between 10^4 and 10^6 cells L^{-1}), mainly species of *Bacteriastrum*, *Rhizosolenia*, *Pseudonitzschia* and *Chaetoceros*. It is not clear what caused the termination of the *Phaeocystis* sp. bloom on the Catalan Coast; potential mechanisms include colony sedimentation, microzooplankton and viral infection. A bloom of ciliates (predominantly *Laboea* sp.), observed in April 2006 on the Barcelona coast [4], suggests that ciliate grazing could have been one of the main causes for the *Phaeocystis* bloom termination.

Based on colony morphology (Fig. 4a), *Phaeocystis* sp. was first identified as *Phaeocystis globosa* [5]. However, scanning and transmission electron microscopy observations revealed neither the typical body scales described for the flagellate stage of this species (Fig. 4b and c) nor filaments of any kind. The morphological characteristics of both the colonies and the flagellated and non-flagellated free cells agree with those of a new species of *Phaeocystis* found in Mediterranean waters (*Phaeocystis* sp. 2 in [6]). However, genetic information is needed to confirm whether the *Phaeocystis* from Catalonia is cospecific with *Phaeocystis* sp. 2.

The occurrence of the *Phaeocystis* bloom on the central coast of Catalonia was probably due to an intrusion of surface offshore aged Atlantic waters into the zone, a phenomenon related to the hydrographic changes observed in the whole NW Mediterranean during 2005 and 2006 as a consequence of two consecutive unusually dry, cold and windy winters. Information on how this hydrographic forcing affected the nutrient and phytoplankton dynamics on the Barcelona coast, as well as more details related to the bloom conditions, can be found in [7].

Acknowledgements

We thank O. Chic, J.M. Fortuño and G. Forlani for assistance, and Adriana Zingone for her help on *Phaeocystis* identification. Funded by the Catalan Water Agency, EU project MARPLAN (MarBEF) and grant 2009 SGR 588 of the Generalitat of Catalonia.

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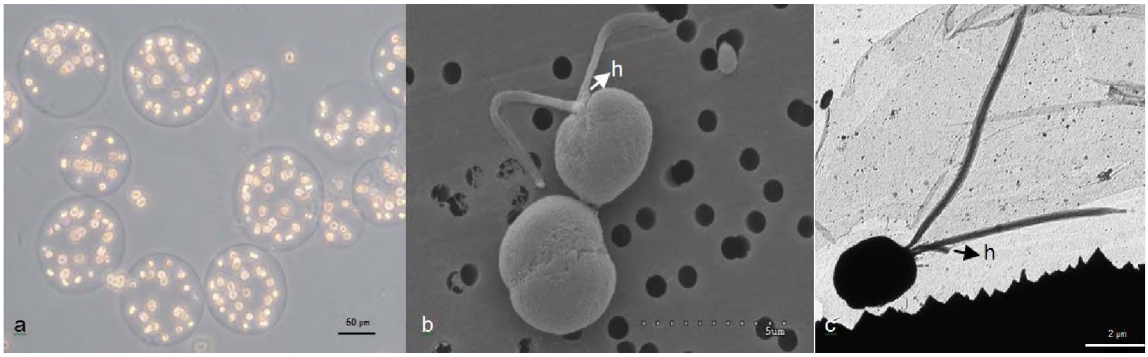

Fig. 4. Phaeocystis *sp. colonies and cells observed at different magnifications a) Small new colonies, Light microscopy; b) Free non-flagellate and flagellate cells, SEM (Photo: Fortuño and Arin); c) Free flagellate cell, TEM (Photo: Forlani); h = haptonema.*

PHENOMER: Better Knowledge of HAB with the help of Citizen observations

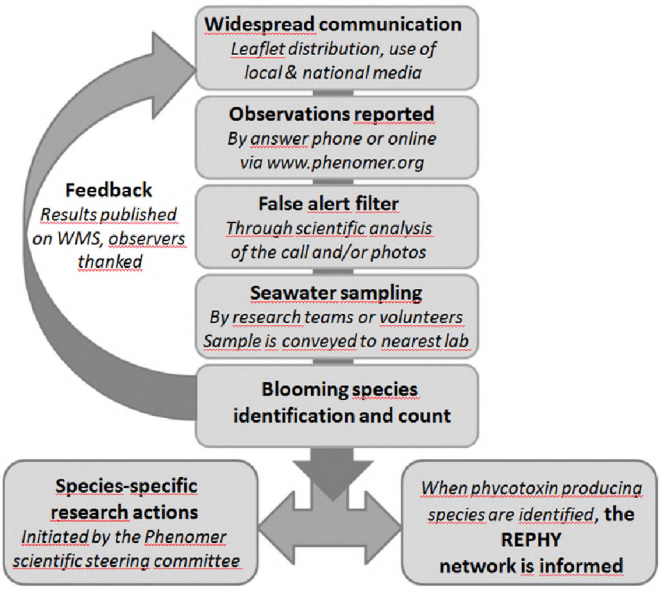

Fig. 1 The Phenomer HAB reporting process.

A citizen monitoring programme of water discoloration observations was launched across Brittany (France) waters in 2013, in parallel with the ongoing phytoplankton and biotoxin monitoring network REPHY [1]. This project, named PHENOMER (an acronym for 'visible phenomena at sea', combined with 'phenology', in French) is carried out by Ifremer and scientific and non-government organization partners and has as objectives a) to increase general public awareness of Harmful Algal Blooms (HABs) and b) to provide scientific data for current and future research.

To our knowledge, PHENOMER (www.phenomer.org) is a unique and new initiative in European waters. Volunteer HAB monitoring programmes were launched along the United States west coast in the early 1990's [2], along the east coast of the U.S.A. since 2001 via the NOAA-sponsored Phytoplankton Monitoring Network [3], and very recently along Canada's west coast due to an outbreak of diarrhetic shellfish poisoning [4]. The PHENOMER pilot study area is the Brittany coastline, a region frequently subject to microalgae blooms in its many tideways and small coastal rivers. Examples of high biomass HAB species already reported in Brittany are *Alexandrium minutum, Lepidodinium chlorophorum, Noctiluca scintillans* and *Mesodinium rubrum*.

Citizens are invited to report events they may observe (water discoloration, foam, or faunal mortalities) (Fig.1). The observations are reported either by phone or via an on-line form, and are subsequently analysed by the scientific team on-call in order to discard false alerts. In case of phytoplankton discolorations, either the nearest research teams of the project network promptly go and sample the bloom, or the volunteers are asked to follow a sampling protocol. Sample are then taken to the nearest laboratory (there are 6 dotted around Brittany's 2730 km of coastline) for analysis. The species responsible for the blooms are identified and feedback is spread both to the scientific community and to the general public on the internet site and via social media.

Prompt and very localised reports of water discolorations across a wide spatial scale can provide information on the distribution and extension of these phenomena, as well as on the biogeography of the causative organisms, including harmful species. Ecosystems where water discolorations are more likely to occur can be better identified on the basis of a monitoring system based on a theoretically infinite number of sampling points (via public observations). This would eventually favour the managing and monitoring of areas at risk as well as the realisation of new research projects. From a practical point of view, the PHENOMER network allows for rapid bloom sampling. Depending on



Fig. 2. Photograph of a Noctiluca scintillans *bloom sent in by a volunteer. Taken in July 2013 in the Bay of Biscay. Source: Maxence Loridan/Phenomer.*

the blooming species and the ongoing research programmes, the water samples and subsequent cultures can be dispatched to different research teams. By combining human observation data from PHENOMER with chlorophyll *a* satellite imagery and results from ongoing water quality programmes, we hope to shed new light on the cell concentrations at which high biomass HABs can be observed.

Citizen science has influenced both the scale of ecological research that is being done and the relationship between ecologists and the public [5]. It can be very effective for engaging people with how science works and for increasing their awareness of environmental issues and their local environment [6]. Projects including citizen participation must cross national boundaries, necessitating partnerships between governmental and non-governmental organisations to achieve relevant geographic scope. We therefore hope to extend PHENOMER to the rest of mainland France's coastline, before developing partnerships with neighbouring countries.

Acknowledgements

The authors gratefully acknowledge the PHENOMER participants who shared their bloom observations. We would like to warmly thank the scientific and environmental association partners (http://www.phenomer.org/Phenomer/Partenaires). Support for Phenomer was provided by the "Quels littoraux pour demain?" tender of the Fondation de France, the Loire-Brittany Water Agency and the French Ministry of Environment.

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The UK Marine Climate Change Impacts Partnership launches the 2013 annual report card

The United Kingdom Marine Climate Change Impacts Partnership (MCCIP) brings together scientists and policy makers from universities, government, government agencies and non-governmental organisations to provide co-ordinated advice on the impacts of climate change in UK waters. One of the main activities of the MCCIP is the production of an annual report card supported by scientific documents which have been peer-reviewed. This report card provides an update on the understanding of the impacts of climate change on the UK and Republic of Ireland (RoI) marine ecosystem and associated services. The MCCIP has recently launched the 2013 annual report card. Peer reviewed scientific reports support the 33 topics discussed. These include temperature, salinity, ocean acidification, eutrophication, plankton, harmful algal blooms (HABs), non-native species, aquaculture and fisheries.

Plankton and HAB highlights from the supporting scientific reviews reveal a decrease in dinoflagellates in the north east Atlantic and North Sea areas as well as changes in the timing of some plankton production. HABs remain a concern in both the UK and RoI with blooms of *Karenia mikimotoi* continuing to impact areas with an Atlantic influence. Closures of shellfish harvesting areas are still enforced in both UK and RoI due to high concentrations of algal toxins in shellfish flesh. Since the previous report card in 2011, the first closures of shellfish harvesting areas as a result of concentrations of azaspiracids (AZA) above the EU closure limit have also been enforced in Scottish waters. Prior to this, closures for AZA had only been enforced in the RoI.



2013 MCCIP Report Card

The MCCIP annual report card can be found at http://www.mccip.org.uk/media/18758/mccip-arc2013.pdf. The individual supporting scientific reviews can be found at http://www.mccip.org.uk/annual-report-card/2013.aspx/#Supporting. For further information about the MCCIP please contact office@MCCIP.org.

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ICHA NEW ZEALAND 2014

THE 16TH INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

27 – 31 October 2014 • Wellington, New Zealand

The theme of the conference is "**Advancement through Shared Science",** which reflects the multidisciplinary nature of the field and the immense value of international collaboration. Topics include:

- HAB biogeography, regional events and population dynamics (modeling and prediction)
- Harmful and nuisance algae in marine and freshwater benthic environments (including diatoms, cyanobacteria and ciguatera)
- Toxin chemistry and analytical methods
- Toxicology and international regulation of algal toxins in food
- Taxomony, functional genomics and genetic diversity of HABs
- Sensors and new technologies for cell and toxin detection
- Impacts of HABs on ecosystems, aquaculture, fisheries and public health
- Climate change, eutrophication and HABs (including macro-algae)
- Monitoring and remote sensing, management and mitigation of HABs

CRITICAL DATES

First circular and call for papers:	6 Jan 14
Paper submission deadline:	15 May 14
Notification to authors of acceptance:	15 Jul 14
End of early-bird registration:	15 Aug 14
Conference:	27-31 Oct-14

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International Society for the Study of Harmful Algae ISSHA



EASTHAB-VIII Symposium held in Seoul, Korea, November 2013

The EASTHAB-VIII Bi-annual Conference was held on November 7-8, 2013 at Hanyang University, Seoul, Korea with more than 50 scientists and experts from the four member countries, China, Japan, Philippines, and Korea. Each country presented its national report on harmful algal blooms (HABs), focused on outbreaks and recent trends. There were also 20 scientific reports including 12 oral presentations and 8 posters. Three scientific sessions were established, on 1) bloom environments and monitoring, 2) ecology, physiology, and oceanography, and 3) benthic dinoflagellates and mitigation, to understand the present situation and to find out collaborative procedures for more efficient monitoring.



Some participants in the EASTHAB-VIII Symposium, 7 Nov. 2013

According to these reports, a total of 43 HABs occurred in Korean coastal waters in 2013, of which, 28 events were caused by *Cochlodinium polykridkoides*. *C. polykridoides* blooms occurred both on the southern and eastern coasts from mid-July to early September, with a massive fish kill (about 24.7 billion Korean won, equal to US$ 25 million), the second largest fish kill since 1995. It was assumed that the large-scale blooms in 2013 were closely related to low rainfall, high water temperature, high salinity, and the strong influence of the Kuroshio warm current on Korean coasts in the summer season (Dr. Changkyu Lee).

Seventy three HAB events were reported in Chinese coastal waters in 2012. HABs occurred mainly during May and June. The main species responsible were *Prorocentrum donghaiense, Alexandrium tamarense, C. polykrikoides, Karenia mikimotoi, Aureococcus anophagefferen* and potentially toxic species such as *Karlodinium veneficum, Heterosigma akashiwo*. SOA recorded a large mortality of abalone caused by *K. mikimotoi* in the East China Sea, with an economic loss of over 2 billion Chinese Yuan (Dr Douding Lu, Dr Xinfeng Dai).

In Japan in 2011-2012, serious HAB problems for aquaculture have continued, mainly in western Japan. The causative organisms are diatoms, dinoflagellates, and raphidophytes. *K. mikimotoi* blooms occurred widely in western Japan and caused great damage, particularly in the western part of the Seto Inland Sea and Bungo Suido. Blooms of *Heterocapsa circularisquama* in Lake Kamo (Niigata Prefecture) and *C. polykrikoides* in the west caused significant economic loss (Shigeru Itakura, Kazumi Matsuoka).

In the Philippines, *Pyrodinium bahamense, Gymnodinium catenatum,* and *Alexandrium* spp. have caused PSP events. *C. polykrikoides* caused a fish kill on the western coast of Palawan (Rhodora Azanza).

Following the provisional agenda of the host country, three topics were discussed, 1) operation of the terms of reference (ToRs) of EASTHAB, 2) future activities for 2014-2015, 3) the EASTHAB-XI meeting venue. The EASTHAB International Office is to revise and enlarge ToRs to accommodate recent changes. Dr. Hak-Gyoon Kim was reelected Secretary General to serve a four year term from 2014.

With regard to Future Activities for 2014-2015, a Scientific Steering Committee (SSC) was established based on the revised ToRs. Twelve SSC members comprised of three scientists from each of the four countries were nominated as follows: Dr. Mingjiang Zhou (IOCAS), Dr. Douding Lu (SIO-SOA), Dr. Songhui Lu (Jinan University China), Dr. Goh Onitsuka (Fisheries Research Agency), Dr. Mitsunori Iwataki (Yamagata University), Dr. Ichiro Imai (Hokkaido University Japan), Dr. Changkyu Lee (NFRDI), Dr. Myungsoo Han (Hnyang University), Dr. Joongki Choi (Inha University Korea), Dr. Rhodora Azanza (UP-MSI), Dr. Ulysses Mentojo (NFRDI Philippines), Dr. Aletta Yniguez (UP-MSI Philippines).

A work plan for a collaborative cruise to clarify the initiation and subsequent transport routes of fish killing HABs was proposed by NFRDI-Korea. The plan would include keeping track of simultaneous HABs such as fish killing blooms of *Cochlodinium polykrikoides*, for the purpose of HAB prediction, especially in the East China Sea and adjacent regions. When the cruise is funded and implemented, an interim meeting including a workshop or forum will take place in 2014. One option to improve public attention to the EASTHAB scientific reports, is an agreement to publish scientific articles in special issues of scientific journals. Creation of an EASTHAB web site and ways of fund raising were also discussed. More in-depth issues pertaining to this work are to be discussed at the EASTHAB-IX.

Dr. Songhui Lu (Jinan University) offered to host the next EASTHAB-IX Meeting, to be held November 2015 in Guangzhou (Canton).

Dr. Hak Gyoon Kim, Secretary General, EASTHAB, Pukyong National University, LED-Marine Convergence Technology R&BD Center, San 100 Yongdand-dong, Nam-gu, Busan, 608-739, Korea

National Shellfish Sanitation Programme in Argentina

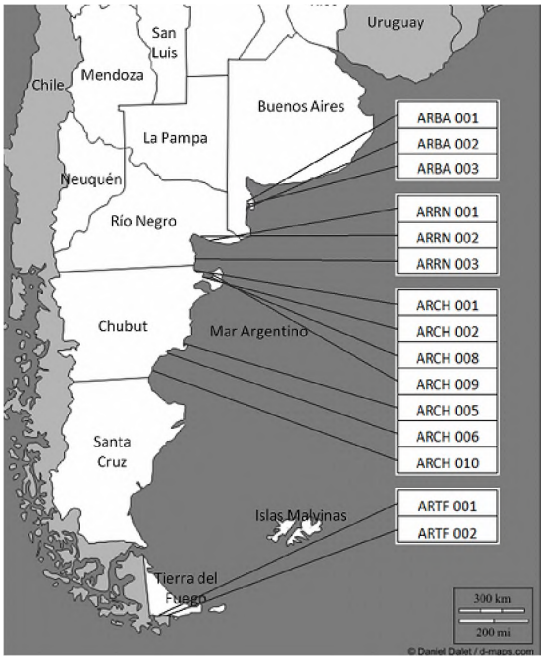

Fig. 1. Classified Shellfish Production Areas in the Argentinean coasts.

In 2005 Argentina began a National Programme to classify shellfish harvesting areas, to provide a framework for the development of molluscan shellfish aquaculture and harvesting activities in coastal areas. One of the first steps was the establishment of a covenant between the "National Subsecretariat of Fisheries and Aquaculture" and the "National Service for Food and Agriculture Quality Control and Sanitation", both belonging to the Ministry of Agriculture, Fisheries and Food (MAGyP). Activities were coordinated by the Aquaculture Directorate from the same ministry.

National regulations for classification of shellfish production areas (Fig. 1) were modified and implemented after meetings with the provincial authorities and taking into account the different kinds of exploitation. Training courses were organized for phytoplankton and shellfish sampling, good procedures for handling shellfish, and identification of harmful algae species, and followed by the application of these common criteria to the whole country. In addition, technical staff had open workshops with shellfish growers and fishermen.

Advances in standardization required coordination between the central government and the competent maritime authorities (ACP), in gulfs and embayments and in coastal waters up to the 12 miles limit in their respective jurisdictions (Table 1). Shellfish production areas are classified as A, B, or C on the basis of their levels of chemical pollutants and microbiology. The National Food Security Agency, SENASA, is the authority which classifies the areas and audits results provided by the provincial authorities. All coastal areas in Argentina are classified as A, and shellfish harvests do not require depuration before going to market. Specific regulations to control a variety of resources are published in resolution 829/2006 of the Secretary of Agriculture, Fisheries and Food.

The Pacific oyster (*Crassostrea gigas*), an exotic species successfully adapted to northern Argentina, is harvested from shellfish beds and cultures in Bahía Anegada, south of Buenos Aires Province. Purple clams (*Amiantis purpurata*), mussels (*Mytilus edulis platensis*), geoduck (*Panopea abbreviata*) and "cholga" (*Aulacomya atra*) are the main shellfish resources in the province of Río Negro; the same species, (except the purple clam), and also scallops (*Aequipecten tehuelchus*), white clams (*Protothaca antiqua*) and razor clams (*Ensis macha*), are exploited in the southern provinces of Chubut and Santa Cruz. Mussel (*Mytilus edulis*) cultivation predominates in Tierra del Fuego, Antártida and the South Atlantic Islands, and "cholga" and deep-water scallops (*Zygochlamys patagonica*) are harvest in the Beagle Channel.

Provincial authorities have implemented monitoring of harmful algae, and provide information to consumers on the risks involved in eating seafood unregulated by official controls, and the need to establish shellfish harvesting bans to protect public health in all areas associated with commercial exploitations or recreational shellfish collection. Some provinces (e.g. Chubut) have a long history of HABs and shellfish monitoring, but others have started monitoring quite recently. Monitoring is carried out by the ACP in cooperation with universities, such as the National University of La Plata in Buenos Aires and the University of San Juan Bosco in Patagonia, and staff trained by the administration for these specific tasks. Provincial inspectors are responsible for phytoplankton and shellfish sampling and for their custody such that products sent to SENASA laboratories can be traced.

Biweekly analyses of phytoplankton samples with a focus on HAB species

Table 1. Competences of the Provincial (ACP) and National (ACN) Authorities for Molluscan Shellfish Safety in Argentina.

COMPETENT PROVINCIAL AUTHORITY (ACP)	COMPETENT NATIONAL AUTHORITY (ACN)
1. ESTABLISHES MOLLUSCAN SHELLFISH PRODUCTION AREAS	1. ENDORSES PROPOSED PRODUCTION AREAS
2. UNDERTAKES STUDIES FOR TO CLASSIFY	2. KEEPS NATIONAL REGISTER OF THE AREAS
3. DEFINES SURVEILLANCE CONDITIONS FOR EACH AREA (MONITORING PROGRAMME) AND PREPARES PROTOCOLS.	3. CARRIES OUT AUDITS TO VALIDATE COMPLIANCE WITH REGULATIONS AND EFFICIENCY
4. ENFORCES SHELLFISH HARVESTING BANS	4. NEGOTIATES SHELLFISH EXPORTS TO THIRD COUNTRIES
5. ORGANIZES CAPACITY BUILDING WORKSHOPS	
6. SETS THE REQUIREMENTS FOR SHELLFISH COLLECTION AND CULTURES AND UPDATES RECORDS	



Fig. 2. Participants in the First National Workshop on Marine Biotoxins and Harmful Algal Blooms, Puerto Madryn, Argentina, 23-27 September 2013

are carried out in most coastal provinces. Results and early warnings of the occurrence of toxigenic species are sent to the provincial authorities and to the national network of laboratories. The latter perform the testing and calibration required by regulation ISO 17025, and are responsible for toxin analyses of shellfish collected at the same time as plankton samples. PSP toxins are analyzed according to the standard mouse bioassay (AOAC 959.08), and lipophilic toxins by the procedure of the EU Reference Laboratory for Marine Biotoxins (CRLMB 2011). ASP toxins are analyzed by HPLC. Results are communicated to the provincial authorities who enforce harvesting bans/openings. Recently SENASA has acquired an LC-MS that will be used to solve uncertainties in lipophilic toxin results obtained by mouse bioassay.

The First *National Workshop on Marine Biotoxins and Harmful Algal Blooms*, convened by the MAGP through the Subsecretariat of Fisheries and Aquaculture, was held in Puerto Madryn, Chubut, 23-27 September 2013 (Fig. 2). Researchers from Universidad Nacional de La Plata, Universidad de la Patagonia San Juan Bosco, and INIDEP, technical officers from the MAGP coastal laboratories and from SENASA, as well as officers from the Aquaculture Directorate and two invited speakers from Galicia, Spain (Ángeles Moroño, INTECMAR and Beatriz Reguera, IEO) participated in this meeting to coordinate ongoing activities of HAB research and monitoring in Argentina, and to identify the main impediments to progress.

The first day of the workshop was open to representative fishermen and shellfish grower associations. Protocols and results obtained in phytoplankton and shellfish toxin monitoring programmes carried out in each maritime province were presented. Special attention was focused on regulations for classification of shellfish production areas, emerging toxins, and the way European laboratories, such as INTECMAR, are adapting their existing control to recent European Union regulations for marine phycotoxins. On the second day, three round tables were arranged to discuss: i) harmful algal blooms in Argentina; ii) the main problems met by laboratories in charge of toxin analyses; iii) management of classified production areas.

The group recognized the success of the workshop in facilitating contact between professionals dedicated to different aspects of HAB-related problems in Argentina. They underlined advances in recent years concerning implementation of monitoring programmes and identification of the main agents of toxic events. They directed attention to new issues on regulated and unregulated emerging toxins (azaspiracids and fast acting toxins) that seem to be widely distributed on Argentinian coasts. Future goals identified were: i) to establish a communication network including all participants in HAB-related activities; ii) news dissemination; iii) updates on harmful phytoplankton species identification; iv) periodic meetings to coordinate all HAB and biotoxin monitoring programmes in the country. The group on toxin analyses considered essential: i) common purchase of imported toxin standards; ii) to set as soon as possible the analytical procedures with the new LC-MS/MS system to resolve uncertain results of lipophilic toxins obtained by mouse bioassay; iii) to ensure participation of missing provincial laboratories on the National Laboratories Network. In the context of classification of production areas, there is a need to revise current regulations including: i) checking the time gaps between sampling and analysis; ii) communication of harvesting bans; iii) sampling frequency for different toxins; iii) review of the applied system for classification of areas.

Participants in the meeting had the opportunity to visit the HAB monitoring laboratory of the National University of Patagonia (Trelew) and the provincial laboratory of the Health Ministry in Chubut. In addition, the invited speakers met with authorities from the National Fisheries and Aquaculture Directorates, and visited the SENASA Laboratory in Buenos Aires.

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News from Latin America



Participants in the RLAN (Latimerican Meeting on Harmful Algae) meeting. Florianápolis, Brazil, 7-9 October 2013.

Researchers from Latin American countries met in Florianópolis, southern Brazil for the Latin American Meeting on Harmful Algae (RLAN). The meeting between 7 and 9 October 2013 was attended by about 60 participants from Argentina, Chile, Uruguay, Ecuador, Peru, and Mexico,as well as colleagues from Sweden and Spain. The main objective of RLAN is to meet and discuss the harmful algae theme, recent advances in the field and future perspectives. This event also aimed to discuss strategies for organization as a regional initiative of the 17th International Conference on Harmful Algal - ICHA, the International Society for the Study of Harmful Algae, which will take place in Florianópolis in 2016.

The RLAN was very productive and brought together old colleagues with the new generation of researchers in the region. There were 3 days of discussions, with presentation of oral and panel contributions and several roundtables. These activities were joined by people involved in bivalve mollusk production in Santa Catarina. The meeting strengthened the idea of 17 ICHA as a regional event with the effective participation of Latin American colleagues in the organizing committees. The event is scheduled to take place between 9 and 14 October 2016.

During the RLAN, the *Latin American Association for Harmful Algae Studies* (AlEAN) was created, with statutes and philosophy inspired by the International Society for Harmful Algal Studies, ISSHA. The association will work with ISSHA to strengthen their common goals and prepare a great event in 2016. More details about plans for the 2016 ICHA will be presented to the society at the meeting in Wellington, New Zealand.

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GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

GEOHAB Activities and Synthesis Plans

It has been an active year for GEOHAB. The Core Research Project on HABs in Stratified Systems held an Open Science Meeting in 2012. The outcomes from that meeting are published and available at www.geohab.info (Advances and Challenges for Understanding Physical-Biological Interactions in HABs in Stratified Systems) as a GEOHAB Report. This workshop directly led to a special issue in *Deep Sea Research II.* Guest editors include R. Raine, E. Berdalet, M. McManus, and H. Yamazaki. The special issue "Harmful Algal Blooms in Stratified Systems" is already published. It includes 21 peer-reviewed manuscripts and a preface. GEOHAB has also participated in several international meetings and workshops, including co-sponsoring the "Harmful Algae Blooms in a Changing World" workshop with ICES and PICES (see accompanying article), and participation in the Group on Earth Observations (GEO) Oceans and Society: Blue Planet Symposium, held in Sao Paolo State, Brazil. GEOHAB was also asked to participate in the recent Joint Programming Initiative for Health and Productive Seas and Oceans Initiative (JPI-Oceans).

The most exciting activity, however, was the successful Open Science Meeting (OSM) held in Paris, France, April 24-26, 2013. This meeting attracted an international group of scientists to share their insights regarding GEOHAB past, present, and future activities. This meeting had special relevance not only for the GEOHAB programme, but also for the entire international scientific community of researchers and managers engaged in the advance in harmful algal bloom research and mitigation of impacts. This synthesis OSM had two objectives, first to review scientific advances accomplished under GEOHAB since its inception, second to identify a near-future roadmap of GEOHAB-like activities to be pursued beyond 2013. As part of the meeting, GEOHAB solicited submission of concept papers focusing on potential activities that could be completed within the next 5 years, and asked the international HAB community to envision what opportunities and challenges we face over the next decade.

A total of 15 concept papers were presented and discussed. These form a core list of potential science objectives moving beyond the 2014 end-date for the GEOHAB Programme. Meeting participants identified several positive outcomes from GEOHAB, including the success of the various Open Science Meetings and workshops, the process for endorsing programmes and projects from the community, and the emphasis on technology development, capacity building, and training of young scientists.

The primary outcome from this meeting was the proposal to initiate a new International HAB Programme, GlobalHAB, as a follow-on to GEOHAB. This programme focuses on the theme of "HABS in a Changing World" and is to be based on the GEOHAB Science Plan. A new Science Steering Committee will be formed, and an Implementation Plan will be developed for GlobalHAB. This will build on the success of GEOHAB, but will provide the opportunity to address areas identified in the GEOHAB Science Plan but not previously implemented, such as a science-based focus on HAB toxins, socio-economic impacts, and HABs at the land-sea interface.

Immediately following the Paris OSM, this plan was presented to the Intergovernmental Panel on Harmful Algal Blooms (IPHAB). The plan was endorsed by the International Oceanographic Commission (IOC), with an invitation to the Scientific Committee on Ocean Research (SCOR), the co-sponsor of GEOHAB, to continue their involvement.

In preparation for the termination of the GEOHAB of synthesis documents and publications, while also maintaining our active involvement with relevant international groups and agencies. We look forward to providing updates on both the GEOHAB Synthesis and the launch of the GlobalHAB Programme at the 16th International Conference on Harmful Algae in New Zealand!

Raphael Kudela, GEOHAB-SSC Chair, Department of Ocean Sciences, University of California, Santa Cruz, CA 95064, USA.



"We all came to Paris because we recognize a fundamental problem (HABs), and cannot solve this problem in our individual laboratories. This requires an international approach." Participants in the OSM on GEOHAB Synthesis, UNESCO, Paris 24, 26 April 213

Harmful Algal Blooms in a Changing World

There are projections that the process of climate change will lead to increased frequency and severity of Harmful Algal Blooms (HABs). Indeed there is evidence that climate change already may be causing shifts in phytoplankton community composition, but the projections on climate-increasing the HAB impact remain, at this point, largely speculative. Although there are many intuitive linkages, these scenarios are founded on limited and often conflicting experimental data, so that scientific debate at this time cannot establish a link between HABs and climate change, let alone how dramatic any change in HABs might be in the future. Moreover, the field of HAB research lacks the rigorous organization structure of the Intergovernmental Panel of Climate Change, where scientists worldwide work in collaboration to quantify and evaluate trends in climate impacts. HAB scientists need to proactively identify the fundamental parameters and research infrastructure needed to effectively address this important question if we are to have those data we need when called upon to forecast or explain changing HAB patterns.

It is important to recognize that the issue here is not simply whether a pattern might develop where fluctuations in HAB frequencies become more prevalent in the future. Instead, based on limited ecophysiological insights, it is reasonable to postulate there will be fundamental changes in the distributions of HABs, bringing ecosystem and human health threats to extensive new regions, perhaps compounding current problems. There also likely will be increased "windows of opportunity" for existing regional HABs, intensifying existing threats at a time when humans will be relying more on coastal resources for food security. As it stands, the HAB research community is woefully unprepared to provide solid insights into the changes that will define the next generation's access to many marine resources.

A workshop of international experts convened in March, 2013 at the Whiteley Center, Friday Harbor Laboratories, University of Washington (funded by PICES, GEOHAB and NOAA) to develop a preliminary assessment of where the field of HAB research stands in terms of addressing HAB/climate change, and the directions it needs to move over the next decade.

Three broad classes of HABs were considered: toxic HABs that impact human health, fish-killing HABs where the causative organisms affect both wild and cultured fish populations, and high-biomass HABs, whether caused by natural or anthropogenic processes, leading to hypoxia, foam, and other negative impacts. The key underlying consideration surrounding climate related distributional changes in HABs is three-pronged: HAB species "getting there", being adapted well enough to "remain there" over the course of the season, and ultimately "staying there" for multiple seasons. There is much in the invasive species research field that could be applied to investigate HAB/climate change links.

The workshop deliberations used as a foundation observed and predicted climate changes in the physical and chemical conditions in aquatic systems identified in the AR4 IPCC Synthesis Report on climate change, and combined what is known about how these affect the physiology of both general phytoplankton as well as HAB species. The central questions asked were: what do we know about how a given parameter affects HAB species, what do we not know of importance in terms of this parameter's impacts, which of these unknowns are the most pressing questions, and how should we go about addressing them?

Topics considered included *temperature* and it's effects on cellular growth rates, nutrient uptake rates, toxin production and cellular lipid composition, and *stratification* and its impact on vertical nutrient flux, physical and chemical stability of the system and the prolonging of HAB windows of opportunity. Similarly, the effect of ocean *acidification* was examined in terms of success of HAB species and cellular toxin synthesis and accumulation, along with the effects from altered *nutrient inputs* associated with changing precipitation characteristics (e.g., pulsed terrestrial riverine flows) and facilitated transport of culturally-derived nutrients. While each of these four broad parameters have known impacts on HAB species, it will be synergistic interactions among them that will determine the overall impact on HAB species success in phytoplankton communities, interactions that, realistically, there is at present no evidence with which to inform.

The workshop participants felt that current insights on how climate change may influence grazing, and the effects of light on HAB species, are entirely insufficient. Many HAB species are both grazers and prey, but there is very limited information on how the balance of these processes might deviate as the ocean environment changes. There are model projections that climate change will alter patterns of cloud cover, and thus change light fields spatially in surface waters. While light is a key parameter affecting phytoplankton communities, and different species are know to be better light or shade adapted candidates, there is no indication so far that HAB species will be affected differently from non-HAB species.

An overriding theme during the workshop discussions was consideration of how environmental changes will impact HAB species relative to non-HAB species. Participants recognized that much of the HAB research to date has focused on the specifics of HAB organisms, which though essential for understanding the ecophysiology of the organisms provides limited insight as to how HAB organisms interact within the broader phytoplankton communities. One of the key workshop findings was the need for a shift in the strategy of HAB research towards more comparative investigations that inform on the thresholds for shifting balance among HAB and non-HAB species in the context of climatically driven changes in coastal and oceanic environments.

The workshop participants also considered what new research tools would help move the science forward most quickly. The primary need at this time is organizing long-term sampling programs to collect HAB-centric datasets of relevant parameters across diverse geographical and oceanographic regimes. While regional and national monitoring programs already exist, none are sufficient to provide the data streams to assess changes in HABs in new, currently



Participants in the PICES-GEOHAB-NOAA workshop on HABs in a Changing World, Whiteley Center, Friday Harbor Laboratories, University of Washington, March, 2013.

unaffected regimes. The vast majority of these HAB data also lack the essential calibrated oceanographic data to link HAB changes to climate-dependent parameters.

Participants agreed that the most productive means to initiate these data collection streams is to collaborate with existing coastal and offshore oceanographic monitoring sites, to add a limited list of parameters (e.g. phytoplankton composition, toxins, etc.) to establish HAB "observer sites". Recognizing that laboratory facilities and expertise in many areas of interest are limited, a shorter list of key parameters that are easily obtained with simple sampling approaches was developed to facilitate new outpost-type monitoring sites.

A second kind of sentinel site was envisioned by participants, termed "super sites", that while limited in number, would be equipped with sophisticated monitoring equipment to enable high-frequency sampling of phytoplankton species composition along with a wide range of environmental parameters. Their purpose would be to enable *in situ* investigations of the ecology of HABs in relation to non-HAB species. The goal of both "observer" and "super" sites is to ensure adequate datasets for statistical assessment of long-term change across multiple coastal and oceanic regimes.

New investigative approaches will also be needed to address the HAB/climate change issue. It is recognized that isolates of given phytoplankton species differ in their growth responses to different stimuli, so there is a strong need to understand these localized differences in projecting climate change effects. One novel approach is to organize "common garden" style culture experiments, where many laboratories around the globe conduct identical experiments using precise established procedures to test the effects of one or more parameters on a single species isolated from their local waters. This approach provides the ideal mechanism for evaluating species responses as well as characterizing inter-strain variability. Other methods included cross–sectional research programs such as mesocosms and other enclosures using standardized methods, design, analysis, and assessment. Enclosed basin study sites, where HABs are a repetitive feature, were also cited as promising instruments for gauging HAB competitive interactions in unrestricted coastal waters.

Participants also identified a strong need to develop measures for dealing with HAB outbreaks, including proactive (avoidance), abatement (halting) and mitigation (reduced impact) strategies. In addition, virtual assessment approaches to understand past outbreaks and forecasting future outbreaks built upon detailed conceptual or heuristic models were felt to be under-utilized.

Participants felt that next logical step in focusing HAB/climate change research was to convene an Open Science Meeting on global change impacts on marine and freshwater HABs. The goals of this symposium would be to promote research in the topics along the lines identified by the workshop participants to be of high priority over the next decade, to bring new people and expertise into the field (i.e., to make climate change researchers aware of the HAB issue, and how their expertise and methods may find rich ground for research), and to focus community efforts towards identifying the data needed to unequivocally demonstrate a linkage between HABs and climate change.

The workshop findings are now being integrated into a manuscript intended for publication in the international journal *Harmful Algae.* The findings will help provide a broader view for the HAB research community on some of the key gaps in our understanding, and to help focus global research efforts on addressing HABs and climate change.

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GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

Participation of GEOHAB in "CSA Oceans UN/International Organisations Consultation Workshop", Brussels, June 11th 2013

GEOHAB was invited to participate to the CSA Oceans UN/International Organisations Consultation Workshop (June 11, 2013). The activity was part of a broad mapping exercise being conducted by the European Commission FP7 Coordinated Support Action (CSA) Oceans to the Joint Programming Initiative on Healthy and Productive Seas and Oceans (JPI Oceans, www.jpi-oceans.eu). Inputs provided in the framework of this workshop and a subsequent extensive questionnaire filled by the participants are analysed to inform the European Commission on the development of the JPI Oceans Draft Strategic Research and Innovation Agenda.

Participants provided their views on the gaps and knowledge needs that could potentially be relevant to JPI Oceans and how best to interface with other mechanisms for European international coordination and cooperation on marine and maritime research. In particular, JPI Oceans aims to add value at a European level to make better use of R&D related national investments and resources, including human capacities and infrastructures. The document elaborated by GEOHAB emphasized pressing issues and promising research, technology and innovation potentials, in a 20-30 years perspective, related to the seas and oceans in general. In addition, the major challenges related to harmful algal blooms (HABs) in European waters were highlighted, such as the need for an operational oceanography of HABs events, technical advances to develop sustained long term and high-resolution observing systems for biological processes (in general), to ascertain the impact of climate change on HABs (including benthic ones), to implement sustainable and secure fisheries, aquaculture and tourism, organization of common and efficient ocean data bases, etc. JPI Oceans was also informed in detail about the scientific GEOHAB programme and its achievements as a platform to coordinate and foster international research on HABs since its launch in 2000. In the future, GlobalHAB, recently endorsed by IOC/UNESCO in the last IOC Assembly, will continue to work with the principle that the fundamental problem of HABs cannot be solved in individual laboratories and requires an international approach. The delivered document indicated that collaborative links between GlobalHAB and JPI Oceans will contribute to the structuring of efficient and sound marine research in Europe.

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In Memoriam - Grethe Rytter Hasle

Grethe Rytter Hasle, 93, passed away quietly while sleeping between Nov 8 and Nov 9. She was born Jan 3, 1920. She was buried in Haslum near Oslo, Norway.

Grethe was active until the very last. She drove her car for the last time a week before she died. She had her last scientific discussion 1½ week before she died, thus scientific ideas that she could not explore herself were passed on. Her photos, slides and a huge collection of samples will be transferred to the Natural History Museum of Oslo to form the core of a newly established algal collection.

Grethe Hasle was a dedicated, inspiring and productive scientist. She published several important pioneering papers, and has influenced science and scientists throughout the world in the field of algal taxonomy. Grethe started her career at Oslo University studying the vertical migration of dinoflagellates and the reliability of single observations in phytoplankton surveys. Her subsequent work was on the taxonomy of coccolithophorids and ciliates. Her 1968 Ph. D. dissertation was entitled "An analysis of the phytoplankton of the Pacific Southern Ocean: abundance, composition and distribution during the 'Brategg' Expedition, 1947-1948".

Stimulated by a visit to Dr. Friedrich Hustedt in Bremen, Grethe turned to diatom taxonomy, ranging from pennate (*Nitzschia, Fragilariopsis*) to centric



(*Thalassiosira, Cymatosira*) genera. As a pioneer in the combined use of light, scanning and transmission electron microscopy, she became an internationally renowned expert on diatom ultrastructure, and described the strutted and labiate processes of Thalassiosiraceae. She has supervised students from all over the world, and her dedication has inspired diatomists everywhere. Grethe Hasle was nominated a Full Professor in Marine Botany at the University of Oslo in 1977, the third female professor in Natural Science at the university.

The high quality of Grethe's work is illustrated by her 1965 publication in "Skrifter utgitt av Det Norske Videnskaps-Akademi", on the group *Pseudo-nitzschia*. She paid careful attention to details of the number of striae and fibulae, rows of poroids on the striae membrane, the presence/absence of a central interspace, as well as to diatom type material. This ground-breaking work includes a description of *Nitzschia pungens* forma *multiseries* (now *Pseudo-nitzschia multiseries*), which in 1987-88 was found to be responsible for the production of domoic acid, causing Amnesic Shellfish Poisoning in Prince Edward Island, Canada. Grethe Hasle's research is thus a prime example of the practical importance of basic science. Grethe helped researchers from all over the world with the difficult taxonomy of this domoic-acid-producing genus, culminating in her 2002 review in "Harmful Algae" on the global distribution of toxigenic *Pseudo-nitzschia* species.

Grethe continued her prolific publication output throughout her eighties. She worked until the very last with manuscripts still in progress, and had her last paper published only a few years ago. She was elected to the Norwegian Academy of Science and Letters in 1980, and at the time she was the only woman in the Science Class. In 1999, she received the Excellence Award from the Phycological Society of America, and in 2002, she was awarded the Yasumoto Lifetime Achievement Award by ISSHA. She is honoured by the names of the diatoms *Haslea, Nanoneis hasleae, Praethalassiosiropsis hasleae, Thalassiosira hasleae* and *Pseudo-nitzschia hasleana,* and the dinoflagellate *Dinophysis hasleae*.

Nina Lundholm, Natural History Museum, University of Copenhagen, Denmark
Gustaaf Hallegraeff, Institute for Marine and Antarctic Studies, University of Tasmania, Australia

In Memoriam - Professor Zhu Mingyuan

We are sad to communicate the passing away of our dear colleague, Professor Mingyuan Zhu.

Professor Mingyuan Zhu, was an outstanding Chinese expert on phytoplankton and HAB studies, at the State Ocean Administration of China, First Institute of Oceanography, Qingdao City. He suddenly passed away without any warning while attending the PICES 2013 annual meeting in Nanaimo, BC, Canada. He was 70. Prof. Zhu was elected to many academic positions. He was Secretary General of the Chinese Committee of SCOR, Vice Chairman of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, Head of the Regional Working



Group (RWG) on Investment, and Vice Head of the RWG on Ecosystems, UNDP/GEF Yellow Sea Large Marine Ecosystem Project, as well as Vice-Chairman of the Chinese Committee of the SCOR-IOC Working Group on Harmful Algal Blooms, and many others. He was very active both in Chinese ocean science and in regional and international programmes and projects such as LME, GEOHAB, and GLOBEC. His death is a great loss for the Chinese and worldwide phytoplankton and HAB communities. This has been a shock not only for his numerous former students, many of them now in key positions in China and elsewhere, but also for the many colleagues who have had close cooperation with him and his research team.

Ian Jenkinson & Ming Jiang Zhou, Chinese Academy of Sciences, Institute of Oceanology, 7, Nanhai Road, Qingdao 266071, China

Future Events



ICES Annual Science Conference 2014

A Coruña, Spain, 15-19 September 2014
Call for Abstracts now open

ICES Theme Session H: Harmful Algal Blooms in Aquac Culture and Fisheries ecosystems: prediction and societal effects

ICES 2014 ICES Annual Science Conference

Conveners: Beatriz Reguera, Spain (beatriz.reguera@vi.ieo.es), Juan Blanco, Spain (juan.blanco@cimacoron.org) and Bengt Karlson, Sweden (bengt.karlson@smhi.se)

We invite you to contribute with oral/poster presentations on the following topics:

- Advances in the ecology and oceanography of HABs in the ICES domain
- Improvements in HAB forecasting – coupled physical-biological, and toxin uptake-detoxification models.
- HABs and their impact on wild fisheries and shellfisheries
- Emerging benthic HABs and their toxins.
- Advances in automated HAB observing systems, biosensors and toxin-detection methods.
- Mitigation strategies
- Supporting information for the end-users

Deadline abstracts submission: 14 April 2014

The ICES IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

The working group, chaired by Bengt Karlson, Sweden, will meet in IJmuiden, Netherlands, 29 April – 2 May 2014 to:

a) Report on new findings in the area of harmful algal bloom dynamics;
b) Deliver National Reports on harmful algal events and bloom dynamics for the year 2013;
c) Summarize the harmful algal bloom events 1990-1999 and in 2000 to 2009 in the ICES region based on decadal maps using HAEDAT with a view to investigating if data are of a quality that allows inter-decadal comparisons;
d) Review progress regarding the entering of data onto the HAEDAT data-base and review synthesis stories (HAEDAT entries from start to date) submitted in advance from each country with a view of drafting a 'synthesis story' about HAB events in the ICES area;
e) WG should report on Automated Harmful Algal Bloom in situ Observation Systems;
f) Finalize draft a review document quantifying on the scale nature and extent of the problems associated with fish killing algae in the ICES region;
g) Review progress and advise the conveners on the planned session on Harmful Algal Blooms in Aquaculture and Fisheries ecosystems: prediction and societal effects at the 2014 ICES Annual Science Conference in La Coruna, Spain;
h) Review progress and advice the scientific steering committee for the planned joint ICES-PICES-IOC scientific symposium on Climate change and harmful algal blooms. The symposium is planned to be arranged in 2015;
i) Contribute to the development of a Global Harmful Algal Bloom Status Report.

The Working Group is open to participation by experts also from IOC Member States outside the ICES region. If you or experts you wish to involve in the WGHABD are interested in participating they should communicate their interest to the IOC (H. Enevoldsen) and with copy to the Chair of the Group (B. Karlson). Participation is at national/institutional/personal expense.

Priorities for International Cooperation on HAB set for next two years

In April 2013, the Eleventh Session of the IOC Intergovernmental Panel on Harmful Algal Blooms met at UNESCO Headquarters in Paris to review actions completed during the past two years by IOC and partners in the area of HABs. Major achievements reported included: (i) developments within GEOHAB including the launch of the GEOHAB Research Plan for the Core Research Project in Benthic Systems and the development of a revised international coordinated research plan for HABs; (ii) development of regional activities within ANCA, FANSA, HANA and WESTPAC-HAB; (iii) implementation of more than ten training courses and training-through-research projects; (iv) results from the ICES-IOC WGHABD and ICES-IOC-IMO WGBOSV; (v) continued development of the integrated IPHAB-IODE Harmful Algae Information System (vi) continued publication of the IOC Harmful Algae News; (vii) and IOC co-sponsorship of international HAB conferences.

The Panel made decisions concerning (i), a Scientific Symposium on Harmful Algae and Global Change with ICES and PICES (ii) Development of a Global HAB Status Report, (iii) an initiative on Harmful Algae and Desalination of Seawater, (iv) Regional HAB Programme Development, and revised terms of reference for the (v) Task Team on Biotoxin Monitoring, Management and Regulations, (vi), Task Team on Algal Taxonomy, and (vii) Task Team on Harmful Algae and Fish Kills.

The Panel made recommendations (which were endorsed by the IOC Assembly June 2013) concerning (i) a new global approach to HAB research to meet societal needs, (ii) an increased coordinated international focus on ciguatera, (iii) a summary of the Decisions and planned intersessional activities into a Work Plan and budget for the IOC HAB Programme 2014-2015, and (iv) continuation of IPHAB and time of next Session, in April 2015. Dr. R. Magnien (USA) was re-elected as Chair and Dr. Gires Usup (Malaysia) was re-elected as Vice-Chair.

A list of current members of IPHAB can be found at http://hab.ioc-unesco.org, and individuals or institutions who have issues or ideas for international cooperation that they wish to bring to the attention of IPHAB, are welcome to contact the IOC Secretariat (hab.ioc@unesco.org) or the Chairs are of IPHAB (see web site).

Highlights and new initiatives

Scientific Symposium on Harmful Algae and Global Change: IOC, PICES and ICES will convene an open scientific symposium on harmful algal blooms and climate change in 2015 to:

a. provide examples of locations and events where climate change may be affecting HABs and their impacts;
b. identify and promote research on critical topics/aspects of the broader field of HAB research to advance our knowledge of the impacts of climate change on the global scale;
c. attract and retain new expertise from other scientific disciplines;
d. evaluate the use of new technologies for the collection and analysis of long term data on appropriate parameters;
e. develop the HAB component of global climate observing systems;
f. foster framework activities to facilitate identifying and responding to climate change-driven effects on HABs, including risk assessment with associated , probabilities and uncertainties.;
g. develop best practice recommendations for research and monitoring to fill critical knowledge gaps;

Development of a Global HAB Status Report: IOC will with partners (such as IAEA, ICES, PICES, etc) develop a periodic Global Harmful Algal Bloom Status Report to:

a. provide a global status and overview of HAB events and their societal impacts;
b. provide a global overview of the occurrence of toxin producing microalgae;
c. assess the status and probability of change in HAB frequencies, intensities, and range expansions resulting from global change;

Harmful Algae and Desalination of Seawater: IOC will work with partners to organize a meeting on impacts and management of toxic and harmful algal blooms (HABs) at desalination plants and related seawater facilities in Muscat, Oman, 16-17 April 2014 to:

a. Review the state of knowledge on the impact of HABs on desalination plants and other facilities that utilize large volumes of seawater in commercial or industrial applications
b. Explore the engineering and operational strategies that are used, or could be used to mitigate the impacts of HABs and other planktonic threats to these types of facilities
c. Produce a manual of operations containing information on HABs and their impacts and a series of recommendations on plant design and operations in areas potentially impacted by HABs
d. Explore the potential risk for human health from chronic exposure to HAB toxins in drinking water, with due attention to approaches used to remove cyanobacterial toxins in freshwater from drinking water reservoirs.
e. Produce a report summarizing the conference and its findings and recommendations including research priorities to fill knowledge gaps and to inform policy decisions in this subject area.

HABs in a Changing World: A New Global Approach to HAB Research to Meet Societal Needs: Based on the synthesis of SCOR-IOC GEOHAB (www.GEOHAB.info), a follow-up international program will be developed on the foundations of the GEOHAB Science Plan, focusing on understanding HABs in the context of global sustainability, with the new name of GlobalHAB. A GlobalHAB Scientific Steering Committee will be established with partner organizations and will develop an Addendum to the GEOHAB Science Plan extending the scope to include human and ecosystem health, and socio-economic impacts of HABs to enhance sustainable management of the oceans and coastal zone, and will develop an Implementation

Plan that describes a decadal program focused on HABs in a Changing World.

Ciguatera, A Plan for Improved Research and Management: IOC will assess the establishment of a coordinated IOC-FAO-WHO effort on CFP to combine the capabilities of those agencies and that of ecologists, toxin chemists and medical researchers to:

a. Develop a coordinated ciguatera strategy
b. Improve organism detection and sampling strategies
c. Improve toxin detection
d. Improve epidemiological data collection, reporting and assessments

IPHAB recommends that the IOC and its Member States make the CODEX Committee for Fish and Fishery Products and its member countries aware that IPHAB prioritizes efforts on ciguatera, and also recommends the World Health Organization to assess if there is a basis for classifying CFP as a neglected tropical disease.

Acronyms

ANCA
IOC HAB working group for Central America and Caribbean Sea
FANSA
IOC HAB working group for South America
HANA
IOC HAB working group for North Africa
IAEA
International Atomic Energy Agency
ICES
International Council for the Exploration of the Seas
PICES
North Pacific Marine Sciences Organization
SCOR
Scientific Committee on Oceanic Research
WESTPAC/HAB
HAB Project of the IOC Sub-Commission for the Western Pacific

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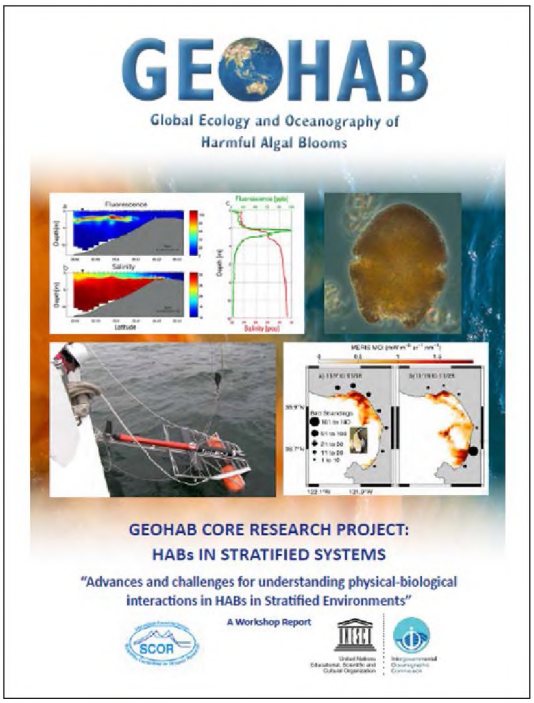



This publication can be downloaded from www.geohab.org

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

International Conference on Harmful Algal Blooms and Desalination

The First International Conference on Harmful Algal Blooms and Desalination was held in Muscat, Oman on April 17-18, 2014. This conference was organized to identify the knowledge gaps that heighten the concerns and challenges to sustainable production of drinking water by desalination in areas subject to harmful algal blooms (HABs).

In many arid regions, countries are increasingly reliant on seawater desalination to supply drinking water for rapidly growing coastal populations. There are currently more than 14,000 desalination plants in more than 150 countries worldwide. In 2008, the installed capacity was 52.3 million m^3/d. The desalination market is expected to grow by 12% per year reaching a capacity of 94 million m^3/d by 2015.

An emerging threat to the desalination industry is from HABs. In recent years, toxic or high-biomass blooms have caused serious impacts on the operation of desalination plants, in part because of an expansion in the number of HABs in some regions and in part because of the transition from thermal desalination to reverse osmosis (RO), the treatment process that is most affected by HABs. Impacts include the cessation of operations due to clogging of filters, fouling of surfaces, damage to RO membranes, as well as taste and odor problems. In the case of toxic HABs, there has been concern that toxins could persist in the treated water and thus represent a threat to human health [1]. Recent evidence suggests that these toxins should largely be excluded during freshwater production with suitable pretreatment [2] well-maintained membranes or with other desalination procedures such as multi-stage flash evaporation [3]. However, not all toxins have been assessed, and the breakthrough of some low molecular weight organic compounds (e.g., taste and odor components) has occurred, so it is unclear whether toxin exclusion under operational conditions is universal. The concern is that even low-level



Fig. 1 Omani government officials at the opening session for the HABs and Desalination Conference, Oman, April 17-18, 2014.

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Fig. 2 Two of the three Co-Chairs of the Conference: Don Anderson, WHOI, and Shannon McCarthy, MEDRC

toxin penetration into treated waters, if persistent, could be a chronic exposure threat to human health.

A recent HAB of the dinoflagellate *Cochlodinium* is a clear example of the risk posed by these phenomena. That outbreak, which lasted over 8 months in the Arabian Gulf-Arabian Sea region [4], closed or restricted the operation of multiple desalination plants, one for as long as 55 days. With little reserve water storage or alternative sources, this was a major threat to the region.

The initial impetus for the conference came in part from the IOC's Intergovernmental Panel on HABs (IPHAB), which passed a resolution recognizing the problem and the need for an international conference. IPHAB appointed Don Anderson to lead the effort and appointed a scientific steering committee with representatives from multiple countries. Concurrently, and independently, the Middle East Desalination Research Center (MEDRC) recognized the importance of this issue and held a small workshop in 2012 that also set the stage for the 2014 conference.

The two-day conference had 130 participants from 18 countries. This was the first major meeting to bring HAB experts together with desalination scientists, engineers and operators, as well as government officials responsible for water supply and public health. Toxic and high biomass HABs are increasingly common in the Arabian/Persian Gulf, Gulf of Oman, and the Red Sea as well as many other areas of the world where desalination occurs, and these restricted basins also are experiencing greater eutrophication and other anthropogenic stresses, making Oman an ideal setting for an inaugural meeting on this topic.

Held under the patronage of H.E. Mohammed al-Mahrouqi, Chairman of the Public Authority for Electricity and Water (PAEW), Oman, the conference was co-chaired by Don Anderson (Woods Hole Oceanographic Institution), Shannon McCarthy (Middle East Desalination Research Center) and Hamed Al Hasni (PAEW). The meeting had a number of corporate and public sponsors and multiple partner organizations and media partners. The complete list of sponsors, as well as the program, are available on the MEDRC website: *http://www.medrc.org/home/habd/* .

The meeting began with background presentations on HABs and on desalination to help all participants understand basic concepts. Then, through a combination of invited and contributed talks, sessions were convened on dissolved and colloidal organic materials, pretreatment processes, desalination plant design and operations, HAB distribution and dynamics, HAB control, and technologies for HAB detection and forecasting. The conference concluded with a plenary discussion that addressed research priorities and that also highlighted the strong consensus for another meeting on this topic, probably in two-three years. Research needs can be broadly categorized as those leading to improved operational practices at desalination plants, and forecasting of HAB effects. These two categories overlap in that better knowledge about the underlying causes of operational problems (e.g., membrane fouling) will help inform about the conditions that must be forecast. The research priority summary will be presented to the IPHAB at its next meeting in 2015.

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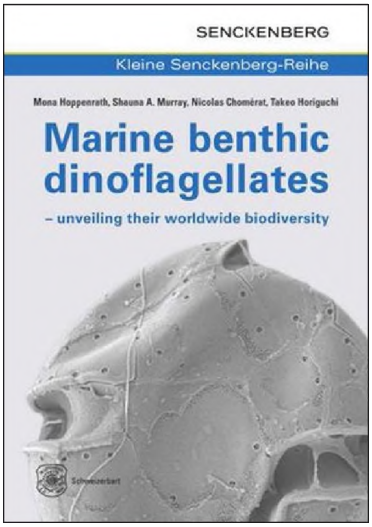

This publication presents the first summary of our knowledge of benthic dinoflagellate species. Information on the book content, instructions for purchase,etc can be found at: http://www.schweizerbart.de/publications/detail/isbn/9783510614028/Kle

International Workshop on the Ecology and Taxonomy of Benthic Marine Dinoflagellates: Project Report

Inspired by the GEOHAB Open Science Meeting on HABs in Benthic Systems, a Workshop on the Ecology and Taxonomy of Benthic Marine Dinoflagellates was held in Malaysia in May 2012. This Core Research Project was sponsored by GEOHAB-IOC and funded to commemorate the "Living Ocean and Coast" theme of the Expo 2012 in YEOSU ("beautiful water"), Korea. This multiyear project incorporated:

- a hypothesis driven field effort,
- followed by a taxonomic workshop,
- a number of individual project proposals to implement sampling in different habitats as a proof of concept for a new sampling method and
- a reporting session culminating in presentations at WESTPAC in April 2014 and a publication on BHAB collecting methods.

With the recent resurgence of interest in the BHABs, there is a concern that the lack of a standardized sampling protocols reduces the potential for meaningful comparison between studies [1]. Benthic harmful algae bloom (BHAB) species occupy complex habitats. Some can be found on a variety of substrates, for example turf algae, macrophytes, sea grasses or on coral rubble and in sand. The objective of the field effort and workshop was learn to identify BHAB species and to compare sampling protocols for benthic species. Both natural and artificial substrates were tested in a number of different habitats.

Existing sampling protocols for BHABs included: (a) collecting substrates in plastic bags and dislodging cells of interest by shaking, then normalizing the resultant cell counts on a per gram wet or dry weight basis [2-3], (b) scraping turf algae off substrates into collection tubes and normalizing the final cell counts to surface area or removing cells from substrate surfaces using suction devices (slurp guns) or vacuum systems, and normalizing final cell counts to surface area [4] and (c) placing artificial substrates, such as pieces of fiberglass screen, in benthic habitats to collect cells with subsequent recovery of cells via shaking and filtration [5]. One of the core research projects identified by the GEOHAB-BHAB Report [1] is a quantitative evaluation of sampling methods. Of particular interest is whether estimates obtained from collecting cells from natural substrates (macrophytes) are comparable to those obtained from artificial substrates. To begin addressing this issue, the workshop, *Ecology and Taxonomy of Benthic Marine Dinoflagellates,* was held in Malaysia 21-31 May 2012. Dr. Gires Usup from the National University of Malaysia and Dr. Patricia Tester, National Centers for Coastal Ocean Science, National Ocean Service, NOAA, lead sixteen participants in a field exer-



Fig. 1. Participants in GEOHAB International Workshop on the Ecology and of Benthic Marine Dinoflagellates, 21-31 May 2012, Malaysia. First row, seated, left to right: Dr. Leaw Chui Pin, Dr. Lim Po Teen, Dr. Mitsunori Iwataki, Prof Dr. Sharim Ahmad, Dr. Wayne Litaker, Prof Dr. Gires Usup, Prof Dr. Mazlan Ghaffar, Dr. Asmat Ahmad, Dr. Patricia Tester, Dr. Jacob Larsen, Dr. Normawaty Mohd Noor, Middle row, standing from left to right: Jeeva Moganarajo, Moime Walican, Hikmath Thoha, Intan Ernieza Farhana, Nguyen Ngoc Lam, Grace Joy Chin Wei Lie, Grace Abdala, June Moh Hwei Yieng, Hua Zhang, Nguyen Ngoc Tuong Giang, Ho Van The, Gan Hui Shan, Mimi Nora Mansor, Sitti Ryah, Berdiri Barisan Belakang, Top row, standing, from left to right: Shereen Yim, Nguyen Chi Thoi, Praderm Uttayarnmanee, Arief Rachman, Gan Jia Cheng, Mark Skinner, Hong-Chang Lim, Hii Kieng Soon, Tan Toh Hii, Teng Sing Tung, Thamrin Thamrin

cise to compare BHAB sampling methods (Figs 2-4).

The group departed Kuala Lumpor for a six hour bus ride to reach the southeast coast of Malaysia at Johor. Then a short boat ride to the field base on Tinggi Island provided an ideal location to test the hypothesis, "Are benthic HAB species and the cell abundances collected on artificial substrate correlated with benthic HAB species and cell abundances collected on adjacent macrophytes?" A week of field work provided data to test the hypothesis as well as material to examine during the second week of the course at the National University of Malaysia. There Drs. Gires Usup, R. Wayne Litaker, Jacob Larsen, Mitsunori Iwataki, Lea Chu Pin, Lim Po Teen and Normawaty Mohd Noor lectured on the taxonomy of BHAB species especially Gambierdiscus, Prorocentrum, and Ostreopsis. During this second week the field participants were joined by twelve others; all were introduced to the molecular techniques currently used in BHAB species identification (Fig. 5) [6].

Preliminary results indicated a positive correlation between the species and cell abundance collected on the artificial substrate and natural substrate (macrophytes). At the conclusion of the workshop the participants were invited to write proposals that would allow them to test the artificial substrate sampling method back in their coastal areas and compare BHAB species and cell abundances found on natural substrates like macrophytes. Successful proposals were submitted by Hikma Thoha and Thamrin Thamrin from Indonesia, Lim Po Teen, Normawaty Mohd Noor and Gires Usup from Malaysia, Ho Van The from Vietnam and Lu Song Hui, Peoples' Republic of China. Their collective results are being assembled for analysis, report-out and discussion in a web-facilitated session planned for March 2014. To date a manuscript using the artificial sampling method taught in the workshop has been published [7] and a methods manuscript incorporating the study results [8]. Abstracts from the IOC-GEOHAB-YEOSU Project were submitted to the WESTPAC meeting 22-25 April 2014 in Nha Trang, Vietnam for presentation of the project results. Project related publications are:

- Tan, T.-H., P.-T. Lim, A. Mujahid, G. Usup, C.-P. Leaw. 2013. Benthic harmful dinoflagellate assemblages in a fringing reef of Sampadi Island, Sarawak, Malaysia. *Marine Research in Indonesia* 38 (2) 11-21.



Fig. 2. Sampling off Tinggi Island (W. Chris Holland, Steven R. Kibler and Gan Jia Cheng).



Fig. 3. Sample preparation (Steven R. Kibler and workshop participants).



Fig 4. Field cell counting stations (Teng, Sing, Hikmah Thola and Arief Rachman).



Fig. 5. Arief Rachman, Praderm Uttayarnmanee and Hii Kieng Soon examining the morphology of BHAB dinoflagellates in the laboratory of Prof. Usup at the Universite Kebangsaan.

- Tester, P.A., S.R. Kibler, W.C. Holland, G. Usup, M.W. Vandersea. C.-P. Leaw, P.-T. Lim, J. Larsen, M. Mohammad-Nor, M.A. Faust and R.W. Litaker. 2014. Sampling harmful benthic dinoflagellates: Comparison of artificial and natural substrate methods. *Harmful Algae* 39:8-35.

Acknowledgements

We would like to thank Henrik Enevoldsen, Head of the IOC Science and Communication Center on Harmful Algae, for his support of this project. Funding was provided by the YEOSU Project to honor the "Living Ocean and Coast" theme of the Expo 2012 in YEOSU ("beautiful water"), Korea.

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NF-POGO AWI-CofE Regional Training Programme

Detection of HABs in Southeast Asia by Remote Sensing: Operational Warning and Regional Monitoring Protocols

Harmful algal blooms, HABs, represent a concern in Southeast Asian (SEA) countries particularly where the frequency and severity of HAB occurrences have increased with anthropogenic factors such as eutrophication and climate change. HABs are associated with a variety ecosystem impacts, e.g., fish kills as well as financial impacts, e.g., bans on shellfish harvests. The causes and consequences of HABs vary from country to country, yet a common set of detection methods and protocols exist that could be implemented, regardless of the region, for the detection of HAB events in the early stages as well as for the development of an early-warning system. Remote sensing data and integrated modelling, combined with holistic monitoring programmes specific for each country, provide an operational framework for a set of protocols that can be implemented in each region to help understand and detect HAB events, enabling appropriate mitigation and management responses.

In response to these broad needs, an intensive hands-on three-week training programme was offered to students with basic or no prior experience with satellite remote sensing and modelling techniques. This was held at the Bolinao Marine Laboratory, Pangasinan, Philippines of the Marine Science Institute, University of the Philippines from 24 February to 15 March 2014. The programme provided the students with a fundamental interdisciplinary knowledge of HAB dynamics, capability to assess HAB sites using satellite remote sensing technology, design of standardized monitoring protocols, initial skills and tools to begin to develop integrated HAB models, and assistance in the development of an early-warning system for HABs in their own countries.

Twenty-four trainees were selected from a large number of applicants. These participants came from six countries: India (3), Indonesia (4), Malaysia (3), Philippines (9), Thailand (1), and Vietnam (4).

The local organizers and hosts of the Philippine Regional Training Programme were Drs. Aletta T. Yñiguez and Laura T. David from the Marine Science Institute, University of the Philippines, Diliman (MSI-UPD). Mr. Joseph Dominic Palermo and Mr. Aldwin Almo, also from MSI-UPD, also supported the Training Programme as did Dr. Rhodora V. Azanza (MSI-UPD), who provided organizational input as well as HAB biology expertise.

Five local scientists from MSI-UPD who are actively investigating HABs through the Department of Science and Technology-funded programme on HABs in the Philippines provided their time and expertise: Drs. Cesar L. Villanoy, Gil Jacinto, Maria Lourdes San Diego-McGlone, Fernando P. Siringan, and Mr. Chris Mendoza. International experts in HABs, remote sensing and modelling shared their expertise: Dr. Kazumi Matsuoka from Japan, Dr. Chui Pin Leaw from Malaysia, Dr. Stewart Bernard from South Africa, Dr. Liew Soo Chin from Singapore and the Drs. William Cochlan and Raphael Kudela from the USA. Dr. Gerald Plumley, a representative from the Centre of Excellence in Observational Oceanography (CofE)-AWI also attended the training programme.

The CofE at the Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, was the main external sponsor of the Philippine Regional Training Programme; the CofE-AWI is sponsored by and supported through Nippon Foundation – Partnership for the Observation of the Global Oceans (NF-POGO).

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Dr. William Cochlan conducting a hands-on activity for the chemical oceanography of HABs module.



Group photo at the end of the three week training.

IOC Qualifications in Identification and Enumeration of Harmful Microalgae

Since 1993 the IOC has conducted training courses in identification/taxonomy and enumeration of harmful microalgae. The advanced course held at University of Copenhagen includes 100 hours of teaching and is divided into two parts, an internet part consisting of 50 hours of teaching prior to the practical part also consisting of 50 hours of teaching. The first part of the course is an internet teaching programme mainly for self-study of background material of the various groups of harmful algae, while the second part is a practical course in species identification. The course finishes with a 3-hour practical examination in species identification which qualifies for an IOC CERTIFICATE OF PROFICIENCY IN IDENTIFICATION OF HARMFUL ALGAE. Certificates are issued to participants who have at least 70% correct answers.



IOC Training Course on Harmful Marine Microalgae 2014

This year's practical course was held 12-21 August and the participants came from Colombia, Costa Rica, El Salvador, Hong Kong, Jamaica, México, Nicaragua, South Africa, Spain and the United Kingdom and was an engaged and hard working group enjoying the wisdom and guidance of Jacob Larsen, Santiago Fraga, Øjvind Moestrup, Nina Lundholm and Helene Annadotter.

News

Only four weeks before the next 16th International Conference on Harmful Algae in Wellington, New Zealand!

There are more than 400 participants already registered and an exciting programme you can check on-line.

Go to the ISSHA website (*www.issha.org*) and directly to the conference website to get more information on invited speakers, social events, etc.

The 15th ICHA Proceedings

These will be published as:

Kim, H.G., B. Reguera, G. Hallegraeff, C.K Lee, M.S. Han and J.K Choi. (eds). Harmful Algae 2012, Proceedings of the 15th International Conference on Harmful Algae. International Society for the Study of Harmful Algae 2014, ISBN 978-87-990827-4-2.

(All the accepted manuscripts are on line on the ISSHA website)



ISSHA Council Elections

Deadline for nominations for a new President and Secretary and replacing Council members is on 26th September. IISHA members can vote on line.

Venue for the 18th ICHA Conference (2018)

Bids have been presented from France and Germany to host the 18th ICHA in 2018. ISSHA members will be able to vote on-line and/or during the conference in New Zealand after listening to the bids presentations on Monday 27 October.

An exceptional *Dinophysis* driven toxic algae event in the Scottish Shetland Islands

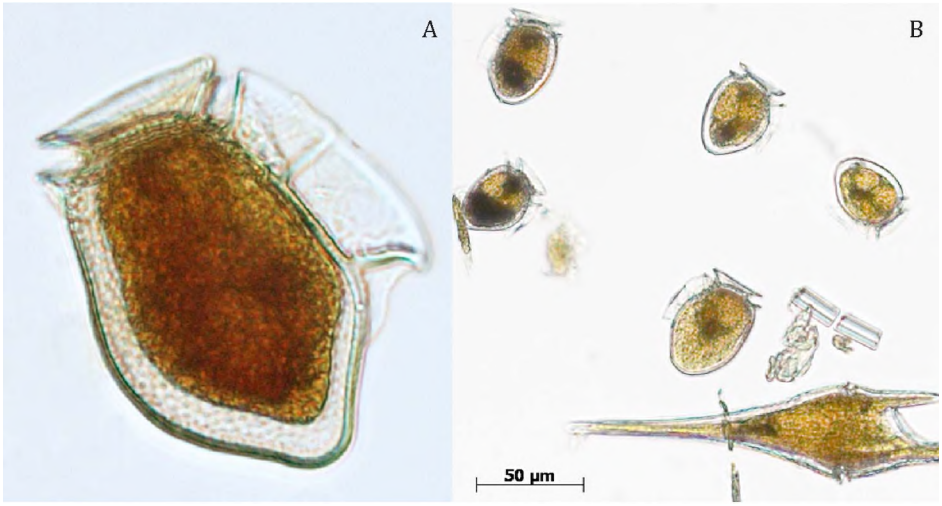

Fig. 1. Dinophysis from field populations in the Shetlands. A. Dinophysis acuta; B. Dinophysis acuminata complex

Harmful algal blooms (HABs) of the genus *Dinophysis* are common in UK waters (Fig. 1), and are spatially and temporally variable, with marked differences between years. In common with other EU shellfish harvesting countries, human health is protected by regulatory monitoring of both the concentration of toxins within the shellfish flesh and the abundance of the potentially causative biotoxin- producing phytoplankton in the water column. In Scotland the regulatory authority is the Food Standards Agency, with the phytoplankton component of the monitoring being conducted by SAMS Research Services Limited (SRSL), the commercial arm of SAMS.

Historically, there have been a number of UK human health incidents linked to *Dinophysis* mediated diarrhetic shellfish poisoning (DSP). For example in June 2006, 171 people became ill after consuming mussels from a Scottish site [1].

In general, and certainly in recent years, monitoring has been very successful in preventing contaminated shellfish from reaching market. However, during the summer of 2013, 70 people in London and the South East of England contracted DSP after consuming mussels (*Mytilus edulis*) harvested in the Shetland Islands, Scotland (Fig. 2). The mussels had been supplied to a number of restaurants, some through a number of intermediary suppliers. Customers reported illness after eating at: Belgo in Covent Garden, Holborn, Clapham and Bromley; Zero Degrees in Blackheath and Reading; The Phoenix near Hook, Hampshire; Boulevard Brasserie in Covent Garden; and Pig's Ears in Richmond. The vast majority of cases occurred between 13th and 15th July. While there were no known severe or long-lasting health impacts, the negative impact of the event for the industry was significant, making headlines on both local and national news.

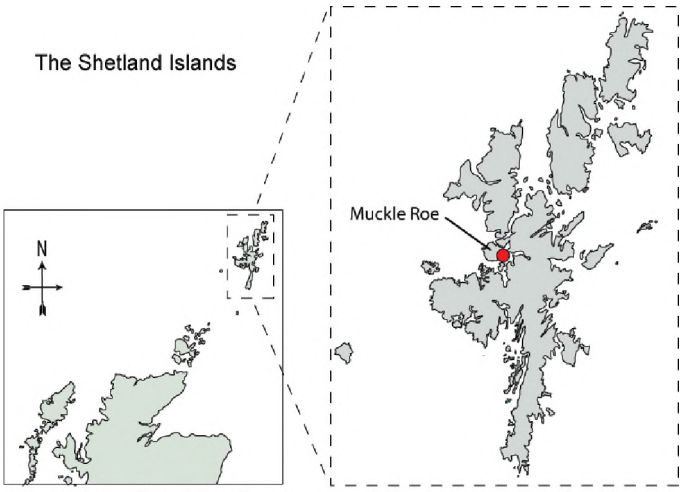

Fig. 2. Map of location showing sampling sites mentioned in the text.

Although the numbers of *Dinophysis* observed in the waters around Shetland had exceeded the regulatory monitoring programmes warning levels for several weeks preceding the event, toxicity testing indicated concentrations of biotoxins, although finite and rising, were below the regulatory threshold.

Two days after the event, however, the monitoring programme indicated that its numbers had risen very rapidly to unexpectedly large values in the waters around the islands, with associated high shellfish toxicity. Action was immediately taken to suspend operations in the area but, unfortunately, not before one of the farms harvested and shipped some of the affected mussels. While this incident was exceptional, it is instructive to analyse the causes behind it and determine whether current early warning methods could be improved.

In summer, regulatory monitoring occurs weekly, a frequency that is typically sufficient to give one or two weeks warning of the increase in harmful algal cells to densities above regulatory threshold that may result in shellfish toxicity. However, in summer 2013 the rate of increase in *Dinophysis* abundance in the Shetland Islands was unprecedented, going for example at one site, from 340 (cells/l) to 6,280 (cells/l) in the space of only one week (Fig. 3).

A plot of the median *Dinophysis* abundance value for each day of the year (for the period 2006 to 2013) demonstrates that the 2013 bloom markedly exceeded that in previous years in terms of magnitude (Fig. 4), with a Chi Square test showing that this difference was significant. This rapid increase, to an exceptionally high abundance, resulted in an increase of shellfish toxicity from a below threshold value of 150 (µg/kg) recorded at Muckle Roe on 3rd July to a value markedly above threshold of 3,452 (µg/kg) by 10th July.

The observed rapid increase in cell abundance was greater than that which could be explained by *in situ* growth alone, and hence we examined other potential environmental factors that might have caused the rapid bloom development. As *Dinophysis* can happily grow in dynamic, offshore locations such as a shelf front [2], it was decided

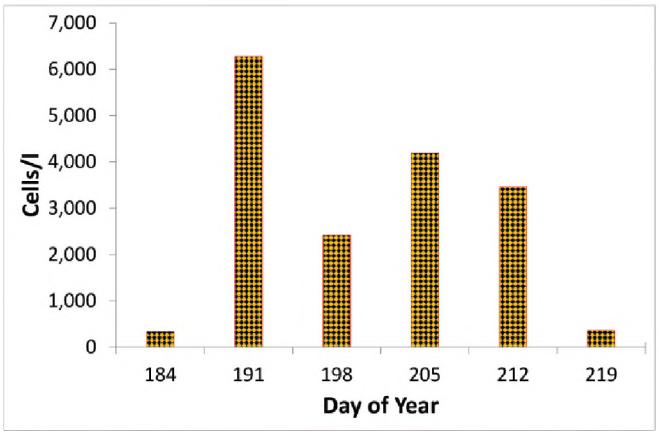

Fig. 3: Plot of Dinophysis abundance increasing with time.

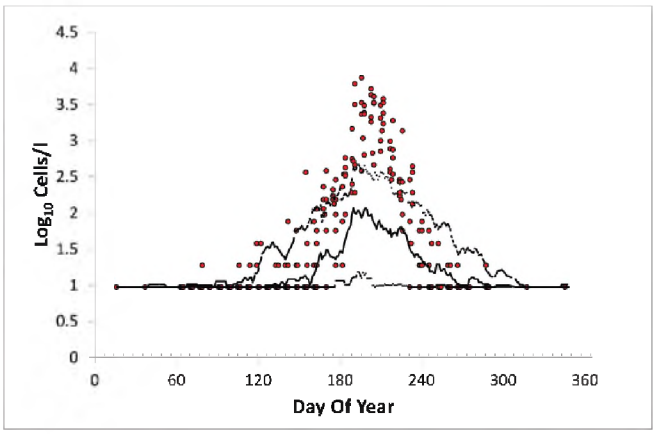

Fig. 4: Dinophysis counts recorded in Shetland during 2013 (circles) plotted against median values of Dinophysis recorded in Shetland between 2006 and 2013 (solid black line). Dashed lines represent the 5th and 95th percentiles of the data collected between 2006 and 2013.

to look at the local wind patterns. Data was obtained from the British Atmospheric Data Centre (BADC) [3], and used to create a climatology of mean wind directions in Shetland between 2006 and 2013. When compared with the wind direction observed during the summer of 2013 it was noticed that, while the usual mean, prevalent wind direction during June, July and August is from the South East, during the summer of 2013 the wind direction changed and blew predominantly from the West. This suggested that the large numbers of *Dinophysis* observed were blooming out at sea and then being carried into the bights and voes of Shetland where they were accumulating.

Rigorous monitoring is still the safest way to ensure that consumers can eat shellfish with confidence and without worrying about food poisoning. However, as the recent toxicity event illustrates, the possibility for contaminated shellfish to enter the food supply still exists. While phytoplankton monitoring picked up the 2013 event, a sampling frequency of at least bi-weekly would have been required for the rapid rise in biotoxins to have been spotted in time for appropriate action to be taken.

Given the costs and logistical difficulties of such high frequency monitoring, this is not a viable option for the future. Rather, enhanced early warning methodologies are required. To this end SAMS are currently producing weekly "bulletins", first developed within the EU FP7 project ASIMUTH (ww.asimuth.eu) that provides a summary of the current HAB/biotoxin status of the area and risk assessment of the likelihood of harmful blooms in the following week. Inclusion of wind data in these bulletins allows the local industry to better assess the risk of future rapid wind driven HAB events (Fig. 5).

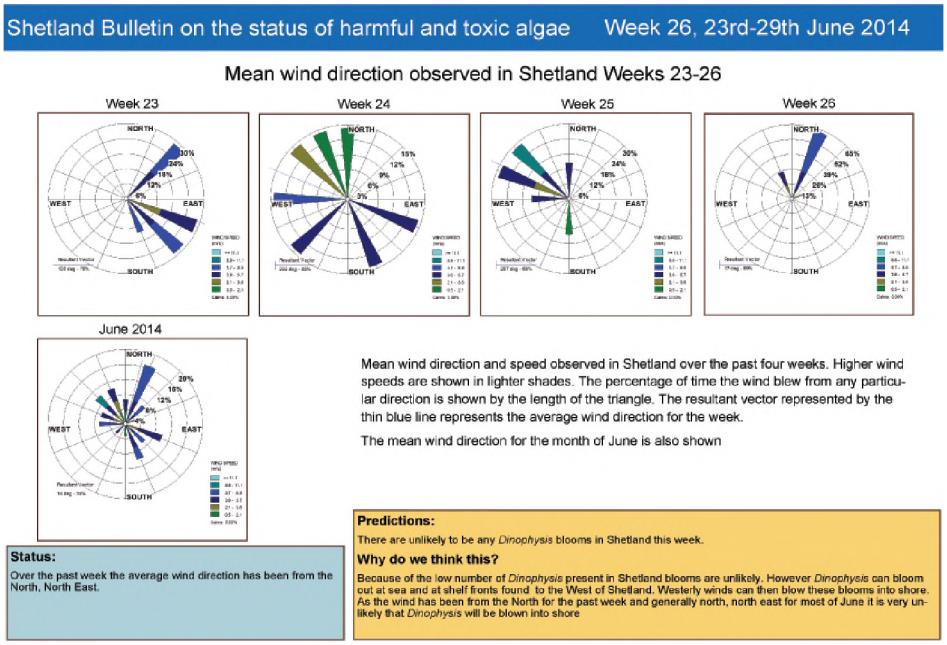

Fig. 5: Example of a page of the Shetland bulletin with the wind rose.

Acknowledgements:

The authors would like to acknowledge the NERC PURE research programme and the EU FP7 project ASIMUTH who supplied the funding for this study. They would like to thank the following for their assistance in identifying and enumerating the phytoplankton samples: Debra Brennan, Christine Campbell, Eilidh Cole, Joanne Field, Sharon McNeill, Elaine Mitchell and Rachel Saxon. They would also like to thank the British Atmospheric Data Centre and the Food Standards Agency for the generous use of their data.

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An unprecedented bloom of *Gonyaulax polygramma* in Kota Kinabalu, Sabah, Malaysian Borneo

The west coasts of Sabah, northern Malaysian Borneo, have been experiencing harmful algal blooms annually for the past four decades since the first incident of paralytic shellfish poisoning (PSP) was reported in 1970 [1]. Since then, harmful algal monitoring has been undertaken by governmental agencies (Fisheries Research Institute, and The Department of Fisheries, Sabah) to safeguard seafood safety, in particular in shellfish mollusks harvested from the region. Seasonal blooms in the area were always found to be associated with the highly toxic dinoflagellate *Pyrodinium bahamense*. The blooms, however, occasionally were followed by unarmored harmful dinoflagellates, *Cochlodinium polykrikoides* and *Gymnodinium catenatum* [2].

In a field survey conducted in April 2014, we observed a bloom of a heavily thecated dinoflagellates with maximum cell densities of $9 \pm 0.36 \times 10^{-5}$ cells L^{-1}. The bloom was found to form patches between the southern part of Sepanggar Island and eastern Gaya Island (Fig. 1).

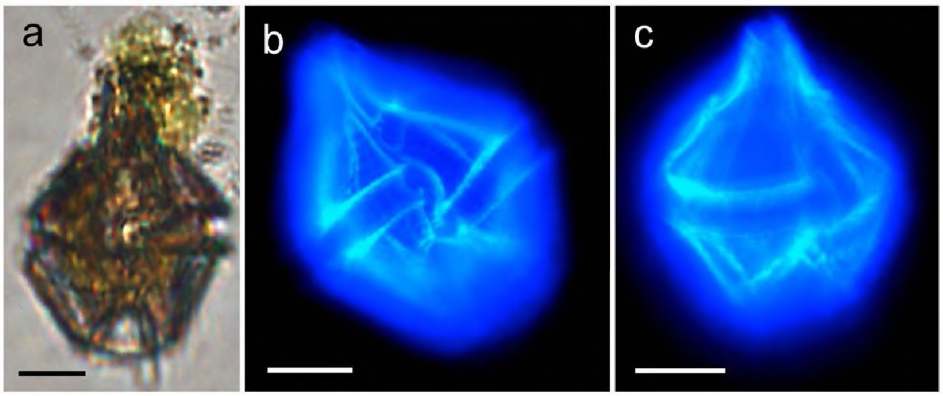

Fig. 2. Light and epi-fluorescence micrographs of Gonyaulax polygramma, bar = 10 µm.

Based on light and epi-fluorescence microscopic observations, the dinoflagellate was identified as *Gonyaulax polygramma* (Fig. 2). *G. polygramma* blooms were previously reported in Japan [3], South Africa [4] and the South Sea of Korea [5], but there were no previous records from Southeast Asia. Some *Gonyaulax* species are known to produce yessotoxin (YTX), i.e. *G. spinifera* that caused YTX contamination in greenshell mussels (*Perna canaliculus)* in New Zealand [6]. Despite well-studied bloom-forming harmful marine dinoflagellates in Kota Kinabalu, this represents to our knowledge the first record of a *G. polygramma* bloom accompanied by water discoloration in the area (Fig. 1). Fortunately neither fish kills nor intoxicated shellfish were reported during this bloom.

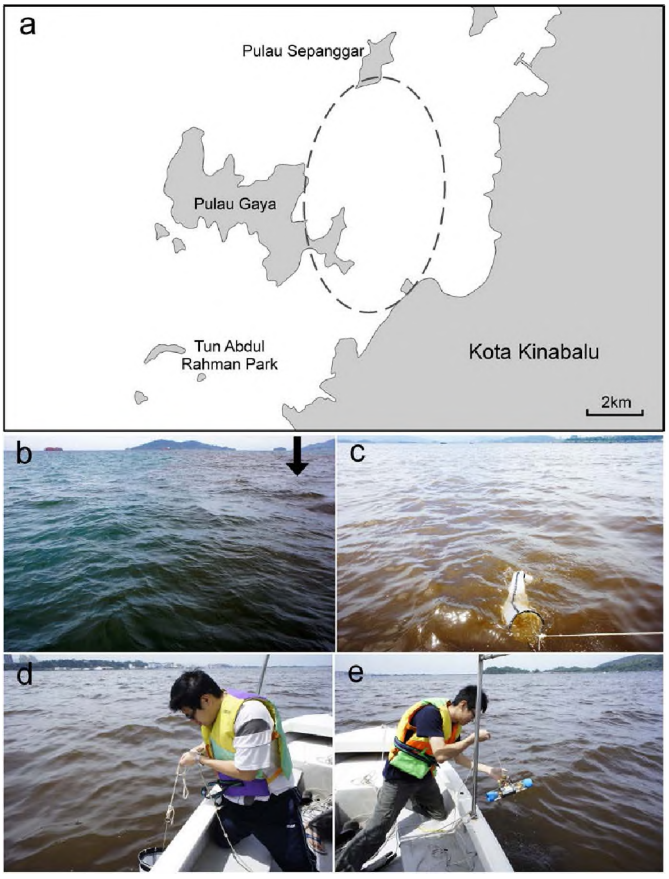

Fig. 1. (a) Sampling sites at northwest Kota Kinabalu coast. Dotted circle indicates blooms location. (b) Blooms forming patches [arrow]. (c) Red discoloration. (d-e) Research scientists collecting water samples using plankton net and Van-Dorn water sampler.

Acknowledgements

This work was funded by the Malaysian government through the Ministry of Education under JSPS COMSEA Matching Fund (GA002-2014); the Ministry of Science, Technology and Innovation under Science Fund (04-01-09-SF0092); and University of Malaya under UMRP (RU006-2014).

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First report of *Pyrodinium bahamense* cysts in marine sediments from the Gulf of Fonseca, El Salvador

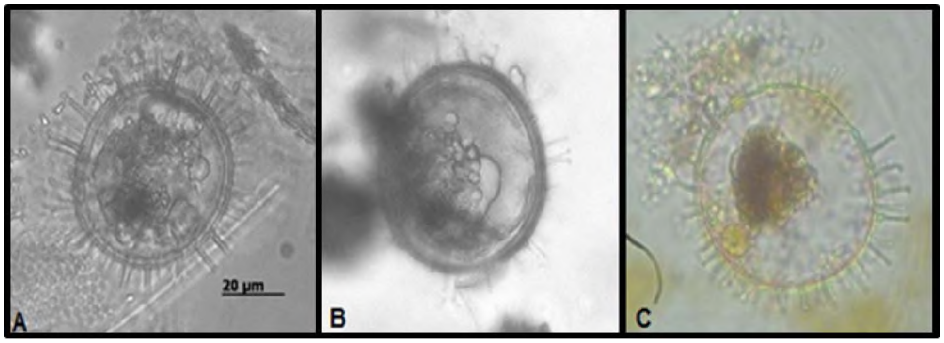

Fig. 1. Pyrodinium bahamense cysts. Micrographs of specimens found at the 0-2 cm section in core GF01 at 32X (A-B) and 40X (C) taken with a manual camera.

The PSP toxin producer P*yrodinium bahamense* has been reported in tropical and subtropical estuaries and coastal areas from the Atlantic and western Pacific oceans [1]. Dense blooms of this species, reaching concentrations up to 106 cells · L^{-1}, have been reported in Malaysia [2], Papua New Guinea, The Philippines, Guatemala and Costa Rica [3, 4]. *Pyrodinium bahamense* poses a threat to public health and the exploitation of artisanal fisheries in El Salvador, a country with an economy that largely relies on coastal resources for its subsistence. Blooms of this species have reached densities of $3.3 \cdot 10^4$ cells · L^{-1} in April 2010 [5]. Further blooms occurred in August 2011, with $1.4 \cdot 10^4$ cells · L^{-1} and a toxicity of 1872 µg equiv STX · $100g^{-1}$, and in September 2012, with 730 µg equiv STX · $100g^{-1}$ detected in oysters and death of sea turtles associated with the event [5].

The Gulf of Fonseca (13° 15' N, 87° 45' W) covers an area of about 3,200 km^2 with a coastline that extends for 261 km of which 185 km re in Honduras (northwest and east), 40 km in Nicaragua (south), and 29 km in El Salvador (northwest). During March and August 2012, fifteen sediment cores were collected at the Gulf of Fonseca, El Salvador to look for the occurrence of dinoflagellate cysts following the method of Matzuoka and Fukuyo [7]. Samples were collected with an Uwitec A-5310 gravity corer at an average depth of 8 m. Cyst analyses were carried out of the top 10 cm of each core sample subdivided in 5.2 cm thick transversal slices. A total of 75 core sediment samples were sonicated using a Branson Sonifier 450 and sieved through 125 and 20µm meshes. Analyses were performed at the Marine Toxins Laboratory of the University of El Salvador (LABTOX-UES).

Thirteen cysts of *Pyrodinium bahamense* were detected in 6 out of 15 sediment cores, confirming the occurrence of the most recurrent producer of toxic (PSP) HABs in the study area. The cysts, with a mean diameter of 48µm, had a pale or transparent wall, a cytoplasm with yellowish granules and a lateral red spot that was used as a taxonomic criteria (Fig. 1). This is the first report on the occurrence of *Pyrodinium bahamense* cysts in coastal waters from El Salvador.

In addition, 178 cyst morphotypes, belonging to the genera *Protoperidinium, Pyrodinium, Scrippsiella* and *Alexandrium*, with a maximum concentration of 31 and 40 cysts · ml^{-1} (in cores GF07 and GF14 respectively), were found in the top 10 cm of sediment (Fig. 2). Of these, 7 were identified at species level. Species cyst reported for the first time in El Salvador were *Protoperidinium subinerme, Stelladinium reidi, Protoperidinium oblongum, Protopedinium conicoides, Protoperidinium* sp1 (Fig. 3, 4).

Only one specimen of the genera *Alexandrium* and *Scrippsiela* were found. Further cyst analyses in samples collected along the coasts of El Salvador are needed. The low density of *P. bahamense* cysts found in Gulf of Fonseca casts doubts on whether these act as inoculum of the blooms. Dense patches of the dinoflagellate were detected offshore in 2009 before a PSP event affected coastal shellfish resources [8].

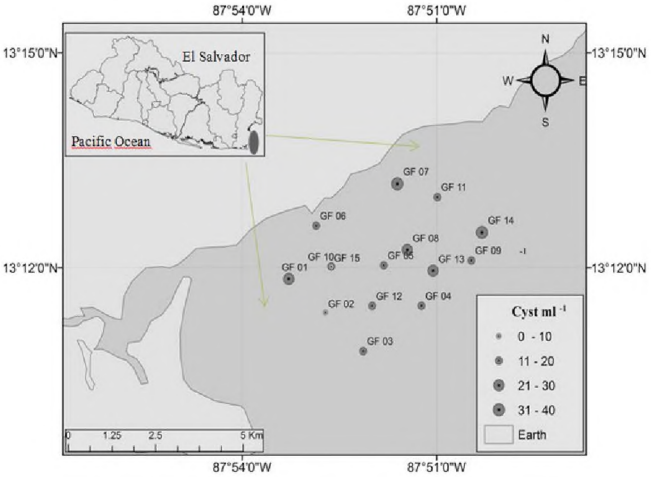

Fig. 2. Dinoflagellate cysts density (cysts • mL^{-1}) in core sediment samples from the Gulf of Fonseca, El Salvador.

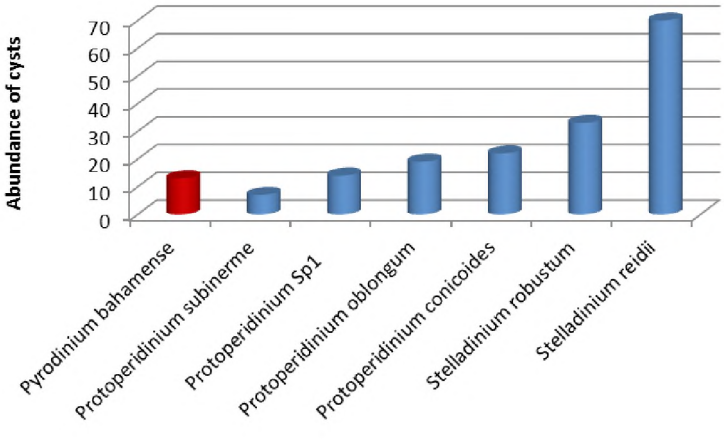

Fig. 3. Frequency (%) of the most abundant species found on the top 10 cm in sediment cores from the Gulf of Fonseca.

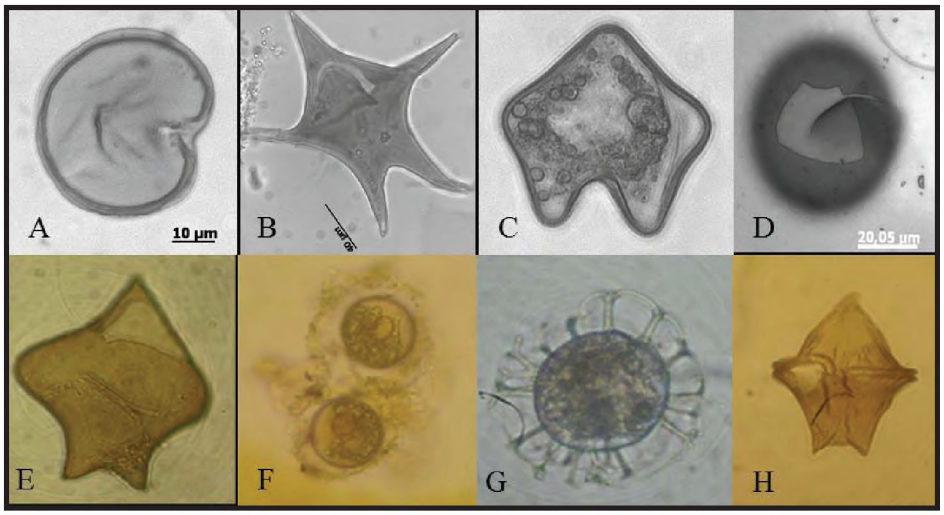

Fig. 4. Micrographs of other species cysts found in samples collected at the gulf of Fonseca: A) Protoperidinium subinerme; B) Stelladinium robustum; C) Protoperi-dinium oblongum; D) Protoperidinium conicoides; E) Protoperidinium sp; F) Alexandrium sp; G) Scrippsiella sp and H) Protoperidinium sp.

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Forthcoming Events

11th Advanced Phytoplankton Course - Taxonomy and Systematics Stazione Zoologica Anton Dohrn (SZN), Naples, Italy, 4-24 October 2015. Contact: Adriana Zingone (*zingone@szn.it*). Application form at *http://www.szn.it*

PICES 2014 Annual Meeting. Yeosu, Republic of Korea , 16 - 26 October 2014. HAB-Section Meeting 18 October. Contact: Vera L Trainer (*vera.l.trainer@noaa.gov*)

16th International Conference on Harmful Algae (16th ICHA), Wellington City, New Zealand, 27–31 October 2014. *http://www.icha2014nz.com/*

COI/FANSA Curso avanzado de entrenamiento sobre microalgas marinas nocivas: Biología y Toxicidad. Chubut, Argentina, 10 - 22 November 2014. Contact: Martha Ferrario (*meferra@fcnym.unlp.edu.ar*)

ICES - IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD), Lisbon, Portugal, April 2015 (t.b.c.) Contact: Eileen Bresnan (*Eileen.Bresnan@scotland.gsi.gov.uk*)

12th Session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB), UNESCO, Paris, France, 21-23 April 2015 (t.b.c). Contact: Henrik Enevoldsen (*h.enevoldsen@unesco.org*)

Bloom of *Dolichospermum flos-aquae* in a coastal lagoon in the Southern Gulf of Mexico

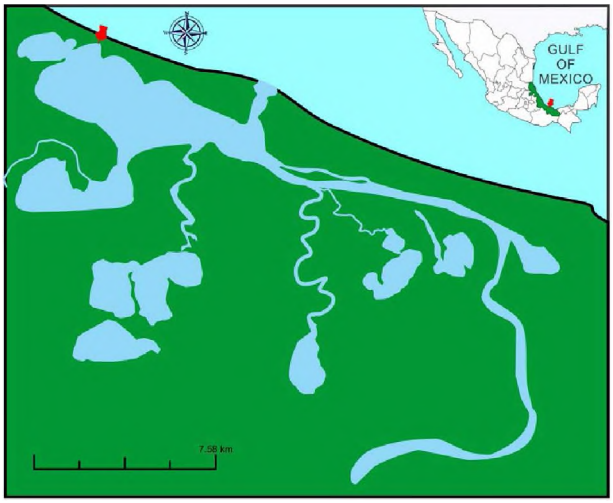

Fig 1. Map of Alvarado lagoon and location of sampling station (in red).

Alvarado lagoon (Fig. 1) is a coastal lagoon permanently connected to the Gulf of Mexico. This lagoon has a mean salinity of 5.59 psu [1]. The low salinity values are due to the influence of many important rivers that discharge into the lagoon which is the final part of the watershed called Papaloapan, one of the most important watersheds in Mexico. The mean salinity may decrease in the rainy season due to increased rain and riverine inputs.

At the beginning of October 2013, discoloured water (yellowish) was noticeable in most of the area of Alvarado lagoon. On October 3rd 2013, a water sample was obtained from the surface at the site called "Arbolillo", 10 km distance from Alvarado City (Fig. 1). The sample was fixed with acetate-lugol. Counting of filaments and cells was done using a Sedgwick-rafter chamber.

The species that caused the water discoloration was *Dolichospermum flos-aquae* (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek (synonym: *Anabaena flos-aquae* Brébisson ex Bornet & Flauhault) (Fig. 2). Counting chains resulted in 1938 filaments ml^{-1}. Based on the mean number of cells per filaments, a cell density of 91357 cells ml^{-1} was estimated. No other phytoplankton species occurred except *Phacus* sp. with a density of 2 cells ml^{-1}. Chains of cells (filaments) formed regular-irregular spirals and were composed of 5-217 cells (mean=47.14, n=86). Cells were spherical with a diameter of 4.9 µm or slightly constrained in the center (maybe due to cellular division). Heterocysts occurred in variable number and in long filaments we observed from 3 to 5. Akinetes were not observed.

Salinity when the bloom occurred was 2 psu and temperature was 28°C. Although Alvarado lagoon is an oligohaline-mesohaline system, during August-September 2013 the lagoon received an unusual amount of freshwater. In addition to the rainy season, two tropical depressions affected Veracruz coasts and the Central States of Mexico. The tropical storm "Fernand" caused more than 200 mm of rain in August 24-26th; hurricane "Ingrid" hit North of Veracruz on September 16th, and brought rain throughout the region with values of 100-150 mm in the center and south of Veracruz. Papaloapan River is one of the most important rivers of Mexico and discharges into Alvarado lagoon. This river and others caused a noticeable increment in the level of Alvarado lagoon.

This is the first report of a bloom of this cyanophyte in Alvarado lagoon, although in a similar system close to it, *Dolichospermum flos-aquae* caused a rare bloom in 2006. We believe that that the unusual rainfall in this year is a factor that contributed to the bloom in Alvarado. The Papaloapan watershed comprises the states of Puebla, Oaxaca and Veracruz, and agriculture is one of the main economical activities in the watershed. The lower watershed is subject to flooding every year [2] in the rainy season, and this is enhanced by tropical depressions, so most of the fertilizers drain into Alvarado lagoon during this period. The tropical depressions "Fernand" and "Ingrid", besides bringing an unusual fresh water supply, might have increased the availability of nutrients in the lagoon and thus favoured the *Dolichospermum flos-aquae* bloom.

Alvarado lagoon is an important region for shrimp and oyster fisheries in Mexico [3] and because of the discoloration of water, sanitary authorities established vigilance those days. No toxic effects were recorded in the lagoon, nor in the community of Alvarado, but it is known that *Dolichospermum flos-aquae* produces toxic microcystins and nodularins [4, 5]. Because of the occurrence of this potentially toxic species in a bloom, caution is important in the detection of future water discolouration in Alvarado lagoon.



Fig 2. Dolichospermum flos-aquae specimen from Alvarado Lagoon, Mexico (scale bar = 10 µm).

Acknowledgements

Sample and information were provided by the Mexican Health Department, 8th jurisdiction and Department of Sanitary Risk Protection from Veracruz.

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Which Lugol's is the best 'solution'?

Lugol's iodine solution (Lugol's) is generally recognised as being the most suitable preservative to use when studying phytoplankton taxa, in part because it is less toxic than alternatives such as formaldehyde and gluteraldehyde [1]. Each preservative is more suited to a different range of taxa but in general Lugol's is preferred for most 'wild' samples. Table 1 shows three different versions of Lugol's which we decided to compare against each other to determine which might be best used to preserve various phytoplankton taxa.

From April 2013 to February 2014 we conducted a series of degradation experiments on a range of algal species, specifically chosen to represent a wide range of taxon groups.

For each representative species, the sample was homogenised and divided equally into three subsamples which had been spiked with 2ml of L_{acid}, C_{acid} or C_{neut}. The mean count of cells in three 5ml aliquots of each sample were enumerated after 1 day of preservation using the Utermöhl technique [2]. This process was repeated after 3 days, 1 week, 2 weeks, 1 month, 2 months, 4 months, 6 months and 8 months.

The comparative degradation of cell counts in each Lugol's solution was highly variable across the different species tested. Figures 1 and 2 are revealing examples of the variability found.

The full results of these experiments are currently been prepared for publication. It is already apparent that L_{acid}, C_{acid} or C_{neut} may each be the most or least efficient Lugol's preservatives, depending on the taxa being studied. Determining the overall 'best' solution may prove very difficult, the 'lag time' between preservation and analysis is certainly a key factor in this decision.

It is clear that due to the lack of peer reviewed papers comparing the different Lugol's solutions; further work in this area will be of great utility to those conducting monitoring programmes or working with data derived from preserved samples.

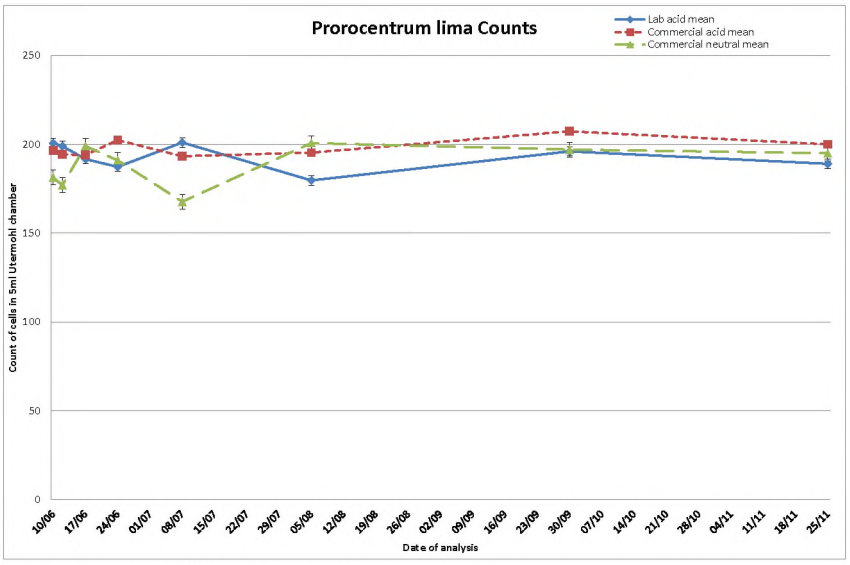

Figure 1: Results of Prorcentrum lima experiment

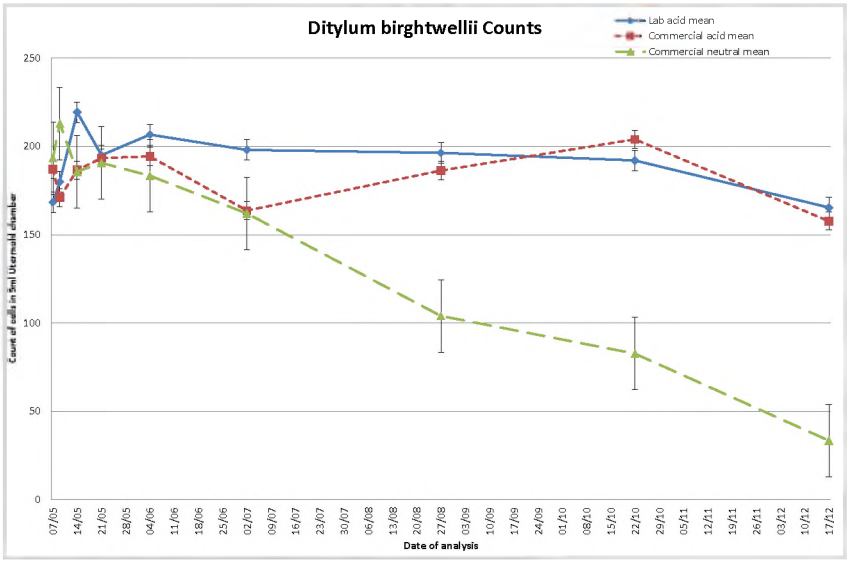

Figure 2: Results of Ditylum brightwellii experiment

Table 1: Details of Lugol's solutions used

Preservative name	Source	Concentrations
Lab Acid (L_{acid})	Produced 'in lab' by Cefas staff	8% potassium iodide / 4% iodine / 8% concentrated acetic acid / 84% distilled water
Commercial Acid (C_{acid})	Purchased commercially	0.68% potassium iodide / 0.34% iodine / ~98.98% distilled water / <1% acetic acid
Commercial Neutral (C_{neut})	Purchased commercially	0.68% potassium iodide / 0.34% iodine / 98.98% distilled water

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We thank Dr. R. Pipe and M. Jutson of The Plymouth Culture Collection, Marine Biological Association, Citadel Hill, Plymouth, PL1 2PB, UK and D. Sivyer, Dr. V. Creach, R. Beckett, C. Crisp, S. Milligan, N. Travell and A. Walton of Cefas.

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Introducing a web-based interactive identification key to species of *Pseudo-nitzschia* (Bacillariophyceae)

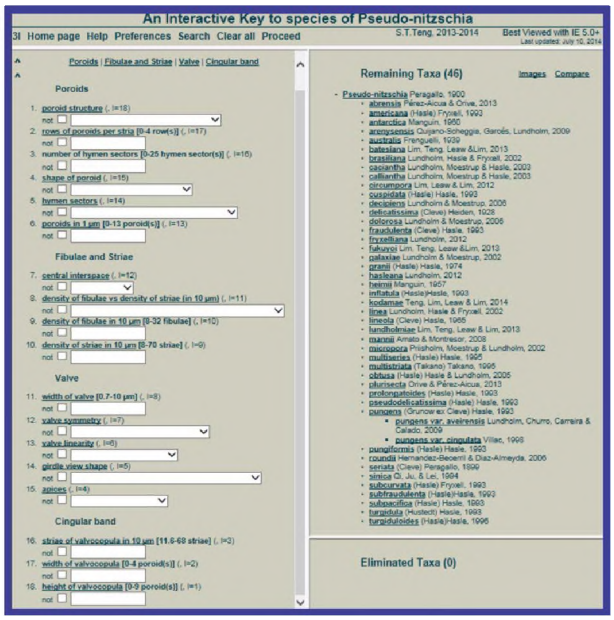

Fig. 1. A page from the web site on interactive identification of Pseudo-nitzschia spp

Pseudo-nitzschia is one of the most important genera of harmful algae because of the ability of some species to produce the neurotoxin domoic acid, responsible for amnesic shellfish poisoning (ASP) [1, 2]. This diatom genus is distributed worldwide [3, 4], with 44 valid species currently described [5]. Identification of *Pseudo-nitzschia* species relies heavily on minute differences in ultrastructural features on the diatom frustule. However, this morphometric information can only be obtained by advanced electron microscopy, including transmission electron microscopy (TEM). In addition, complexes and varieties of *Pseudo-nitzschia*, including cryptic species (that are morphologically identical, or too similar to be distinguished, but genetically different) and pseudo-cryptic species (that have minor ultrastructural differences) continue to be discovered, making species identification difficult.

Here we present a comprehensive taxonomic database of the 44 valid species and two varieties (*P. pungens* var. *cingulata* and *P. pungens* var. *aveirensis*) of *Pseudo-nitzschia* (total of 46 taxa) and an interactive key for species identification (Fig. 1), *http://imperialis.inhs.illinois.edu/dmitriev/key.asp?key=Bacillariales&lng=En&i=1&keyN=2.* This web-based interactive key was created using the 3I (Internet-accessible Interactive Identification) Interactive Key and the Taxonomic Database Software Package [6]. This interactive key is designed to assist researchers in identifying *Pseudo-nitzschia* species. An alternative computer-assisted identification of *Pseudo-nitzschia* species, not available on the Internet, was designed by Ehrman and Kaczmarska [7].

The database contains 18 morphological characters, grouped into four aspects of the diatom frustule: 1) poroids; 2) fibulae and striae; 3) valve; and 4) cingular band (the valvocopula) (Fig. 1) [8]. Half of the morphological characters are numerical, based on morphological measurements; the others are multiple-state characters. Examples of each of these characters are obtained by clicking on a link, where the character states are defined and micrographs illustrate how to measure the numerical characters. In addition to taxonomic information, this web-based database also gives the species' global distribution and a list of literature references.

The main window of the interface presents the list of characters, with a drop-down box of a set of states (Fig. 1). Species identification may start with any character by choosing a state from the drop-down box. Each character has a hyperlink to illustrative images. The <Proceed> button is then pressed after the states of one or several characters have been chosen. This will update the list of taxa fitting the search criteria, and display a list of "Eliminated taxa" and "Characters useful/no longer relevant for further identification". If a character has multiple states and there is uncertainty about which one to choose, the "not" box can be checked (Fig. 1); the taxa not having this state will remain in the list. For characters with numeric values, the range of valid values is shown in square brackets.

An exciting auto-generated function, <Compare>, is available to compare two or more species. <Compare> features include illustrative "Images"; "Similarities"; "Differences"; "All Characters"; and "Diagnosis". A phenetic tree of selected taxa of *Pseudo-nitzschia* can be reconstructed using the complete linkage method of cluster analysis. Finally, the interface links to the NCBI databases Taxonomy Browser and GenBank.

This web-based interactive key to species of *Pseudo-nitzschia* provides a taxonomic database platform to assist species identification. The species list, global distribution, and literature references will be updated periodically. The interactive key and database are at the beta-test stage, and under-going validation. This key can also be accessed from *www.harmfulalgae.info/psn.html*, where one can find more detailed instructions. Please direct feedback, with any queries or comments, to Sing Tung Teng (*tst861208@hotmail.com*) or Chui Pin Leaw (*cpleaw@um.edu.my*).

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Red Tides dissemination in Ushuaia, Argentina, an area with record observations of PSP toxicity

This could be just another story about raising awareness on the red tides phenomenon. It tells you how a group of experts from the southernmost tip of the American continent, when changing their dissemination work strategy, met the most moving learners from our society, the kids.

Here we are in the city of Ushuaia, capital of the province of Tierra del Fuego (Argentina). Ushuaia is by the Beagle Channel, the place where the first fatal case of paralytic shellfish posisoning within the Yamanas aborigines was reported by Dr. Polidoro Seguers in 1886. Cases of shellfish poisoning were not reported again until summer 1991-1992, when record levels of PSP toxins in shellfish (127,000 µg STXeq/100gr) where estimated in association with a bloom of the dinoflagellate *Alexandrium catenella*. Since then, the Marine Environment Laboratory from Tierra del Fuego carries out a monitoring for the presence of shellfish toxins in the Beagle Channel region. Population awareness-raising activities (dissemination talks, seminars, expositions, brochures) started about 5 years ago. Our activities were not always welcome, there was not a genuine interest to learn and sometimes people even questioned the existence of PSP events in the Beagle Channel.

As an alternative, we decided to create characters representing different shellfish vectors of the toxins to present them in an attractive manner to kids. This initiative developed and ended with the creation of a "Red Tides" comic which is currently used for dissemination schools and educational centres in the whole province (see the comic, in English and Spanish, in the following pages). We started our first visits to kindergartens and primary schools in Ushuaia feeling hesitant about how our new educational tool would be received. With pride and delight we realized we had succeeded to strike deep into the children's mind. Children amazed us with their open minds about a subject that turned out to be so complex for many grownups. For them it was a simple and amusing story! Half an hour talking, drawing, writing and having fun was enough to make parents go the next day to the school to ask what their kids had been learning about. It was such a positive experience, that in some cases the school children wanted to continue studying about red tides with the participation of their parents, the same ones that had stared at us before as if we were weird creatures. Some of the children's work were presented at provincial and national science fairs and even received prizes for their excellent results. We attach our "Red Tides Comic" (English and Spanish). We hope our experiences are of interest to you. Greetings from the end of the world!

Information on the Yamanas tribes and Tierra del Fuego can be found at the World's End Museum (Museo del Fin del Mundo) website *www.mfm-ushuaia.com.ar.*

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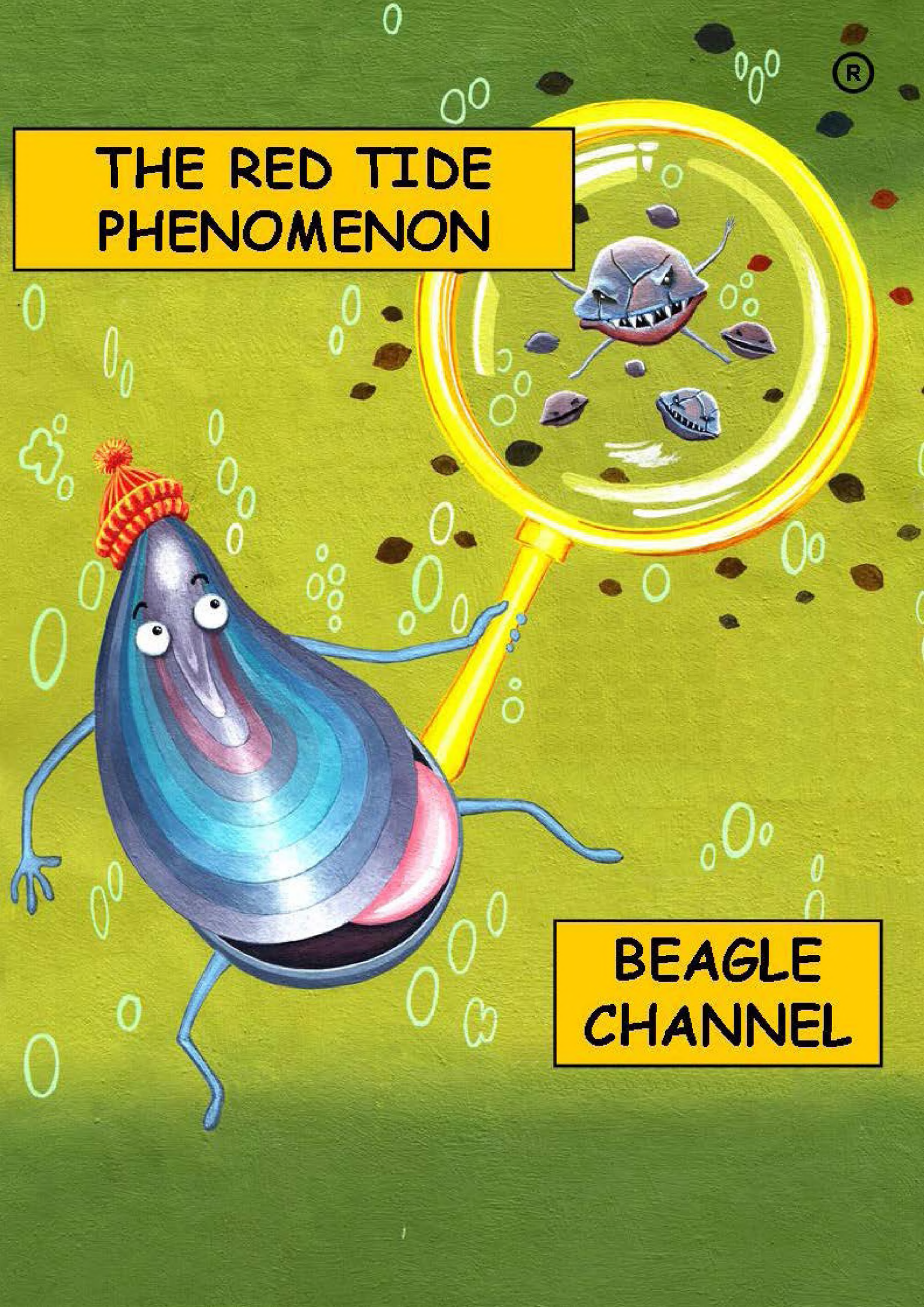
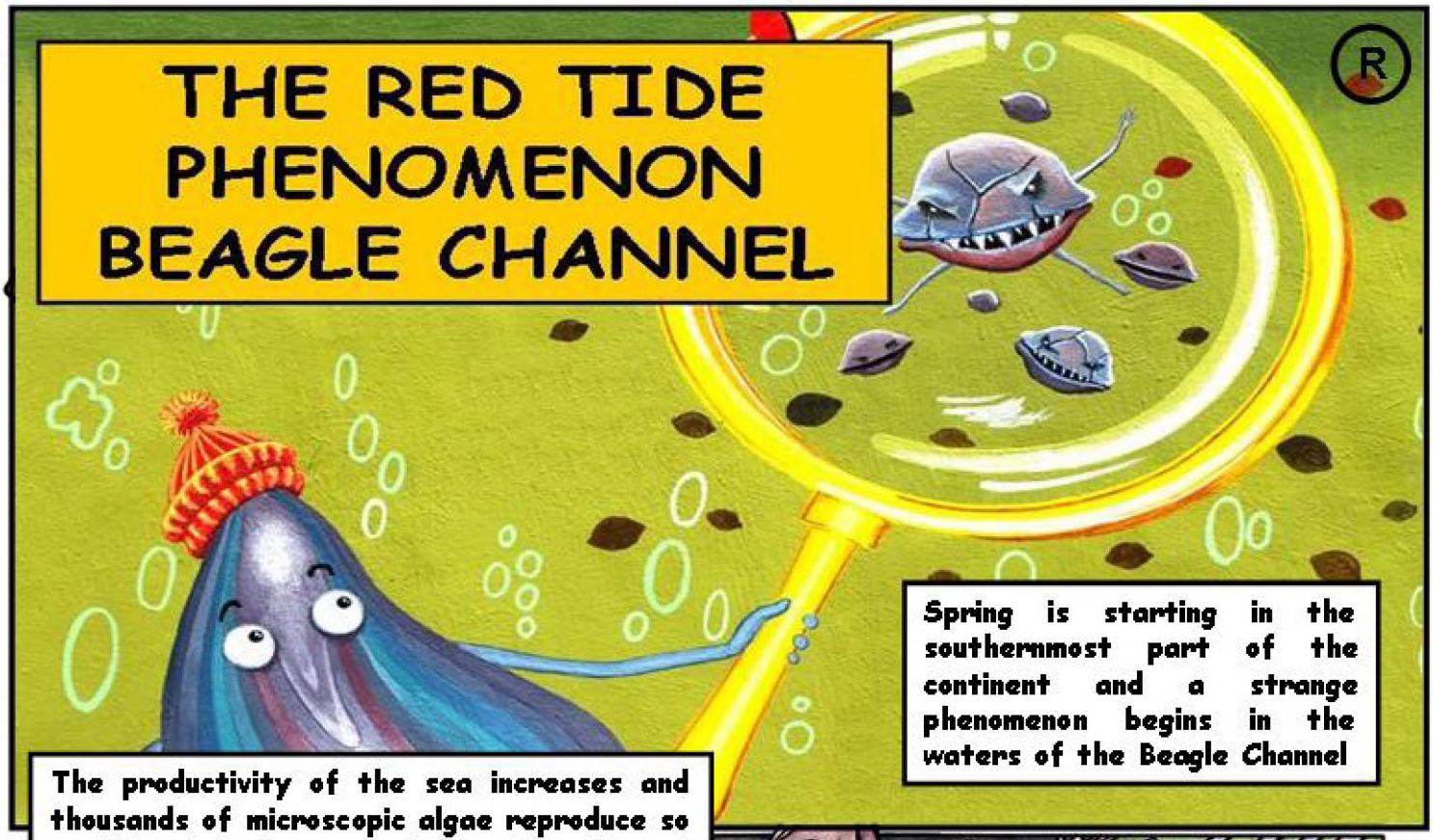
Fig. 1. Les Éclaireurs lighthose, Beagle Channel, Argentina

THE RED TIDE PHENOMENON



BEAGLE CHANNEL

THE RED TIDE PHENOMENON BEAGLE CHANNEL

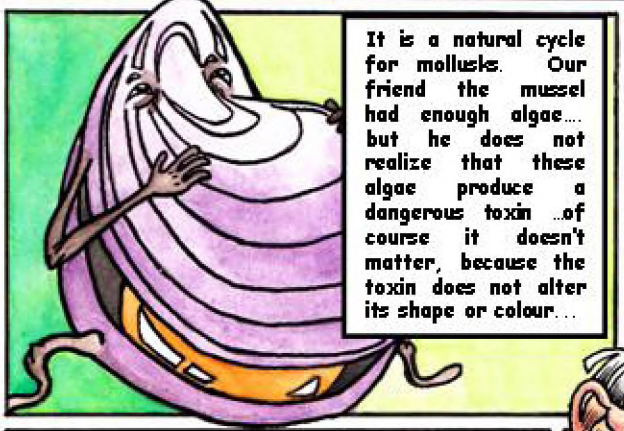


Spring is starting in the southernmost part of the continent and a strange phenomenon begins in the waters of the Beagle Channel

The productivity of the sea increases and thousands of microscopic algae reproduce so rapidly, that they may change the colour of sea water to yellow-brown or red.

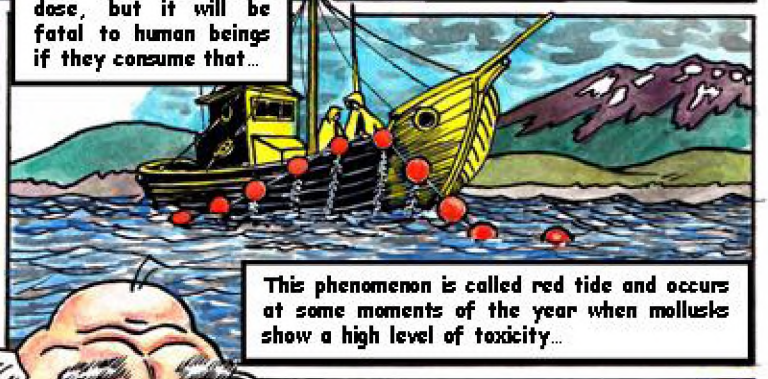


At the same time, mussels feed by filtering these algae, dragged by current...



It is a natural cycle for mollusks. Our friend the mussel had enough algae.... but he does not realize that these algae produce a dangerous toxin ...of course it doesn't matter, because the toxin does not alter its shape or colour...

It's a very small dose, but it will be fatal to human beings if they consume that...



This phenomenon is called red tide and occurs at some moments of the year when mollusks show a high level of toxicity...



...if they are not properly controlled by sanitary authorities...



This can be very dangerous for people

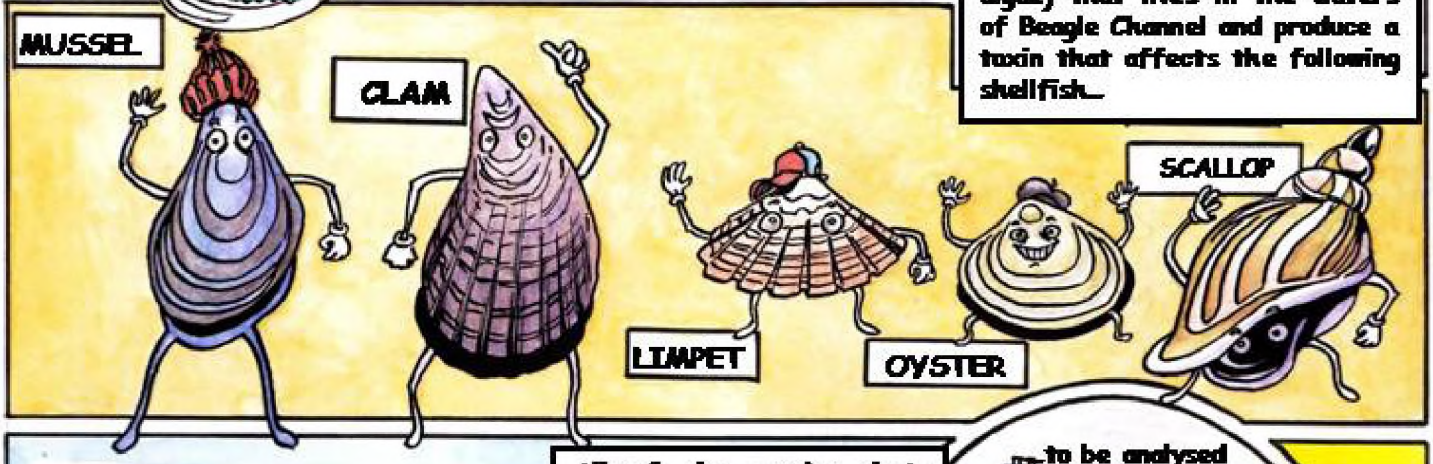
Something terrible is about to happen...

The culprit...



Within 5 to 30 minutes a fatal intoxication occurs. In moderate cases, there is a slight tingling and numbness around the mouth, headache, dizziness and nausea. If the intoxication is severe, it can cause death...

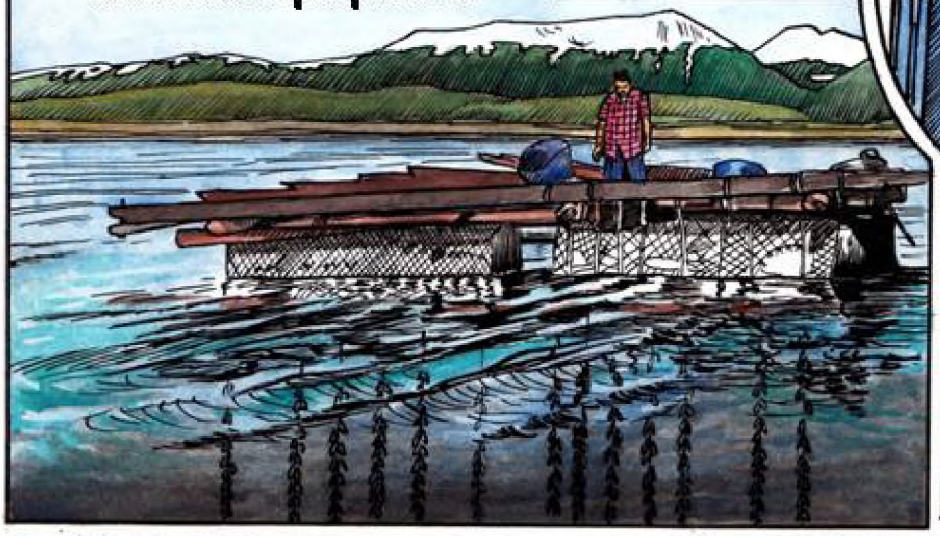
a phytoplankton (microscopic algae) that lives in the waters of Beagle Channel and produce a toxin that affects the following shellfish...



Fresh mussels are often collected by local producers for commercial purposes...

All of them take their products to the Laboratory of Marine Toxins and Microbiology of Ushuaia...

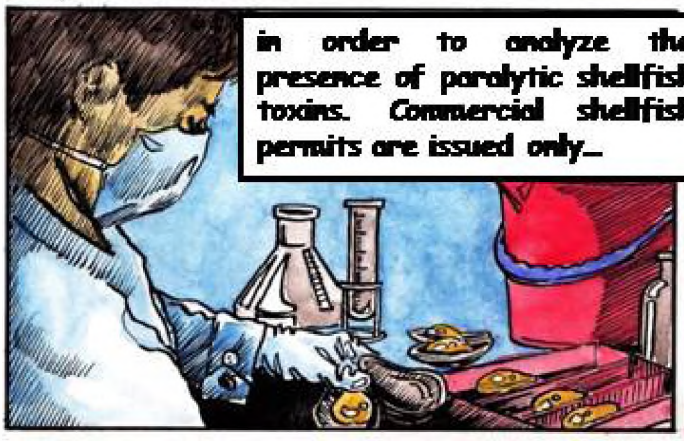
...to be analysed



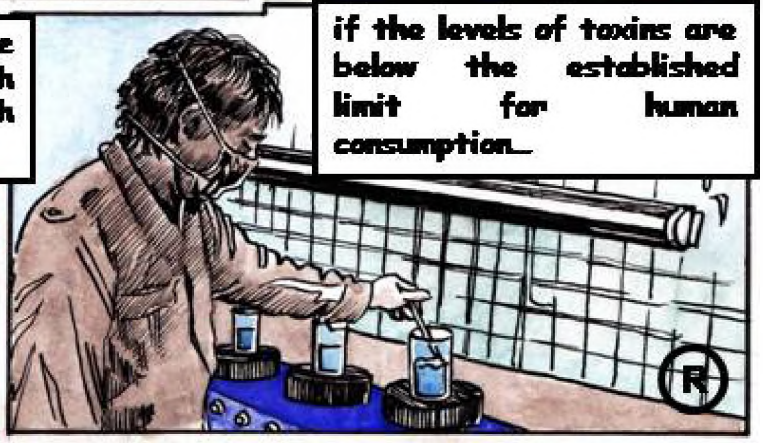


laboratory staff take a sample for testing...

in order to analyze the presence of paralytic shellfish toxins. Commercial shellfish permits are issued only...



if the levels of toxins are below the established limit for human consumption...



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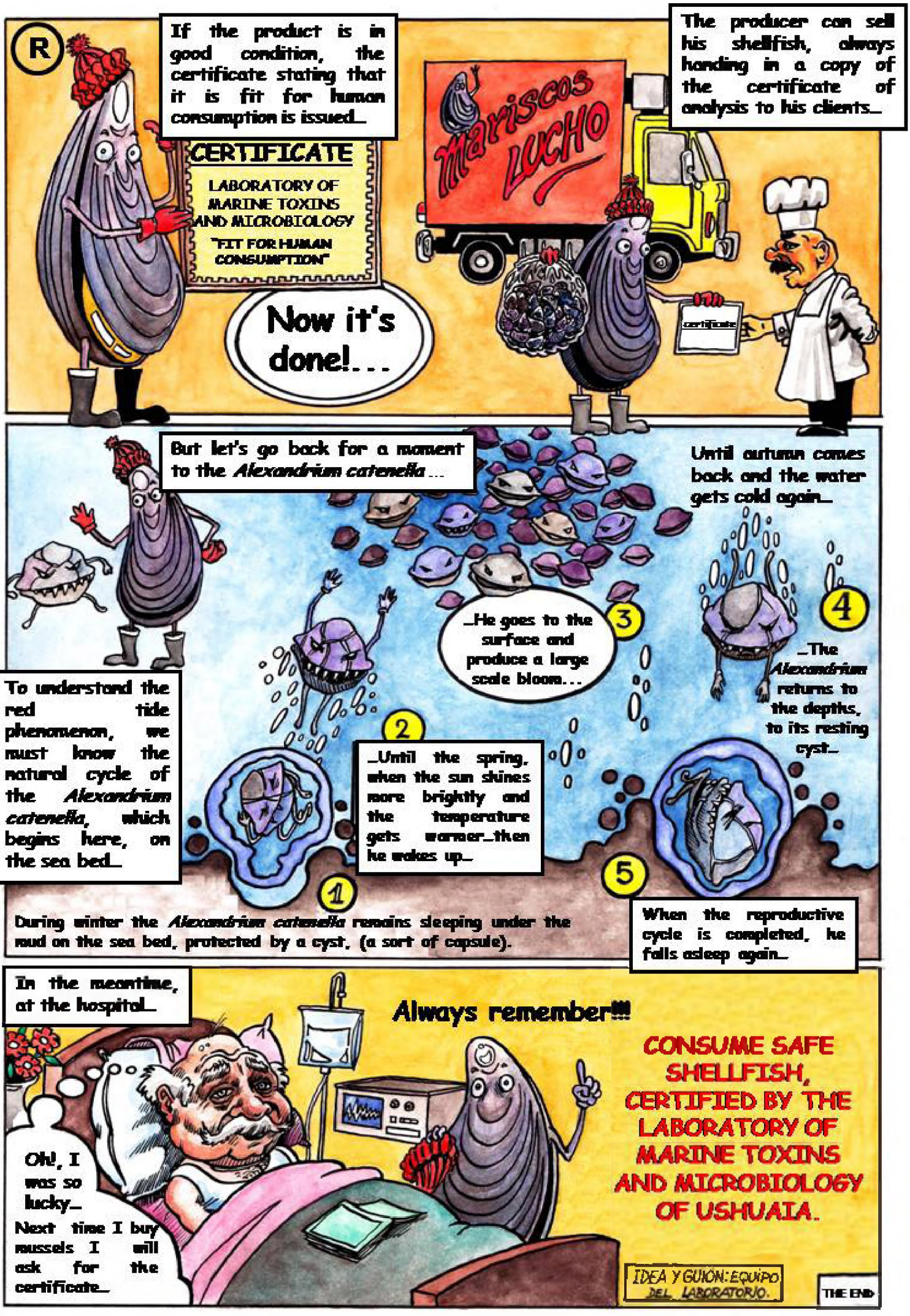
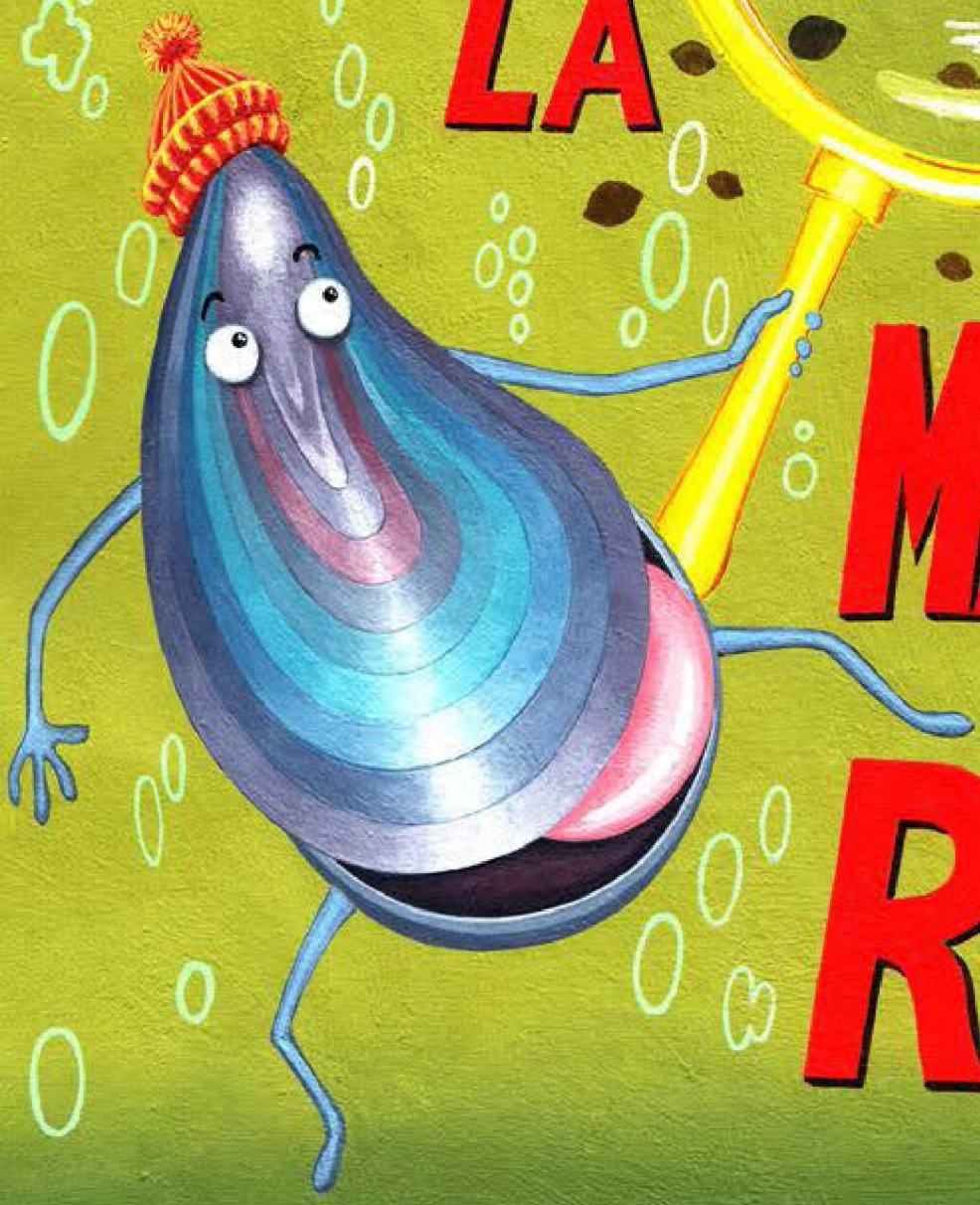
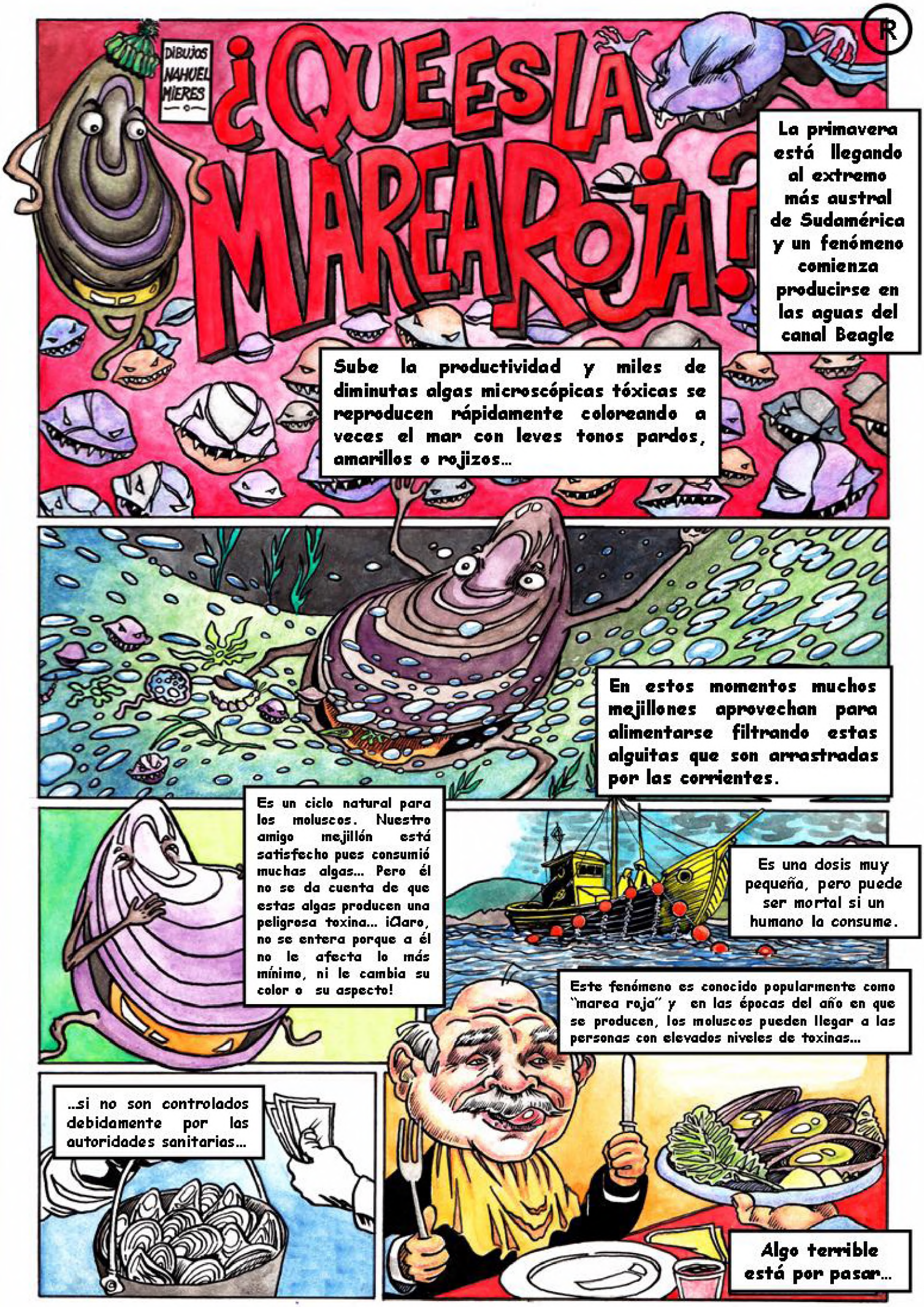

R
If the product is in good condition, the certificate stating that it is fit for human consumption is issued...
CERTIFICATE
LABORATORY OF MARINE TOXINS AND MICROBIOLOGY
"FIT FOR HUMAN CONSUMPTION"
Now it's done!...
The producer can sell his shellfish, always handing in a copy of the certificate of analysis to his clients...
Mariscos LUCHO
certificate
But let's go back for a moment to the *Alexandrium catenella*...
To understand the red tide phenomenon, we must know the natural cycle of the *Alexandrium catenella*, which begins here, on the sea bed...
1
During winter the *Alexandrium catenella* remains sleeping under the mud on the sea bed, protected by a cyst, (a sort of capsule).
2
...Until the spring, when the sun shines more brightly and the temperature gets warmer...then he wakes up...
3
...He goes to the surface and produce a large scale bloom...
4
...The *Alexandrium* returns to the depths, to its resting cyst...
5
When the reproductive cycle is completed, he falls asleep again...
Until autumn comes back and the water gets cold again...
In the meantime, at the hospital...
Oh!, I was so lucky...
Next time I buy mussels I will ask for the certificate...
Always remember!!!
CONSUME SAFE SHELLFISH, CERTIFIED BY THE LABORATORY OF MARINE TOXINS AND MICROBIOLOGY OF USHUAIA.
IDEA Y GUION: EQUIPO DEL LABORATORIO.
THE END

EL FENOMENO DE LA MAREA ROJA




DIBUJOS NAHUEL MIERES
¿QUE ES LA MAREA ROJA!
La primavera está llegando al extremo más austral de Sudamérica y un fenómeno comienza producirse en las aguas del canal Beagle
Sube la productividad y miles de diminutas algas microscópicas tóxicas se reproducen rápidamente coloreando a veces el mar con leves tonos pardos, amarillos o rojizos...
En estos momentos muchos mejillones aprovechan para alimentarse filtrando estas alguitas que son arrastradas por las corrientes.
Es un ciclo natural para los moluscos. Nuestro amigo mejillón está satisfecho pues consumió muchas algas... Pero él no se da cuenta de que estas algas producen una peligrosa toxina... ¡Claro, no se entera porque a él no le afecta lo más mínimo, ni le cambia su color o su aspecto!
Es una dosis muy pequeña, pero puede ser mortal si un humano la consume.
Este fenómeno es conocido popularmente como "marea roja" y en las épocas del año en que se producen, los moluscos pueden llegar a las personas con elevados niveles de toxinas...
...si no son controlados debidamente por las autoridades sanitarias...
Algo terrible está por pasar...

Entre 5 y 30 minutos se produce una intoxicación que si es leve, los síntomas empiezan con un cosquilleo en la boca y a veces vómitos y mareos; si la intoxicación es grave puede llegar a causar la muerte...

El responsable...

Alexandrium catenella

un alga microscópica del plancton que vive naturalmente en aguas del Canal de Beagle y produce una toxina que se acumula en los siguientes productos de mar...

Mejillón

Cholga

Lapa

Almeja

Caracol

Periódicamente productores y recolectores de moluscos de la isla cosechan mariscos frescos para su comercialización...

Todos ellos trasladan su mercadería hasta el Laboratorio Regional de Toxinas Marinas y Microbiología de Ushuaia...

...para ser analizada

Un agente del laboratorio toma una muestra representativa de la carga para ser controlada...

...se analiza la presencia de toxina paralizante y solo se permite la comercialización del producto...

...si los niveles de toxinas se encuentran por debajo de límites seguros para el consumo humano...

®

Si la mercadería está en óptimas condiciones se entrega el certificado de apto para consumo humano...

CERTIFICADO
LABORATORIO REGIONAL USHUAIA DE TOXINAS MARINAS Y MICROBIOLOGÍA
"APTO PARA CONSUMO"

¡AHORA SI!...

El productor sale a vender sus moluscos entregando siempre a los clientes una copia del certificado del análisis...

Mariscos LUCHO

Pero volvamos por un momento al *Alexandrium catenella*...

Para comprender el fenómeno de la marea roja debemos conocer el ciclo de vida de *Alexandrium*, que comienza aquí en el fondo del lecho marino...

1

2

...Hasta la llegada de la primavera, cuando la luz y temperatura se tornan más agradables... y es allí cuando despierta...

3

...Entonces germina, sube a la superficie y se reproduce a gran escala...

Hasta que regresa el otoño, el agua vuelve a enfriarse, la luz del sol se debilita...

4

...y *Alexandrium* retorna a las profundidades en forma de quiste...

5

Y cumplido su ciclo reproductivo se duerme nuevamente...

Alexandrium permanece durante todo el invierno bajo el fango del fondo del mar en forma de quiste, una especie de cápsula o "semilla" que le permite dormir tranquilo.

Mientras tanto en el hospital...

¡Uf que suerte tuve!

La próxima vez que compre mejillones voy a solicitar el certificado...

Y recuerde siempre!!!

Consumir moluscos seguros certificados por el laboratorio Regional de Ushuaia de Toxinas Marinas y Microbiología

IDEA Y GUIÓN: EQUIPO DEL LABORATORIO.

FIN

A Global HAB Status Report

For decades the HAB community and various HAB groups have discussed the need for and the difficulties of a global appraisal of HAB species occurrences and their impacts. In April 2013 the IOC Intergovernmental Panel on HABs (IOC-IPHAB) decided to develop a 'Global HAB Status Report' with the aims of compiling an overview of HAB events and their societal impacts; providing a worldwide appraisal of the occurrence of toxin-producing microalgae; and assessing the status and probability of change in HAB frequencies, intensities, and range resulting from environmental changes at the local and global scale. Linkages will be sought established with the IPCC reporting mechanism which increasingly is focusing on the biological impacts of climate change.

The project now being launched by the IOC, with the support of the Government of Flanders, will compile data on toxic algae occurrence and blooms, focusing on species included in the IOC Taxonomic Reference List/ WoRMS/ HAB (*http://www.marinespecies.org/hab/index.php*). This initiative will happen within the IOC International Oceanographic Data Exchange Programme IODE and its components HAEDAT (*http://haedat.iode.org/*) and OBIS (*http://www.iobis.org/*), in close partnership with the International Atomic Enegy Agency (IAEA), the International Council for Exploration of the Sea (ICES), the North Pacific Marine Science Organisation (PICES) and the International Society for the Study of Harmful Algae (ISSHA).

The Project will thus complement and complete global data sets already in OBIS, HAEDAT and WoRMS/ HAB on the distribution and impact of harmful algae with the view to publish an IOC UNESCO Global Harmful Algal Bloom Status Report. These efforts will take time to complete and we expect it will take up to 2 years to develop the first status report.

There are two main lines of data compilation: species occurrence and HAB events.

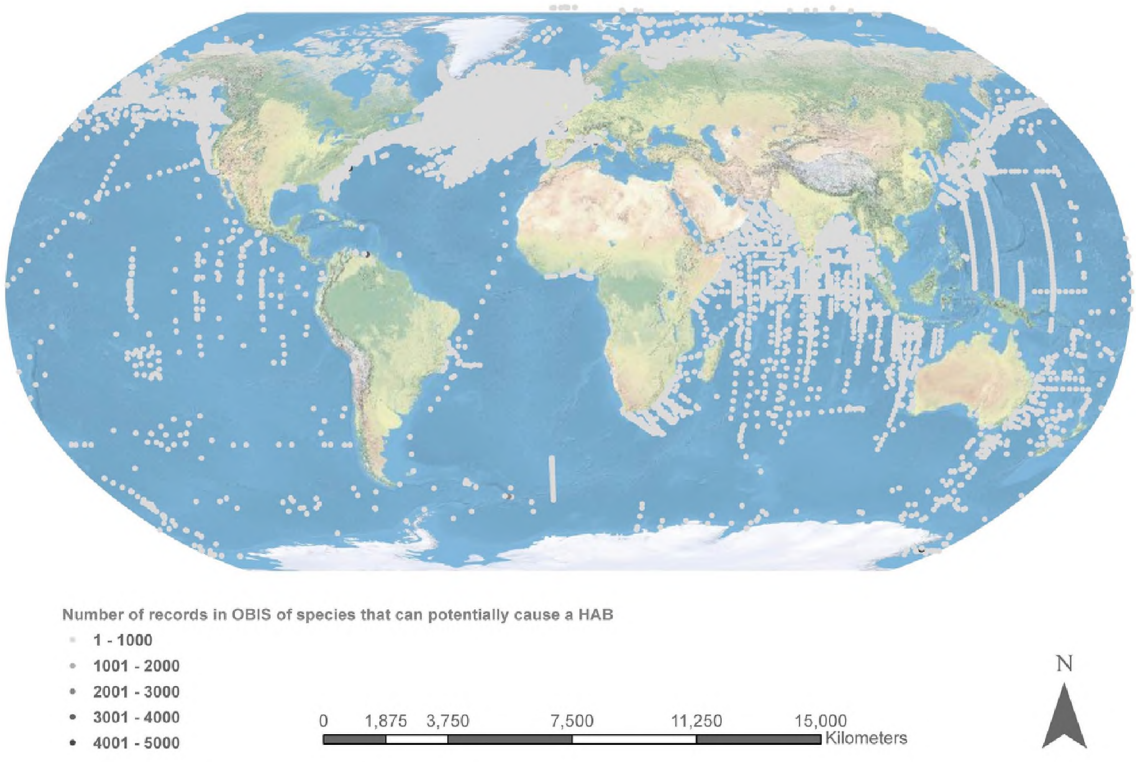
For the species occurrence, information will consist of documented records extracted primarily from the published literature. Data will be stored in OBIS. This activity was first introduced several years back in ISSHA and several scientists from around the world volunteered to this in the context of the IOC-ISSHA Project HABMAP. Some have already compiled part or most of the data. However, funding to proceed systematically has not been available until now. We are now in the process of confirming a list of editors for regional data compilations. A monograph in preparation by Lassus et al will be a significant contribution to this effort.

As for HAB events, data are already compiled systematically in some regions and collected in HAEDAT, while a new effort is required for other regions . The collaboration with many agencies and institutions holding monitoring data will be required and we hope for broad interest and back-up. The new data will also be stored in HAEDAT.

A Task Team established at the last IPHAB meeting (Paris 2013) will oversee the work and develop an outline of the Global Harmful Algal Bloom Status Report (further nomination from IPHAB member states still possible). The Task Team will also recommend a review mechanism; establish a periodicity and a regular process for preparation of a Global Harmful Algal Bloom Report, and for a dissemination and communication strategy. The Task Team is co-chaired by Drs. Gustaaf Hallegraeff (Institute for Marine and Antarctic Studies, University of Tasmania, Australia) and Adriana Zingone (Stazione Zoologica Anton Dohrn, Naples, Italy).

The first activities in the project work-plan include a training workshop for the researchers who will compile and upload data from each region, organized by IOC in collaboration with IAEA. A side information meeting is also planned at the 16th International Conference on Harmful Algae (ICHA) in Wellington, New Zealand, next October.

If you are more interested, please do not hesitate to contact us at *h.enevoldsen@unesco.org*



Lora Fleming awarded with the *Dr Edouard Delcroix* prize

The non-profit organisations HYDRO and the Flanders Marine Institute (VLIZ) have established the Dr Edouard Delcroix (1891-1973) Prize in honour of the Belgian orthopaedic surgeon and pioneer in thalassotherapy. This international triennial scientific prize, with a value of 25000 €, is awarded to a researcher or a research team for an original scientific study on the links between oceans and human health (website: http://www.vliz.be/delcroix)

In 2013, the IOC and ISSHA nominated Prof Dr Lora E. Fleming, European Centre for Environment and Human Health, University of Exeter Medical School, UK, for the Scientific Prize Dr E. Delcroix 2013. On Friday 27 June 2014 the Hydro VZW and the Flanders Marine Institute VZW, presented in Oostende, Belgium the Prize to Dr. Fleming for her work 'Harmful algal blooms, microbial pollution of marine waters, and climate change beyond the state of the art (Oceans and Human Health)'.

Prof. Fleming's work over the past decade as both an interdisciplinary physician and epidemiologist in environment and human health, led in the creation of the new scientific discipline of Oceans and Human Health (OHH), bringing together biomedical and oceanographic investigators (including toxicologists, biological/physical oceanographers, health educators, veterinarians, microbiologists, engineers, economists, physicians, and epidemiologists) to do research and training at the frontier in diverse areas: harmful algal blooms, microbial pollution of marine waters, and climate change beyond the state of the art. She was the Director of one of 4 US National Science Foundation (NSF)-National Institute of Environmental Health Sciences (NIEHS) funded interdisciplinary Oceans and Human Health Centres at the University of Miami. In addition to supporting cutting edge science and training in the modelling of Oceans and Human Health, she has lead several research groups in the area of human exposures and health effects associated with interactions with the oceans and ocean health. Since Jan 2011 she became Director of the European Centre for Environment and Human Health (www.ecehh.org) at the University of Exeter Medical School UK, where she is involved in promoting active engagement in interdisciplinary approaches to the complex challenges of environment and human health.

We congratulate Dr. Fleming with the price and hope to see an increased focus on HABs in the context of Oceans and Human Health (see also 'Message from Betruthan" on pages xx).

H. Enevoldsen/IOC UNESCO



Prof. Dr Lora Elderkin Fleming receives the Prize Dr Edouard Delcroix 2013 © VLIZ (Verhaeghe, Els)

Message from Bedruthan









Unanimous call for a coordinated, transnational and interdisciplinary Oceans and Human Health research programme in Europe

On 20 & 21 March 2014, a group of 50 experts from a broad range of disciplines gathered in Bedruthan (Cornwall, UK) to discuss the complex interactions between the marine environment and human health. The workshop was convened by the **European Centre for Environment and Human Health** (University of Exeter) and the **European Marine Board**, with organizing support from **Ifremer**, the **Scottish Association for Marine Science** (SAMS) and **Plymouth Marine Laboratory**.

Human health and wellbeing are intrinsically connected to the seas and oceans.

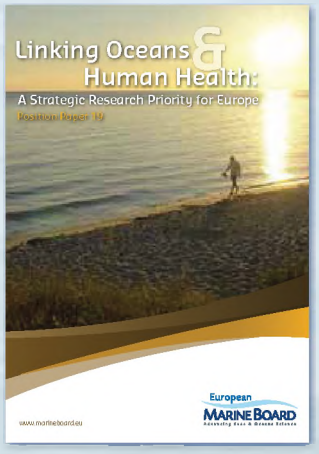
This relationship is becoming increasingly important in light of rapidly growing coastal populations and climate change. Europe can achieve significant public health benefits through a better understanding of highly complex marine environment and human health interactions.

Oceans and Human Health – An integrative research field addressing societal needs.

"Oceans and Human Health" (OHH) represents a relatively new and integrative research field, drawing from expertise across the natural, social and economic sciences, including public health and medicine. A beneficial step-change in scientific understanding, evidence-based policy, public awareness and human behaviour is possible through:

- coordinated interdisciplinary research;
- building OHH communities and capacities;
- engaging with stakeholders; and
- managing effective knowledge transfer and science policy interfaces.

The Bedruthan workshop unanimously called for a coordinated, transnational and interdisciplinary Oceans and Human Health research programme in Europe. Research should be solutions-oriented, supporting health and wellbeing promotion and disease treatment, and informing maritime, environment, public health and innovation policy.



European Marine Board (EMB) position paper

The Message from Bedruthan follows publication of EMB position paper 19, **"Linking Oceans & Human Health: A Strategic Research Priority for Europe"**. The EMB is a partnership of 36 major national research institutes and funding agencies from 19 European countries. The paper represents a compelling endorsement from this large European research network of the societal importance for Europe of supporting coordinated interdisciplinary OHH research.

Download at www.marineboard.eu

UNIVERSITY OF EXETER MEDICAL SCHOOL

European Centre for Environment & Human Health

EUROPEAN UNION Investing in Your Future
European Regional Development Fund 2007-13

convergence for economic transformation

European MARINE BOARD Advancing Seas & Oceans Science

PML Plymouth Marine Laboratory

SAMS

Ifremer

NEW: "Harmful Algae in the Gulf of Maine: Oceanography, Population Dynamics, and Toxin Transfer in the Food Web"

A special issue on harmful algae in the Gulf of Maine published in May 2014 in Deep-Sea Research Part II: Topical Studies in Oceanography (Vol 103). This special issue, available for free downloading until 31 January 2015, consists of 26 papers describing the results of a 5-year multi-investigator research program, Gulf of Maine Toxicity (GOMTOX), formulated and funded through the US-NOAA Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) program. Led by Dr. Donald Anderson at Woods Hole Oceanographic Institution, GOMTOX was a partnership between researchers, federal agencies, state managers, and representatives from the shellfish industry. *http://www.journals.elsevier.com/deep-sea-research-part-ii-topical-studies-in-oceanography/news/free-online-access-to-new-special-issue-on-harmful-algae-in/*

The Gulf of Maine (GOM) in the northwest Atlantic Ocean harbors a valuable shellfish resource that is often impacted by algal blooms of the toxigenic Alexandrium fundyense and Pseudonitzschia spp. responsible for paralytic shellfish poisoning (PSP) and amnesic shellfish poisoning (ASP) respectively. While intensive monitoring efforts and continuous adjustments to harvesting closures in state waters has allowed for the safe harvest of shellfish inshore, vast areas of federal waters offshore had been closed to bivalve molluscan shellfish harvest for decades due to the potential risk of PSP and the lack of understanding of bloom dynamics and associated toxicity in this region. The aim of the GOMTOX was to establish a comprehensive regional-scale understanding of HAB dynamics, transport pathways, and associated toxicity and to use this information and relevant technologies to assist managers, regulators and industry to fully access nearshore and offshore shellfish resources with appropriate safeguards for human health.

One of the many noteworthy outcomes from the GOMTOX program was the development of a novel biotoxin management strategy that led to the reopening of a portion of Georges Bank to the harvest of bivalves, a multi-billion dollar resource, after being closed for 22 years (*http://www.fda.gov/forconsumers/consumerupdates/ucm368533.htm*). Researchers from the Food and Drug Administration (FDA) led the efforts to:

(1) modify PSP test kits for use by fishermen at sea to determine when product was safe for harvest and

(2) evaluate the performance of the management strategy via a pilot study that permitted one fishing vessel access to harvest surf clams and quahogs on Georges Bank while following the management strategy, known as the onboard screening dockside testing (OBSDT) protocol. Shellfish product landed under the protocol is verified as safe via an approved laboratory method before it is released into commerce.

Success of the pilot study resulted in the safe harvest of $2.7 million in surf clams in 2010, and in 2013 a region of Georges Bank was reopened for the harvest of shellfish to fishermen that follow the protocol. Access to this fishery has resulted in the doubling of the number of quahogs available on the market and a 40% increase in surf clams.

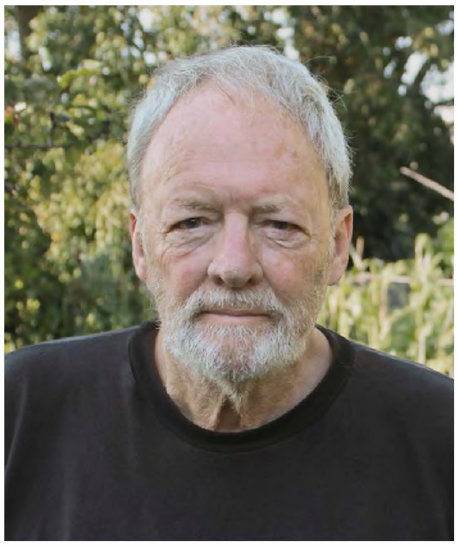
Interested readers can learn more about the OBSDT management strategy and other GOMTOX project outcomes in the special issue. Scientific themes included in this volume are:

(1) cysts and sediment dynamics;
(2) ecology and bloom dynamics;
(3) the physical/chemical setting;
(4) toxin dynamics, food web transfer, and monitoring; and
(5) management implications.



Thanks Tim!

When Harmful Algae News (HAN) was launched in 1992 it was agreed from the start that Tim Wyatt would be the Editor. With the engagement of Tim, the IOC had an experienced editor and serious scientist to help launch and develop the newsletter. In the early days the Editor of HAN was even paid a fee for the work, but over the years this translated into travel support for the ISSHA Conferences, a beer at the ISSHA Conferences and the honour, all as a consequence of the changed framework conditions for IOC and UNESCO. Every second year the IOC has asked Tim about his willingness to take another term and for the past 22 years Tim has been willing to continue. During the spring of 2014 Tim felt it was time to let someone else take over. The IOC Secretariat and all involved with the IOC HAB Programme and IPHAB wish to extend their sincere gratefulness and thanks to Tim for his unusually stable commitment and his efforts which have implied significant time to encourage contributions, help with article writing and editing. It has always been a true pleasure to work with Tim and the manuscripts for HAN have always been delivered with the same high standard of editing. The, at the same time broad and deep, scientific insight of Tim has been very valuable for HAN and his synthetic and personal summaries from several of the ISSHA conferences has been enjoyed and appreciated by the broad HAB community and HAN readers. We hope Tim may still appear as a contributor in HAN. The big question then came, how to find somebody who wished to follow on to Tim? Well, it became clear that there was a wish to have a more formal network of regional-editors and as these ideas were developed the person that knows Tim better than any, Beatriz Reguera, come up with a model where she and Eileen Bresnan acts as Eds-in-chief and will work with a group of regional editors. After a process of soliciting for volunteers and asking key people we are now proud to introduce the new editor's team for HAN:



Tim Wyatt

Coming deadlines

HAN 50: 15 November 2014
HAN 51: 15 February 2015
HAN 52: 15 May 2015
HAN 53: 15 August 2015

Deadlines to be repeated the following years

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The ambition of the new team is for HAN to become a more regular newsletter, i.e. to appear every 3 months and have fixed submission deadlines for each issue. Also it is the plan in a near future to prepare a HAN index. There are many historic pieces of information that appeared first in HAN and it would be valuable to be able to find and cite them easily.

Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

The publication of Harmful Algae News is sponsored by the Department of Biology, University of Copenhagen

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Harmful Algae News

AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Highlights of the XVI International Conference on Harmful Algae

Aotearoa ("land of the long white cloud") was the name given to New Zealand in the maori language. As a result of its remoteness, 1500 km east of Australia and 1000 km south of New Caledonia, this South Pacific country was the last major habitable land mass to be occupied by humans. New Zealand's economy is largely based on primary products for export to international markets, a growing tourist industry attracted by its beautiful and varied natural parks (distributed in a range from subtropical to alpine climate). The hospitality of this egalitarian country that has set an example in terms of democracy, standard of living and environmental protection. Shellfish and aquaculture are important components of New Zealand's primary sector and seafood is an important component in their local cuisine. Not surprisingly, the country has devoted important efforts to research and management of their marine and freshwater resources. Innovative and dynamic R+D centres were set up more than 20 years ago along with the increasing seafood exports, and strict controls are imposed on visitors to make sure they do not introduce undesirable pests.

The 16th International Conference on Harmful Algae was held in Wellington (New Zealand) in October 27-31, 2014. The theme of the conference was *Advancement Through Shared Science* in recognition of the multidisciplinary nature of the field and the important role that international collaboration has played in the understanding of HAB phenomena and the mitigation of their effects. More than 400 participants of 41 countries from all five continents flew thousands of miles to attend this event. This is the third (Tasmania 2000, South Africa 2004) time the conference has been held in the Southern hemisphere, but not the last (the 17th ICHA will be held in Brazil).

There were two key note (**Moestrup**, **Paerl**) and 7 plenary (**Burford**, **Kilroy**, **Munday**, **Murray**, **Suzuki**, **Tester**, **Turnbull**) speakers, 196 orals and 150 poster communications, including one innovation in the ICHA series: two sessions with 10 posters each presented as 4-min speed talks. The presenters of those rose to the challenge magnificently and gave a series of succinct, well prepared and interesting talks.

It was obvious throughout the conference that almost every single detail had been carefully planned in advance by the local organizing committee, chaired by Lincoln Mackenzie (Cawthron Institute). The facilities of the modern *Michael Fowler* Conference Centre were excellent. A key component of the success of the conference was the great job done by the professional conference organisers *Conferences and Events*. Special thanks are due to Michelle Kane, Amy Abel and Francie Gaiger who ensured that handbook and abstracts were well presented, that the



Government and institutional authorities singing during the traditional maori Powhiri opening ceremony (Photo J. Camp)



Maori dancers at the Conference Banquet (Photo B. Reguera)

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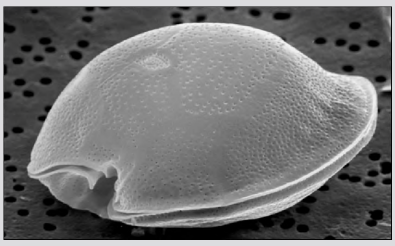

conference ran smoothly, and delegates were well fed and watered.

Conferences always get the imprint of the host country. New Zealand is close to Polynesia, where the most intense Ciguatera Fish Poisoning outbreaks occur. The study of freshwater HABs is also of paramount importance in a country with a tourist industry focused among other activities on angler-fishing and canoeing in pristine rivers and streams. For these reasons, Ciguatera and Freshwater HAB issues attracted more communications than ever before in the ICHA conference series. There were four full sessions devoted to Ciguatera research and management, and three others to the biology and ecology of freshwater (including benthic) HABs. Many of us heard for the first time about "Didymo" (*Didymosphenia geminata*), or "rock snot", a cold, low nutrient-water diatom native to the northern hemisphere, and considered an invasive species in Australia, New Zealand and Chile. Didymo does not pose a human health risk, but is associated with massive production of extracellular polymeric substances (EPS) through its branching stalks which form multi-layered structures that are resistant to degradation. The plenary speaker (**Kilroy**) and presenters from New Zealand and Chile illustrated well the threat of this species and its impact in recreational waters, ecosystem disruption and irrigation problems.

Taxonomic designations, as usual, were the protagonists of hot debates. This time it was about *Alexandrium catenella,* a species name that some authors proposed should be reduced to a synonym of *Alexandrium fundyense*. The Linnaean classification collides with post-Darwinian evolutionary (cladistic) systematics!

But above all, the 16th ICHA witnessed a blooming of molecular biology developments (next generation sequencing, metagenomics, transcriptomics) which combined with other advanced instruments were applied to solve multiple scientific and managerial questions. New and maturing monitoring high technology (e.g. robots, *in situ* fluid imaging, receptor binding assays, near universal acceptance of LC-MS) were also illustrated. A review of the highlights from the main thematic sessions follows. The programme of the conference and abstracts can be downloaded from *http://www.icha2014nz.com/*

Once more, the ICHA conference provided a unique atmosphere for scientific exchanges and social activities. These included a reception at the "Beehive", the eye-catching New Zealand parliament building, mid-conference excursions ("Lord of the Rings" tour, Wellington Seal Safari and others), a great Conference Banquet at the National Te Papa Museum, and the ISSHA Society auction (see ISSHA's Corner).

Remember March 1, 2015 is the deadline to submit your contribution for the 16th ICHA Proceedings to *icha2014@confer.co.nz*. More information and the template for your submission can be found at the ISSHA website (*www.issha.org*).

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Taxonomy and Genetics

In his opening address Prof. Moestrup presented microalgal taxonomy as a very complex topic. Some of the questions he posed were: "Is taxonomy getting easier because of the new modern tools?; Is taxonomy to be taken seriously?; Should we aim at a taxonomy which can be used outside taxonomist's narrow circles?" The many problematic and so far unsolved cases described in this talk suggest negative responses to the three questions. Nevertheless, many resolved cases and deeper insights into species delimitation presented at ICHA 16 demonstrate that taxonomy is still alive and useful, especially in this transitional age between purely visual and purely molecular approaches. Cases of integrative taxonomy, where functional aspects are linked to morphology and taxonomy, were widespread in both oral communications and posters. These were clear examples of the fundamental role of taxonomy as a backbone needed to attach the information collected about organisms. As David Mann stated at the 12th ICHA in Copenhagen, *organisms lacking a formal name are generally ignored* [1]. *If the names are unknown, knowledge of the things also perishes ...*", Carolus Linnaeus wrote in *Philosophia Botanica* 1751. More important than its infrastructural role, taxonomy, nowadays largely supported by molecular phylogenetics, aims at tracing the evolutionary history of organisms.

Problems related to frequent changes in species name can be understood if we accept that, rather than mere labelling, taxonomy is a science and as such it is 'work in progress' [2]. In this respect knowledge about *Alexandrium* species taxonomy has been evolving from conference to conference, at least since 1989, when experts at the 4th ICHA in Lund reached a *consensus* about the genus name. At the previous, 15th ICHA, *A. ostenfeldii* was shown to be a species complex. This time it was the turn of the *A. tamarense* species-complex, within which 5 taxa were separated. Two of them were assigned to *A. tamarense* and *A. fundyense*, while for the others the new names of *A. pacificum*, *A. mediterraneum* and *A. australiense* were established (**John**). Within this new scheme, the name *A. catenella* was not unequivocally usable for any of the recognized taxa, and so was superseded. Yet, some taxonomists strongly disagree with the latter decision.

The winners of the competition for the most taxonomically studied organisms at ICHA 16 were the small naked dinoflagellates, for a long time confined to a bulk categorization (e.g. *Gymnodinum/Gyrodinium* spp.) but now calling for detailed research (**Reñé**). New results were shown for *Amphidinium* (**Sasai**), woloszynskiod species (**Taka-**



Opening address by Prof. Øjvind Moestrup

hashi), Kareniaceae (new *Karlodinium gentienii*, **Nézan**), and *Gymnodinium* (new *G. smaydae*, **Kang**). Surprises and new species are also coming from small thecate *Azadinium* (**Tillmann, Proença**), and larger benthic *Ostreopsis* (**Giussani**), *Gambierdiscus* (*G. scabrosus*, **Nishimura**) and *Peridiniopsis* (**Prabowo**). Two new *Prymnesium* species (**Seoane**) and a new raphidophyte close to *Heterosigma* were also reported (**Engesmo**).

Intraspecific diversity reflects relevant functional and ecological differences. This concept clearly emerged from studies coupling molecular and physiological approaches in *Alexandrium ostenfeldii* (**Kremp**) and *Pseudo-nitzschia pungens* (**Kim**), the latter displaying three physiologically distinct ITS-clades. Two haplotypes and 7 microsatellite genetic markers were used to depict *'A. catenella'* (to be reconciled with the new taxonomy, see above) intra-specific diversity across coastal seas (**Nagai**). For *Gonyostomum semen*, an array of different tools was applied to study intraspecific diversity and spatial distribution patterns (**Lebret, Sassenhagen**), including a new genomic approach, RADtag sequencing (**Rengefors**). Using microsatellites, a study from the Baltic revealed a clear spatial structure during a bloom of *Skeletonema marinoi* (**Godhe**), while rapid genetic changes were reported during blooms of *Alexandrium minutum* (**Dia**) along French coasts.

Cryptic and pseudocryptic diversity has widely been demonstrated across microalgal taxa. Molecular tools nowadays support traditional optical methods and allow a sounder appraisal of species present at one place. This coupled approach effectively contributes to both a better knowledge of local diversity and a reliable definition of the geographical range of harmful species (**Tester**). New surveys with such tools were presented for *Pseudo-nitzschia* in Chinese (**Li**) and Singapore waters **(Peng),** harmful dinoflagellates in Arctic waters **(Tilman),** fish-killing dinoflagellates in the Baltic **(Eskola),** *Gambierdiscus* species in the Florida Keys and Virgin Islands (**Pitz**), and inThai waters (**Tawong**).

In terms of identification of local diversity and biogeographical ranges, a novelty at ICHA 16 was the explosion of studies based on total environmental DNA analysis, through clone libraries but, mainly, next generation sequencing (NGS) platforms (**Smith, Shaw, Le Bescot, Edvardsen, Ruggiero, Karlson, Taylor**). Only one such study, on *Azadinium*, was presented during the previous ICHA 15. Clearly, this is a very promising and increasingly applied approach, which offers both adequate resolution for biodiversity assessment and a new tool for ecological studies of species otherwise difficult to trace in the environment. Some concern arises from the methodological differences among the studies, including the marker sequence chosen and the instrumentation used, mostly driven by the rapid technological evolution of NGS platforms. Definitely there is a need for the harmonization and intercalibration of NGS methods in order to ensure full comparability of results obtained at different places.

Moving towards functional aspects of genetic diversity, many research groups are now studying the transcriptome of harmful species (**Jones, Ki, Dong, Di Dato, Guo**), often with a comparative approach (**Campbell**). Many of these studies are taking advantage of the transcriptomics data provided by the Moore Foundation through the (Marine Microbes Transcriptome Project (MMTP). The whole genome of *Pseudo-nitzschia* species is also being investigated, while the possibility to genetically modify these species offers new models for functional studies in diatoms (**Ferrante**). Genome and transcriptome analysis in toxin producing species may help identifying genes responsible for toxin production (**Kohli**), as well as functional and niche differences among species (**Coyne, Gobler, Jason-Smith, Jenke-Kodama, Kim)**. Certainly at the next conference these recently begun studies will be developed further.

1. *Mann DG & KM Evans 2008. In: Moestrup O. et al (Eds) Proceedings of 12th International Conference on Harmful Algae. ISSHA and IOC of UNESCO, Copenhagen, pp. 262-68.*
2. *Zingone A 2006. Harmful Algae News 32:1-3*

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HABs Biology, Life Cycles and Parasites

The role and contribution of sexuality to the life cycles of HAB species remains unclear, but there is no doubt about their relevance in understanding algal population structures, bloom dynamics and recurrence, and their susceptibility to control by parasite infections. However, step-by-step, we are reaching a better understanding about their real contributions to HAB species life cycles, and the number of species for which sexual resting cysts are described is increasing. At this congress we learnt more about the factors contributing to sexual induction and meiosis in *Pseudo-nitzschia* (**Montresor**). Additionally, the resting cysts and germination patterns of *Akashiwo sanguinea* were described for the first time (**Tang**), and modifications to previous life cycle models of *Alexandrium* species (from haplontic to biphasic) were proposed (**Figueroa**).

Most of the diversity in parasites infecting HAB species has yet to be explored. Three new parasite species infecting *Alexandrium tamarense* have been discovered (**Yamaguchi**), and *Amoebophyra* has been found infecting at least eight dinoflagellate species (including *Alexandrium* sp., *Gonyaulax spinifera* and *Prorocentrum minimum*) from Chinese coastal waters (**Li**). Parasites have been suggested as a control factor in HAB population dynamics. However, laboratory experiments with the parasite *Parvilucifera* cannot accurately reproduce host-parasite interactions in the field. This parasite reaches high infection rates in laboratory cultures but seems to have a lower potential to control dinoflagellate populations in the field. Dimethylsulphide (DMS) appears to induce parasite activation, working as a density-dependent cue to the presence of potential hosts (**Alacid**). Therefore, the role of HAB parasites in the real world will probably continue to be a debated topic in future HAB conferences.

Given the huge genome of many HAB species, genomic insights into the biology of these species are being achieved through transcriptomics. During the two sessions devoted to transcriptomis we learnt about "Eco-transcriptomics" as a new tool to deepen our understating of the molecular biology of harm-



Ostreopsis ovata *mats on a macroalgal turf dominated by* Jania *spp. (Cala San Basilio, Gulf of Naples, Italy). Winner of the ICHA16 photo competition (Photo D. di Cioccio, Stazione Zoologica di Napoli).*

ful algae, and in particular the factors regulating toxin production (**Gobler**). Transcriptome analyses are shedding light also on phytoplankton responses to environmental conditions (**JS Ki, Wohlrab, Jin)**, community succession (**Coyne**), competition strategies (**HY Li**), and toxin biosynthesis pathways in relevant dinoflagellate HAB groups (**Campbell**, **Kohli**, **GJ Smith**). Furthermore, we are finally approaching the development of specific mitigation strategies, because increased understanding of gene regulation is allowing us to target key specific pathways in algal proliferation.

Regarding resting cysts, the role and regulation of endogenous clocks controlling their germination is still under intense research; new data support the view that they are a key to the life strategy and environmental adaptation of some *Alexandrium* species (**Fischer, Greengrove**). Resting cyst diversity reveal a lot about species biogeography. An unexpected high diversity of cysts, not previously reported in plankton sampling, was found at some Mediterranean sites (**Satta**).

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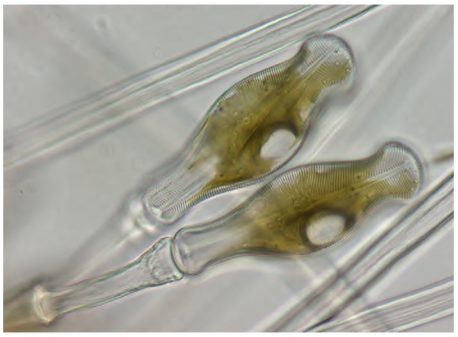
Freshwater HABs

A session was held on freshwater HAB Biology and Ecology. This included two plenary talks by Prof Hans **Paerl** (University of North Carolina, USA) and Michele **Burford** (Griffith University, Australia). Paerl talked about the relative importance of controlling nitrogen versus phosphorus in systems dominated by cyanobacteria. He gave examples of studies demonstrating that both nitrogen and phosphorus promote cyanobacteria, and hence management of both is required. Burford examined nutrient utilisation by a toxic cyanobacterial species, *Cylindrospermopsis raciborskii*, and demonstrated the need to incorporate ecological, physiological and molecular findings on nutrient responses by the species and individual strains into algal growth models. There followed a series of interesting talks on *Microcystis* ecological studies in a New Zealand lake with a focus on fine scale variability in toxin production, and the role of strain dominance (**Hamilton**), *Microcystis* photosynthesis in a surface bloom (**Puddick**), and response of *Microcystis* in mesocosms and modelling approaches (**Borges**). Interestingly, there was a report of accumulation of freshwater algal toxins in marine mussels on the west coast of USA (**Preece**).



Didymosphenia geminata *cell (~ 250 µm) and stalk (Photo C. Kilroy)*



Didymosphenia geminata *mat cut open to show the mass of stalk material cells at the surface. The mat is about 5 cm thick (Photo C. Kilroy)*

Later in the week there was also a session on freshwater benthic HABs. Dr Cathy **Kilroy** (NIWA, New Zealand) in her plenary talk summarised the history of research on the freshwater invasive diatom species, *Didymosphenia geminata* in New Zealand. She illustrated the site of its probable introduction and subsequent spread throughout the South Island of NZ. This work has also lead to a greater recognition of the world-wide invasion of this diatom. Surprisingly, this species appears to prefer low dissolved phosphorus concentrations. A session followed with a focus on *Didymosphenia* and the benthic cyanobacterial species, *Phormidium.* Both species have major impacts on New Zealand river systems. Current research is focusing on understanding the ecology and habitat of these species to assist in bloom prediction and toxin production.

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Ciguatera

Ciguatera Fish Poisoning (CFP), caused by ciguatoxins produced by *Gambierdiscus* spp. and bioaccumulated in reef fishes is the most common phycotoxin-related illnesses. Globally, CFP affects the greatest number of victims and often with significant long-term health effects. Although traditionally considered as a tropical sickness, CFP is also being recently reported on subtropical

waters and the Mediterranean Sea, and new species of *Gambierdiscus* have been identified in these areas.

During the 16th ICHA, Ciguatera at last occupied the place it deserved. **Rob Magnien**, Chair of the IOC Intergovernmental Panel on HABs (IPHAB) presented a Global HAB Strategy supporting the science and management of ciguatera fish poisoning. The World Health Organization (WHO) will be contacted to designate CFP a "neglected tropical disease".

In her plenary session, **Pat Tester** reviewed the state of knowledge on taxonomy, genetics and biogeography of the increasing list of CFP species. Originally thought of as a monotypic genus (*G. toxicus*) there are now at least 13 species described (*G. silvae* and *G. scabrosus* in 2014), and based on DNA sequence data, two of the previous species (*G. yasumotoi* and *G. ruetzleri*) have been re-adscribed to the new genus *Fukuyoa*. New results on the light physiology of *Gambierdiscus* were presented (**Adachi**) and a new FISH assay has been developed which allows identification of four *Gambierdiscus* species (**Pitz**). *Gambierdiscus* substrate attachment dynamics on altered reef ecosystems was described (**Parsons**); artificial substrates were recommended as standard monitoring protocol for benthic HABs (**Kibler**). The long term exposure of groupers to CTX added useful information to the effects of *Gambierdiscus*/CTXs (**Mak**) as did the first widespread study of the CTX toxicity of lionfish in the Caribbean (**Litaker**). An overview of both *Gambierdiscus* and *Ostreopsis* from the Cook Islands and the risk of CFP from New Zealand sea food provided a management perspective (**Rhodes**).

There have been considerable advances in more and more sensitive LC-MS technologies and considerable efforts devoted to obtain toxin standards to be distributed worldwide. Further, new vectors of CTXs have been identified (see section on Toxins).

A French Polynesian outbreak of CFP occurred in 2010 in Rapa, a remote area previously known to be CFP free (**Chinain**). There were two fatalities in this small community but comprehensive science and education outreach provided information on what had happened. The residents of Rapa have been provided some safer fishing alternatives and their awareness of CFP is now quite high. Toxins other than CTX were considered in this study but 77% of the 250 fish samples tested positive for CTX. Epidemiological data from 2011-2012 indicate altered fishing practices has helped reduce the risk of CFP. A quite innovative and badly needed self-reporting website for CFP victims was developed so data can be shared across the vast French Polynesian archipelagos (**Gatti**).

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HABs in a Changing World

This topic, which first received considerable attention during ICHA 14 in Crete, continued ticking over in New Zealand; the slow pace of progress undoubtedly a result of coming to grips with the daunting scale of the problem. Attempts are continuing to link HAB time series to e.g. ENSO events (**Guzmán**), or use palaeoecological records to resolve species shifts in Chinese coastal waters (**Lu**). Potential range expansion of *Alexandrium* into the Arctic (**Cembella**) was raised, as well as Australian dust storms adding mycotoxins to seafood security problems (**Hallegraeff**). Genetic diversity and phenotypic variability provide physiological plasticity to different Baltic populations of *Alexandrium ostenfeldii* and suggest they should be able to adapt to changing climate conditions (**Kemp**). Ciguatera featured prominently at this conference, partly because of increasing concerns that this syndrome will be a winner of climate change (**Tester**). Multi-stressor laboratory experiments with the fish-killer *Heterosigma akashiwo* were tentatively extrapolated to future climate scenarios (**Matheson**). Increasing attention was also focused on threats to inland water quality from *Prymnesium* (**Brooks**) and cyanobacteria (**Pearl**), where climate change tends to render the synergy between anthropogenic nutrient loading and hydrological regimes more favorable to the development of HABs.

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Ichthyotoxic HABs

Fish-killing HABs, identified as a priority issue during the last IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB-XI, April 2013), may cause much more economic damage than HABs producing shellfish toxins but remain poorly studied and understood. The 16th ICHA devoted two sessions to different fish-killing genera: *Heterosigma* **(Trainer, Ikeda, Ueki),** *Karlodinium* **(Cosgrove**), *Prymnesium* **(Andersen)**, *Karenia* **(Sakamoto)**, *Alexandrium* **(Mardones**), *Chattonella/ Pseudochattonella* **(Haigh).** The increased use by different research groups (US, Australia, Europe) of plate-reader based assays using comparable fish-gill cell lines has allowed important progress (**Dorantes-Aranda**), but the full calibration of these results against adult fish bioassays has not yet been conducted. For some fish-killers (e.g. *Heterosigma*, *Karlodinium*) lysis is a key factor. The precise nature and relative importance of the active molecules involved (ion-channel activators, prymnesins, PST, KTX, ROS) remains disputed.

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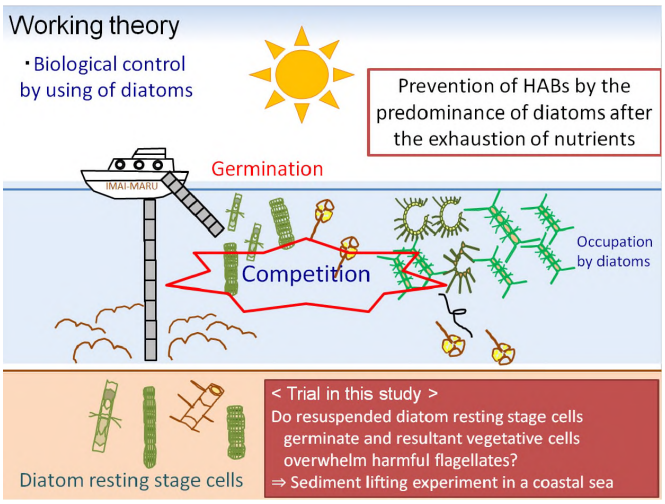
HABs Mitigation

Ultimate HAB control remains reducing nutrient loads into watersheds but mandated load reductions remain impossible to implement so band-aid temporary fixes (mitigation) remain as the research and management foci.

Traditionally, phosphorus (P) input reductions were prescribed to control CyanoHABs. This is because P limitation is widespread and some CyanoHABs can fix atmospheric nitrogen (N_2), thereby satisfying their nitrogen (N) requirements. However, eutrophying systems are increasingly plagued with non-N_2 fixing CyanoHABs that are N and P co-limited or even N limited. In many of these systems N loads are increasing faster than P loads. Therefore N and P input constraints are likely needed for long-term CyanoHAB control (**Paerl, Sellner**).

There is continued exploration of clay application techniques for removing toxins (**Seger**) and taxa (**Jung, Rivera**) from mariculture and natural ar-



Scheme of mitigation practice (Photo I. Imai)

eas experiencing blooms. Lytic bacteria (**Moon**), terrestrial plant extracts (**He**), and facilitated aerobic-anaerobic operational (**Lee**) approaches continue at small scales and in controlled settings.

'Lifting' and dispersing bottom sediment-associated diatom assemblages into surface waters is a recent attempt to enhance euphotic zone diatom growth instead of motile HABs that routinely dominate stratified surface waters in mariculture-rich areas of Japan (**Imai,** attached figure). In a similar approach, deployment of structures in euphotic depths that facilitate periphyton growth (**Ahn**) is an attempt to reduce ambient nutrient pools and HAB prevalence.

Freshwater HABs control seems realistic in systems where increased rates of hydraulic flushing are possible and low-cost barley straw can be deployed early in the HAB growing season (**Japareng, Sellner**).

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HABs Modelling and Forecast

A major goal is to achieve operational forecast models. In some areas this is beginning to get closer to reality. A near operational model for *Pseudo-nitzschia* spp and associated toxicity in California was presented (**Kudela**). This model uses statistical approaches that get round the need for difficult to measure driving variables (nutrients etc). Illustrations of it can be seen at *http://www.cencoos.org/sections/conditions/blooms/habforecast/*. A model-based forecasting system for *A.catenella* in New Zealand waters is being developed (**Knight**). It draws on the work already carried out for the Gulf of Maine, USA, but also employs an unstructured grid-based physical model that allows detailed resolution of the fjord of interest, similar to an approach, presented during 15th ICHA (**Aleynik**) for the Scottish fjords. Given that much aquaculture occurs in fjords or other sheltered embayments, such fine resolution modeling approaches are likely to become increasingly important.

An innovative approach was the use of remotely piloted aircraft "drones" to help with the survey of freshwater benthic microalgae (**Milne**). This approach, which requires significant development, is taking off in many areas of ecology and looks to have particular application for high biomass blooms in freshwater, but may also help to overcome the issues with cloud cover related to satellites when one wants to observe in marine waters.

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HAB Technologies

In the last decade, powerful new technological developments in optical instruments combined with species-specific probes have altered the way HABs can be monitored and opened new avenues for experimental research [1]. Likewise, the recent discovery of nuclear encoded genes for saxitoxins [2,3] now makes it possible to target the specific genes (e.g. *stxA*) controlling PSP toxin production in dinoflagellates and cyanobacteria. During 16th ICHA there were four full sessions devoted to "HAB Technologies" where attendants could learn the latest exciting developments on: i) new instruments and sensors and ii) new methods for cell and toxin detection.

Progress on the development of universal microarrays and other products to detect HAB cells and their toxins (from previous EU projects MIDTAL and *μ*aqua) is leading to high-density microelectrode arrays (MEA) that can detect up to 100 targets. This development is ultimately intended for use on *in situ* buoy sensor systems (**Medlin**). A qPCR test that simultaneously identifies and quantifies the presence of cyanobacterial 16srRNA along with three genes responsible for four different toxins, (microcystin/nodularin (mcyE), clylindrospermosin (cyrA), saxitoxin (sxtA4)), was presented (**Van Asten**). The *Phytoxigene CyanoDTec* test can be completed within 3 hours and represents a new tool for monitoring of toxin producing species and their toxins. Other novel methods for cell and toxin detection were based on preshaped sensor elements with a natural affinity for algal biotoxins (**Richter**). These were isolated from tunicates and are termed xenobiotic-activated nuclear receptors, XANRs.

Within the world of imaging particle analysis system, FlowCAM is now introducing its next generation FlowCAM *SV*, with new classification software, biovolume estimation, and expanded particle properties (**Nelson**). A 'Cyano'CAM for freshwater plankton analysis is coming soon. The FlowCAM was also used with Fluorescent *in-situ* Hybridization (FISH) and species-specific probes to quantify *Alexandrium* abundance in plankton samples (**Sirois**).

A new solar-powered, multi-sensor platform for remote deployments of the



Photo D.M. Anderson



Scandinavian ladies of the Prodiversa network (NordForsk 2011-2014) having an opportunistic meeting. (Photo D.M. Anderson)



View of a poster session, Fowler Center, Wellington.

Imaging FlowCytoBot (IFCB) and other instruments was presented that allows high-frequency, high-resolution detection of fusing gametes, planozygotes, and other life cycle transitions during an *Alexandrium* bloom (**DM Anderson**). The IFCB has been used for a successful early warning of *Karenia* blooms weeks earlier than visible signs (water discoloration and dead fish) appeared (**Campbell**); it is also used to characterize the phytoplankton community composition (*Karenia* is not always dominant during bloom conditions)(**Anglés**).

The Environmental Sample Processor (ESP) is now being used in multiple HAB research programs. In the US Pacific northwest, five ESP deployments totaling ~100 days provided advanced warning of HABs in the inland coastal waters of Puget Sound to support proactive fisheries management efforts (**Moore**). Deployment sites included a tribal shellfish and finfish hatchery and a commercial shellfish farm. The ESPs were configured to sample daily and detect *Heterosigma akashiwo*, *Alexandrium* spp., and *Pseudo-nitzschia* spp. along with bacterial targets and microbial source-tracking indicators. In another application of the ESP technology, excellent agreement was found between estimates of shellfish toxicity based on ESP cell count measurements and flesh testing results at downstream shellfish monitoring stations (**DM Anderson**). This instrument has also proven to be a good research tool for resolving HAB bloom dynamics in complex systems when used in conjunction with AUVs and other observing assets (**Marin**). A smaller, 3rd generation ESP is now under development that can be deployed in a small AUV. Other advanced technologies to test just the toxins are discussed in the Toxins section.

Anderson DM 2014. In: Harmful Algae 2012, Kim HG et al (eds), ISSHA 2014, pp. 3-17 (available on line at www.issha.org, Conferences)
*Stüken A et al 2011 PLoS One **2011**, 6, e20096*
Hacket et al 2013 Mol Biol Evol 30: 70–78

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TOXINS

New Toxins: Improved performance of liquid chromatography-coupled to mass spectrometry equipments (LC-MS/MS), now replacing mouse bioassays in many countries, is leading to increased detection of different toxins in new parts of the world ("the more you look the more you find"). Azaspiracids (AZAs) have now been detected in New Zealand, Brazil and even in Greenland. Pinnatoxins (PnTX-G), presumably from *Vulcanodinium* spp., was found in kelp (*Saccharina latissima*) from Norway (**de la Iglesia**). Two new toxin groups, brevisulcenals (KBTs) and brevisulcatatoxins (BSXs), isolated from *Karenia brevisulcata* were described (**Satake**). In addition, new analogues of already known toxins were reported. These included new analogues of AZAs, DTXs, six new spirolides from *Alexandrium ostendfeldii* and five karlotoxins from *Karlodinium veneficum* (**Krock, Place**); of ovatoxins: OvTX-g and a new PLTX analogue from *Ostreopsis* cf *ovata* (**García-Altares**) and OvTX-h (**Hess**); of yessotoxins: 22-OH-YTX from *Gonyaulax spinifera* (**Chikwililwa**), and gymnodimine analogues from *A. ostenfeldii* (**Harju**).

New vectors: Tetradotoxins (TTXs) were found in slugs (Wood), and ciguatoxins (CTXs) in sharks (**Arnich**), giant clams (**Roue**) and lionfish (**Litaker**).

Detection methods, structure elucidation and reference materials.

Highly sensitive ultraperformance liquid chromatography coupled to mass spectrometry (UHPLC-MS/MS) methods are being increasingly used for toxin detection, e.g. for CTXs/MTXs (**Selwood**), and HILIC (hydrophilic interaction) UPLC-MS/MS for STXs (**Boundy**). UPLC with fluorimetric detection (FLD) has also been used for STXs (**Watanabe**). LC-MS/MS methods have been optimized for analyses of CTXs/MTXs related to Caribbean/Pacific *Gambierdiscus* species (**Lewis**), and a turn-key multitoxin method for marine cyanobacterial toxins is in development (**Quilliam/McCarron**). Passive sampling (SPATT) has been widely used (**Dumalan**, **Hess**, **Roué**, **Frangopoulos**, **Chinain**, **Li**, **Qu**, **Lian**), not only for lipophilic toxins but also for hydrophilic toxins, including STXs. Mass spectrometry applications are more and more used for structure elucidation: LC-TOFMS (**Yasumoto**) and (LC-HRMSn) (**Dell'Aversano**) for palytoxin and ovatoxin congeners from Japanese and Mediterranean *Ostreopsis* spp. Semi-synthesis of acyl-esters of AZA1, PTX2 and cyclic imines was achieved (**de la Iglesia**). Increased efforts are being invested at the Japan Fisheries Research Laboratories (**Suzuki**) and Japan Food

Research Laboratories (**Kato** for CTX) in addition to those at the Canadian NRC (**Quilliam**, **McCarron**) to isolate, purify and certify reference materials for toxin analyses.

Effects in human health

Epidemiology of *O. ovata* blooms showed that impacts in humans coincided with the transition from exponential to stationary growth phase (**Berdalet**). Following experiments with *Artemia salina* nauplii and with ephyrae of *Aurelia aurita*, an innovative model organism, suspicions were raised that the toxins are actually delivered with the mucus (**Giussani**) but the relationship between the blooms and the aerosol/contact problems are still elusive. Intestinal absorption and toxic effects of PnTXs from *Vulcanodinium rugosum*, and their cellular nicotinic acetylcholine receptors (nAChR) were studied in depth (**Fessard**, **Molgó**, **Hellyer**). The effects of brevetoxins (BTX) in mammals have been compared with those in freshwater turtles used as a model (**Cocilova**). Other studies showed that domoic acid (DA) causes learning impediments and hyperactivity in mice at sub-acute dose levels (**Lefebvre**). Cytotoxic assays with toxin mixtures showed a potential synergy between AZA1 and YTX (**Ferron**). New toxicity equivalence factors (TEFs) have been established for some STX-analogues, e.g. NEO=2.54 x STX (**Munday**). The revision of the risk assessment of saxitoxins and other toxins groups will have an impact on the revision of regulatory levels.

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Preparations for the 17th International Conference on Harmful Algae (ICHA) have started

Following the successful meeting at Wellington, NZ, preparations for the 17th edition of ICHA, to be held in Florianópolis, the beautiful island and capital of Santa Catarina State, South Brazil, have started. Local organization of the conference will be carried out by the Latin American Association for Studies on Harmful Algae (ALEAN) and by the Federal Institute of Santa Catarina (IFSC). The 17th ICHA will be held from 9 to15 October 2016 at the *CentroSul* convention center. The FANSA regional committee is formed by colleagues from Brazil, Uruguay, Argentina, Chile, Peru and Ecuador and is now discussing major issues to bring to the conference.

The logo of the conference uses strong but friendly colours, inspired by the South-American region. The logo also recalls the astonishing dinoflagellate illustrations by Ernst Haeckel (1834-1919). The detailed cell wall, ornamented with pores, where information flows connecting the inside to the outside, resembles a network-shaped world of information and knowledge.

Registration for the event will be opened at the ICHA Brazil 2016 web site (to be launched). See you at ICHA Brazil 2016!

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ICHA
The 17th International Conference on Harmful Algae
Brazil 2016

The 18 ICHA logo (Photo L. Proença)



The ISSHA flag has arrived safely to Itajaí, its home for the next 2 years. It will be proudly guarded by young brave and hard working scientists until next ICHA in Florianópolis 2016 (Photo L. Proença)

IFREMER will host the 18th International Conference on Harmful Algae (ICHA) in Nantes, France

After submitting an application to the International Society for the Study of Harmful Algae (ISSHA) in July 2014, a 17-member strong French delegation led by Philipp Hess (IFREMER, Nantes) presented the French bid at the last ICHA conference in October 2014 in Wellington, New Zealand. Votes have confirmed Nantes (France) as the venue for the 18th edition of this conference in 2018.

The French initiative is supported by scientists from over 20 scientific institutions, including IFREMER, the French research institute on marine topics. The proposal arose from the recently created French research network *GdR PHYCOTOX* (*http://www.phycotox.fr*), coordinated by Hélène Hegaret (CNRS, LEMAR, IUEM, Brest) and Philipp Hess (IFREMER and IUML, Nantes). The City of Nantes and the Region Pays de la Loire have already committed to lend support to the initiative, as has the French Ministry for Food Agriculture and Forestry. The 25-strong scientific committee will draw its members from a wide range of research institutes and universities representing the two main scientific communities around harmful marine algae and freshwater cyanobacteria. Allan Cembella, from the competing-bid institution (Alfred Wegener



The French delegation celebrates winning the bid to host the 18 ICHA in Nantes, France, 2018 (Photo J. Camp)

IFREMER: Institut Français de Recherche pour l'Exploitation de la Mer/ French Research Institute for Exploitation of the Sea ;
CNRS: Centre National de la Recherche Scientifique/National Center for Scientific Research
LEMAR: Laboratoire des Sciences et de l'Environnement Marin/Marine Environmental Science Laboratory
IUEM: Institut Universitaire Européen de la Mer/European Institute for Marine Studies
IUML: Institut Universitaire Mer et Littoral

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Institute, Germany) will be an honorary member of this committee. The local organizing committee will include IFREMER staff and representatives from local stakeholders such as the University of Nantes and the industrial cluster organization *Atlanpole Blue Cluster* (Pôle Mer Bretagne-Atlantique).

Workshop on quantitative PCR techniques for harmful algae

In association with the 16th International Conference on Harmful Algae, a two day workshop on qPCR techniques for harmful algae was held at the Cawthron Institute in Nelson, New Zealand (23-24 October). It was attended by 22 people from Australia, the United States, Germany, the Netherlands and New Zealand. The workshop combined presentations with demonstrations of qPCR assays for *Gambierdiscus* species and toxin genes in the new molecular facility at the Cawthron Institute. Topics discussed included target genes, advantages/disadvantages of detection systems, generation of standard curves, DNA extraction methods and sample storage issues. Workshop presenters were Wayne Litaker (NOAA, USA), Shauna Murray (University of Technology, Sydney), Uwe John (Alfred Wegener Institute, Germany) and Mark van Asten (Diagnostic Technology, Australia).

If you are interested to receive the hand-outs and presentations from the workshop, please contact Kirsty Smith).

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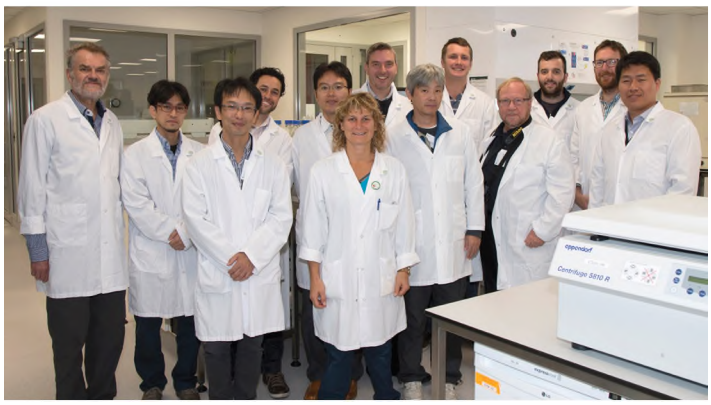
Cawthron visitors post ICHA meeting

In the week after the ICHA conference in Wellington, New Zealand, the Cawthron Institute enjoyed hosting about 20 delegates representing at least 10 countries who chose to extend their New Zealand trip to include a visit to the beautiful South Island. The Cawthron Institute is situated in Nelson, in the north of the South Island. It was a busy couple of days with visits to the various Cawthron laboratories, including the new Envirotech complex, an excursion to the Cawthron Aquaculture Park, a barbeque and various collaborative meetings. Many also enjoyed the nearby Abel Tasman National Park and the rugged Kaikoura Coast.

Tim Harwood and Lesley Rhodes are co-leaders of the government-funded Safe New Zealand Seafood programme.
Email: Lesley.Rhodes@cawthron.org.nz



Visitors to the new molecular laboratories at Cawthron Institute, Nelson, New Zealand.

Eutrophication and Harmful Macroalgal Blooms in Florida's Indian River Lagoon

The Indian River Lagoon (IRL) along Florida's east-central coast is a shallow (~ 0.8 m) and narrow (~3 km) bar-built estuary extending 248 km between New Smyrna Beach and Jupiter, Florida. As a result of its overlapping position along the temperate to subtropical ecotone, the IRL is regarded as one of the most species diverse estuaries in North America, and includes 7 species of seagrasses and >225 species of benthic macroalgae. Because of the limited number of oceanic inlets and the addition of several man-made causeways, tidal influence is restricted to several kilometers around each inlet and residence time can be high within the inner basins of the IRL. The significant freshwater and nutrient loads associated with urban and agricultural land uses, combined with restricted flushing and dilution, make the IRL highly susceptible to eutrophication [1].

Historically, macroalgae were not an important component of the flora in the IRL. However, with increasing nutrient availability in the water column, the IRL now supports extensive blooms of unattached, drift macroalgal communities (Fig. 1) and the attached rhizomatous alga *Caulerpa prolifera* (Fig. 2). Unlike toxic phytoplankton blooms, macroalgal blooms generally lack direct chemical toxicity but typically have a broader range of ecological impacts including: i) oxygen depletion; ii) loss of habitat and biodiversity; iii) alteration of biogeochemical cycles and iv) die-off of important keystone species, such as seagrasses and corals. Increasingly, macroalgal blooms foul beaches and shorelines important to local tourist economies and require expensive biomass removal programs.

Blooms of red macroalgae (Rhodophyta) have been especially prevalent in the IRL in recent decades. In comparison to seagrass light requirements, experimental studies of the growth rate-irradiance relationship of an abundant red drift macroalga in the IRL – *Gracilaria tikvahiae* – showed that when growing at the seagrass compensation irradiance of 0.2 I_0 (I_0 = full natural irradiance) under nutrient-replete conditions, this alga can grow at 0.1 doublings·d^{-1}, corresponding to a doubling of biomass in 10 days; when growing at I_0 such as in shallow waters, this macroalga can grow at 0.37 doublings·d^{-1}, or a doubling of biomass in only 2.7 days [2]. Increased nitrogen availability and reduced irradiance lead to elevated chlorophyll *a* and phycobiliprotein pigments in *G. tikvahiae*, increasing photosynthetic efficiency under low irradiance. As a result, *G. tikvahiae* is capable of high productivity under the increasingly eutrophic environmental conditions in the IRL where light attenuation (K) is relatively high and where seagrass growth is limited or absent.

To examine the role of nutrient enrichment in eutrophication and HABs in the IRL, we sampled a network of 20 fixed stations throughout the entire IRL to document spatial and temporal variability in nutrients, chlorophyll *a*, and seagrass/macroalgae communities during 2011/2012. Following a prolonged drought between 2006 and 2010 and heavy rainfall between 2011 and 2013, unprecedented phytoplankton blooms developed in the northern IRL in the beginning of our study. A "super bloom" (the green alga *Resultor* and small cyanobacteria; ~ 100 µg/l) developed in summer 2011 in the Banana River [3], which was followed by brown tides (*Aureoumbra lagunensis*, >200 µg/l) in the Mosquito Lagoon and northern IRL in summers of 2012 and 2013 [4]. Total dissolved nitrogen (TDN) in both the wet and dry seasons increased from the southern to the northern IRL, with mean concentrations exceeding 70 µM in the northern IRL segments where the phytoplankton blooms developed. This high TDN concentration largely reflects dissolved organic nitrogen (DON), which could support the emerging brown tides in Mosquito Lagoon, as similar brown tides in northeastern and mid-Atlantic U.S. estuaries are known to utilize DON. The nitrogen build-up in the northern IRL was also reflected in a high TDN:TDP ratio, which increased from ~28 in the southern segments to >59 in the northern segments, suggesting shifts towards P-limitation in

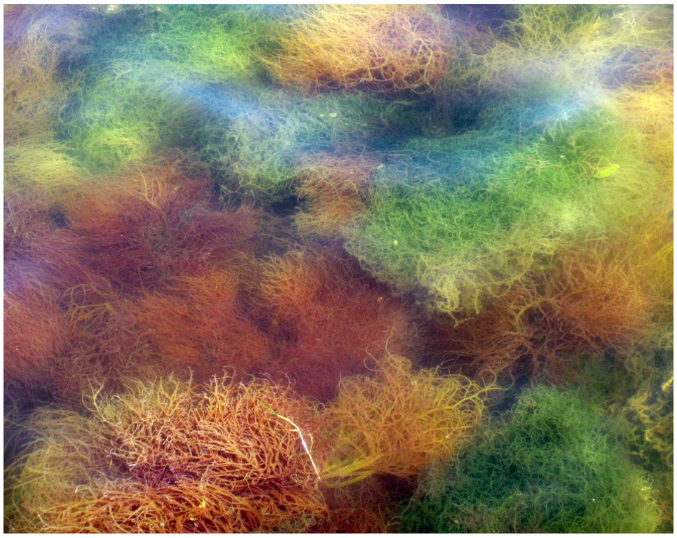


Fig. 1. Blooms of drift macroalgae in the Indian River Lagoon, including the rhodophytes Hydropuntia secunda *and* Gracilaria tikvahiae, *and the chlorophyte* Chaetomorpha linum. *Photo courtesy of St. Johns River Water Management District.*



Fig. 2. Bloom of the attached, rhizomatous chlorophyte Caulerpa prolifera. *Photo courtesy of St. Johns River Water Management District.*

the northern IRL segments. This could be ecologically significant, as the brown tide organism *Aureoumbra lagunensis* outcompetes other phytoplankton under P-limited conditions [5].

The dense phytoplankton blooms in 2011 and 2012 caused extensive seagrass loss (~ 60%) and coincided with unusual mortality events (UME) involving manatees, dolphins, and pelicans [6]. Necropsies of dead manatees showed evidence of abundant macroalgae, including *Gracilaria* spp., in their stomachs, along with severe intestinal irritation and bleeding, suggesting an apparent "toxic shock syndrome." This raised concerns about possible intoxication of the manatees following the HAB events. During the period of unusually high manatee mortalities in the northern IRL in spring and summer 2013, we sampled *Gracilaria tikvahiae* and associated drift macroalgal communities, which became a primary food source for manatees following seagrass die-off, at a manatee mortality "hot spot" at Shorty's Pocket in the Banana River in May and July, 2013. The mixed macroalgal communities were dominated by red drift algal species including *G. tikvahiae*, *Acanthophora spicifera*, *Hypnea musciformis* and *Hydropuntia secunda*, but also included conspicuous blooms of the green alga *Chaetomorpha linum* (Fig. 1) as well as cyanobacteria mats (Fig. 3).

Although these drift macroalgae communities in the IRL are generally not considered toxic, human intoxication due to consumption of *Gracilaria edulis* (now *Polycavernosa tsudai*) did affect 13 people who ingested the raw seaweed in Guam, 3 of whom died [7]. A novel glycosidic macrolide, polycavernoside A, was isolated from the *G. edulis* and was considered responsible for the poisoning [8]. A sewage outfall at 18 m depth to the north of the reef flat where the *G. edulis* was collected, was considered a possible factor by the Guam Environmental Protection Agency; to date, however, no evidence exists that sewage pollution was a direct cause of the seaweed toxicity in Guam.

In the IRL, however, stable nitrogen isotope values ($\delta^{15}N$) of macroalgae tissue sampled during our 2011/2012 studies averaged +6.3 $^{o}/_{oo}$, a highly enriched value similar to that reported for macroalgal blooms in a variety of sewage polluted estuaries and coastal waters [9]. With the exception of periodic wet weather discharges, all wastewater treatment plants in the IRL watershed are presently in compliance with current water quality protection codes, rules, and statues [10]. Despite the elimination of point-source sewage inputs (i.e. outfalls) to the IRL through the IRL Act of 1990, non-point source sewage pollution from septic tanks (on-site sewage treatment and disposal systems, OSTDS) has continued to expand and remains a serious environmental and human health concern [11]. Federal and state agencies have long recognized the harmful impacts of OSTDS to groundwaters and surface waters in Florida, including the IRL [10, 12]. Currently, there are ~300,000 OSTDS installed in the counties adjacent to the IRL. Contamination of groundwaters with nutrients derived from non-sewered human wastewater has long been known to contribute to eutrophication of lakes, estuaries, and coastal waters [13, 14] and could be an important pathway for nutrient pollution of the IRL. Such groundwater-borne nutrient inputs would negatively impact the seagrass communities and biodiversity of the IRL by fueling both macroalgae and phytoplankton [4, 11, 15] HABs.

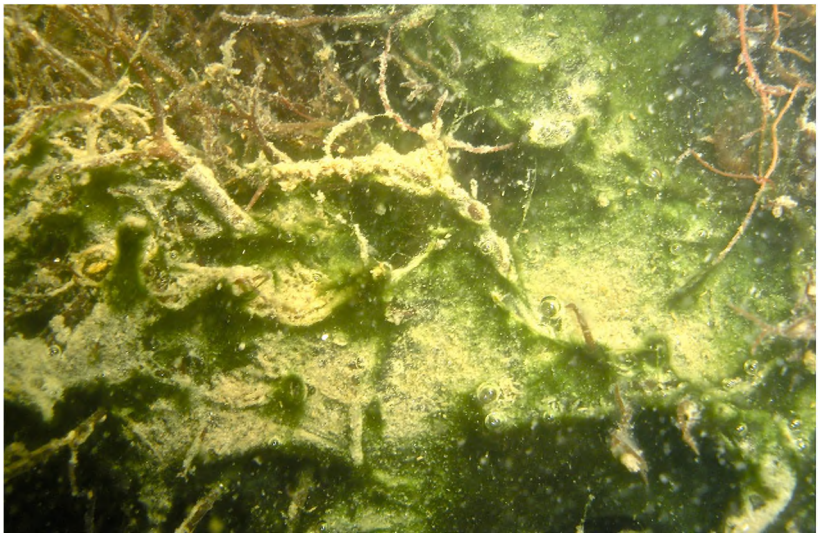


Fig. 3. Bloom of the rhodophyte Gracilaria tikvahiae *with epiphytic cyanobacteria at Shorty's Pocket, Banana River, Florida.*

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Conspicuous blooms of *Chaetomorpha linum* in the International Biosphere Reserve Seaflower, SW Caribbean

Blooms of ephemeral macroalgae are a well-known consequence of nutrient enrichment and eutrophication in coastal waters, especially in oligotrophic tropical and subtropical waters, where their frequency has increased over the last decades [1, 2]. These blooms may have detrimental effects on seagrass and coral reefs, overgrowing coral colonies and seagrass beds, inhibiting the recruitment of juvenile corals, reducing light availability and leading to hypoxia [1, 3, 4].

These ephemeral blooms usually involve one or more species of early successional, opportunistic green algae, from the genera *Ulva*, *Chaetomorpha* or *Cladophora* [2]. In the Caribbean, recurrent blooms of the green algal species *Chaetomorpha linum* have been reported in Jamaica, and on seagrass beds in the Florida Keys [1]. Generally, these blooms have been attributed to nitrogen enrichment of the coastal waters [1, 3, 5].

San Andres island is part of the International Biosphere Reserve *Seaflower* in the Southwestern Caribbean, and, with a resident population of 70069 habitants [6], equivalent to 2803 hab/km^2, it is one of the most populated islands in the Caribbean [7]. Furthermore, tourist affluence is high and constant throughout the year, increasing the anthropogenic pressure on the nearshore coastal ecosystems. From February to May 2013, and again from September to October of the same year, San Andres experienced unusual blooms of *Chaetomorpha linum* (Fig. 1), which smothered seagrass beds (Fig. 2) before being washed ashore (Fig. 3).

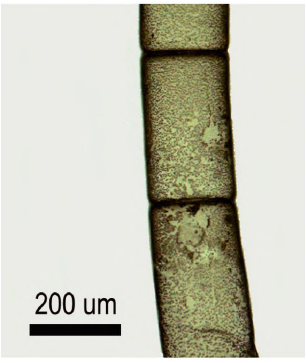

Fig. 1. Filament of Chaetomorpha linum. *Scale bar: 200 mm*

The alga formed large mats or "pillows" with sediment trapped within (Fig. 2, arrows). These moved and attached to seagrass for several days, before ending up on the beaches. The sediment-coated mats (Fig. 2) may have a scouring effect on seagrass leaves. The algae were not floating on the surface, instead they were always found tumbling along the substrate, sometimes attaching to seagrass leaves and shoots. This behavior has already been reported for this species in other locations [8]. On the adjacent coral reefs, the presence of the green alga was not observed.

In Jamaica, the first observed macroalgal blooms in shallow coastal waters were mainly due to *Chaetomorpha linum*, which smothered coral reefs and seagrass [9]. Those events have been directly related to an increase of nutrient pollution from raw sewage. According to [10] green tides composed of *Chaetomorpha linum* expanded in areas most influenced by sewage discharge.

Although we did not perform nutrient analysis of seawater, it is probable that the progressive nutrification of the coastal waters of the island, which has occurred for at least the last15 years [11] is responsible for these conspicuous blooms. The wastewater disposal is highly inefficient, and does not include any treatment prior to the sewage discharge at sea [11]. In order to prevent the appearance of these blooms and their negative effects on the nearshore ecosystems in the future improvements to the management of wastewater discharge and reduction of nutrient input on the coastal waters are urgently required.

Acknowledgements

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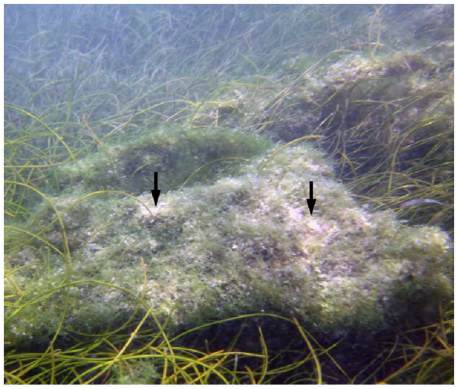

Fig. 2. Mats of Chaetomorpha linum *with sediments (arrows) smothering seagrass leaves.*



Fig. 3. Chaetomorpha linum *washed ashore. Spratt Bight, San Andrés Island, Colombia, May 10, 2013.*

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Unusual biomass of pelagic Sargassum reached the Southwestern Caribbean



Fig. 1. Map showing location of San Andrés Island in the Caribbean Sea (modified from www.worldatlas.com)

Offshore from the coast of Florida, northeast of the Great Caribbean Basin, the Sargasso Sea, an extensive pelagic ecosystem is sustained by two species of holoplanctonic *Sargassum*: *S. fluitans* and *S. natans*. Under the influence of the Trade Winds blowing from a Northeast to Southwest direction in the second part of the year, drift algae coming from the Sargasso Sea are a common site from September to January in the Gulf of Mexico and the Antilles [1]. Periodically, atypical quantities of *Sargassum* have been observed in the Gulf of Mexico, where sightings have been very abundant followed by a decline in the biomass reaching the coast some years[2]. Despite the traditional belief that these floating seaweeds originated from the Sargasso Sea, it was recently demonstrated that the biomass flux is transported in the opposite way, i.e., the algae grows at the beginning of the year in the Gulf of Mexico, and during spring-summer is transported, by the Loop Current and Gulf Stream, to the Sargasso Sea [3].

In 2011, the above mentioned phenomenon reached dimensions never previously reported. Huge quantities of pelagic *Sargassum* were washed ashore along all the eastern coastline of the Caribbean Sea, and crossed the Atlantic to reach the western coast of Africa, from Sierra Leona to Ghana [4, 5] in an unprecedented event. The biomass observed in the Caribbean was estimated to be 200-fold higher than the average seasonal peak and it was the first time drift *Sargassum* reached the coast of Africa in living memory [5]. It was demonstrated that the seaweeds originated in an area north of the estuary of the Amazon River, offshore the coast of Brazil [4, 6]. This region, in the past, had never been associated with *Sargassum* growth, and scientists suggest a link with global warming and perhaps nutrient load from the Amazon River [4]. In 2012, less striking, although atypical, sightings of drifting *Sargassum* have been reported from Cuba [1].

San Andrés Island lies in the Southwestern Caribbean, about 280 km off the coast of Nicaragua (Fig. 1). Its coast is periodically subjected to drifting biomass during winter months, generally from November to January, when Trade Winds are strong and great amounts of drift are driven to the coast. The drift reaching the island is mainly composed of seagrass species (*Thalassia testudinum* and *Syringodium filiforme*), and a diverse community of seaweeds, including a reduced biomass of *Sargassum natans* and *S. fluitans* [7]. Most of the drift annually washed ashore is local in origin, coming from seagrass and coral ecosystems nearby.

During September 17-20 2014, an unusual tide of *Sargassum* (Figs 2, 3), composed by the planktonic species *S. fluitans* and *S. natans*, reached the northeastern coast of the island, covering an estimated surface of 32.520 m^2 [8]. Meanwhile, reports of floating algae reaching the coastline of the Lesser Antilles have been documented between August and September of this year [9]. During the 2011 event, San Andrés was not affected by the anomalous event impacting the Eastern Caribbean.

Although the origin of this 2014 event has yet to be identified, the drift in San Andrés was observed shortly after the passage of hurricane *Edouard* towards the Atlantic coast of United States, and currents deriving from this atmospheric event may have blown seaweeds to San Andrés Island [10].



Fig. 2. Floating Sargassum *along the coast of San Andrés island.*



Fig. 3. Beached Sargassum *on the coast of San Andrés Island.*

Acknowledgements

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First record of the harmful dinoflagellate *Cochlodinium polykrikoides* in Lampung Bay, Indonesia

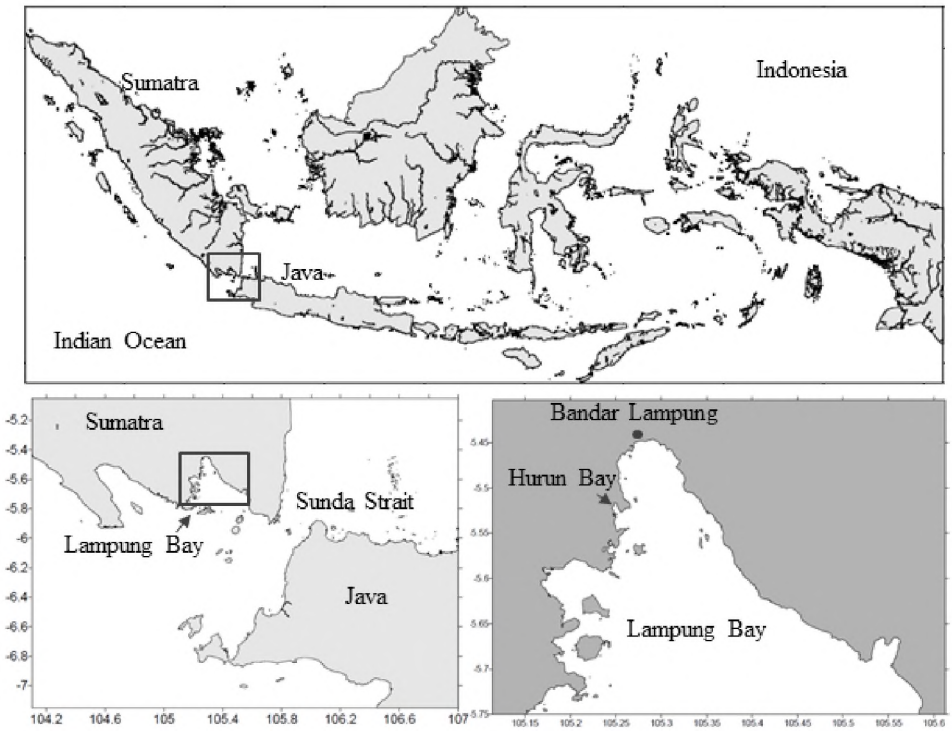

Fig. 1. Location of sampling site at Lampung Bay, southeast Sumatra coast

The Millennium Ecosystem Assessment (2005) [1] pointed out the indisputable impact of invasive alien species (IAS) on island ecosystems. One of the major causes of loss of biodiversity in coastal areas, these species are implicated in changes to the structure and functioning of these particularly sensitive ecosystems. Some harmful algal bloom (HAB) species are considered by the *International Union for Conservation of Nature (*IUCN) to be invasive. Their expansion is indisputably worrisome.

In Asia, where intensive aquaculture endeavors to meet the increasing need for food, thousands of poisonings have been attributed to HABs since the 1980's [2]. Indonesia, with more than 253 million inhabitants, has a vast maritime territory, with nearly 108,000 km of coastline. Although no detailed information is available, it is recognized that the number of HAB events has been increasing in recent years and now affects most Indonesian coastal waters, especially in semi-confined areas [3, 4].

Lampung Bay, located southeast of the island of Sumatra, facing the Sunda Strait (Fig. 1), hosts numerous economic activities (aquaculture, shellfish farming, pearl farming, fishing and harbour activities). Bandar Lampung, the main town in eastern Sumatra, is on the bay which is in a strategic location and is absolutely vital for thousands of people.

In October 2012, the surface coastal waters off Bandar Lampung became dark brown (Fig. 2 a). The phytoplankton bloom progressively spread in the southern part of the bay (Fig. 2 b). In November 2012, mass mortalities occurred in many fish-farms in Lampung Bay resulting in the loss of millions of dollars (Fig. 2 c). This phenomenon continued over the following months. During this phytoplankton bloom, vegetative cell densities reached 7.4 x 10^5 cells L^{-1} in December 2012. After microscopic observations, it appeared that the unarmoured dinoflagellate *Cochlodinium polykrikoides* was the species involved in this fish killing event. Based on the position of the sulcus and shape of chloroplasts the causative species was identified as *C. polykrikoides* and clearly differentiated from the similar species *C. fulvescens* [5]. In October 2013, the abundance of this species ranged from 8.1 x 10^5 to 1.05 x 10^8 cells · L^{-1}. Fish kills associated with *Cochlodinium polykrikoides* bloom were reported in Indonesia for the first time. The economic losses for mariculture industries and fishermen around the bay were unprecedented.

This species was then scarcely observed in the water column. The putative explanation of such sudden events is the existence of numerous cysts beds of *C. polykrikoides* in the bottom sediments. Numerous cysts were isolated from sediment samples collected in May 2014, and their morphology was very similar to that described by Tang and Gobler [6] and somewhat different from the forms described by Li et al. [7] (Fig. 3 a, b). An exact identification will be possible after cultivation. In October 2014, *Cochlodinium polykrikoides* was again observed in the waters of Lampung Bay (Fig. 3 c, d, e, f).

The harmful unarmoured dinoflagellate *Cochlodinium polykrikoides* (Gymnodiniales, Dinophyceae) displays features of an invasive species: cosmopolitanism, geographic discontinuity and a wide ecological spectrum. It is considered as an invasive alien species according to DAISIE (Delivering Alien Invasive Species Inventories for Europe, DAISIE European Invasive Alien Species Gateway (*http://www.europe-aliens.org*). Kudela and Gobler described a complete inventory of its expansion and its ecological strategies [8]. Before 1990, blooms of this species were observed mainly in Southeast Asia. Then, in the 1990s, the South Korea's fishing industry suffered annual losses of more than 100 million US dollars. Since then, blooms have been seen throughout the Asian area and on European and North American coasts. Iwataki et al. identified three *C. polykrikoides* sub-clades or ribotypes: the East Asian ribotype (which refers to the original Japanese-Korean clade), the American/Malaysian ribotype and the Philippine ribotype [9]. This latter species has been observed in the Mediterranean Sea, where the Italian coast is affected since the late 1990s, and in the Black Sea since 2001 (see references cited in [8, 10]. Until recently, it had rarely been detected on the Catalan coast, but in 2011, concentrations up to 2x10^4 cells L^{-1} were recorded in the port of Arenys, North of Tarragona [10].

Investigations will be conducted to study the cysts beds distribution of this harmful species in Lampung bay



Fig 2. (a) Cochlodinium polykrikoides *bloom forming dark brown patches off Bandar Lampung. (b) Mrs Muawanah, research scientist from BBPBL, collecting water samples and (c) mass groupers mortality in BBPBL fish farm, in Hurun Bay, November 2012*

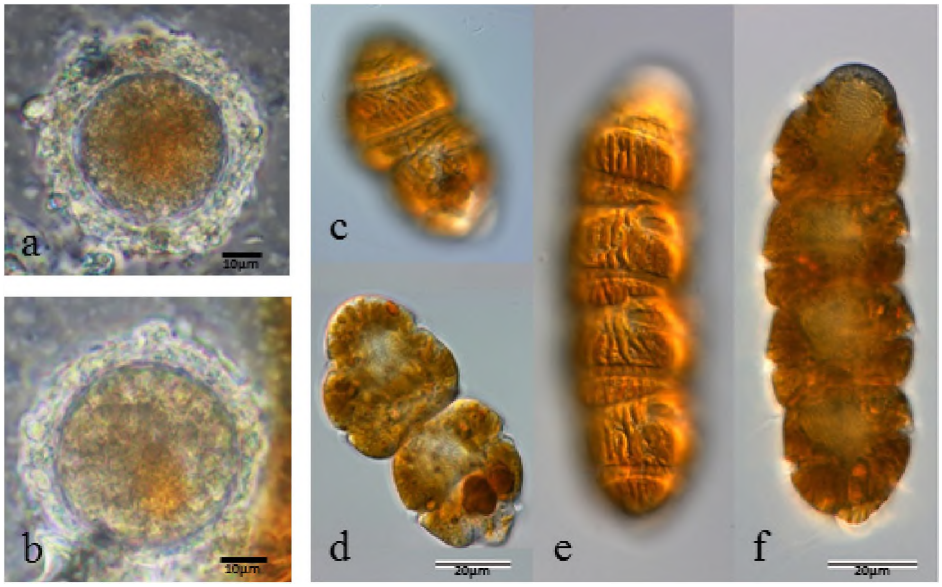

Fig 3. (a, b) Cochlodinium polykrikoides*-like cysts collected in 0-5 cm of the surface sediment sampled in May 2014 off the city at Lampung Bay, where the highest cell abundance of* C. polykrikoides*, from 2012 to date, was recorded; (c, d) Two-celled and (e, f) four-celled chains of* C. polykrikoides *collected in Lampung Bay, October 2014*

and molecular identification will allow us to determine the sub-clade of the *C. polykrikoides* isolated and help us to understand its expansion.

Acknowledgements

This work was supported by the Research Center for Oceanography-LIPI and the Balai Besar Perikanan Budidaya Laut Lampung (Indonesia), the Institut de Recherche pour le Développement and the University of Montpellier (France) and the Grants-in-Aid for Scientific Research, JSPS KAKENHI 25304029 and the JSPS ACORE-COM-SEA program (Japan).

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Observations of *Heterosigma akashiwo* bloom and associated wild salmon lethargic behavior in Cowichan Bay, Canada, 2014

Heterosigma akashiwo is the most well-known and widely distributed fish killer and the main killer of farmed salmon in the Pacific Northwest region of Canada [1; Nicky Haigh, pers. com.]. In recent years (2009 - 2012), harmful algal blooms (HABs) caused $16 million of direct economic losses in farmed British Columbia (BC) salmon, with *Heterosigma* being the causative agent for the majority of these losses [2]. While the direct effects of *Heterosigma* on farmed BC salmon are evident, wild salmon losses and fish stress responses during *Heterosigma* blooms are currently understudied [3].

Over the past 20 years, there have been dramatic declines in the abundance of wild Chinook Salmon and Coho Salmon in the Strait of Georgia, BC. In response, the Pacific Salmon Foundation (PSF), an independent non-governmental organization, initiated the *Salish Sea Marine Survival Project (SSMSP)*. The SSMSP is a five year project of ecosystem research and habitat restoration designed to improve fisheries management and enhance economic and cultural benefits for Salish Sea communities. The current multi-disciplinary project team consists of over 20 federal and provincial agencies, First Nations tribes, academics and non-profit organizations [4]. The project seeks to improve our understanding of the causes of salmon mortality in the Salish Sea and explain wide fluctuations in salmon returns over the years. As part of the project, we initiated a pilot study to monitor HABs and investigate their possible effects on juvenile salmon (wild and hatchery) in the Cowichan Bay estuary and outer bay.

From the beginning of May to mid-July 2014, a team of technicians from PSF and Fisheries and Oceans Canada (DFO) collected fish samples using both beach seines (Fig. 1) and purse seines (Fig. 2). We also collected corresponding surface water samples at each set location for phytoplankton analysis. After collection, water samples were preserved with Lugol's Iodine solution; subsequent phytoplankton analysis was performed on a Sedgewick Rafter slide and dominant and harmful algae species were identified and enumerated under a light microscope.

During the late spring to mid-summer period, we observed three high phytoplankton biomass events or blooms. The first and third bloom were caused by *Pseudo-nitzschia* spp. (May 20, 21) and *Skeletonema costatum* (July 8, 9). During these blooms, the water was brown but the captured fish did not appear to be affected by the blooms. The second bloom was recorded during the first week of June and was caused by *Heterosigma* (Fig. 3); below we describe in detail this bloom development and associated changes in fish behavior.

The first few cells ($\leq$10 cells mL^{-1}) of *Heterosigma* were recorded on the north side of Cowichan Bay on May 26-27 while purse seining (Table 1, Fig. 4). Much higher concentrations (mostly in the range of 100-1000 cells mL^{-1}) were observed the next day when beach seining.

In the first week of June, *Heterosigma* concentrations did not exceed 100 cells mL^{-1} in both beach and purse seines (Table 1, 2).

On the beach seining day of the second week of June, all near shore areas were covered in a highly visible, thick orange bloom. *Heterosigma* concentrations reached 3-4 x 10^{4} cells mL^{-1} at the north area of the Bay (Table 2, Fig. 4). During sampling, we did not notice any unusual fish behavior at these locations, though very few fish were caught at that time.

In the third week of June the water was brown/orange in color and most of Cowichan Bay was covered in swathes of highly visible phytoplankton bloom. Subsequent laboratory analyses of these water samples confirmed high concentrations of *Heterosigma,* ranging from 800 to 12 x 10^{3} cells mL^{-1} on June 16, and 3.2-8 x 10^{3} cells mL^{-1} on June 17. The highest concentration of 12 x



Fig. 1. Beach seine in Cowichan Bay, 2014.

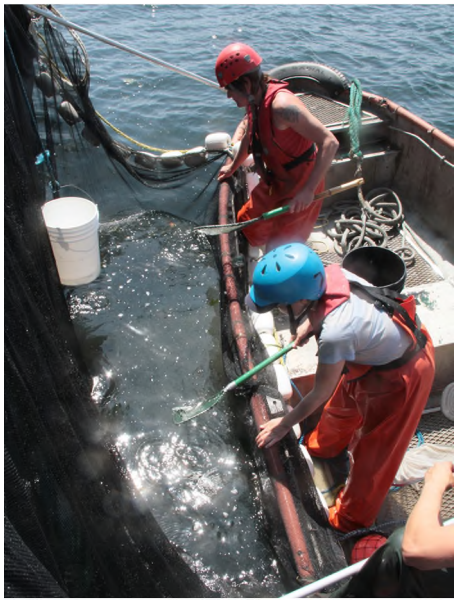

Fig. 2. Purse seine in Cowichan Bay, 2014.

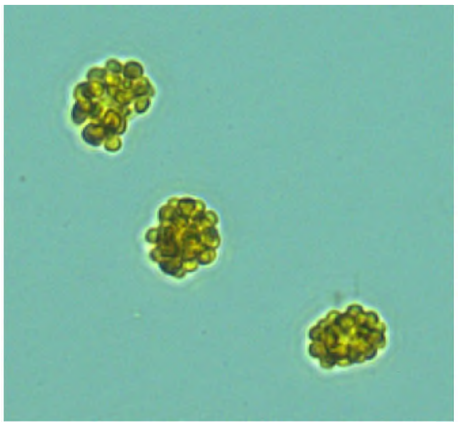

Fig. 3. Heterosigma akashiwo *cells from the bloom in Cowichan Bay, June 2014.*

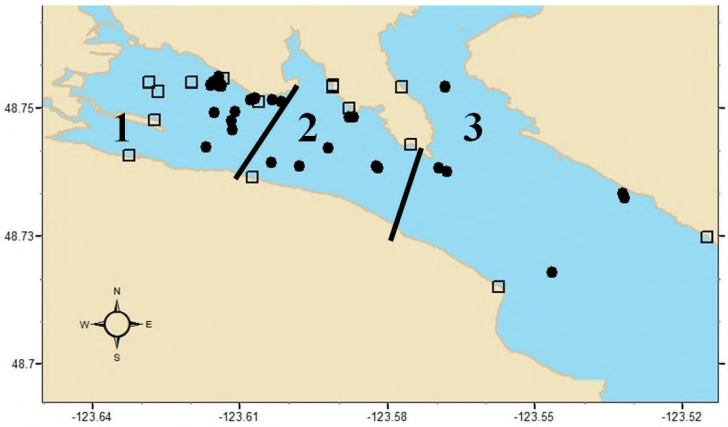

Fig. 4. Map showing purse seine (circles) and beach seine (squares) locations in Cowichan Bay, British Columbia, Canada from May 26 to June 18 of 2014.

10^3 cells mL^{-1} was collected from the north side of the Bay.

On these days we observed unusual behavior in the salmon that were captured in the net. In general, fish appeared to move more slowly; salmon species (Chum, Chinook, Coho, Sockeye) appeared to be lethargic (*i.e.* inactive and disoriented). We observed differences in behaviour between salmon species - while Chum Salmon were also slowed in their responses, the lethargy was very pronounced among Chinook Salmon in the net. Chinook Salmon did not exhibit any avoidance response when approached with a dipnet; several of them rolled over after approximately a half hour of sampling even without being handled. It should also be noted that on these two days of seining, we were collaborating with a team doing a Passive Integrated Transponder (PIT) tagging pilot project in which all captured Chinook Salmon were lightly sedated, PIT-tagged and carefully released upon full revival. The team's historical mortality rate associated with tagging has been about 1%; on this day the mortality rate rose dramatically to 25%. Some of these post-tagging morts were retained and frozen on dry ice for further laboratory analyses.

To test the hypothesis that the bloom could be the source of the apparent physiological stress condition of salmon, we selected one site away from the visible bloom and observed their behavior. Fish at this site did not appear lethargic; however causal associations must be qualified by the fact that Chinook Salmon catches were low at that site. (Note: subsequent laboratory analyses confirmed that *Heterosigma* concentrations at that site were the lowest recorded that day – 800 cells mL^{-1}.)

On June 18, beach seine samples had significantly lower concentrations of *Heterosigma* than those recorded the previous days of purse seining and only two sites had *Heterosigma* counts exceeding 10^3 cells mL^{-1}. That was also the last day that we observed *Heterosigma* in Cowichan Bay that summer and in all following samples, *Heterosigma* was absent.

In summary, over the period ranging from late spring to mid-summer, we observed the development and decline of *Heterosigma* bloom in Cowichan Bay. *Heterosigma* cells first appeared on May 26 in very low numbers (<10 cells mL^{-1}) in offshore waters. It reached maximum concentrations of 4×10^4 cells mL^{-1} at a near shore site on June 11 and 1.2×10^4 cells mL^{-1} at an offshore site on June 16. High concentrations of *Heterosigma* at open water sites were associated with lethargic fish behavior, with Chinook Salmon appearing to be the most affected. *Heterosigma* cells were last seen on June 18th in high concentrations (>1,000 cells mL^{-1}). All later samples from June 25 to July 16 did not have *Heterosigma* cells. Based on these observations, the north side of the Bay appeared to be a

Table 1. Purse seine locations and Heterosigma akashiwo *cell mL-1 at surface water samples.*

Day	Area	Number of sets	*Heterosigma akashiwo* (cell mL^{-1})	*H. akashiwo* (cell mL^{-1}) (average per area)
26-May	1	5	0-5	1
	2	1	0	0
	3	1	0	0
27-May	1	4	0-5	2
	2	1	0	0
2-Jun	1	2	0	0
	2	3	0-10	5
	3	2	0-8	4
3-Jun	1	3	30-60	43
	2	1	20	20
	3	1	30	30
16-Jun	1	3	2500-5000	3500
	2	2	800-12000	6400
17-Jun	1	3	3200-5000	4067
	3	2	7000-8000	6500

Table 2. Beach seine locations and Heterosigma akashiwo *cell mL-1 at surface water samples.*

Day	Area	Number of sets	*Heterosigma akashiwo* (cell mL^{-1})	*H. akashiwo* (cell mL^{-1}) average per area
28-May	1	1	220	220
	2	3	120-850	490
	3	1	10	10
4-Jun	1	3	0-15	5
	2	3	0-3	1
11-Jun	1	5	900-3200	2300
	2	3	4000-40000	24667
18-Jun	1	3	20-2500	907
	2	3	170-2000	957
	3	2	300-800	550

"hot spot" with consistently higher *Heterosigma* concentrations than other areas. Sampling will continue in Cowichan Bay for another three years and the cumulative data will provide insights into the effects of *Heterosigma* blooms on local juvenile wild salmon.

Acknowledgments

We are grateful to all the contributors and financial supporters who made Salish Sea Marine Survival Project possible. We thank Dennis Woloshuk, captain of the fishing vessel *Ocean Venture* and his crew for providing smooth and safe sailing. We thank Ewa Sniatycka for volunteering her sampling skills. Very special thanks to *SSMSP* technicians - Chelsea Adams and August Jones for making the sampling process enjoyable and setting an excellent example for future sampling crews.

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Book review

Marine benthic dinoflagellates – unveiling their worldwide biodiversity

Mona Hoppenrath, Shauna Murray, Nicolas Chomérat & Takeo Horiguchi

Kleine Senckenberg-Reihe, Band 54, Schweizerbart, Stuttgart, Germany 2014, 276 pp., 93 Figs (more than 200 colour images, approximately 150 scanning electron micrographs, and more than 250 drawings), 8 Tables, in English. ISBN 978-3-510-61402-8, paperback, 19.90€.

In 1974 Georg Thieme Verlag published the book *Marines Phytoplankton* by Gerhard Drebes, which was based on photographs of living organisms when, at that time, the usual focus on taxonomy work was on dead parts of the cells. A new updated and enlarged version of that book was published in 2009 by Schweizerbart on the collection Kleine Senckenberg-Rehie and was authored, in addition to Gerhard Drebes, by Mona Hoppenrath and Malte Elbrächter. Now, using the same collection, Mona Hoppenrath, this time together with Shauna Murray, Nicolas Chomérat and Takeo Horiguchi, has published *Marine benthic dinoflagellates – Unveiling their worldwide biodiversity*. Benthic dinoflagellates have been overlooked for a long time or even included as phytoplankton in many works. With this new publication, these authors give the benthic dinoflagellates the importance they merit. For many years within HABs studies, the focus of interest was on blooms of planktonic species. Nevertheless, given the importance of the Ciguatera syndrome caused by benthic dinoflagellate blooms, and the increasing number of benthic dinoflagellates identified as toxin-producers, the SCOR-IOC GEOHAB programme decided to include benthic HABs as a new distinct Core Research Project in addition to the initial four projects (Upwelling-, Eutrophic-, Stratified Systems, and Fjords and Coastal Embayments).

This book, which is devoted to all benthic dinoflagellates, both harmful and benign, fills a gap on this important topic and gives an appropriate context for the studies on benthic HABs independent of the phytoplankton literature. For this reason it was endorsed by the GEOHAB Steering Committee in the category "Framework Activity" related to the core research project on Benthic Harmful Algal Blooms (BHAB).

The book is based on astonishing light and electron microscopy images but the abundant ink drawings are a valuable tool for a better understanding of the photographs. As there is much work still to be performed on the phylogeny of benthic dinoflagellates, the genera are presented on alphabetic order. Due to the huge information provided by the book, it will be a must beside the microscope and it will induce many phytoplanktologists to work with benthic dinoflagellates. Although it is full of colour pictures and very good quality printing, it has a very affordable price of less than 20 €. More information in: *http://www.schweizerbart.de/publications/detail/isbn/9783510614028/Kleine_Senckenberg_Reihe_Nr_54_Marine_benthic_dino?l=EN*

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First report of a mass-poisoning outbreak following the consumption of *Tectus niloticus* (Gastropod) in French Polynesia: a novel pathway of Ciguatera Shellfish Poisoning?

Marine products represent a major food source in many French Polynesian islands. The high risk of seafood poisoning, well known in several of these islands has the potential to pose significant health issues to local populations [1]. Ciguatera Fish Poisoning (CFP) is a toxic syndrome classically related to the consumption of tropical fish contaminated with ciguatoxins produced by dinoflagellate genus *Gambierdiscus*. CFP is the most prevalent ichthyosarcotoxin reported in French Polynesia [2], with an average incidence rate of 19/10,000 inhabitants reported annually, although this number may reach 1,800/10,000 in the areas most severely affected by this disease. These data are even more worrying based on the general assumption that actual reports may represent only 10% of the cases [3].

Moreover, reports of atypical forms of ciguatera tentatively designated as Ciguatera Shellfish Poisoning (CSP), and associated with the consumption of various marine invertebrates highly popular among local populations (e.g. giant-clams, sea urchins), have also been documented in French Polynesia [4]. Until very recently, these CSP incidents, which represent less than 10 cases/year were known to affect only the Australes (Tubuai, Raivavae and Rurutu islands) archipelago.

On June 2014, nine tourists suffered from a CFP-like syndrome following the consumption of several specimens of a gastropod, *Tectus niloticus* ("troca", Fig. 1), collected in Anaho Bay, Nuku-Hiva Island (Marquesas archipelago, French Polynesia, Fig. 2). All patients displayed clinical symptoms similar to those of CFP, e.g. gastrointestinal (diarrhea, vomiting), neurological (paresthesia, cold allodynia) and cardiovascular (bradycardia, hypotension) disorders. However, the rapid (2 h) onset of symptoms associated to a burning/tingling sensation of the mouth and throat, their unusual severity (requiring the hospitalization of 7 of the patients) and the long-standing persistence of gastrointestinal symptoms, suggested the involvement of other toxic compounds in addition to ciguatoxins (CTXs). At the time of going to press, the two most severely intoxicated patients were still under medical surveillance in the Poison Control Centre of Pavia (Italia) and are presently subjected to a 5-month follow-up protocol. Other tourists agreed to answer a comprehensive medical questionnaire. The results of this clinical survey will be presented elsewhere.

To further investigate the etiology of this novel form of poisoning, 20 specimens of trocas from the incriminated fishing area in Anaho Bay were sampled in July 2014 with the assistance of the Sentinel Network of the Public Health Directorate and the Marine and Mining Resources Directorate of French Polynesia. Following their extraction using the protocol previously described by Pawlowiez et al. [5], RBA- and CBA-based toxicological analysis were conducted. Preliminary results confirmed the presence of both lipophilic ciguatoxin-like compounds and hydrophilic toxins in the extracts, whose identification by LC-MS/MS techniques is currently underway.

Although there were previous reports of CSP incidents in several South Pacific islands [6], the present report is the first to document a cluster directly associated with the consumption of *T. niloticus* in French Polynesia. Following our laboratory investigations, the local population was recommended by local health authorities not to consume trocas collected from the incriminated area.

It should be noted that *T. niloticus* is classified as a protected species in French Polynesia. However, its exploitation by the local population for shell trading and meat consumption is possible once a year through an official permit issued by the Ministry of Marine Resources. Therefore, increased outreach about the potential health risk associated with the consumption of trocas will undoubtedly benefit future CFP/CSP risk management plans not only in French Polynesia, but also in any PICT (Pacific Islands Countries and Territo-

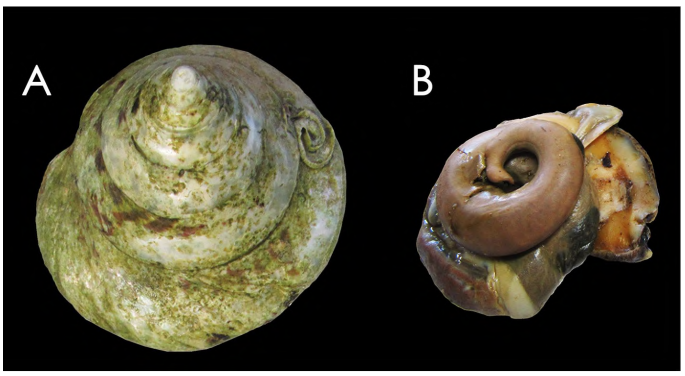

*Fig. 1. A: Shell and B: mollusk flesh from a "troca" (*Tectus niloticus*).*

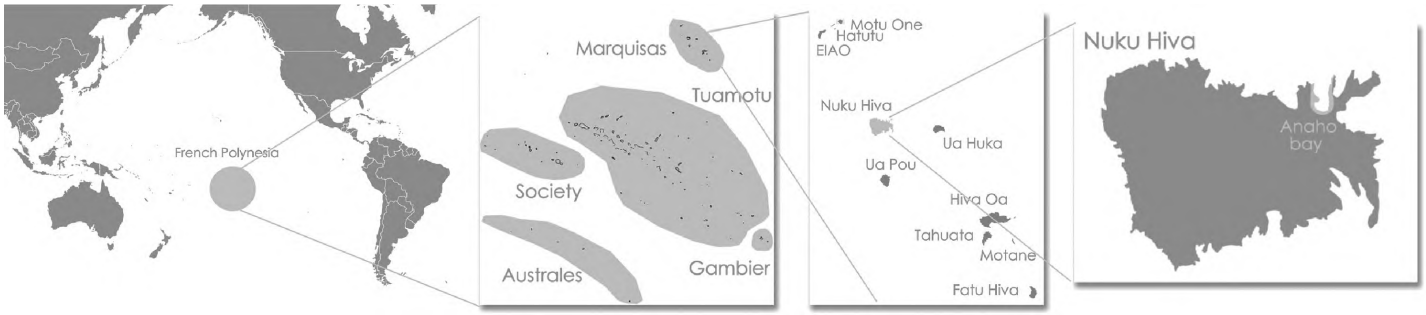

Fig. 2. Map of Anaho Bay in Nuku-Hiva Island (Marquesas archipelago, French Polynesia)

ries) where they constitute a significant subsistence and economic resource.

Acknowledgements

We express our gratitude to Stéphanie Nouel from the "Cellule de Promotion de la Santé" of Nuku Hiva, and Arsène Stein from the Marine and Mining Resources Directorate of French Polynesia for providing the troca samples for toxicological analysis purposes. Our sincere thanks also go to the nine tourists for their active cooperation in the present study.

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First report of *Gambierdiscus* in Moroccan Atlantic waters

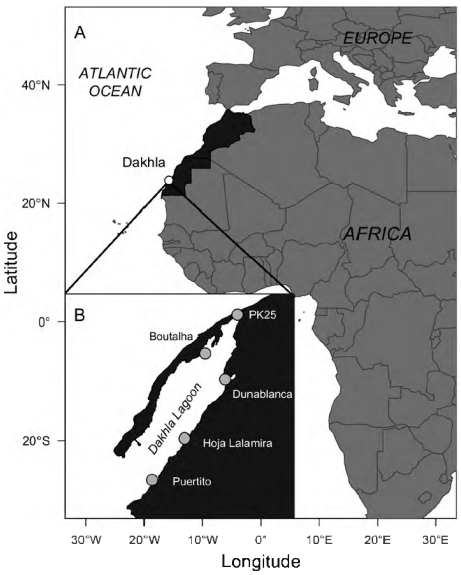

Fig. 1. Dakhla shores on the Atlantic Morroccan coast

During summer 2012, *Gambierdiscus*-like cells were observed in water samples collected from shellfish growing areas on the Bay of Dakhla, on the Southern Atlantic coasts of Morocco (Fig.1). Cell morphology was observed using light microscope and scanning electron microscopy. The taxonomic study is ongoing, but the morphological analysis showed the presence of the first plate (P1) and the apical pore complex (P0) corresponding to the genus *Gambierdiscus* (Fig. 2).

This finding should alert managers of fisheries resources and highlights the need to include *Gambierdiscus* spp. in the list of potentially toxic microalgae monitored in Moroccan waters.

Gambierdiscus species have been described as epiphytic and benthic dinoflagellates living on tropical reefs and attached to macroalgae surface, algal turf, detritus and sand [1-3]. Some species/strains of this genus have been shown to produce ciguatera fish poisoning (CFP) toxins [4]. These toxins cause gastrointestinal, neurological and cardiovascular disorders in humans [5].

Different species of toxin-producing dinoflagellates are present in the Atlantic coasts of Morocco, but *Gambierdiscus* spp. had never been reported [6,7]. Here we report the first detection of *Gambierdiscus* species on the Atlantic coast of Morocco.

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Authors

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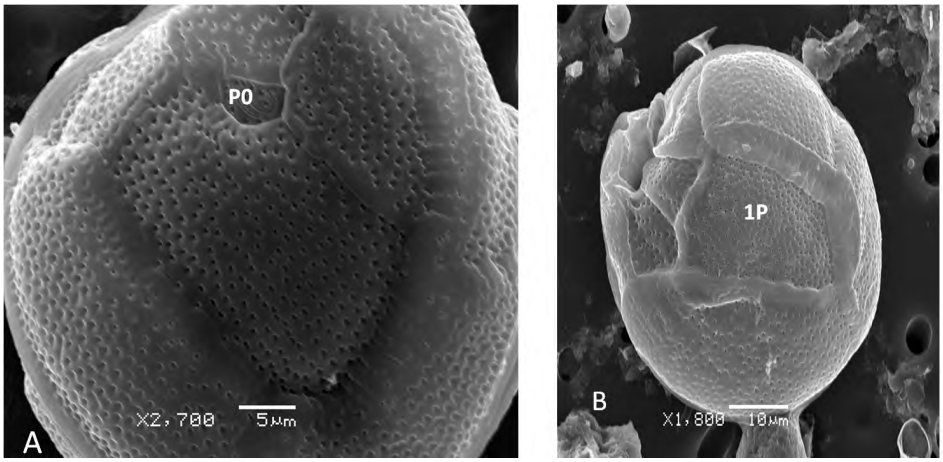


Fig. 2. Gambierdiscus *sp.detected in Dakhla bay in Moroccan Atlantic coast: Scanning electron micrograph of epithecal plates with P0 (A) and 1 P (B) in apical view.*

PACE-Net+ Think Tank: Coastal ecosystem disturbances, fish and shellfish poisoning and their socio-economic implications

Secretariat of the Pacific Community (SPC), Nouméa, 18-20 November 2014

In the Pacific, epidemics and food poisoning caused by fish consumption are widespread and can lead to severe illness and death. Their effects jeopardise food security and local economies. Population growth and climate change are likely to make these events more common. There is therefore an urgent need to conduct relevant research and find new ways of restricting these occurrences.

To help produce proposals in this regard, from 18 to 20 November 2014, the Secretariat of the Pacific Community, with European Commission, French Pacific Fund and ACIAR support, organised a think tank session on the theme of *Coastal ecosystem disturbances, fish and shellfish poisoning and their socio-economic implications.*

Four thematic areas were discussed: marine toxins, the effects of climate change and environmental disturbances, health and society, and cultural and economic aspects.

Some fifty experts (scientists, senior government officers, industry representatives) from the Pacific (Australia, Fiji, Cook Islands, Marshall Islands, Solomon Islands, New Caledonia, New Zealand, Papua New Guinea, French Polynesia, Samoa, Tonga, Tuvalu) and Europe (Germany, Denmark, Spain, France) actively contributed to the meeting.

After constructive discussions, the participants selected a number of priority research areas, highlighting the need to consolidate regional data bases so as to enhance the knowledge available and enable enlightened decision-making. The need to use traditional knowledge was also emphasized. The innovations seen as necessary by participants included easy-to-use diagnosis kits to identify fish and affected persons.

Some ten new project ideas designed to find solutions were also outlined. These project concepts were based on cooperation between the European and Pacific partners present over the 3 days. As always with PACE-Net+, relationships were formed and paths opened up for new networks with future potential.

Joint endeavors of this kind are fully consistent with European policy. Indeed, in his introduction at the think tank, Mr Efstratios Pegidis, European Bureau Chief for the Pacific Overseas Countries and Territories, stressed the importance of scientific and technical cooperation between Europe and the Pacific, in a setting where challenges such as climate change and food security are global in nature.

Lastly, the participants drafted recommendations that will inform regional and bi-regional political dialogue on scientific and technical cooperation and innovation, to be encouraged by PACE-Net+ with the main research players and decision-makers.

The outcomes of the Think Tank will be presented at the bi-regional dialogue to be held in Auckland on 10 and 11 December 2014.

For more information on PACE-Net+ and this Think Tank: *http://pacenet.eu*



ISSHA

International Society for the Study of Harmful Algae

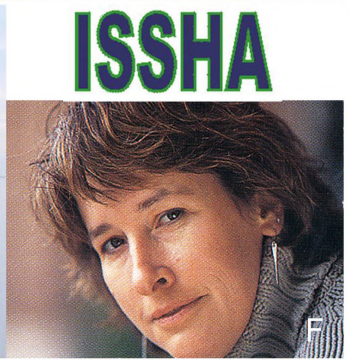














A&B: Frantic activity at the ISSHA Auction, coordinated by Esther, with Robin Raine as auctioneer.
C. A beautiful Takayama carving is given to Lincoln Mackenzie in recognition of a well done job organizing the ICHA 16.
D. Vera Trainer, newly elected ISSHA President, gives a present to the past President, Beatriz Reguera.
E. Anke Kremp (Finland), new ISSHA Secretary. F. Vera Trainer (USA), new ISSHA President
G. ISSHA Council meeting 2014 held on November 1 after the conference.

ISSHA Corner

The International Society for the Study of Harmful Algae (ISSHA) convened the 16th International Conference on Harmful Algae (ICHA 16), held in Wellington, New Zealand, 27-31 October 2014.

The ISSHA Council had two opportunistic meetings: the first before the conference on Sunday 26 October and the second after the Conference, on Sunday 1 November morning. The ISSHA General Assembly was held on Thursday 30 October from 18.30 to 20.00, followed by a generous wine and snacks party which preceded the traditional ISSHA Auction. Robin Raine did a superb performance as our new ISSHA auctioneer. Bids (from France and Germany) to host the ICHA 18 (2018) were presented in plenary on Monday 27 October at the end of the first day of scientific presentations.

The 9th ISSHA General Assembly was opened by Beatriz Reguera and minutes from the previous assembly were approved. Karin Rengefors (Secretary) announced the results of the elections for Council members. The President (B. Reguera) and the Secretary (K. Rengefors) had to step down because their period of service for the same office was due. The newly elected President is Vera Trainer (USA), and the new Secretary, Anke Kremp (Finland). Two new council members are Luis Proença (Brazil) and Catherine Lebret (Sweden). The others were re-elected members and Gustaaf Hallegraeff was elected as Vicepresident to replace Minjiang Zhou (who had to resign for personal reasons). Committee chairs summarized activities and achievements in the previous two years. There were 27 travel awardees (ISSHA funds plus the generous support from FAO and SCOR); publication of the 15th ICHA Conference Proceedings was on-line and freely distributed through the ISSHA website.

During the conference closing ceremony on 31st October, the new achievement awardees (Yasumoto Award; Maureen Keller student awards) were announced. Luis Proença (Brazil), on behalf of the local organizing committee, presented the venue for the 17th ICHA to be held in Florianápolis (Santa Catarina, Brazil) in 2016. The French bid was the winner, so the ICHA 18 will be held in Nantes (France). Finally the beautiful hand-embroidered ISSHA flag, a gift from the Korean hosts of the previous ICHA 15, was passed from Lincoln MacKenzie (Chair of the 16th ICHA local committee) to the future Brazilian organizers.

Officers and Council members 2014

Officers

President: Vera Trainer (USA)
Vice-Presidents: Robin Raine (Ireland) & Gustaaf Hallegraeff (Australia)
Secretary: Anke Kremp (Finland)
Treasurer: Henrik Enevoldsen (Denmark)
Past-President: Beatriz Reguera (Spain)

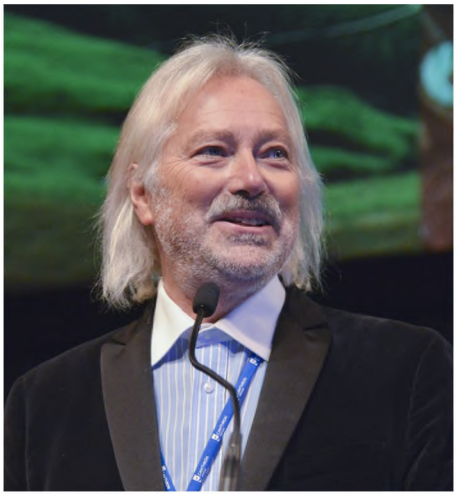
Council

Aligizaki, Katerina (Greece)
Amorin, Ana (Portugal)
Band-Schmidt, Christine (Mexico)
Davidson, Keith (United Kingdom)
Estrada, Marta (Spain)
Garcés, Esther (Spain)
Godhe, Anna (Sweden)
Hess, Philipp (France)
Jenkinson, Ian (China, France)
Lebret, Catherine (Sweden)
MacKenzie, Lincoln (New Zealand)
Proença, Luis (Brazil)
Roy, Suzanne (Canada)
Tubaro, Aurelia (Italy)

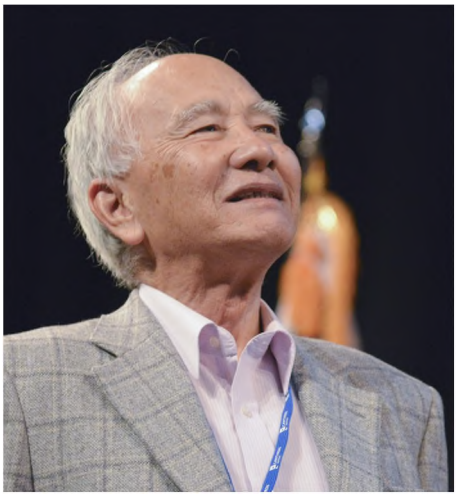
The profiles of the ISSHA officers and council members can be found at *http://www.issha.org/Society/ISSHA-Council-Members*

2014 Achievement Awards

The *Yasumoto Lifetime Achievement Award* was given to Prof. Gustaaf Hallegraeff (University of Tasmania). The *Maureen Keller Awards* for the best student communications were given to: Amanda Neilen (Sydney, Australia), Andres Seger (Tasmania, Australia), Wittaya Tawong (Japan) and Sara Harðardóttir (Denmark).



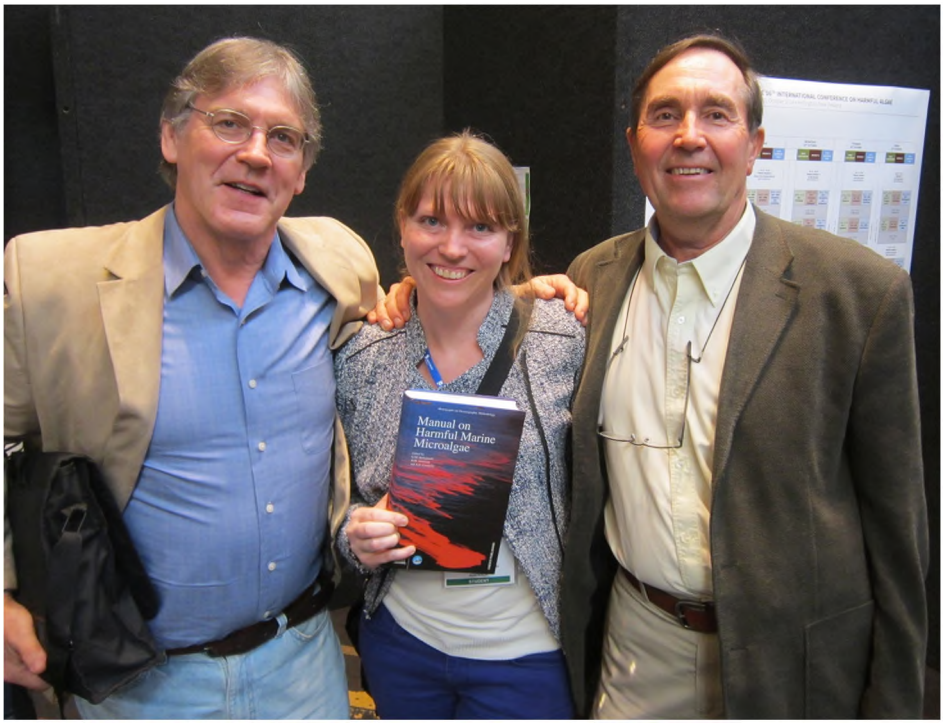
Prof Gustaaf Hallegraeff after receiving the Yasumoto Award (Photo J Camp)



Prof. Yasumoto listens to Hallegareff's speech after giving him the prize named after him (Photo J Camp)

Gustaaf Hallegraeff was born in the Netherlands, received his doctorate from the University of Amsterdam, and in 1978 moved to Australia as a post doc with Dr. S. Jeffrey at CSIRO working on phytoplankton pigments and taxonomy. At this time, he established HAB research in Australia and subsequently in 1992 Gustaaf moved from his Principal Research Scientist role at CSIRO to become a Professor in the UTAS School of Plant Science where he has continued to foster the next generation of HAB researchers. He is currently a Professor at the Institute for Marine and Antarctic Studies, University of Tasmania, Australia, where he has graduated over 30 PhD, 2 MSc. and countless Honours students across a diverse range of HAB topics.

A review of Gustaaf's more than 150 publications demonstrates the breadth of his scientific knowledge, enquiring mind, and even his artistic talent. His expertise in taxonomy, physiology, ecology and algal toxins is evident through many publications, reviews, and book chapters on key HAB species and groups such as *Gymnodinium catenatum*, *Alexandrium* and *Pseudo-nitzschia* species, and ichthyotoxic flagellates. More recently, Gustaaf's research has focused on the impact of climate change on phy-



Amanda Neilen (Sydney, Australia) after getting her HAB Manual gift signed by authors Allan Cembella (left) and Don Anderson (right) (Photo DM Anderson).

toplankton. Just as importantly, Gustaaf established ballast water introduction and global spread of toxic marine as an international issue. Over three decades his research has tackled treatment and control, risk mitigation, ballast water management and regulation, and ultimately prompted development of international policy and regulation of ballast water. The positive impact of this research on marine industries, as well as human and environmental health protection, resulted in the 2004 Eureka Prize for Environmental Research and his election as a Fellow of the Australian Academy for Technological Sciences and Engineering (2005).

Gustaaf has been a member of the Harmful Algal Bloom community and a leader in the field for 35 years. He has served the broader HAB community by authoring and editing many HAB methods and identification guides and state-of-the-science books such as *Manual on Harmful Marine Microalgae* (1995, 2003), *Aquaculturists' Guide to Harmful Australian Microalgae* (2002), and *Algae of Australia: Phytoplankton of Temperate Coastal Waters* (2010). In particular his expertise, research training and leadership has developed or inspired almost all HAB research capacity in the Australasian region. Gustaaf hosted the Ninth International Conference on Harmful Algal Blooms in 2000 in Hobart, Australia, attended by 526 participants from 47 countries. The first Yasumoto Award was given to FJR "Max" Taylor at this meeting. All conveners of such conferences know what sacrifices Gustaaf made to provide such a successful meeting. Gustaaf has always played a very active role in ISSHA devoting considerable time and energy to the publication of the Proceedings, and now serves as Vice-President and on several committees.

Professor Hallegraeff is a gracious, meticulous, hard-working individual. His contributions to the advancement of HAB science, mentoring of students, and support and leadership in the HAB community and to this Society have been truly outstanding over his whole career and fully merit the honor of the Yasumoto Award.

****

Best student oral presentation was awarded to **Amanda Neilen**, from the Australian Rivers Institute, Griffith University, Australia, for her contribution on "*Sources of Dissolved Organic Nitrogen and implications for Harmful Algae Blooms*", co-authored with her supervisors, Michele Burford and Chengrong Chen. The research Amanda presented was produced from her honours degree that was submitted only days before the 16th ICHA conference in Wellington. Amanda had relocated to live in Brisbane city (Queensland, Australia) to join the Australia Rivers Institute, after graduating in 2013 from the University of Sunshine Coast with a Bachelor of Environment Science (minors in chemistry and mathematics). Amanda's keen interest in chemistry and mathematical modeling led her into this exciting world of CyanoHABs, dissolved organic nitrogen and reactive oxygen species (ROS). Fascinated by the dominance of recurring freshwater CyanoHABs in South East Queensland reservoirs and elevated organic nitrogen loads, Amanda 'submerged' herself in her project. The work presented investigated the relationship between land use, nitrogen loads and the associated impacts on *Cylindrospermopsis raciborskii* growth, the results from a series of soil and aquatic microcosm experiments. Amanda's research found that amount of bovine urine applied to the soil affected the concentration and composition of nitrogen that leached from the soil. Leaf litter leachates were a potential source of bioavailable organic nutrients to *Cylindrospermopsis raciborskii* proceeding photolysis reactions. Currently, Amanda is designing a series of field experiments for her PhD to fulfill her interest in catchment land use practices, nitrogen cycling mechanisms and the downstream effect on CyanoHABs.



Andres Seger (Tasmania, Australia)

Honorary mention for students' oral presentation to **Andres Seger**, from the Institute for Marine and Antarctic Studies (IMAS), Tasmania, for his contribution on: "*Mitigating fish-killing algal blooms: clay revisited to remove ichthyotoxins*" co-authored with Juan José Dorantes-Aranda, Marius Müller and Gustaaf Hallegraeff.

Born and raised in Hanover, Germany, Andreas moved to scorching hot Queensland, Australia in 2006, where he visited the last two years of high school. Seeking milder climates more comparable to home, he migrated to Tasmania in 2008 to complete his bachelor of marine science degree at the University of Tasmania in 2010. Thanks to Gustaaf Hallegraeff's teachings, he quickly developed a keen interest in ichthyotoxic algae that led him to complete an honours project with him at the Institute of Marine and Antarctic Studies in Hobart, Tasmania (2011). The project investigated the use of clay to mitigate ichthyotoxic *Prymnesium parvum* blooms in response to fish mortalities at an Australian Barramundi's farm. Hooked on harmful algae, he is now working on his PhD project (supervised by Gustaaf Hallegraeff and Zanna Chase) to further explore the ichthyotoxin adsorptive properties of clay minerals and assess their potential application to other harmful algal species. "I have developed a strong interest in the physiology of fish-killing algae, particularly with regard to their toxic principles and quantification of ichthyotoxicity through cell line assays. I greatly enjoy utilising this knowledge in an applied way to help develop strategies to mitigate fish-killing effects".



Wittaya Tawong (Japan)

Best poster presentation was awarded to **Wittaya Tawong**, Kochi University, Japan, for his contribution on: "Characterization of *Gambierdiscus* and *Coolia* (Dinophyceae) isolates from Thailand based on morphology, phylogeny and toxicity", co-authored with Tomohiro Nishimura, Hiroshi Sakanari, Shinya Sato, Haruo Yamaguchi and Masao Adachi. Born and raised in Chiang Rai (Thailand) in 1982, Wittaya received his bachelor's degree of Fisheries in 2005 and Master of Fisheries Technology in 2008 from Maejo University (Chiang Mai, Thailand). During 2005–2008, he had a great experience as a research assistant with Japanese researchers in the topic of toxic cyanobacteria species and algal toxins in aquaculture ponds. That opportunity was very important to inspire him and improve his knowledge on the interesting topic of harmful algae. Furthermore, he received a scholarship from Naresuan University since 2010 to undertake a doctoral program under the supervision of Masao Adachi at the Laboratory of Aquatic Environment Science (LAQES), Kochi University, Japan. "My Ph.D dissertation was focused on the morphology, molecular biology, distribution, toxicity and growth factors of toxic benthic dinoflagellates causing ciguatera fish poisoning, in particular *Gambierdiscus*, *Ostreopsis* and *Coolia* from Thai coasts. Currently, I am working as lecturer and researcher at Naresuan University and continuing research with the application of molecular tools in the study of marine and freshwater harmful algae species from Thailand".



Sara Harðardóttir (Denmark)

Honorable mention for poster presentation to **Sara Harðardóttir**, University of Copenhagen, for her poster on: toxic diatoms in the Arctic marine food web: "The effect of domoic acid on arctic *Calanus* copepodites grazing on the diatom *Pseudo-nitzschia seriata*", co-authored with Marina Pančić, Anna Tammilehto, Bernd Krock, Torkel Gissel Nielsen and Nina Lundholm. Sara was born in Iceland but migrated to Denmark to undertake her studies. In 2009 she obtained a BSc in Molecular Biology and in Philosophy and Science studies from Roskilde University, Denmark. On a cruise between Tromsø Norway and Nuuk Greenland in 2010, she met Nina Lundholm, her supervisor for the master project and the current PhD project. Since then Sara has been working with phytoplankton from Arctic waters. In 2012 she graduated with MSc in Engineering - Aquatic Science and Technology from the Technical University of Denmark. Sara did her thesis on phytoplankton succession during a *Phaeocystis pouchettii* dominated spring bloom in Disko Bay, west Greenland. The project had an additional focus on succession of sea ice algae in newly forming sea ice and she found and described a new species, *Pyramimonas diskoicola*. Currently she is working on her PhD studies, supervised by Nina Lundholm, Torkel Gissel Nielsen and Uwe John, at the Natural History Museum of Denmark. The main theme of her project is to study toxin (domoic acid) producing diatoms in the arctic marine food web. In her thesis she investigates the interaction between domoic acid-producers *Pseudo-nitzschia* spp. and their grazers.

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

PICES

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

ICESCIEM

International Council for Science SCOR Scientific Committee on Oceanic Research

First announcement

Scientific Symposium on Harmful Algal Blooms and Climate Change

Gothenburg Sweden 19-22 May 2015

A Scientific Symposium on climate change effects on the global distribution, frequency and intensity of harmful Algal blooms will be held 19-22 May 2015, at Gothenburg, Sweden.

The central purpose of this symposium will be to bring together HAB scientists and climate change scientists to ascertain what is known about climate change and its potential linkages to HABs, to identify the most pressing, achievable research goals over the next decade, and to determine the minimal long term monitoring infrastructure needed to provide the data for global HAB assessment in the context of climate change.

The symposium will include invited speakers, oral and poster presentations by participants, with concentration on participant-led break-out sessions on specific topics.

Prepare for registration and submission of abstracts in early 2015.

Conveners: Bengt Karlson, Sweden, Mark Wells, USA, Raphael Kudela, USA and Angela Wulff, Sweden

About the symposium

Climate change will significantly affect marine ecosystems, and it is expected that there will be effects on the character, frequency and intensity of Harmful algal blooms. Examples of the potential drivers of HAB change include:

- warming – changed distribution of HAB species, including range expansion/contraction
- ocean acidification – possibly increased total primary production, positive for some HAB-species
- increased water column stability – increased stratification favour many HAB-species
- changes in rain fall distribution and dynamics - altered nutrient supply and input of humic substances, both potential drivers for change in the occurrence of HAB's
- + several other ways

However, these anticipated linkages to increases or decreases in HAB impacts remain largely undemonstrated, and it is unlikely that all potential avenues of affect are known. Presentations at the symposium will include findings from observations, experimental work and modelling.

A second announcement with information on registration, abstract submission etc. will be distributed in early 2015. Information on symposium fee and possibilities for young scientists travel awards also will be part of the second announcement.

SMHI

GEOHAB
Global Ecology and Oceanography of Harmful Algal Blooms

UNIVERSITAS GOTHOBURGENSIS 1891

Forthcoming events

The Impact of Global Change on Toxic Phytoplankton

Session 011 at the ASLO 2015 Aquatic Sciences Meeting, Granada, Spain, 22-27 February 2015, Aquatic Science: Global And Regional Perspectives—North Meets South.
Session Organizers:
Val H. Smith, University of Kansas, *vsmith@ku.edu*
Dedmer B. Van de Waal, Netherlands Institute of Ecology, *d.vandewaal@nioo.knaw.nl*
Hans W. Paerl, University of North Carolina at Chapel Hill, *hans_paerl@unc.edu*
Programme and abstracts: *http://www.sgmeet.com/aslo/granada2015/sessionschedule.asp?SessionID=011*

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

This group is focused on HABs in the North Atlantic and marginal seas. The next meeting will be held in Lisbon, Portugal, 13-17 April 2015. One task of the group is to gather information about HABs. Please report harmful algal events observed in your nation to your country representative in WGHABD for inclusion in the Harmful Algae Event Database, *http://haedat.iode.org*. Eileen Bresnan, United Kingdom, is the new chair of the group. Bengt Karlson, Sweden, resigned in autumn 2014 after end of term. More information about WGHABD activities: *http://ices.dk/community/groups/Pages/WGHABD.aspx*

Twelfth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB-XII), UNESCO, Paris, 28-30 April 2015.

The Panel (IPHAB) was formed in 1991 to set priorities for and ensure the financial resources for implementation of a global HAB Programme. A primary task is still to identify the required resources, so that the international community jointly can continue to implement activities on capacity building, international cooperative research, and communication networks. The membership of the IPHAB is open to Member States of IOC which have declared to the Secretary IOC their involvement or intention to participate in the development and implementation of the HAB Programme on a global, regional, or national scale. The Provisional Agenda and other documents for the Session is available at *http://hab.ioc-unesco.org/*.

The major focus of the Twelfth Session of IPHAB is tentatively the Global HAB Research Programme; Global Assessments of HAB events; capacity building; formulation and endorsement of specific regional activities; a plan for improved Ciguatera research and management, and Improving HAB forecasting capabilities for more effective mitigation strategies. The Agenda is furthermore open to the priorities of Member States. Based on its discussions the IPHAB will recommend to the IOC Assembly a work plan for the IOC HAB Programme for 2016-2017.

If you wish to know if your country is member of IPHAB you will also find the IPHAB List of Members at *http://hab.ioc-unesco.org/*. If you have questions regarding participation in IPHAB do not hesitate to contact the IPHAB Technical Secretary at *h.enevoldsen@unesco.org*.

IOC Training Course and Identification Qualification in Harmful Marine Microalgae 2015 (with optional workshops on enumeration and/or culture techniques):

Dates: Mandatory E-learning part over 4-5 weeks in the period May-July. Practical course 9-19 August 2015. Optional workshops on enumeration/culture techniques from 19-22 August.
Venue: IOC Science and Communication Centre on Harmful Algae, Department of Biology, University of Copenhagen, Denmark

The course includes 90 hours of teaching and is divided into two parts, each consisting of 45 hours of teaching. The first part of the course is an internet teaching programme, while the second part is a practical workshop on species identification. The Course ends with an examination in species identification.

Part I - Distant learning: The distant learning programme consists of 8 modules. There is a short introductory text to each module, and at the beginning of this text is given a list of suggested reading, which will usually include selected articles or paragraphs of general text books. All exercises are mandatory. This part of the course includes 45 hours of teaching or a work load of about one working day per week for six weeks.

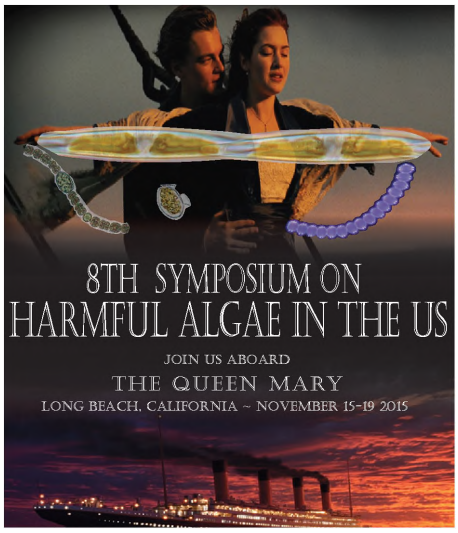
Part II – Practical course on species identification: The workshop will focus on identification of harmful algal species, with particular reference to the 'IOC Taxonomic Reference List on Toxic Plankton Algae'. During the workshop species will be demonstrated as live cultures, mixed samples or as preserved material. Focus will be on light microscopy. All participants will be urged to bring material for the course.

Details and application form: *http://hab.ioc-unesco.org/index.php?option=com_content&view=article&id=32&Itemid=0*

Deadline for applications: 1 April 2015.

Eighth Symposium on Harmful Algae in the U.S.

November 15 - 19, 2015
Long Beach, California
Further details will be posted to the following website, as they become available:
http://www.whoi.edu/habsymposia/



Ciguatera in the South Pacific

The IOC has established a list-server for Ciguatera and HABs in the South Pacific as a service to the community and as a means to facilitate communication.

If you are interested in this region you may subscribe here: *http://iodeweb2.vliz.be/wws/subrequest/picthab* and send messages to the list to *picthab@sympa. iode.org*.

List-servers grow and become useful to the community if used!

As part of its strategy to develop a multi-agency plan for ciguatera the IOC welcome ideas, cooperation, linkages and partnerships with regional organizations and institutions on HABs and Ciguatera.



IOC meeting on Ciguatera in the South Pacific

Eds-in-chief: Beatriz Reguera *beatriz.reguera@vi.ieo.es* and Eileen Bresnan *Eileen.Bresnan@scotland.gsi.gov.uk*

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- Africa: to be identified
- South Pacific: Mireille Chinain *mchinain@ilm.pf* and Lesley Rhodes *Lesley.Rhodes@cawthron.org.nz*

Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Coming deadlines are:
15 February 2015, for HAN 51
15 May 2015 for HAN 52
15 August 2015, for HAN 53
15 November 2015, for HAN 54

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Harmful Algae News

AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

High sea surface temperature, the potential trigger of mass mortality of fish, exceptional toxin producing HABs, and other socio-economic impacts in Uruguay

During February-March 2015 (Austral summer), sea surface temperatures up to 27.8 ºC were recorded *in situ* in Chuy, Department of Rocha, at the northern limit of the Atlantic coast of Uruguay. Values up to 28ºC were also recorded in surface coastal brackish waters from the Departments of Montevideo and Canelones, in the inner parts of Rio de la Plata estuary (10m mean depth). Satellite images of SST (Sea Surface Temperature) and of SST anomalies in relation to the 1971-2000 mean for the area (30º-40º S) [1] showed SST anomalies of +1-2ºC (Figs. 1 and 2). Anomalies were even higher in some coastal areas within Rio de la Plata estuary.

Bright green foam-producing blooms of a *Microcystis*-like cyanobacteria developed in February off Mon-

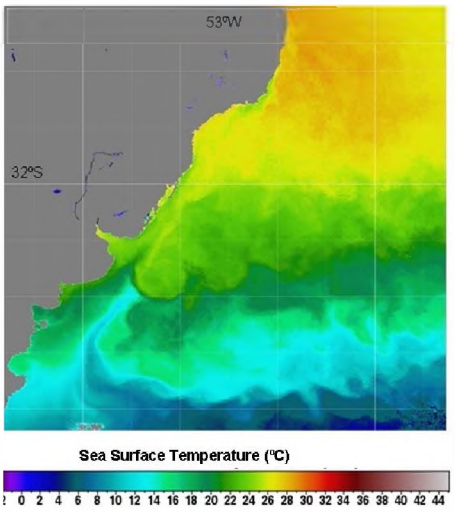

Fig. 1. Aqua MODIS image of sea surface temperature (SST) off the south western Atlantic Ocean (29°-36° S/51-56°W) shelf waters off Brazil, Uruguay and Argentina, March 2015.

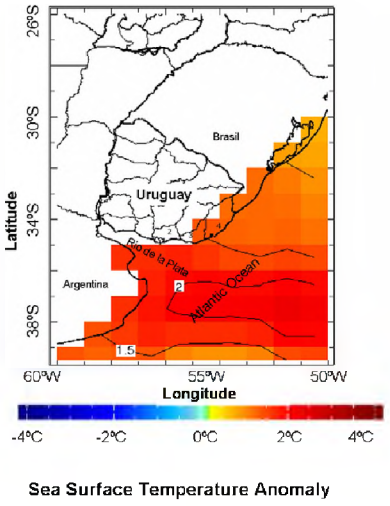

Fig. 2. Distribution of SST anomalies, in relation with the 1971-2000 mean, in Rio de la Plata estuary and shelf waters off Uruguay, March 2015. 1 = Montevideo, 2 = Canelones, 3 = Maldonado and 4 = Rocha.



Fig. 3. Bright green Cyanobacterial blooms in Montevideo coastal waters. Reddish colour is due to resuspended material from the river. (Photo from City Council of Montevideo).

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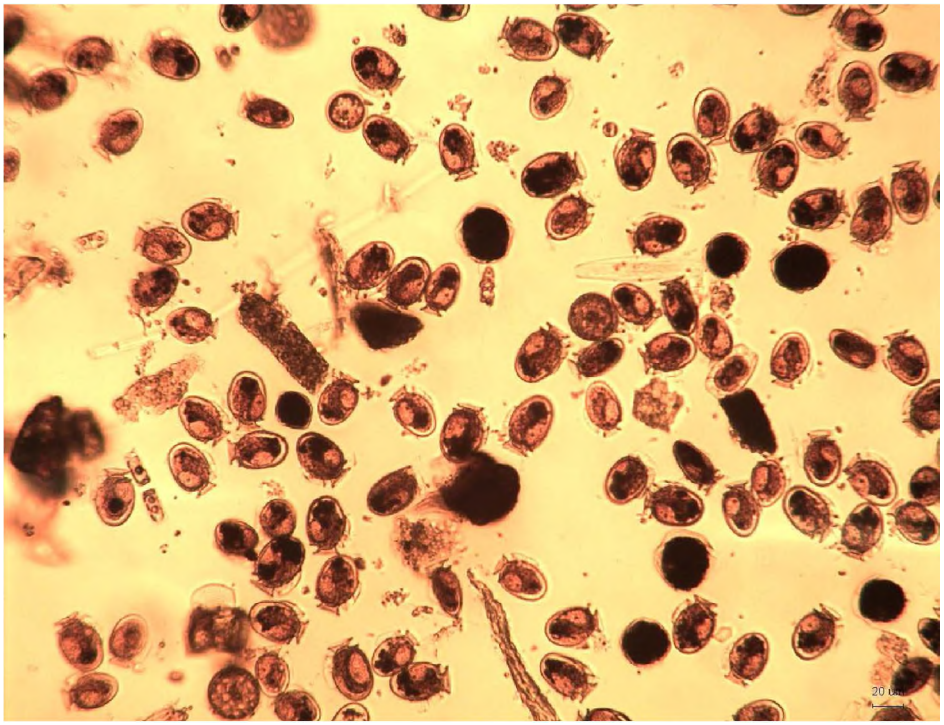

Fig. 4. Micrograph of the almost mono-specific bloom of Dinophysis *cf* ovum *in Uruguay coastal waters, March 2015. (Photo: Silvia Méndez)*

tevideo and Canelones (Fig. 3). Health authorities were prompted to display "sanitation" flags (red coloured with a green cross in the middle) to warn sunbathers about the risk of swimming there. It is worth mentioning that Montevideo beaches are permanently monitored and their water quality certified according to ISO 14001 standards by the City Council of Montevideo.

Late February and March, a massive bloom of the diarrhetic shellfish poisoning (DSP) toxin producer *Dinophysis cf ovum* was found in coastal areas with higher oceanic influence (Departments of Maldonado and Rocha) (Fig 4). Historical maximum densities were found in Punta del Este (83 x 10^3 cells L^{-1} on March 9) at salinity 32 and temperature 20°C and La Paloma (156 x 10^3 cells L^{-1} on March 16), with salinity 31.4 and 23°C in temperature. Shellfish harvesting was banned when DSP toxins were detected in the Departments of Maldonado and Rocha. Still, in the beginning of the bloom, about 20 cases of people, (pers.com. of local mussels vendors) were suspected to be intoxicated, suffering from gastrointestinal disorder after eating mussels. Harvesting closures, which started over 3 months ago, were still enforced at the time this article was written.

On March 5, the beaches of Montevideo and Canelones were covered by stranded dead fish of a single species locally called "Lacha" (*Brevoortia aurea*) (Fig. 5). Over 200 t of dead fish were collected by the City Council. Environmental analyses and examination of dead fish were carried out by the National Direction of Aquatic Resources (DINARA) and the University of the Republic of Uruguay.



Fig. 5. Mass mortality of fish in coastal waters of Uruguay, March 2015. (Photo: L. Carreño)

Harmful algal blooms were not detected in the affected area. *Pfiesteria shumwayae* and *Pfiesteria piscicida* are well known fish killers in United States, Australia and New Zealand. Work has been carried out to track these species with molecular tools in estuarine phytoplankton from 22 countries, including Uruguay, Argentina and southern Brazil; the two species were detected in Rio de La Plata [2]. *Pfiesteria* species are very difficult to distinguish under the microscope because of the multiple forms adopted through their life cycle. To test if these species had been the causative agents of the present fish kill, new sediment samples were taken from three different beaches (Buceo, Carrasco and Shangrilá located at Montevideo (1- Fig. 3) and Canelones (2- Fig. 3) affected by the mortality two weeks after the event and analyzed at the University of North Carolina at Greensboro, USA. Neither *P. piscicida* nor *P. shumwayae* were detected by PCR from the new DNA extractions. Salinity in Montevideo waters that week was 27, which is exceptionally high for the inner estuary waters according to previous studies in the area [3]. During spring–summer periods, conditions in the Rio de la Plata are dominated by offshore winds that deflect the river discharge plume towards the SW [3]. Low rainfall after January 2015 and the predominant NE winds for the season would have facilitated a marine intrusion deeper into the estuary [3]. The fish species affected by this episode is estuarine [4], and probably its optimum salinity space was reduced under this dry summer period. The main hypothesis is that the mass mortality was caused by a decline of dissolved oxygen levels associated with the positive temperature anomaly when fish shoals, pushed to inner parts of the estuary, were very dense.

The events described above were independent and with different locations

Continued on page 9

First report of *Alexandrium globosum* on the Pacific coast of Central America

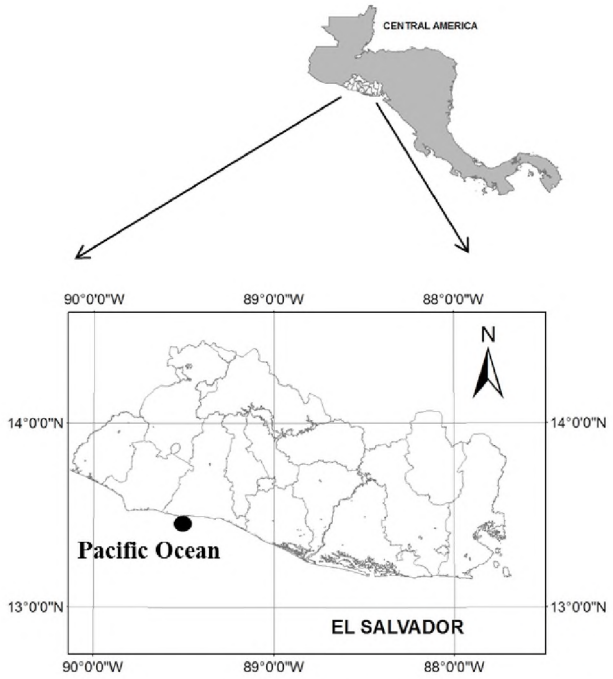

Fig. 1. Sampling area at department of La Libertad, El Salvador.

Harmful algal blooms of the dinoflagellate *Alexandrium* can have considerable socio-economic impacts. Amongst the 30 species described for this genus, more than half are known to be toxic. *Alexandrium* species are unique on their capacity to produce at least three different kinds of toxins: saxitoxins, spirolides and goniodomines [1]. Little is known about the impact of *Alexandrium* in Central America.

Between August and September 2014, an intense bloom of *Cochlodinium* cf *polykrikoides* developed which lasted 21 days and mainly affected the central and western coasts of El Salvador (Eastern Pacific). The observation of reddish-brown patches was associated with fish kills along the rocky shores of La Libertad. In addition, putrid smells from decaying algae affected the tourist industry. Cells of an unidentified *Alexandrium* species co-occurred with this bloom.

Plankton net (20-µm mesh) hauls and 10-L Niskin bottles samples were collected on 2 September 2014 off Puerto de La Libertad (13°29'11.6'' N; 89°19'12.6'' W), fixed with Lugol's solution and taken to LABTOX-UES for analyses. Samples were stained with Calcofluor, observed under the epifluorescence microscope (Zeiss Axio Imager) and cellular density estimated from Sedgewick Rafter chamber counts with an inverted microscope (Zeiss 40CF). *Alexandrium* spp. reached densities over 49 x 10^3 cells L^{-1}.

The genus *Alexandrium* is characterized by the Kofoidean plate tabulation formula: Po, 4', 6'', 6c, 10s, 5''', 2''''. Some important features for morphological classification of this genus are cell shape, size, chain-forming capacity, thecal ornamentation and shape of the cingulum, sulcus, cingular displacement and shape of the apical pore complex (APC), plates 1', 6' and some sulcal plates [2]. Plate tabulation of the cells in this work was Po, 4', 6'', 5''', 1'''', 1sp. The epithecal plate 1' was wide and asymmetrical with no direct connection with the APC (Fig. 2a); a characteristic ventral pore between plates 1' and 4' was present, plate 6' was rectangular and no connecting pores were observed either on the epitheca or the hypotheca (Fig. 2b). Morphology of the *Alexandrium* specimens corresponded to that of *Alexandrium globosum* as described by Larsen & Nguyen [3].

Fish kills were attributed to *Cochlodinium* cf *polykrikoides*. Nevertheless, we cannot rule out that *Alexandrium* cf. *globosum* contributed to the fish kills, because other species of *Alexandrium* have been described as producers of PSP and/or hemolytic toxins devastating fish farms [1].

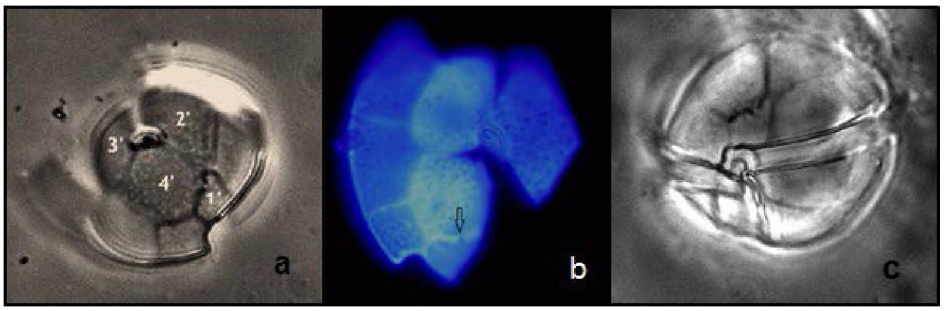

Fig. 2. Micrographs of Alexandrium globosum *a) Epitheca and tabulation of the apical plates; b) Stained cells showing the ventral pore; c) Ventral view. Samples from La Libertad, El Salvador, 2 September 2014.*

Acknowledgements

We thank Jacob Larsen for confirmation of the species identity and CENDEPESCA (La Libertad) for sampling help. This work was supported by the International Atomic Energy Agency (IAEA) projects RLA/7020 and ELS/7007.

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The Bay of Brest (France), a new risky site for toxic *Alexandrium minutum* blooms and PSP shellfish contamination

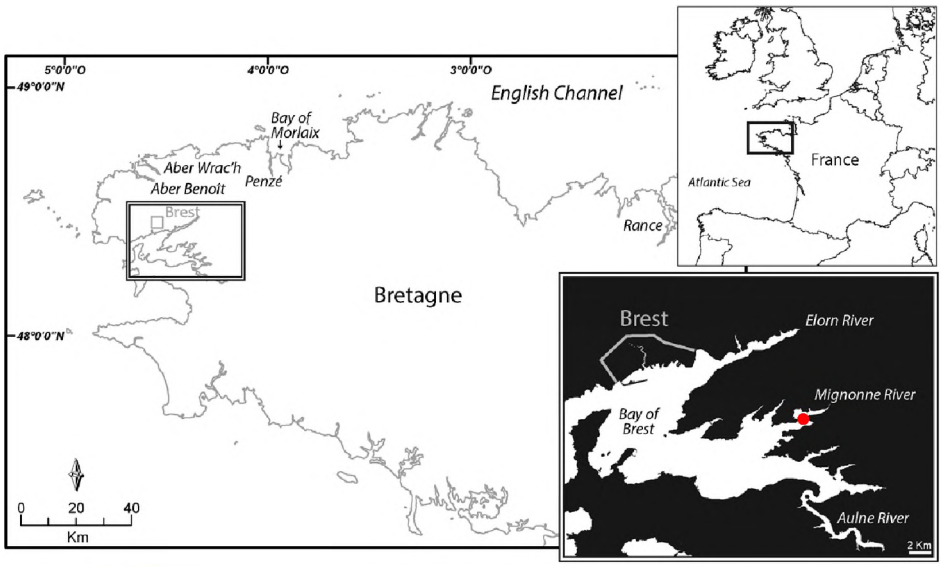

Fig. 1. Map with location of the Bay of Brest and other Breton estuaries where A. minutum *proliferates (● by the Mignonne estuary represents location of the sampling site)*

In summer 2012, a new toxic event occurred in the Bay of Brest (Fig. 1): a major PSP contamination event led to the prohibition of shellfish harvesting. It was the first time shellfish from the Bay of Brest were contaminated with these toxins. The maximal toxin level recorded in shellfish (over 8000 eq STX·kg^{-1}) was 10 times the sanitary threshold and *A. minutum* bloomed up to 41 x10^6 cells L^{-1}. This first PSP event was followed by additional *A. minutum* blooms leading to shellfish toxicities in 2013 and 2014.

A three-year survey of the Bay of Brest (Daoulex project, 2012-2014) has been carried out with water sampling twice a week in the Mignonne estuary around high tide as soon as water temperature exceeded 15°C. Data issued from this survey, associated with those from the phytoplankton and biotoxin monitoring network REPHY [1] (since 1984) and the Oyster network VELYGER [2] (since 2008) have shown that *A. minutum* has been observed in the Bay of Brest since 1990 only. This confirms results of a 1990 sediment survey showing the absence of *A. minutum* cysts in this bay [3]. From 1990 to 2008, *A. minutum* occurred at low densities (lower than the alert threshold of 104 cells L^{-1}). Conversely, since 2009, *A. minutum* has bloomed recurrently, surpassing this threshold and leading to PSP toxicities since 2012 (Fig. 2).

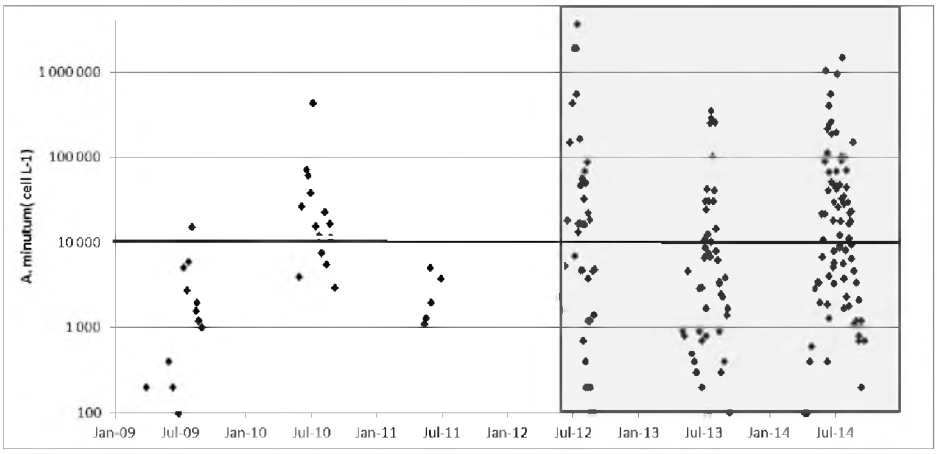

Fig. 2. Alexandrium minutum *abundances (data REPHY, VELYGER, DAOULEX) in the Bay of Brest. Grey box delimits the toxicity period. Black line stands for the alert threshold (10^4 cell L^{-1}).*

The unsafe bloom period (concentrations higher than the alert threshold) extended from mid-May to the end of August. In 2012 *A. minutum* developed from June 15 to August 16 with a maximum density of 41 x10^6 cell L^{-1} detected on July 15. In 2013, the bloom period was shorter (from July 5 to August 5) and with lower densities (maximum 3.6 x10^5 cell L^{-1} on July 22). In 2014, *A. minutum* developed during a longer period from May 26 to September 4 and peaked up to 1.1 x10^6 cell L^{-1} on June 6 and again to 1.5 x10^6 cell L^{-1} on July 22.

Surface water temperature seemed to be the major abiotic signal correlated to increased cell concentration above threshold limits and therefore to the risk period for shellfish contamination (Fig. 3). The temperature of 15°C seems to be a pre-requisite to allow bloom development above the threshold in the Bay of Brest as well as in other Breton estuaries [4]. In fact, 2014 showed initiation of an early *A. minutum* bloom in mid May when the water temperature reached 15°C. In contrast, 2013 showed a cold spring with water temperatures below 15°C until June 1 and the bloom this year initiated only in July. 2012 was an intermediate year for water temperature as well as for bloom initiation, with temperature of 15°C reached by May 24 (Fig. 3).

The main areas impacted by *A. minutum* are the upper estuaries of the Bay of Brest (Mignonne and other estuaries of the south-eastern Brest Bay, the Aulne and Elorn river outflows, Fig. 1). These areas correspond to shallow, sheltered estuaries, strongly influenced by tides. Along the Mignonne estuary, *A. minutum* maximum densities were observed with salinity values ranging from 29 to 34. These are transition waters between nutrient rich Mignonne waters (mean nitrate concentrations of 27 µg L^{-1}, mean phosphates 0.07 µg L^{-1}) and the Bay of Brest waters, which are phosphorus (P) limited for phytoplankton growth during spring and summer [5]. Nutrient concentrations in this estuary are closely linked to river Mignonne run-off and may impact *A. minutum* blooms, notably through phosphorus availability. In 2012, high spring and summer high river discharges (Fig. 3)

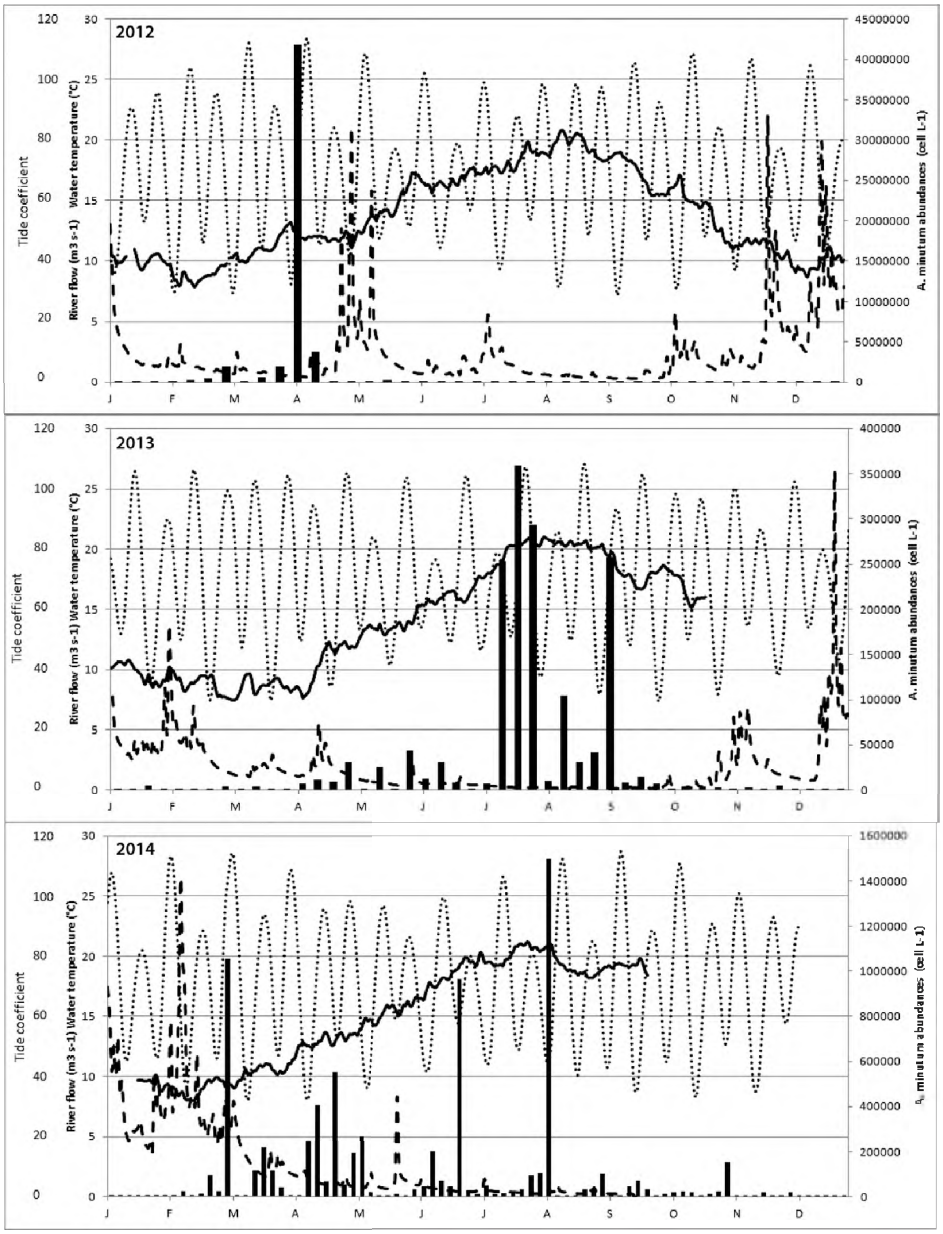

Fig. 3. Alexandrium minutum *densities (vertical bars, right axis), water temperature measurements (black line, left axis), river outflow (dashed line), and tidal coefficient (dotted line).*

may have been linked to the exceptional *A. minutum* bloom (>41 x10^6 cells L^{-1}). In 2013, river outflow was much lower, whereas 2014 presented intermediate river outflow in correlation with intermediate (between those of 2013 and 2012) densities of *A. minutum*.

Another environmental factor that seems to affect *A. minutum* maxima are tides. In fact, maximal abundances are observed when tides coefficients are lower than 80 (neap tides or little spring tides) (Fig. 3). This could be explained by lower bloom dispersion out of the estuary by lower tidal currents, allowing *A. minutum* to stay in its optimal (upper estuary) environmental condition for development.

In conclusion, the Bay of Brest represents today a new risky area for *A. minutum* blooms and occurrence of PSP toxins. These bloom events present similar temporal and geographical patterns as others that have already occurred in estuarine ecosystems of northern Brittany (Penzé, Morlaix, Rance, Aber Benoit and Aber Wrac'h estuaries, Fig.1) [4, 6, 7] as well as in Ireland [8]. They appear in rich-nutrient confined ecosystems, mostly estuaries, from mid-May to August, when nutrients, water temperature and irradiance support sufficient *A. minutum* growth to compensate mortality rates mostly due to tidal dilution.

Acknowledgements

The authors gratefully acknowledge the Region Bretagne which supported the Daoulex project for the in situ survey and the REPHY and VELYGER networks.

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Harmful Algae Blooms in Banderas Bay, Jalisco, México, 2013

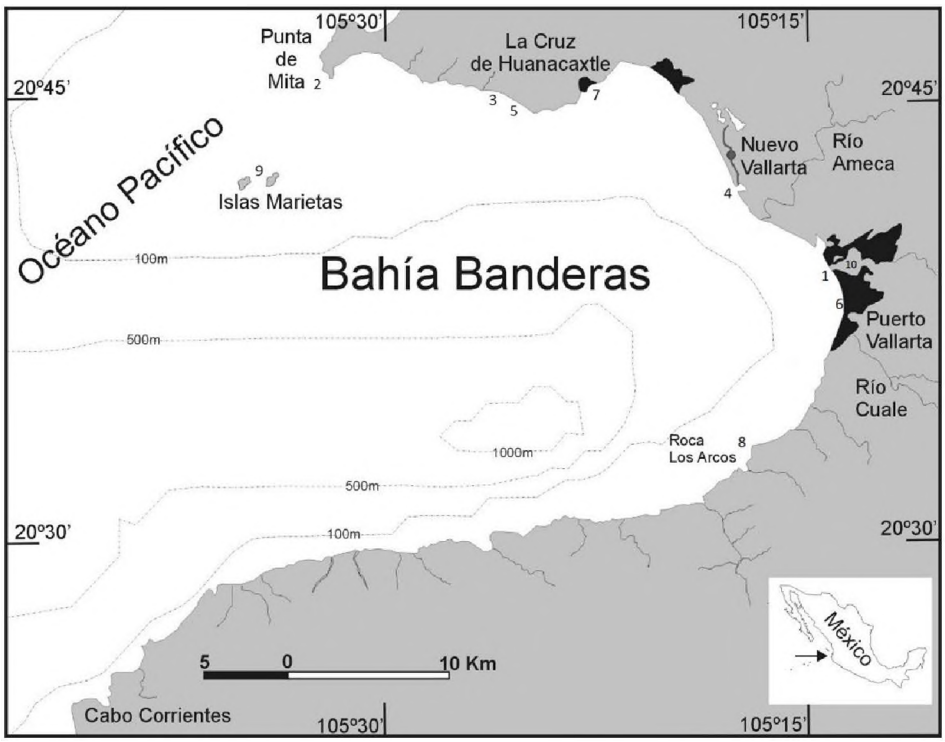

Fig. 1. Study area and sampling stations in Banderas Bay (May to November 2013).

Over the past decade Banderas Bay has been affected by the occurrence of 24 different toxic microalgal species responsible of Harmful Algal Blooms (HABs) [1]. Some of them have caused serious negative impacts to marine life, fisheries and the tourist industry in this locality where mollusks are a very important resource. Here we report data from the routine phytoplankton monitoring programme, carried out by the Centro Universitario de la Costa- Universidad de Guadalajara, during 2013. The main purpose of this HAB program is to monitor the presence and abundance of harmful algal species in Banderas Bay and surrounding areas to provide an early warning of HAB events and anticipate their adverse effect in human health and the environment.

Banderas Bay, the biggest bay in the Mexican Pacific, is on the western Mexican coast (20° 15´ - 20° 47´ N; 105° 15´ - 105° 4´ W) [4]. It has an area of ~ 975 km^2, and is limited by Punta de Mita in the north and Cabo Corrientes on the south (~40 km) (Fig. 1). Mean sea surface temperature (SST) is 26.4°C, with a seasonal range from 23.3°C during winter-spring time, to 30.0°C in summer-autumn. Located in a transitional area, the bay shows a complex hydrodynamics, with internal waves, upwelling pulses and large seasonal and interannual fluctuations. Three current systems converge there: (1) the California Current, which flows southwards bringing cool and low salinity water to the region; (2) the Costa Rica Costal Current, which flows northwards transporting warm waters of intermediate salinity, and (3) the warm dense waters from the Gulf of California, which are transported southwards into Banderas Bay [3].

Sampling areas were established, along the coast of Banderas Bay, in the inner harbor, Los Arcos, Playa Camarones, Estero El Salado, Islas Marietas, La Cruz de Huanacaxtle, Punta de Mita, Nuevo Vallarta, Destiladeras and Veneros. Surface phytoplankton samples from blooming areas were collected in March, April, May, October and November 2013 in 500 mL bottles, preserved with Lugol's solution and counted, with 100x and 400x magnification, using a 1 ml Sedgewick Rafter chamber. Temperature and salinity were measured in situ every 25 minutes using a HOBO (Pendant) data logger thermograph and a salinometer. Micrographs of the main HAB species were obtained using a digital camera.

Our results identified 10 sites where blooms were frequent. Four events causing visible seawater reddish discoloration, three during winter-spring and one during summer-autumn, were directly observed and monitored. The species involved in this outbreaks, such as the toxic dinoflagellate *Gymnodinium catenatum* (potential PSP agent), the ciliate *Mesodinium rubrum*, and the ichthytoxic dinoflagellate *Cochlodinium catenatum* (= *C. polykrikoides*), frequently associated with fish kills in Banderas Bay, are known to form mass occurrences elsewhere [4].

Gymnodinium catenatum showed maximal densities of 0.6 x10^6 cells L^{-1}, and PSP toxins exceeded the limits

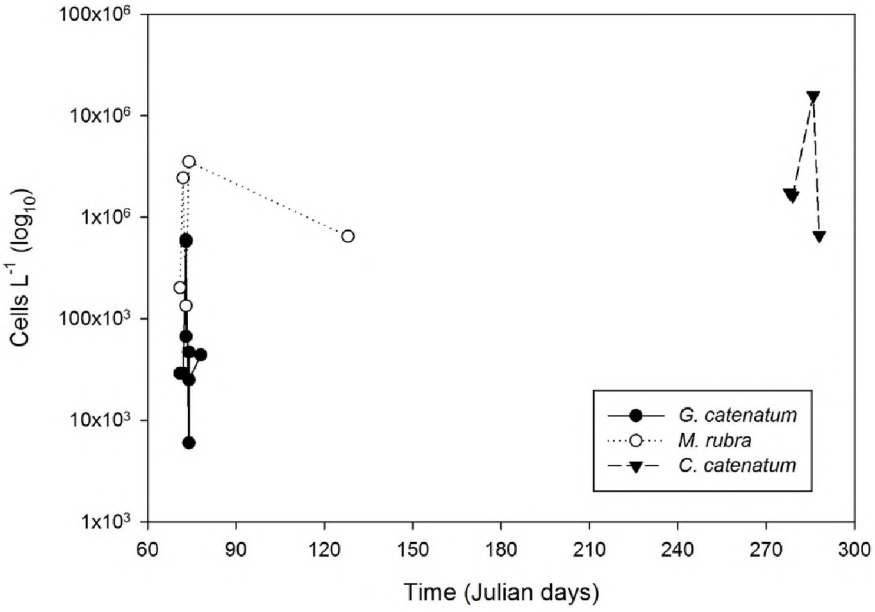

Fig. 2. Concentration of Gymnodinium catenatum, Mesodinium rubrum *and* Cochlodinium catenatum *(cells L^{-1}) in Banderas Bay Jalisco México, from March to November of 2013.*

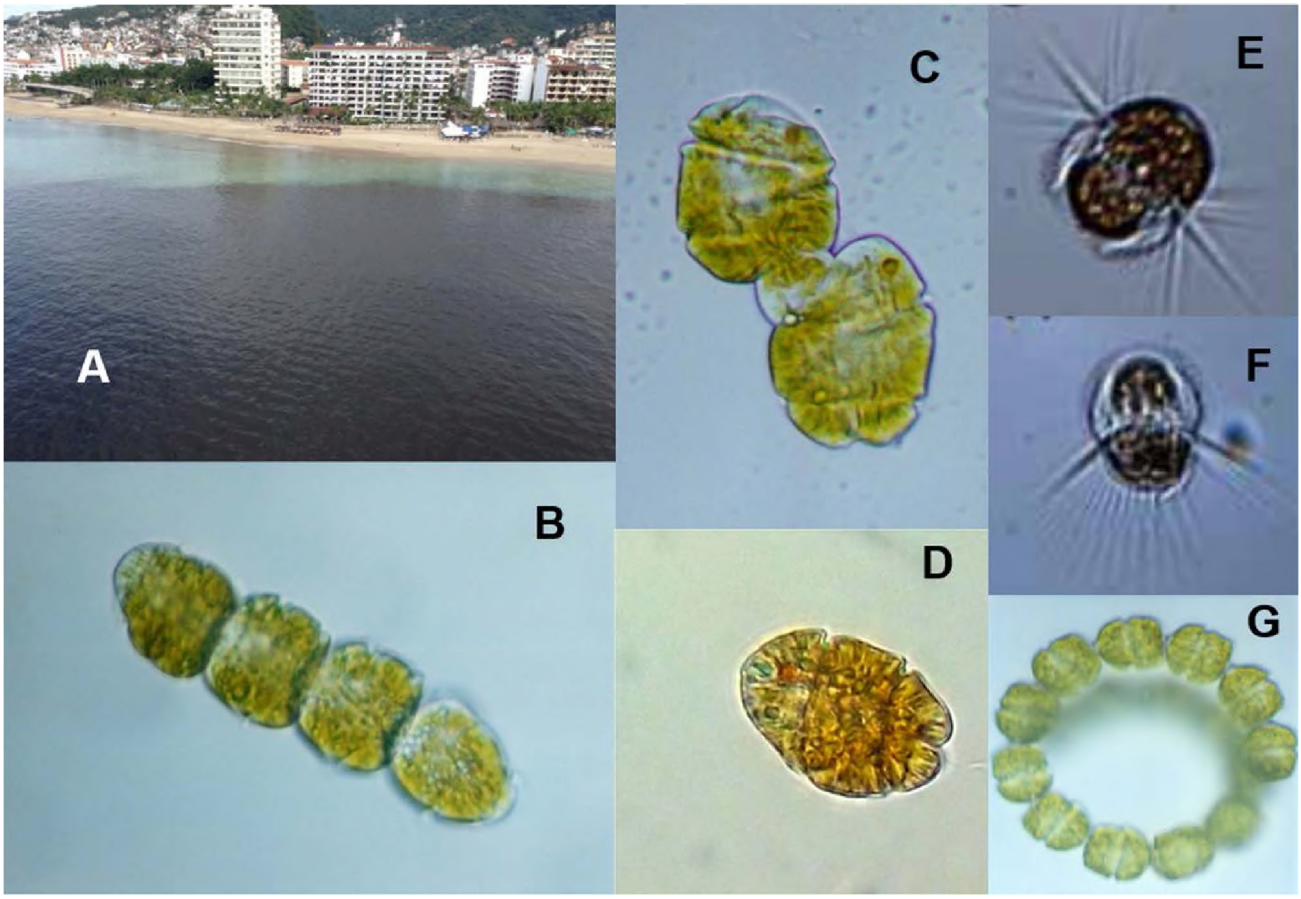

Fig. 3. Harmful microalgal species in Banderas Bay during 2013. A. Discolorations from Cochlodinium catenatum *bloom in November 14, 2013. B-C* Cochlodinium catenatum *(4 and 2 cells). E-F* Mesodinium rubrum*. G.* Gymnodinium catenatum.

established for human consumption. In consequence, COFEPRIS authority, through the formal notification advice COS/DEPE/2/OR/00066/2013 resolved to ban extraction, commercialization and consumption of bivalve mollusks coming from harvesting zones in Banderas Bay. This decision caused a hard impact on local people who make their living from these activities. Likewise, densities of *Cochlodinium catenatum*, which usually occurs after heavy rain, showed record values of 16 x10^6 cells L^{-1}.

Mean sea surface temperature records from the HOBO logger during the bloom in May was 23-24°C, whereas mean values for October-November were 29.12 to 30.03°C. Salinity values (33-35 psu) were within the data range found by other authors in this coastal area. Nevertheless, 2013 was an anomalous year concerning HABs intensity, in particular for the dinoflagellate *Cochlodinium catenatum* which exhibited very high concentrations (up to 15 x10^6 cells L^{-1}) in the coastal zone of Jalisco state throughout the year. There were no reports of affected consumers either within tourists or local residents.

Our results point out the impact of HAB species in Banderas Bay and highlights the importance of its HAB monitoring programme. This programme allows knowing the temporal and spatial distribution of blooming harmful species, providing early warning to decision-makers, such as COFEPRIS authorities, to protect public health, the tourist industry and the exploitation of marine resources.

Table 1. Main blooms reported during the study period.

Sampling date (2013)	Species	Density (cells L^{-1})	Permanence
March 17 - April	*Gymnodinium catenatum* *Mesodinium rubrum*	0.6 x 10^6 2.4-3.5 x 10^6	~20 days
May 8	*Mesodinium rubrum*	0.6 x 10^6	~7 days
October 14 -19	*Cochlodinium catenatum*	1.6 - 1.7 x 10^6	~10 days
November 4-14	*Cochlodinium catenatum*	1.6 x 10^6	~7 days

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First report of a bloom of *Pyrophacus steinii* from the South Eastern Arabian Sea

Pyrophacus Stein is the only genus of the family Pyrophacaceae Lindemann and has three species with very similar cell structure: *Pyrophacus horologium* Stein, *P. steinii* (J. Schiller) Wall and Dale and *P. vancampoae* (Rossignol) Wall and Dale. *P. steinii* cells are round, lens-shaped with an anteroposteriorly compressed theca. *Pyrophacus* species are distributed worldwide and are frequently observed in oceanic, neritic and estuarine waters. However, there is no record on their bloom formation and associated harmful effects, if any, on marine ecosystems in the World Ocean.

Phytoplankton production in the Arabian Sea (West coast of Indian EEZ), usually dominated by diatoms, is influenced by changes in physico-chemical and biological characteristics of the area determined by the two (SW and NE) monsoon cycles. Reports available on HAB events on the Arabian Sea are mainly on the occurrence of *Noctiluca scintillans* [1-3], *Gonyaulax* sp. [4], *Chattonella marina* [5-6] and *Trichodesmium erythraeum* [7-8] blooms. Here we report the first bloom of *Pyrophacus steinii* in Arabian Sea.

P. steinii occurs in very low numbers in Indian waters throughout the year.

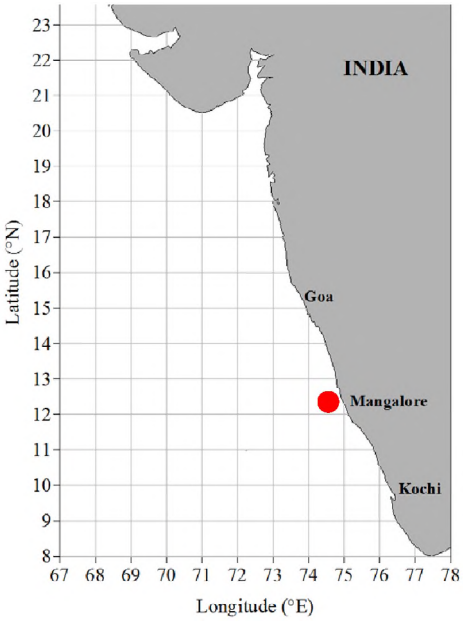

Fig. 1. Study area (red dot represents the bloom location).

A subsurface bloom of this species was observed off Mangalore (12°50.754N; 74°40.354E, Fig. 1) onboard the research vessel *Sagar Sampada* during the wane phase of summer monsoon (end of September, 2009). Hydrographic parameters (Seabird 911 plus CTD) and water samples for phytoplankton, chl *a* and nutrient analysis were collected from standard depths using Niskin bottles attached to a CTD-rosette. For phytoplankton, 30 L were filtered through a 20-µm mesh bolting silk and the samples immediately fixed in buffered formaldehyde-Lugol's iodine solution. Qualitative and quantitative analysis of phytoplankton were carried out using a Sedgwick Rafter counting chamber (Nikon Eclipse microscope) following standard identification keys; Chl *a* was determined by the standard acetone extraction method and nutrients (nitrate, phosphate, silicate) analysed using a segmented flow Auto Analyzer (SKALAR) onboard the vessel following standard procedures.

The bloom was without any noticeable surface water discolouration because the sea surface was slightly rough and the cell maximum (7.75x 10^5 cells L^{-1}) was at base of the pycnocline (10m) (Fig. 3). Almost 94% of the micro-phytoplankton population was composed of *P. steinii*, accompanied by *Chaetoceros* spp., *Thalassiosira* spp., *Rhizosolenia* spp., *Pseudo-nitzschia* sp. and *Ceratium* spp. The e round lens-shaped cells had an anteroposteriorly compressed theca with numerous golden brown chloroplasts. Dividing cysts and planozygotes were also found (Fig. 2). Vertical distribution of physical-chemical properties in the area, known to be well influenced by freshwater influx from the adjoining Nethravathi-Gurupur estuary [9], is shown in Fig. 3.

Intense upwelling during the summer monsoon brings up nutrients along the coastal waters of the South Eastern Arabian Sea through Ekman's transport [10] and high levels of nutrients may be found at subsurface layers [11]. Increased nutrient input and stability as a result of intense upwelling and more brackish waters at the surface may have favored *P. steinii* bloom formation. Mortality of fish and other marine organisms were not observed or reported from the bloom area.

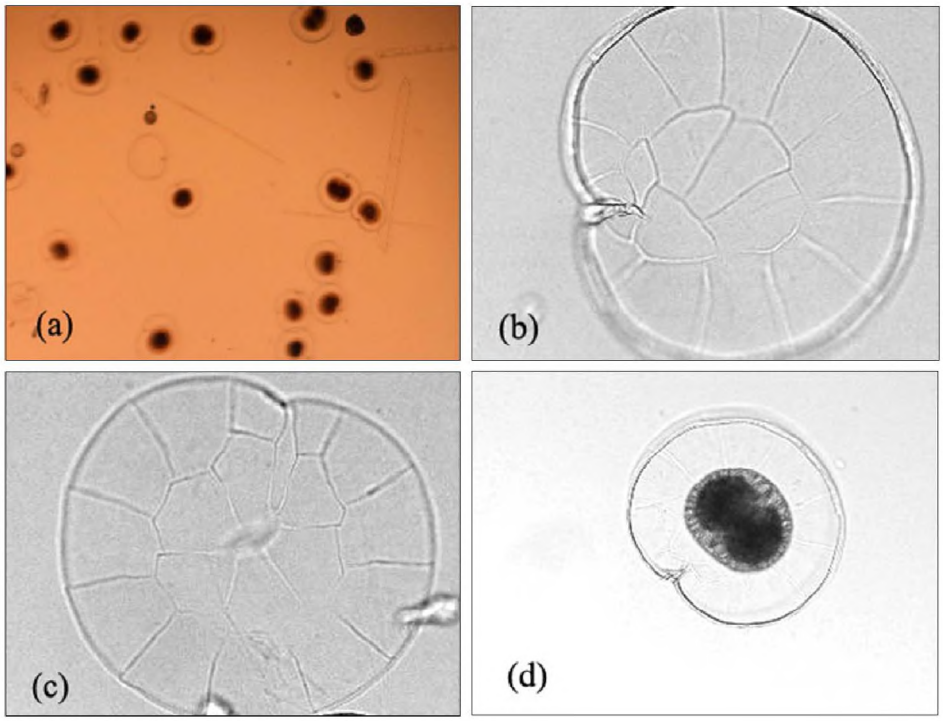

Fig. 2. Micrographs (a) Pyrophacus steinii *bloom (x40);* P. steinii *hypotheca (b) and epitheca (c) (x 400); (d) Binary fission (x 200).*

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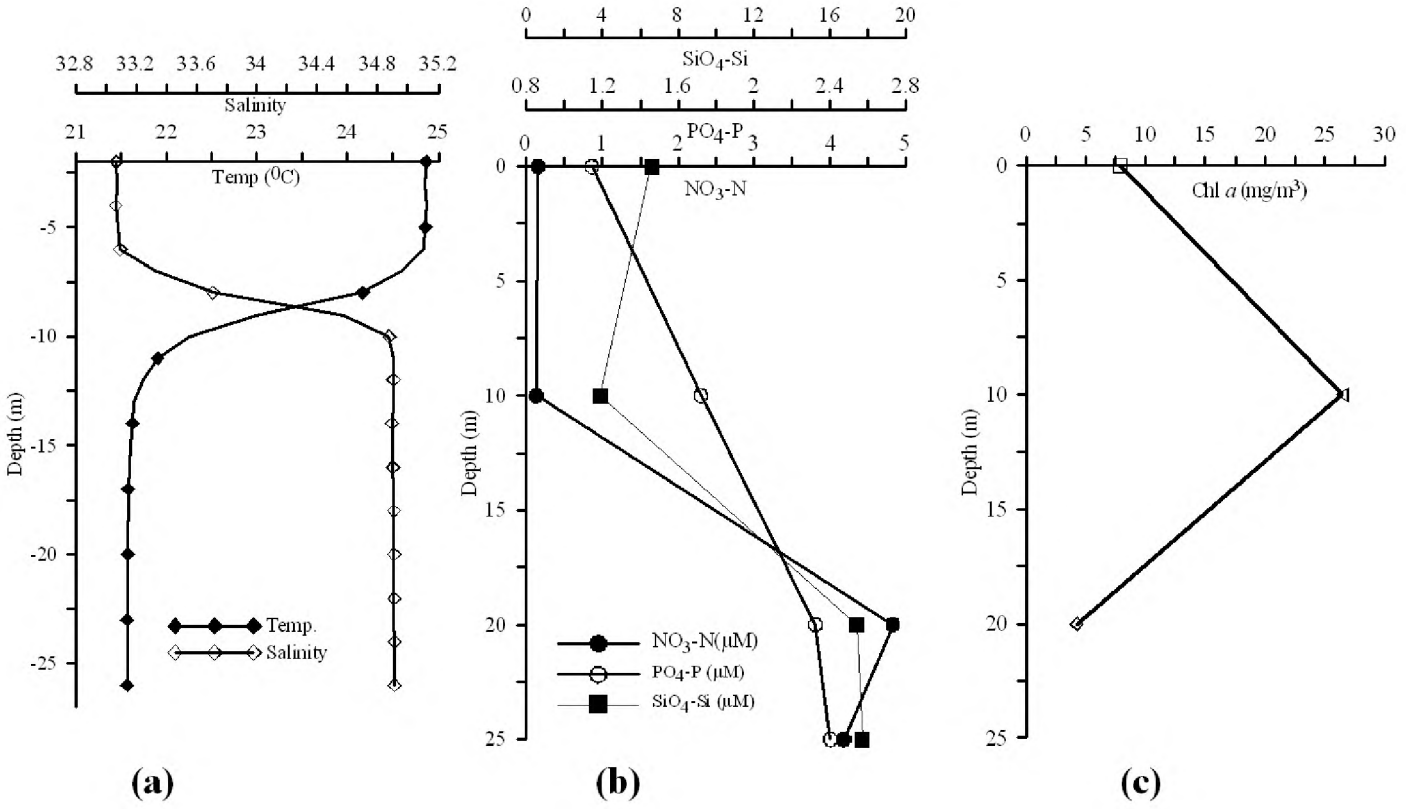

Fig. 3. Vertical distribution of physico-chemical and biological characteristics at the bloom area (a) Temperature and salinity (b) Nitrate, phosphate and silicate (c) Chlorophyll a

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Continued from page 2

and timing. Nevertheless, it is possible they were all triggered by the same environmental anomaly: the anomalous high temperatures observed in coastal waters of Uruguay during Austral summer 2015. Global warming may lead to increased frequency of this kind of events.

Acknowledgements

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French Polynesia Regional Workshop on Ciguatera Fish Poisoning field monitoring for Asia-Pacific countries. A joint IAEA, ILM, NOAA and IOC-UNESCO effort

Ciguatera Fish Poisoning (CFP) is now regarded as a potential threat on a global scale, due to the rapid development of travel, tourism, and fish trade from the tropics, as well as the expansion of the bio-geographical range of *Gambierdiscus* species and toxic fish.

Considering the major impacts of CFP on the health and economy of many countries of the Asia-Pacific region, and the need for capacity building in CFP field monitoring, the International Atomic Energy Agency (IAEA) Technical Cooperation Department organized a regional workshop at the Institut Louis Malardé (ILM), Tahiti (French Polynesia) from March 2-13, 2015 within the framework of the regional project RAS 7/026 project on "Supporting the use of Receptor binding Assay (RBA) to reduce the adverse impacts of Harmful Algae on seafood safety". This event was attended by 17 participants from China, Indonesia, Malaysia, Marshall Islands, Oman, Pakistan, Philippines, Thailand, Viet Nam, and Wallis & Futuna (sponsored by IOC-UNESCO) (Fig. 1).

The training team for this workshop included Jacob Larsen (IOC Science and Communication Centre on Harmful Algae), Patricia Tester (US NOAA-affiliate), Mireille Chinain, Taiana Darius, Clémence Gatti, André Ung (Institut Louis Malardé), Mélanie Roué (Institut de Recherche pour le Développement) and Gires Usup (University of Kebangsaan-Malaysia). The programme comprised a series of lectures coupled with field trips, hands-on training and demonstrations on the following topics: sampling techniques of benthic habitats including the use of passive monitoring devices (window screens, SPATT filters) (Fig. 2), identification of benthic HAB species using light microscopy techniques (Fig. 3), molecular approaches in dinoflagellate taxonomy and detection, isolation of benthic HAB species for culturing purposes, toxin extraction from different types of biological samples (Fig. 4) and toxicity analysis using the radioligand Receptor Binding Assay (RBA).

The training sessions were followed by a five days Project workshop led by IAEA officials Abdulghani Shakhashiro, project management officer (Technical Cooperation program, Vienna) and Marie-Yasmine Bottein, technical officer and research scientist (Environment Laboratories, Monaco), to update the work plan of the IAEA RAS 7/026 project to include new member state participants, to develop a plan for compilation and sharing of HAB event data and HAB species distribution data, and to plan in-detail field sampling of algae and fish and analysis of samples using the RBA. Frances Van Dolah (US NOAA) provided advice and guidance on the RBA work, and Patricia Tester and Mireille Chinain on field sampling of algae and fish. Henrik Enevoldsen (IOC-UNESCO) stressed the importance of HAB data compilation in HAEDAT and OBIS, and Clémence Gatti of implementing an accurate epidemiological survey of CFP towards the development of a Global HAB Status Report. This regional project and similar technical cooperation projects in the Caribbean and Africa using nuclear technics for a sustainable development of IAEA member states will lead to the provision of HAB data, basis for a first ever Global HAB Status Report.



Fig. 1. Group photo during the Tahiti island tour.



Fig. 2. Collection of window screen devices deployed in situ.



Fig. 3. Microscopy identification of benthic HAB species under the supervision of Jacob Larsen.

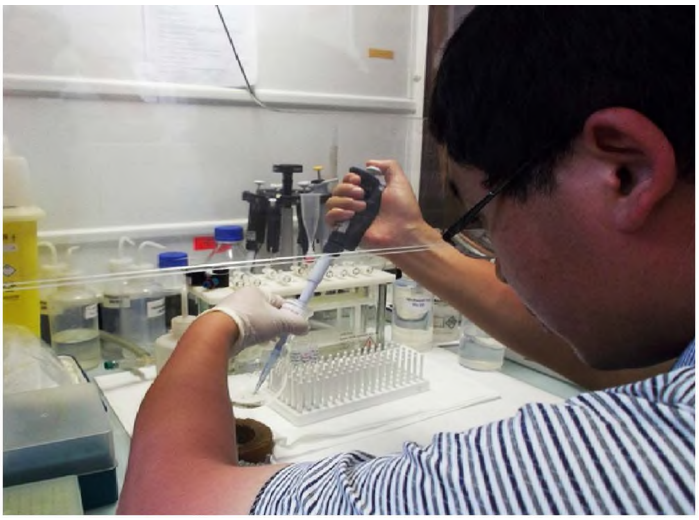

Fig. 4. Toxin extraction training.



la DÉPÊCHE de TAHITI

MALARDE LIVRE SON SAVOIR SUR LA CIGUATERA

Mercredi 4 Mars 2015

Plusieurs chercheurs étrangers venus de douze pays différents de la zone Asie-Pacifique se forment actuellement à l'institut Louis-Malardé, aux techniques de surveillance de la iguatera. (Photo: Jennyfer Rofes)



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sciences

Ciguatera : une préoccupation mondiale

Par Hélène Harte | Publié le 06/03/2015 | 15:22

Ils arrivent de 12 pays de la zone Asie/Pacifique. Ils sont chercheurs, et participent à 2 semaines de formation, organisées à l'Institut Malardé sur le thème de la ciguatera.

Fig. 5. Strong coverage of this regional event by local media.

Acknowledgements

The IAEA is grateful for the support provided to its Environment Laboratories by the Government of the Principality of Monaco.

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HABs and Climate Change: A Report of Two Workshops

Two recent workshops have focused on providing a more rigorous assessment of purported links between anticipated climate-driven changes and HABs. The first was a 5-day international workshop held in Spring 2013 at the University of Washington Friday Harbor Labs in Washington State, USA, co-organized by Mark Wells, from the North Pacific Marine Science Organization Section on Harmful Algal Blooms (PICES HAB-S) and Bengt Karlson representing the International Council for the Exploration of the Sea / Intergovernmental Oceanographic Commission Working Group on HAB Dynamics (ICES/IOC-WGHABD) and jointly sponsored by PICES, US National Oceanic and Atmospheric Administration (NOAA), and Scientific Committee on Oceanic Research/ Global Ecology and Oceanography of HABs (SCOR/GEOHAB). A focused group of internationally-recognized HAB scientists having diverse expertise that bears strongly on climate change/HAB linkages reviewed what is known and unknown about HAB/Climate linkages (summarized in the PICES Press article "Harmful Algal Blooms in a Changing World", Volume 21, Number 2, July 2013). A seminal review paper identifying the keystone parameters and research infrastructure needed to test these purported linkages is accepted pending revisions and will be published in the journal *Harmful Algae* in summer 2015. This paper titled, H*armful algal blooms and climate change: what do we know and where do we go from here?* is authored by Mark L. Wells, Vera L. Trainer, Theodore J. Smayda, Bengt Karlson, Charles G. Trick, Raphael M. Kudela, Akira Ishikawa, Stewart Bernard, Angela Wulff, Donald M. Anderson, and William P. Cochlan.

The 2nd stage of assessment of an exploration of the links between climate change and HABs was a broader International Scientific Symposium on HABs and Climate Change that was held from May 19-22, 2015 in Gothenburg, Sweden, jointly sponsored by ICES, PICES, the Swedish Research Council (FORMAS), SCOR/GEOHAB, the Swedish Meteorological and Hydrological Institute, and the University of Gothenburg, and endorsed by IOC and ICES. This symposium brought together 58 participants from across the globe to discuss how to approach the formidable challenge ahead of ultimately providing a "market forecast" for changes due to climate impacts on HABs. The symposium participants considered what was known about the influences of environmental changes (e.g. nutrient fluxes, ocean acidification, temperature, stratification) on the character and magnitude of primary producers (including HABs) that may result in large-scale changes in HABs. The most likely of these included latitudinal range expansion of warm water HAB species at expense of cold-water species, and changes in seasonal windows of HAB outbreaks. However, substantial knowledge gaps on these and other potential effects prevent any clear assessment at present. Participants also worked to identify the key steps moving forward to improve understanding of HAB effects on fisheries and to develop forecasting abilities of HABs and other disruptive plankton systems. The symposium findings will



The Friday Harbor workshop group: (L to R), Stewart Bernard, Donald Anderson, Vera Trainer, Angela Wulff, Charles Trick, Bengt Karlson, Ted Smayda, Raphe Kudela, Mark Wells, Akira Ishikawa, William Cochlan.



Participants of the HABs and Climate Change conference in Göteborg, Sweden from May 19-22, 2015

be published as a PICES Press article, in a Special Report in the journal *Harmful Algae*, and a short synthesis document in a high-profile journal is being pursued.

There are key knowledge gaps in the underpinning of the intrinsic resilience and vulnerability of marine ecosystems to natural and anthropogenic forcing, how ecosystems of today in the future will respond to natural and anthropogenic forcing, and ultimately how societies will be affected by these altered ecosystems. It is reasonable to argue that the most uncertain of these gaps is constraining how planktonic systems shift from strongly sustaining to disruptive modes. To this end, several international organizations (see below) are continuing their efforts to coordinate observations and trend analysis of plankton data, and link these to climate change science, and to contribute to the newly developing United Nations Educational, Scientific, and Cultural Organization of IOC (UNESCO-IOC) Global HAB Status Report. The Scientific Steering Committee of the newly formed GlobalHAB, the replacement for GEOHAB, GlobalHAB, is being formed, It will include expertise on freshwater HABs, benthic HABs, satellite, observation systems, ecology, oceanography, toxins, human health links, economic links, and will have representation from all regions. Early discussions with IOC delegates have emphasized that PICES needs representation on the small executive committee to ensure a collaborative functioning with global committees that share similar aims, including PICES, IOC, SCOR, Intergovernmental Oceanographic Commission Climate Group (IOCCG), International Atomic Energy Agency (IAEA), and ICES.

Major partners contributing to the strategic focus to this international effort include Intergovernmental Panel on Harmful Algal Blooms (IPHAB), ICES, PICES, Oslo/Paris Convention for the Protection of the Marine Environment of the Northeast Atlantic (OSPAR), Helsinki Commission (HELCOM), Global Ocean Observing System (GOOS) and national and local programs including seafood safety monitoring and phytoplankton monitoring programs. Although long considered more of a unique threat to human health, HABs now are recognized to be a component of a broader array of disruptive plankton systems that influence and shape coastal and oceanic ecosystems. Greater coordination of HAB and Climate Science research will underpin not only the protection of public health in the future oceans, but also greater forecasting of climate change effects on wild and aquaculture fisheries and the broader sphere of ocean health.

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The 2015 meeting of the ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

WGHABD met from 13th – 18th April in Lisbon, Portugal. The meeting was hosted by Teresa Moita, Portugal and chaired by Eileen Bresnan, UK. It was attended by 19 scientists from 11 countries with three scientists from an additional two countries participating by correspondence. The report of the working group is now online and can be found at *http://www.ices.dk/community/groups/Pages/WGHABD.aspx.*

The group addressed 12 terms of reference (ToR) relating to harmful algal bloom dynamics. Members presented national reports on HABs during 2014 (ToR a). Highlights include the first report of concentrations of toxins responsible for paralytic shellfish poisoning (PSP) above the EU closure limit in *Mytilus edulis* Sweden and amnesic shellfish poisoning (ASP) in *M. edulis* in England. Closures of shellfish harvesting areas as a result of high concentrations of the toxins responsible for DSP were enforced in many countries in Europe and also in the USA. Cyanobacteria blooms were recorded in Poland, Finland and Sweden. A bloom of *Concinodiscus conncinus* produced nuisance slimes in Norway. ToR (d) examined the threshold/trigger limits for cell counts of shellfish toxin producing species/genera in phytoplankton monitoring programmes which vary throughout the ICES area. ToR (e) presented new findings on biosensors (Environmental Sample Processor, Imaging Flow Cytobot) in the USA, citizen science and HABs in France, *Vicicitus globosum* in Sweden and modelling *Karenia mikimotoi* in Scotland. Tor (h) reviewed preparations for the ICES-PICES-IOC climate change and HABs symposium in Gothenburg in May 2015. ToR (i) examined molecular methods for the identification of HAB species. Methods discussed included FISH, qPCR, and metabarcoding. ToR (k) addressed the physical and chemical control of different HAB species. This year focussed on the dynamics of *Gymnodinium catenatum* in the Iberian Peninsula. The role of upwelling and cyst beds were included in discussions to explain the variability of this species in this region. ToR (j) reviewed the current status of β-N-methylamino-L-alanine (BMAA) and clarified the confusion between discrepancies in the literature.

ToRs (g) and (l) reviewed the use of HAB/nuisance species for the EU Marine Strategy Framework Directive (MSFD) and JAMP phytoplankton monitoring guidelines. An advice drafting group met at ICES in Copenhagen during June 2015 and incorporated the comments from WG HABD along with other WGs to produce an advisory document which was submitted to OSPAR. Three ToRs contributed towards the work of the IOC Intergovernmental Panel on HABs (IP-HAB). ToR (b) will produce a review of fish killing algae in the ICES area. This manuscript will be completed during the 2015 with a focus on northern Europe. As part of ToR (c) the WG held a one day workshop focussing on the Harmful Algal Event database (HAE-DAT) (*http://haedat.iode.org/*) on Apr 15th. This data is planned to be included in a Global HAB Status Report to be produced by IP-HAB. This workshop flagged issues with historic data to be addressed by group members before the 2016 WG meeting and prioritised issues for computer programmers to address to facilitate data extraction. ToR (f) addressed the production of a HAB status report which could act as an ICES contribution towards the IP-HAB Global HAB Status Report as well as a supporting document for the MSFD. During 2016 the WG HABD meeting will be hosted by Raeffele Siano in IFREMER, Brest, France from 19th -22nd April.

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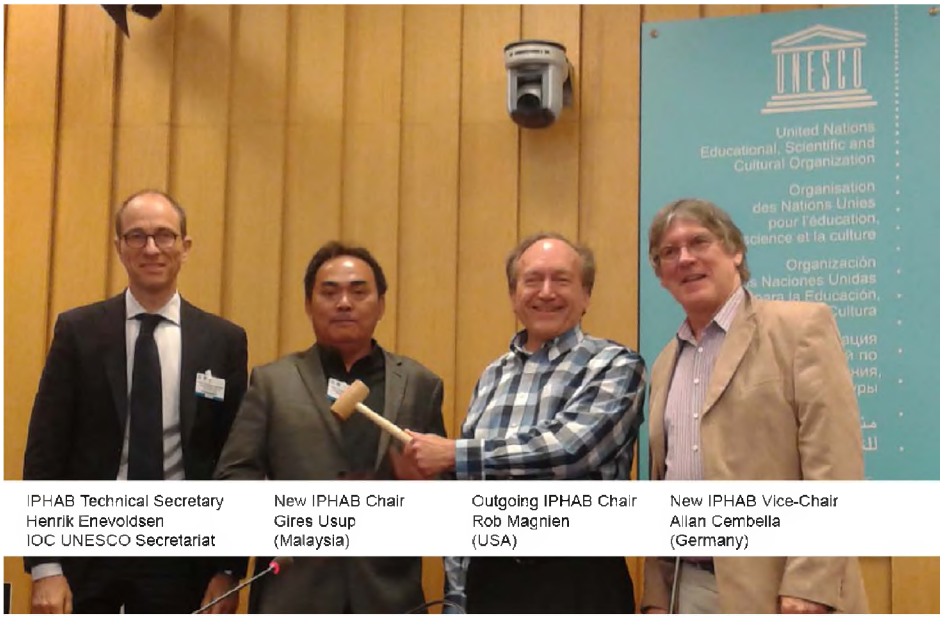
Participants at the ICES-IOC WGHABD meeting, IPMA, Portugal, 2015

XII Session of the Intergovernmental Panel on Harmful Algal Blooms (IPHAB)



IPHAB Technical Secretary Henrik Enevoldsen IOC UNESCO Secretariat | New IPHAB Chair Gires Usup (Malaysia) | Outgoing IPHAB Chair Rob Magnien (USA) | New IPHAB Vice-Chair Allan Cembella (Germany)

Rob Magnien (USA), IPHAB Chair (2011-2015), hanging over responsibilities to the new Chair, Gires Usup (Malaysia) and Vice-Chair Prof. Allan Cembella (Germany). 12th IPHAB meeting,

An Intergovernmental Panel on Harmful Algal Blooms (IPHAB) under auspices of IOC UNESCO has for more than 20 years provided the international framework for regional and global coordination and prioritization of capacity development, research and strategic initiatives on HAB.

The IPHAB met for its 12th session at UNESCO Headquarters, Paris, from 28 to 30 April 2015. The Panel reviewed the many activities and results completed during 2013-2015 and noted the continued broad engagement of the science and management community in activities.

The major achievements presented at IPHAB include: (i) the integrative synthesis of GEOHAB accomplishments; (ii) development of a research and monitoring strategy for Ciguatera; (iii) implementation of more than twelve training courses and training-through-research projects; (iv) continued development of the IPHAB-IODE Harmful Algae Information System; (v) continued publication of the IOC Harmful Algae News; (vi) convening an international conference on HABs and Desalination of Seawater; (vii) development of regional activities within ANCA, FANSA, HANA, WESTPAC-HAB and WESTPAC-TMO; (viii) supporting the work of the ICES-IOC WGHABD and ICES-IOC-IMO WGBOSV; and ix) ensuring the continued vital partnerships with SCOR, ICES, PICES, IAEA and ISSHA.

The IOC-coordinated HAB Programme is the only global intergovernmental effort to understand, manage and mitigate the harmful effects of algal blooms, and has therefore maintained a critical leadership role via IPHAB. Particularly noteworthy is the progess on inter-sessional IPHAB activities, including efforts by Task Teams to maintain an updated taxonomic list of HAB taxa and revisions to the nomenclature, identification and structures of novel phycotoxins, and associated toxicological effects. Combined with IPHAB-sponsored initiatives on mapping biogeographical information on HAB events, these databases are essential to sustain both research and monitoring strategies.

The IPHAB is recognized internationally as more than a static repository of HAB data with an event-reporting mandate. The Panel has shown flexibility and adaptive capacity since its inception, modifying its functions and tasks to address emerging global priorities for ocean research and monitoring as defined by IOC. The IPHAB has aligned its priorities to address the four IOC overarching themes: i. Healthy Ocean Ecosystems; ii. Early Warning for Ocean Hazards; iii. Climate Change Adaptation and Mitigation and iv. Enhanced Knowledge of Emerging Science. Exciting new elements of HAB research have benefited immensely from the recognition by IPHAB of the importance of linkages to ocean observational systems and the effects of global change.

The Panel made decisions for priorities 2016-2017 for international HAB activities to be implemented by the IOC UNESCO and IPHAB partners. The Decisions concern (i) HABs in a Changing World: '*A Global Approach to HAB Research to Meet Societal Needs: GlobalHAB*' (jointly with SCOR); (ii) continued strong regional HAB Programme development; (iii) formation of two new Task Teams, on a Global Strategy on Ciguatera for Improved Research and Management and, on Harmful Algae and Desalination of Seawater; (iv) revised terms of reference for IPHAB Task Team on the development of a Global HAB Status Report, Task Team on Biotoxin Monitoring, Management and Regulations, Task Team on Algal Taxonomy, and a Task Team on Harmful Algae and Fish Kills.

Dr. Gires Usup (Malaysia) was elected as new Chair and Dr. Allan Cembella (Germany) was elected as new Vice-Chair.

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Marine Institute
Foras na Mara

Proficiency testing on the abundance and composition of marine microalgae

Scientists have come under increased pressure in recent years to show that results they obtain arising from their scientific work are quality assured and stand up to scrutiny by independent expert auditors. This has meant that the methodologies used by laboratories involved in making these measurements have to be validated and fit for purpose and has led to the adoption of internationally recognised standard protocols.

These protocols must be underpinned by robust quality systems and must be accredited to an international standard. In order for laboratories to become accredited in particular methods, they have to fulfil a series of prerequisites but a compulsory one is the participation in a proficiency testing scheme. Proficiency testing schemes are independent assessor organisations which coordinate regular inter-calibration and inter-comparative studies between laboratories with a common purpose.

Microalgae are a very important ecological component of the marine ecosystem and have also become important ecological indicators of hydroclimatic change, ocean acidification and eutrophication. Member states of the European Union are obliged to monitor for toxic and harmful algae which can cause problems and devastation in the natural environment, have detrimental effects on human health if contaminated fish and shellfish are eaten, can cause huge economic losses to the aquaculture industry and impact directly in coastal communities.

The Marine Institute, Ireland under the BEQUALM/NMBAQC umbrella from 2005 and in close collaboration with the IOC Science and Communication Center on Harmful Algae, Denmark since 2011 has been producing an annual intercomparison exercise on the abundance and composition of marine microalgae using the Utermöhl cell counting method (Utermöhl et al., 1931, 1958). This collaboration involves the elaboration of a marine phytoplankton taxonomy quiz using an online platform called 'Ocean Teacher' (*http://classroom.oceanteacher.org/*) and the production of samples containing marine phytoplankton species of interest that analysts must identify and enumerate. The exercise is designed to test the ability of analysts to identify and enumerate correctly these organisms. In 2014, 64 analysts from 40 laboratories around the world took part in this intercomparison. The reports of these intercomparison exercises can be found and downloaded from *www.nmbaqcs.org.*

Also, as part of every intercomparison exercise, a training workshop is held annually to discuss the results of the intercomparison exercise and to provide training in some areas of interest on phytoplankton taxonomy to the participants. This workshop is a 2-3 day training workshop and was held in Denmark the 2-4 Dec in 2014.

But first a little bit of the history of the scheme may be helpful. The Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) project, funded by the European Union through the Standards, Measurements and Testing programme of the European Commission, was initiated in 1998 (*www.bequalm.org*). This was in direct response to the requirements of the Oslo-Paris Commission (OSPAR) to establish a European infrastructure for biological effects quality assurance and quality controls (QA/QC) for laboratories contributing to national and international marine monitoring programmes to attain defined quality standards. The original project consisted of twelve work packages (WP) processed by nine partner experts in measurements on biological effects and monitoring techniques, the phytoplankton component (WP 11) was entrusted to the Forschungs und Technologiezentrum Westkuste (FTZ) at Kiel University, Germany. They carried out two interlaboratory comparison exercises or ring trials in 2000 and 2001 (Reckermann and M., Colijn, F., 2000, 2001). These were the first intercomparison exercises in the enumeration and identification of microalgae at European level.

After the 2001 ring trial, there were no more phytoplankton intercomparisons at this level from FTZ. In 2003, INTECMAR in Galicia, Spain, organised a phytoplankton ring trial worldwide. This intercomparison was set up in 2003 under the name Iberia 2003 and the results were published in the proceedings of the IX Iberian meeting on toxin phytoplankton and biotoxins in 2007, Spain (Pazos *et al.*, 2007).

Also, in 2003, BEQUALM adopted the UK NMBAQC (National Marine Biological Quality Control Scheme)



www.nmbaqcs.org as a model to progress the Community Analysis component which included phytoplankton analysis and launched the self-funded programme (participants pay a fee) in 2004. The BEQUALM phytoplankton work package initially comprised two parts: chlorophyll analysis and community analysis. The former was taken forward by QUASIMEME, whilst from 2005, the latter has been re-launched (initially for UK/Eire participants), through the BEQUALM/NMBAQC Scheme via the Marine Institute at Galway.

The participation in this type of scheme is becoming an essential requirement for National phytoplankton monitoring laboratories in order to achieve accreditation to international standards and also to support and assure the quality of their results. Many difficult decisions in policy are made based on quantitative or qualitative measurements made by scientists. It is a good working practise that these results contain some indication of their quality. Since 2008, the participation of individual analysts on the scheme is certified by issuing statement of performance certificates to each participant. The Marine Institute phytoplankton laboratory is accredited to ISO 17025 for marine phytoplankton identification and enumeration since 2005, and it recognizes that regular quality control assessments are crucial to ensure a high quality output of phytoplankton data.

This exercise and workshop has become an important forum for scientists working on phytoplankton monitoring programmes from around the world to convene and be able to discuss taxonomical matters related to monitoring, methodologies and techniques, new advances and finds, taxonomical nomenclature changes, looking at samples from different geographical regions and listen to relevant stories from other laboratories about issues regarding harmful algal events in their areas of great ecological importance.

Registration to this intercomparison exercise is done through the Marine Institute, (*Rafael.salas@marine.ie*). All the information regarding this scheme including past reports and workshop agendas can be found and downloaded from the *www.nmbaqcs.org* website and information on the new rounds of the exercise is also posted in the IOC Harmful algal bloom programme page under training *http://hab.ioc-unesco.org/*

The 2015 exercise is currently underway. If you are interested, keep an eye on the links above or e-mail me and I will add you to our distribution list of intercomparison participants.

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Sigma-Aldrich and Cawthron Institute align to supply Harmful Algal toxins worldwide

Pectenotoxin-11 is the 34-hydroxy analogue of pectenotoxin-2. In fact, at position 34, PTX-11 is chiral and so the additional hydroxyl can be left or right handed. Only the left handed S variant is produced by *Dinophysis acuta*. These subtle chemical realities may or may not be relevant to the biology, ecology and toxicity of Harmful Algal Bloom (HAB) species.

It is difficult for scientists from a range of disciplines to gain access to well characterised pure algal toxins, such as PTX-11, and so the relevance of these important molecular details can be difficult to investigate. The Cawthron Institute aims to make a wider range of toxins available to a global market through a partnership with Sigma-Aldrich to supply algal toxins via their renowned catalogue *www.sigmaaldrich.com*



Fig 1. Andy Selwood, Cawthron Institute

The partnership will focus on providing easy access to a wide range of previously rare and hard to source compounds for use in analysis, toxicology, ecology and other previously impossible to perform studies. The project is the result of improvements in the ability to isolate large quantities of these compounds from micro-algae. Andy Selwood (Fig. 1) is the scientist leading this work and further information can be obtained from Sigma Aldrich.

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ISSHA's Corner

Dear ISSHA colleagues:

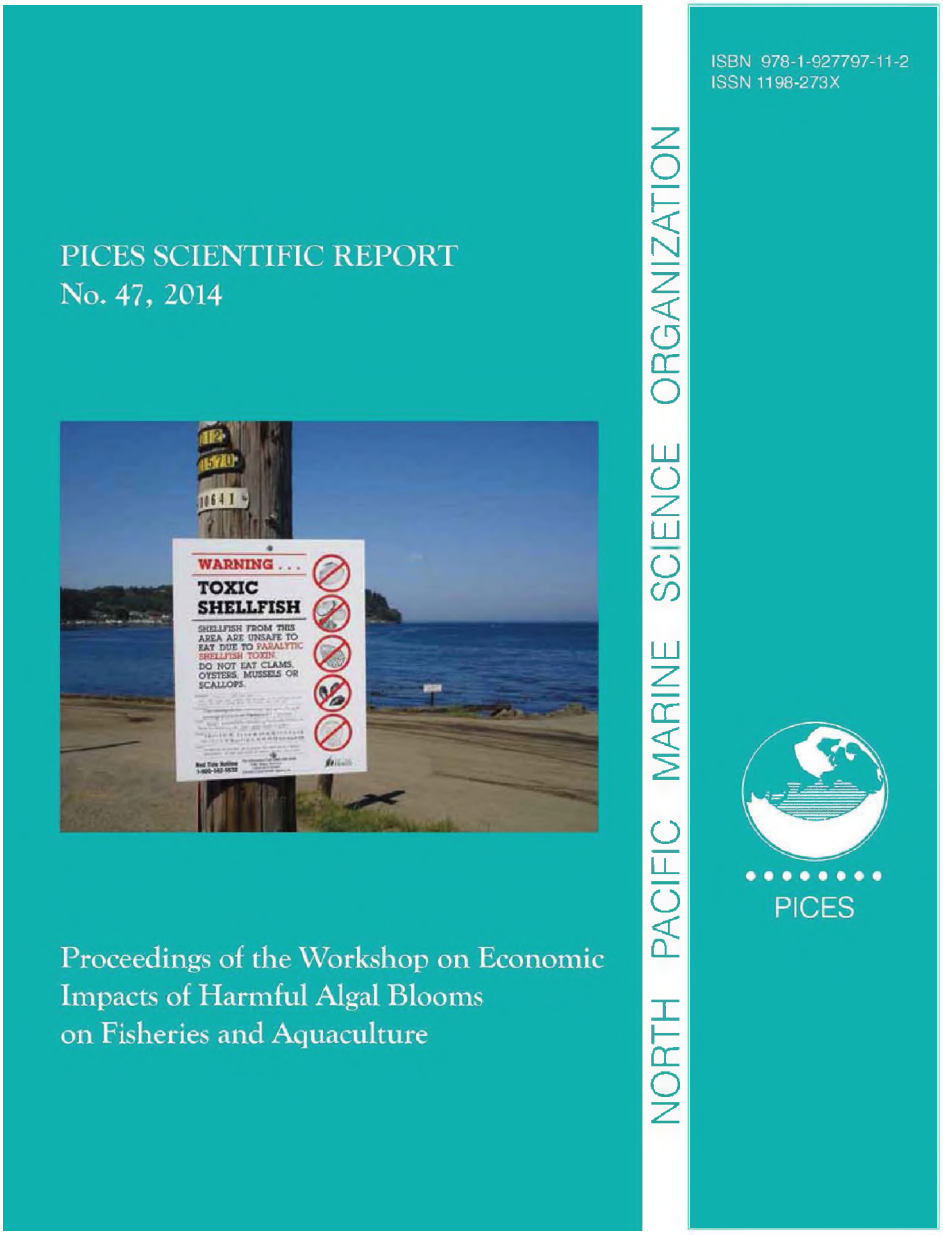
The ISSHA Council is pleased to present the new ISSHA logo! We have tried to incorporate some of the unique features of our past logo, but also to give it a fresh look. In the future, we hope to be able to provide some merchandise to purchase featuring the new logo.

In this HAN issue, you will find an article about a recent international conference in Göteburg, Sweden on HABs and Climate. Look for an article in the journal Harmful Algae that summarizes what we know, what we don't know, and the pathway to the future for HABs and climate change research. We hope that this will provide food for thought and encourage collaborations across the globe to enhance our knowledge on HABs and Climate Change.

As the HAB Section co-chair of the North Pacific Marine Science Organization (PICES), I would also like to bring your attention to a recent Special PICES Scientific Report, "Proceedings of the Workshop on Economic Impacts of Harmful Algal Blooms on Fisheries and Aquaculture" edited by Trainer, V.L. and Yoshida, T. 2014. PICES Sci. Rep. No. 47, 85 pp. This report can be downloaded from *http://pices.int/publications/scientific_reports/* Click on Report No. 47.

Don't forget that the ISSHA website is an important resource for job ads, future workshops and courses, HAB publications, past issues of Harmful Algae News and much, much more. Please visit it at *www.issha.org.*

Vera Trainer. President of ISSHA





Prof. Youssef Halim

(1925 – 2015)

We are sad to communicate that Prof. Youssef Halim (Oceanography Department, Alexandria, Egypt) passed away on Monday 18 May 2015. He was the most senior Oceanographer, a graduate from the first promotion who received its 2-years Diploma from the Oceanography Department, Faculty of Science, Alexandria University in 1950. In 1952, Y. Halim was awarded a French scholarship and moved to the "Station Zoologique" of Villefranche-sur Mer in southern France. Under the supervision of Prof. G. Tregouboff and Prof. L. Fage, he began working on his doctorate thesis, A Quantitative and Qualitative Study of the Dinoflagellates of the Bay of Villefranche. He defended his thesis at the University of La Sorbonne, Paris, in July 1956 and was awarded the degree of "Docteur ès Sciences" with the grade of Very Honourable "avec les felicitations du Jury".

Prof. Halim served as Vice-Dean of the Faculty of Science for Graduate Studies, a position he occupied from 1976 to 1982. He was the National Director and Principal Investigator for the UNESCO/UNDP Project for Aquatic



Environmental Pollution in Alexandria, 1980-1986. He won the UNEP's Global 500 Award in 1997. He published his first paper on this estuarine environment "Observations on the Nile bloom of phytoplankton in the Mediterranean" in 1960. Prof. Halim was the first scientist who studied Harmful algae in Egypt. He described several new species: *Peridinium tergouboffi* 1955, *Histoneis imbricata, H. rampii* 1960, *Ceratium egyptiacum* 1963, *Amphisolenia sigma* 1965, *Peridinium symmetricum* 1968 and *Protoperidinium elipticum* 1969. But the most important one, *Alexandrium minutum* n. gen, n. sp., a dinoflagellate of worldwide concern as a harmful microalgae, was isolated and described in 1960. Several other papers were to follow on the same subject. His last work, in 2012, was concerning benthic harmful dinoflagellates *Ostreopsis* spp. in the Egyptian Mediterranean waters.

Prof. Halim was a member of UNESCO Consultative Panel on Coastal Marine Ecosystems since 1978 (Biennial Workshops) and of GOOS-HOTO Panel. He was also a member of ISSHA (International Society for the Study of Harmful Algae) with many friends in the HAB community around the world. He was the key person to facilitate the establishment of the IOC-HANA Regional Group and its first Chair, from 2007-2012.

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HAB Dissemination

Scientific dissemination is an issue of outmost importance. More than ever, there is a need to communicate our research to a general audience and give back to society some of the resources it has placed at the disposal of the science system.

In 2011 I decided to open a popular science blog, **FITOPASION**, to share the most curious stories I learnt during my career. They do always share a common thread: the fascinating world of microalgae. *Naked or thecate, noxious or not,* all these astounding creatures have their place on this website.

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Coming deadlines are:
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15 November 2015, for HAN 54

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Clinical Use of Paralytic Shellfish Toxins: First evidence of Neosaxitoxin as a long-acting pain-blocker in Bladder Pain Syndrome

The European Society for the Study of Bladder Pain Syndrome (ESSIC) and the International Consultation on Incontinence (ICI) defines Bladder Pain Syndrome (BPS) as a "chronic pelvic pain, pressure, or discomfort of greater than 6 months duration perceived to be related to the urinary bladder, accompanied by at least one other urinary symptom, such as the persistent urge to void urine frequently" [1]. BPS is not a life-threatening illness, but it has recently been acknowledged as a major health issue which seriously affects patients' quality of life, and is often accompanied by sleep and depressive disorders, anxiety and recurrent urinary tract infections. Consequently, ordinary daily activities are usually avoided.

Neosaxitoxin (neoSTX) is a phycotoxin whose molecular mechanism of action shows a reversible inhibition of voltage-gated sodium channels at the axonal level. Consequently, their main physiological effect is linked to impeding both nerve impulse propagation and neuronal transmission over the neuromuscular junction. When it is locally applied there are two clinical outcomes: the control of pain (anesthetic activity) and the control of muscle hyperactivity (relaxing effect) [2].

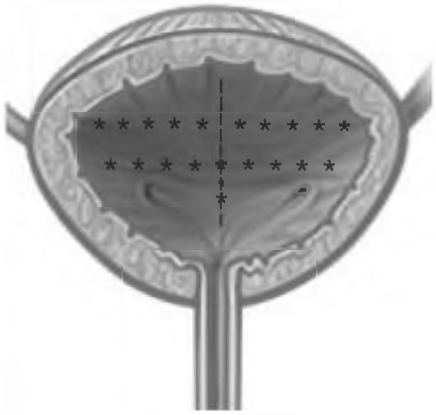

Fig 1. Cystoscopic infiltration protocol model. Cystoscopy was performed to all patients Infusion height was approximately 60 cm above the symphysis pubis. As show in the figure, twenty infiltration points were performed in the bladder submucose. Paint was totally blocked.

A study was designed to evaluate the clinical efficacy of neoSTX as a long-lasting pain blocker in the treatment of BPS. The infiltration was performed via cystoscopy, under spinal anesthesia. Questionnaires were applied immediately before and 7, 30, and 90 days after the procedure to measure the patients' reported pain severity and quality of life. All patients responded successfully to treatment. The analgesic effect lasted for the entire 90 days of follow-up, without the need for a second infiltration. NeoSTX infiltration was shown to be a safe and effective intervention to control pain related to BPS. It was tolerated well by patients who experienced extended pain relief and associated beneficial effects over the 90 days of follow-up. The present findings demonstrate that neoSTX is an innovative new long-acting local pain blocker for BPS with singular potential clinical use [3]. Until now, Neosaxitoxin and Gonyautoxins [4,5] have only been used in clinical trials at the Hospital Clínico de la Universidad de Chile in Santiago, where a pioneering collaboration between basic science investigators and clinicians has demonstrated the therapeutic properties of these biotoxins during a local infiltration are both effective and safe [2].

The paper "First evidence of neosaxitoxin as a long-acting pain blocker in bladder pain syndrome", published by the International Urogynecology Journal this year (DOI: 10.1007/s00192-014-2608-2) was recommended in F1000 Prime as being of special significance in its field.

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HAB genera identified on archival samples from the Continuous Plankton Recorder Survey

Long-term datasets are becoming increasingly relevant in predicting change in marine systems. Many institutes hold archived samples that could provide additional data on species identity and long-term patterns through molecular DNA analysis. However many samples are preserved in ways that are refractory to DNA analysis e.g. formalin or even Lugol's iodine. SAHFOS holds a physical marine plankton archive from the Continuous Plankton Recorder (CPR) survey dating back to 1960 (Fig. 1) [1]. The CPR survey records around 250 phytoplankton entities from offshore waters by light microscopy (LM) as well as a Phytoplankton Chlorophyll index (PCI) – a proxy for phytoplankton. More information can be found at *http://www.sahfos.ac.uk/*. Usually larger plankton cells are captured because of the relatively large mesh size (270 µm), however smaller plankton have been observed especially when phytoplankton is abundant. As a trial to see if smaller plankton could be identified, molecular methods were used to analyse two smaller non-toxic variants of harmful algae species in the Northern North Sea (from Aberdeen in the North East of Scotland to the Shetland Islands – Fig. 2) from 2012-2014; the diatom *Pseudo-nitzschia delicatissima* (23-76 µm length) and the non-PSP toxin producing dinoflagellate *Alexandrium tamarense*, ranging between 22 - 51µm. *P. delicatissima* was recorded by presence or absence [2], whereas *A. tamarense* was also quantified [3]. Both these species are impossible to identify to species-level by LM methods. *P. delicatissima* and similar looking/cryptic species are grouped into the *P. delicatissima* type complex, whilst species within the historic *Alexandrium fundyense/tamarense/catenella* complex are indistinguishable by light microscopy. The CPR survey does not record *Alexandrium* species at all.

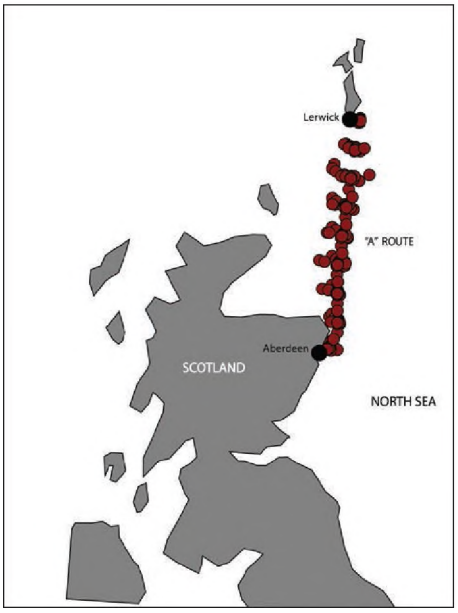

Fig. 2. Map of the "A" route in the North Sea towing the CPR from Aberdeen (north east Scotland) to Lerwick (Shetland Islands) over three years from 2011. Each red dot represents one sample. The shipping route may vary slightly from year to year

Our molecular results showed that both species were identified to variable degrees (see Fig. 3). *A. tamarense*, a widespread European variant as described by [4] was a non-bloom forming population with a near consistent presence at lower quantities. Earlier reports showed a low-moderate presence of the *Alexandrium* genus in this area [5]. By contrast, *P. delicatissima* was seasonal and most frequent in the autumn months of 2012 and 2014,

Continued on page 7



Fig. 1. A CPR Mark II device deployed from ships of opportunity. Left image an empty internal cassette. Right image internal cassette showing silk with captured phytoplankton

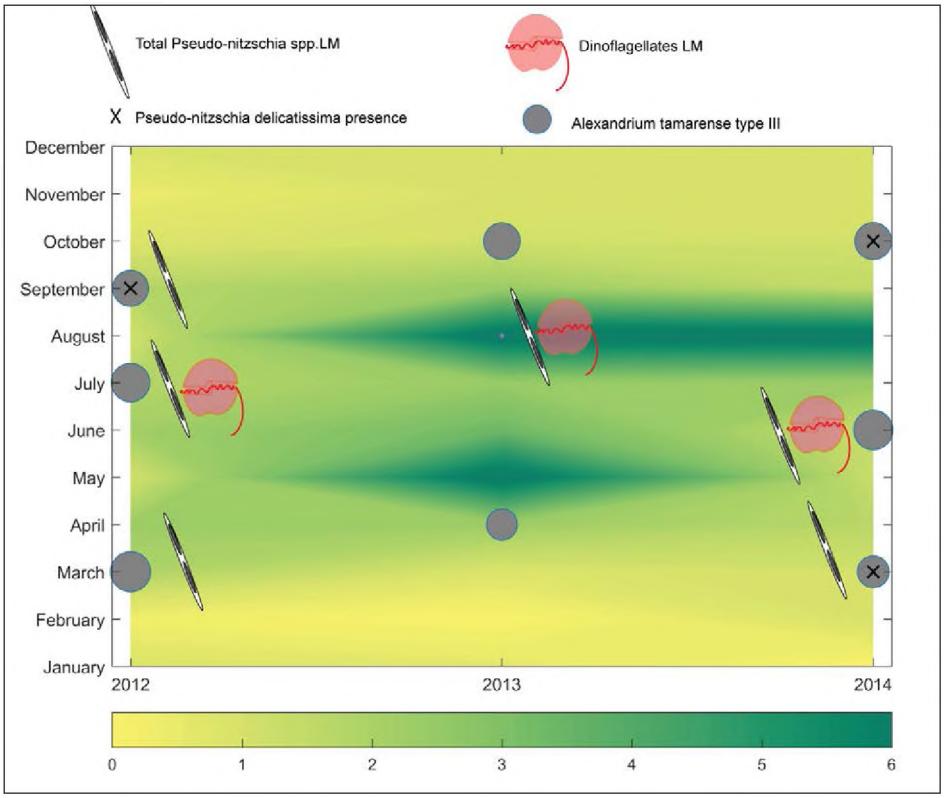

Fig. 3. Three years of Phytoplankton Colour Index (PCI) values (2012-14) were extracted from a series of tows going from Aberdeen to the Shetland Islands in Scotland. For each year, monthly averages were calculated to obtain the dataset used. Three non-consecutive months were interpolated to avoid any missing values. The frequency of occurrence and quantity of DNA of Alexandrium tamarense is represented on the diagram by grey circles where size is proportional to their quantity, while presence of Pseudo-nitzschia delicatissima is represented by a cross

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Persistent golden tides stranding Caribbean Sea in 2014 and 2015

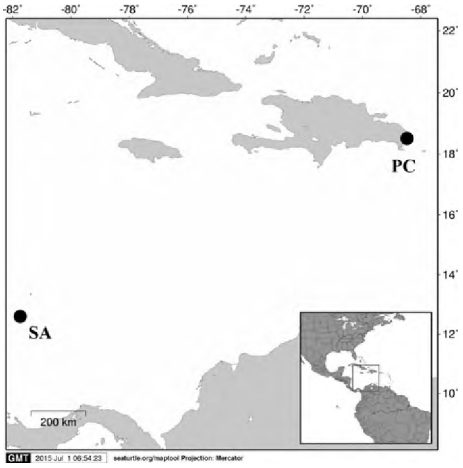

Fig. 1. Map showing the location of Punta Cana (PC) in the SE Dominican Republic and San Andrés (SA) Island (Colombia). Map made from a seaturtle.org/maptool, Mercator projection

Holopelagic seaweeds *Sargassum natans* (Linnaeus) Gaillon and *S. fluitans* (Børgesen) Børgesen coexist in the Sargasso Sea (North Atlantic Ocean) and form an important drifting ecosystem. These species occasionally form golden tides in the eastern islands of the Caribbean Sea and western Africa [1]. Golden tides of Sargassum have also been recently recorded for a few days in September 2014 in San Andrés Island (southwestern Caribbean, 280 km off the coast of Nicaragua) [2,3] (Fig. 1) and drifting thalli reaching the coastline of the Lesser Antilles occurred in August and September 2014 [4]. However, the persistence and extension of the golden tide events in other places of the Caribbean Sea in 2014 and 2015, seem to have been even more severe (Fig. 2). In particular, the golden tides in 2014 in the Dominican Republic shores lasted longer than usual, being observed from May to December. Moreover, the huge biomass of Sargassum reaching the beaches has not stopped at any time during 2015 (Fig. 2). The tides are mainly due to *S. fluitans*, with the contribution of *S. natans* being considerably lower. In the Punta Cana area, the year 2014 was locally referred to as 'the sargasso year' due to the huge biomass accumulated on the beach (50-80% higher than in past events). It must be highlighted that the term 'sargasso year' was previously applied to 2005 where atypical quantities of Sargassum were observed in the Gulf of Mexico [5]. Since tourism represents an important economic resource for the countries of the region, the economic losses during the last event have had a significant impact. It must be highlighted that the most important flow of western tourists takes place from December to March. The impact on tourism is due to three factors: i) the ecological damage involved in the withdrawal of rotten seaweed from the beach; ii) the sand and shores being covered with drifted seaweed that deter swimmers, and iii) the physical damage produced by the contact with seaweeds (dermatitis due to the accompanying epibionts) [6]. For all these reasons, some tourist resorts in Dominican Republic are using, with relative success, contention nets to prevent the stranding of *Sargassum*. However, a global rather than a local measure would be more effective. Different hypotheses have been proposed to explain the occurrence of the golden tides in the Atlantic during the last years. For instance, it has been suggested that global warming and perhaps nutrient load from the Amazon River [7] could be involved in the golden tides recorded in the Caribbean and western coast of Africa because these seaweeds originate in an area north of the river estuary [5,7]. It has also been hypothesized that golden tides in 2014 in San Andrés Island could be associated with the passage of hurricane *Edouard* towards the Atlantic coast of the USA, the currents deriving from this atmospheric event carrying seaweeds [2,3]. However, this last explanation cannot support the golden tides in the Dominican Republic and other Caribbean islands because the hurricane occurred in September and the golden tides appeared several months before.

Whether golden tides of *Sargassum* represent a symptom of anthropogenic global change is an unanswered question but, without any doubt, it is an issue that merits further study because of their ecological and economic significance, especially for the economy of developing countries in the Caribbean region.



Fig. 2. Beached Sarggasum on the coasts of Punta Cana (SE Dominican Republic) in June 2015 (courtesy from P. Guerrero)

Acknowledgements

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Recurrence of the harmful dinoflagellate *Karlodinium australe* along the Johor Strait

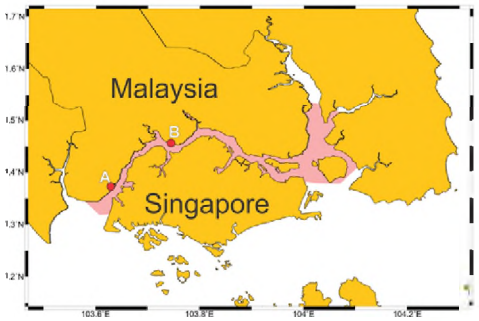

Fig. 1. Sampling sites of this study in Johor Strait. Area shaded in pink was the affected area during the 2015 blooms.

The harmful dinoflagellate *Karlodinium* is well known as a massive fish killer in tropical regions [1,2]. In February 2014, blooms of *Karlodinium* first occurred in the west Johor Strait, Malaysia, and caused massive fish mortality. Following this incident, detailed morphological and molecular studies identified the culprit as *Karlodinium australe* [3].

In February 2015, recurrence of massive fish kills both in fish cages and wild populations (including some demersal fishes and crustacean) were observed along the west Johor Strait. Water discoloration was clearly visible in the area. According to the fish farmers, fish behaved abnormally at night, jumping aggressively out of the cages from 10 pm to 1 am. An aeration system used to aerate all the cages did not seem to improve the fish conditions. Many brownish clots were observed in the mouths and stomachs of the impacted fish. The local farmers claimed that they suffered losses of over 30 t of fish, thus this event was more severe than the previous bloom in 2014.

On the 5th of March, 2015, field sampling was conducted at the western (1.377 °N and 103.641 °E) and central parts of the Strait (1.457 °N, 103.746 °E) (Fig. 1). Water flux was clearly visible near the farm areas because of the tidal movements (Sampling site A, Fig. 2 a-b), however, water movement was limited and nearly stagnant at sampling site B, mainly due to blockage of the causeway across the strait. Fish carcasses were observed in areas with heavy water discoloration (Fig. 2 c-d) where the bloom seemed to be driven by the tidal currents.

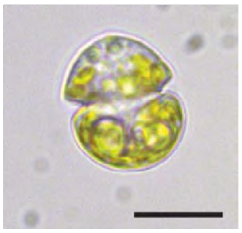

Fig. 3. Light micrograph of Karlodinium. Scale bar = 10 µm.

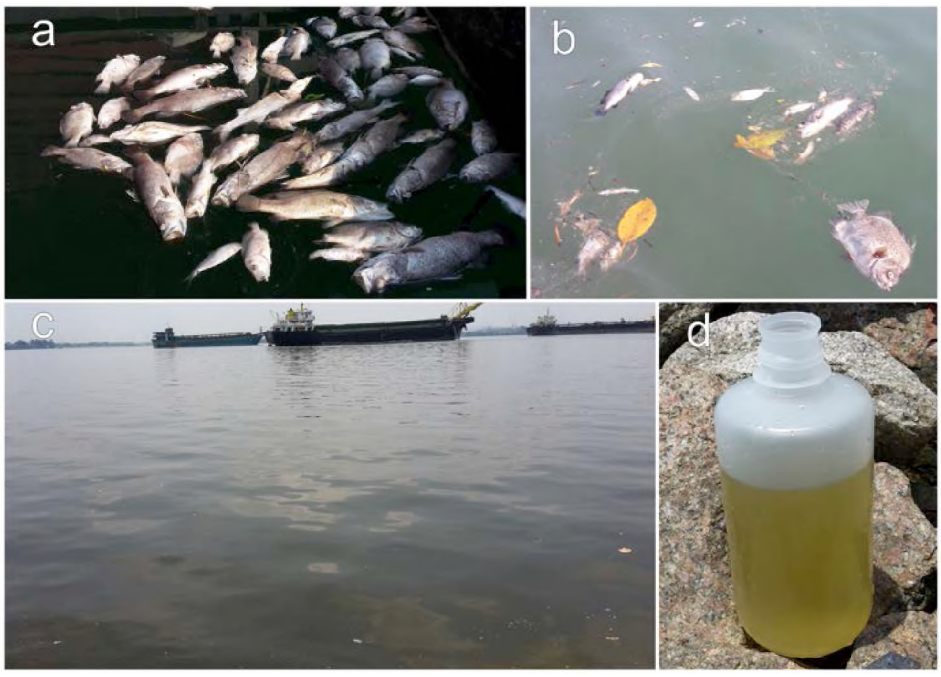

Fig. 2. (a-b) Dead cage-cultured and wild fishes along the west Johor Strait. (c-d) Sampling site B and brownish water samples.

Microscopic observations showed the unarmored *Karlodinium*-like cells (Fig. 3) dominated the samples, with maximum cell densities (up to 2×10^8 cells L^{-1}) higher than in the previous year's bloom [3]. Single cells were isolated from the field samples for molecular analysis. Single-cell PCR [3] was performed to amplify the internal transcript spacers (ITS) region of the rDNA using primer pair, ITS1F and ITSR [4]. ITS sequence data confirmed the species as *Karlodinium australe* [3].

A simple fish kill test was conducted with water samples collected from the bloom area where fish were killed. Fish exhibited behaviours similar to that described by local farmers during the fish kill event. The mechanisms causing the fish mortalities requires further study.

Karlodinium australe was first reported from a lagoon habitat in Australia [5]. The species was also reported from Aman Island, the Straits of Malacca [3] and Singapore [6]. In this survey, we confirmed that *K. australe* was responsible for the fish kill event this year. This is an urgent issue that needs to be addressed by the local relevant authorities to ensure routine monitoring and an early warning system is in place to minimize the losses in aquaculture in the future.

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Gonyaulax spinifera bloom in Thermaikos Gulf

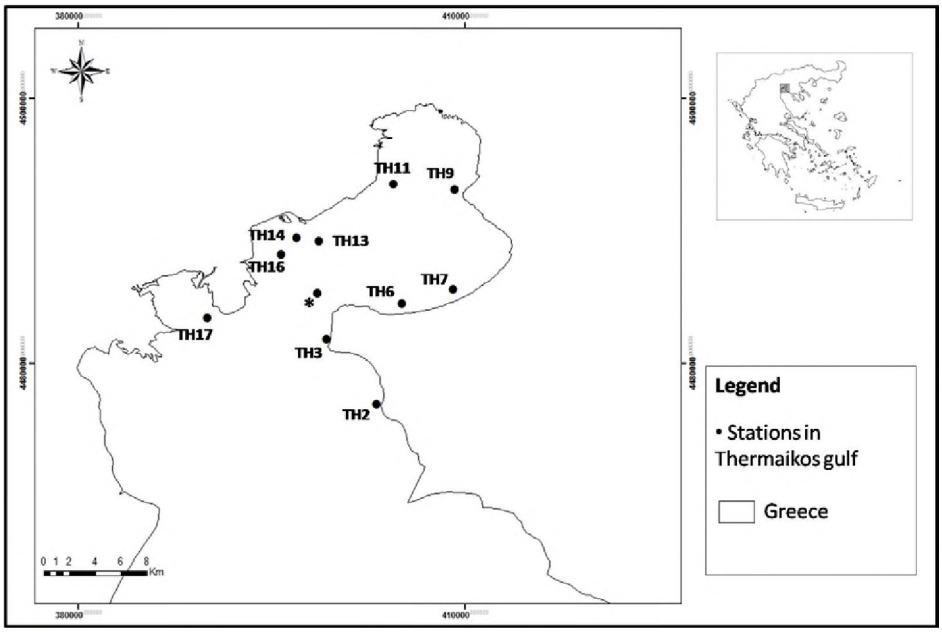

Fig. 1. Map of Thermaikos Gulf showing 10 monitoring sampling stations and the sampling point (*) where surface water discoloration was observed.

Gonyaulax spinifera (Claparède & Lachmann) Diesing 1866 has a world-wide distribution and together with *Protoceratium reticulatum* (Claparède & Lachmann) Butschli, 1885 and *Lingulodinium polyedrum* (Stein) Dodge, 1989 currently constitute the main planktonic yessotoxin producers. Its occurrence in seawater was correlated for the first time with mussel toxicity in New Zealand in 2004 [1]. In 2007, blooms of *G. spinifera* (densities up to 7.1 × 10^5 cells L^{-1}) occurred in the north-western Adriatic Sea [2]. Although there are no reports of human intoxication caused by yessotoxins, a limit of YTX equiv. kg^{-1} shellfish tissue has been set [3, 4].

Thermaikos Gulf is located in the North Western part of the Aegean Sea and constitutes the main bivalve mollusc production area in Greece. A potentially toxic microalgae monitoring programme has operated in the area since 2000 with a network of 19 sampling stations, 10 of which are located towards the inner part of the Gulf (Fig. 1). As part of this monitoring programme, integrated water samples are collected by the local responsible authorities on, at least, a weekly basis and the cell densities are determined using Utermöhl method [5] in the Laboratory Unit of Harmful Marine Microalgae-AUTH (LUHMM-AUTH) [6, 7].

G. spinifera has been recorded in Greek waters [8] and sediments [9] in the past, however not in high abundance. In this report, the first bloom of *G. spinifera* in Greek coastal waters (Thermaikos Gulf, North Western Aegean Sea) is described.

Cells were identified based on morphometrical characters using epifluorescence microscopy (Fig. 2), while individual cells were isolated, cultures were established and the characterization of the morphotypes by molecular phylogeny is also underway.

During October - November 2013, *G. spinifera* was recorded in high densities in Thermaikos Gulf with the same temporal pattern observed at all stations. From middle of October densities increased gradually reaching 113.2 x 10^3 cells L^{-1} at station TH13, located at the western coasts of the inner Thermaikos Gulf, in November (week 45). In all the other stations the maximum cell densities (not exceeding 46.5 x 10^3 cells L^{-1}) were recorded one week later (week 46). The bloom lasted approximately three weeks collapsing rapidly one week later (week 47, Fig. 3).

During the period when maximum densities were recorded at the monitoring stations, a brownish discoloration was observed at the entrance of the inner part of Thermaikos Gulf (*sampling point, Fig. 1) covering an area of approximately 1 km^2 (11/11/2013). Densities of *G. spinifera* in the surface sample collected from that area reached 6.4 x 10^5 cells L^{-1}.

At the same sample, dense *Dinophysis* cf. *ovum* populations (12 x 10^3 cells L^{-1}) were recorded. *D.* cf. *ovum* was present simultaneously with *G. spinifera* from October to November also reaching maximum densities (15x10^3 cells L^{-1}) during week 45, but at station TH7 (Fig. 4), which is located at the eastern part of the inner Thermaikos Gulf.

The recent findings in Thermaikos

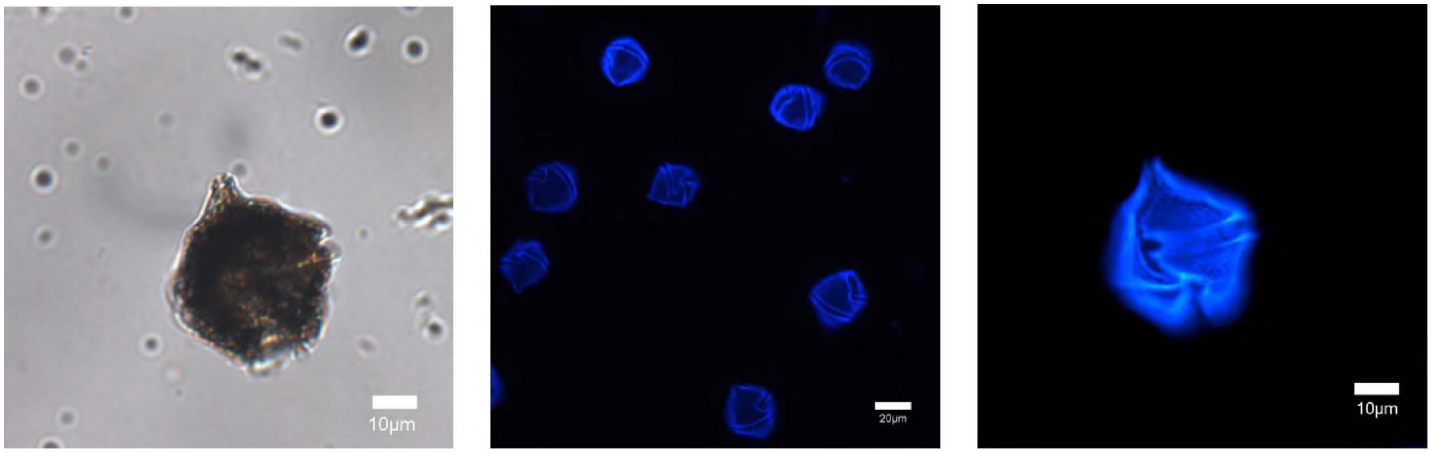

Fig. 2. Microscopy images of G. spinifera cells using (a) light microscopy and (b – c) epifluorescence microscopy

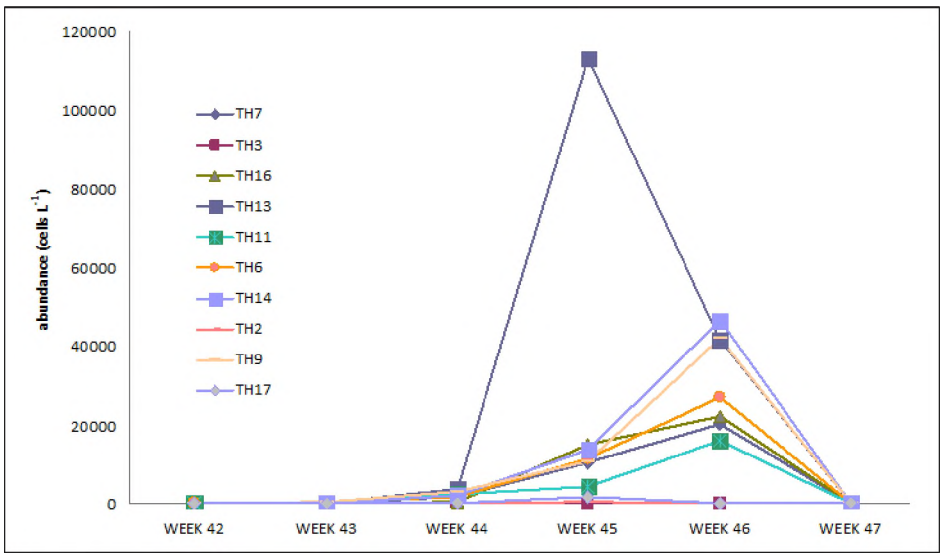

Fig. 3. The Gonyaulax spinifera bloom in Thermaikos Gulf the period October – November 2013 (G. spinifera density in cells L-1).

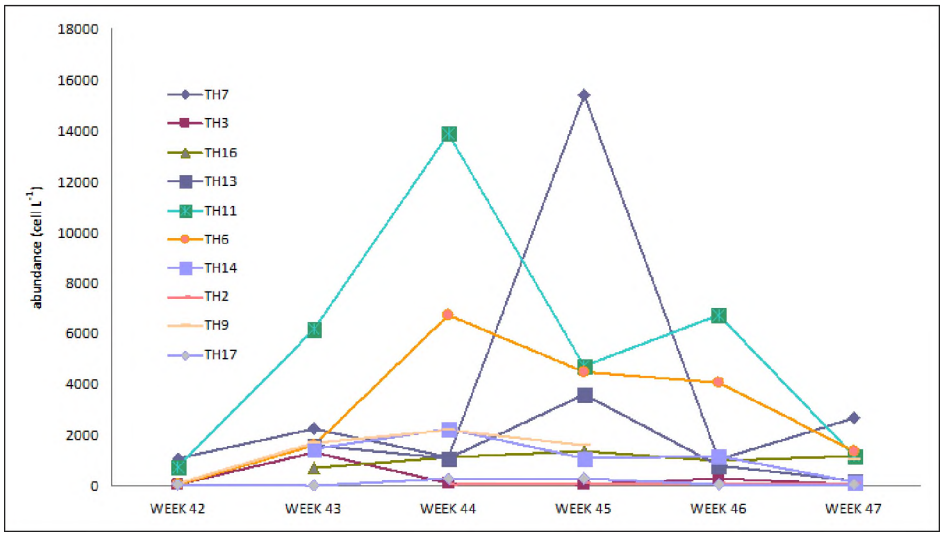

Fig. 4. The Dinophysis cf. ovum bloom in Thermaikos Gulf the period October – November 2013 (D. cf. ovum density in cells L-1).

Gulf add another potential biotoxin threat in the area which could act synergistically to the existing and recurrent phenomenon of toxic *D.* cf. *ovum* blooms.

It must be highlighted that this is the first time since the initial record of *Dinophysis* blooms in Thermaikos Gulf that the *Dinophysis* bloom appears during this period of year. From 1999 to 2012 the period for *Dinophysis* blooms was from approximately mid winter until the end of spring-early summer. This observation together with the first record of such a high density bloom of *G. spinifera* and also the first highly dense bloom (8.5 x 10^3 cells L^{-1}) of *D. sacculus* in the area at mid May (LUHMM-AUTH, unpublished data) indicate possible phytoplankton community changes as a response to meteorological and hydrographic disturbances, a hypothesis that needs to be further investigated.

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Continued from page 2

except for 2013, which appears to be an anomalous year for both species. Neither species showed a consistent relationship with high PCI (Fig. 3) that may boost small-cell capture by blocking the mesh, so these species could prefer different conditions to most other phytoplankton. The molecular-detection of *P. delicatissima* corresponded with LM observations of all *Pseudo-nitzschia* spp. By contrast, *A. tamarense* generally did not occur with other dinoflagellates observed by LM (Fig. 1). These results highlight that despite design constraints, smaller species can be captured on archival CPR samples perhaps as fragments not observed by LM. This information provides additional information about their preferred habitat and temporal distribution and we hope to test more samples for better representation and compare them with other types of datasets.

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Occurrence of *Pyrodinium bahamense* blooms related to cyst accumulation in the bottom sediments in the bays at Ambon, Lampung and Jakarta, Indonesia

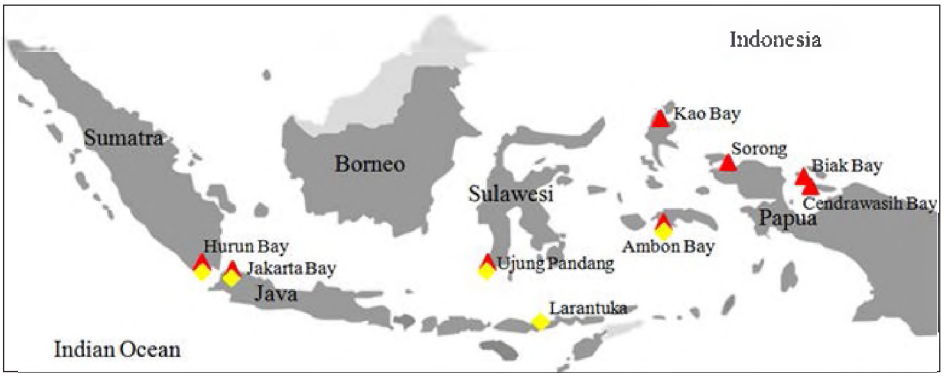

Fig 1. Location of Pyrodinium bahamense var. compressum occurrences in Indonesia. (▲) Bloom events; (●) Cysts observed in the bottom sediments. (according to Wiadnyana et al. 1996 and Matsuoka et al. 1999).

Pyrodinium bahamense var. *compressum* (Pbc) is considered one of the main causative agent of paralytic shellfish poisoning (PSP) in Southeast Asian coastal waters and the Pacific coast of central America [1]. This harmful algal species produces saxitoxins, which may be fatal to humans who consume infected shellfish. Since the first bloom in Papua New Guinea in 1972, Pbc blooms have caused major health security problems and huge economic losses in the Indo-Pacific [2].

In Indonesia, the first confirmed Pbc bloom occurred in Kao Bay (Halmahera Island, East Indonesia) on March 1994. Several months later, a tragedy happened in Ambon Bay. Three children died and 33 persons were hospitalized as a consequence of consuming shellfish [2]. High concentrations of Pbc cells (0.4-1.6 x 10^3 cells and 41% of total cells) were found to be responsible for this tragedy. Since then, Pbc bloom events have spread progressively to other areas in Indonesian waters, such as Piru and Elpaputih Bay (Banda sea), Sorong, Biak, and Cendrawasih Bay (Northern Papua waters), Ujung Pandang (Sulawesi) and Jakarta Bay [2] (Fig. 1).

Since Usup and Azanza [3] published their comprehensive review of *Pyrodinium bahamense* (Pb) in 1998, Pb has become notorious for producing resting cysts which may accumulate on the bottom sediment. It is considered that the accumulation of cysts, so-called cyst beds, has become a potential threat which may initiate blooms of their vegetative cells [4,5]. Later, Mizushima *et al.* investigated the existence of Pbc cysts in Hurun Bay (within Lampung Bay, Sumatra) and Ambon Bay (Maluku province) [5]. Based on sediment-core samples, it was shown that Pbc cysts were continuously recorded from 1830 until recently and their abundance gradually increased. Another study, by Matsuoka *et al.* [6], found cysts of Pb existed in surface sediments at Jakarta Bay (Java), Ujung Pandang (Sulawesi), and Larantuka (Flores Island, East Indonesia) (Fig. 1).

Distribution of Pb/Pbc cysts (Fig. 2a-d) in the surface sediments of the three Indonesian locations previously studied was investigated in 2014. In Jakarta Bay, the maximum density recorded was 144 cysts g^{-1} dry weight sediment. Pb cysts were observed in sites off the Ancol area and Tanjung Priok international harbor. Lampung, Hurun Bay, where Pbc blooms occurred regularly was, as expected, the area where Pbc cysts were most numerous. Ambon Bay was the place where the highest concentrations were observed within all the sampled stations in our study (max value: 1168 cysts g^{-1} dry weight sediment). The maximum relative abundances of Pbc cysts in surface sediment in Jakarta, Lampung, and Ambon Bay, were 13%, 40%, and 66% respectively. Based on these data, there is a serious concern that there is a potential threat for future PSP outbreaks in those areas.

The latest bloom event in Ambon Bay on July 2012, caused the death of thousands of cultured fish and 7 people (2 children and 5 adults) were hospitalised for a week after consuming shellfish [7]. Over 2000 cells L^{-1} of Pbc vegetative cells were observed during that

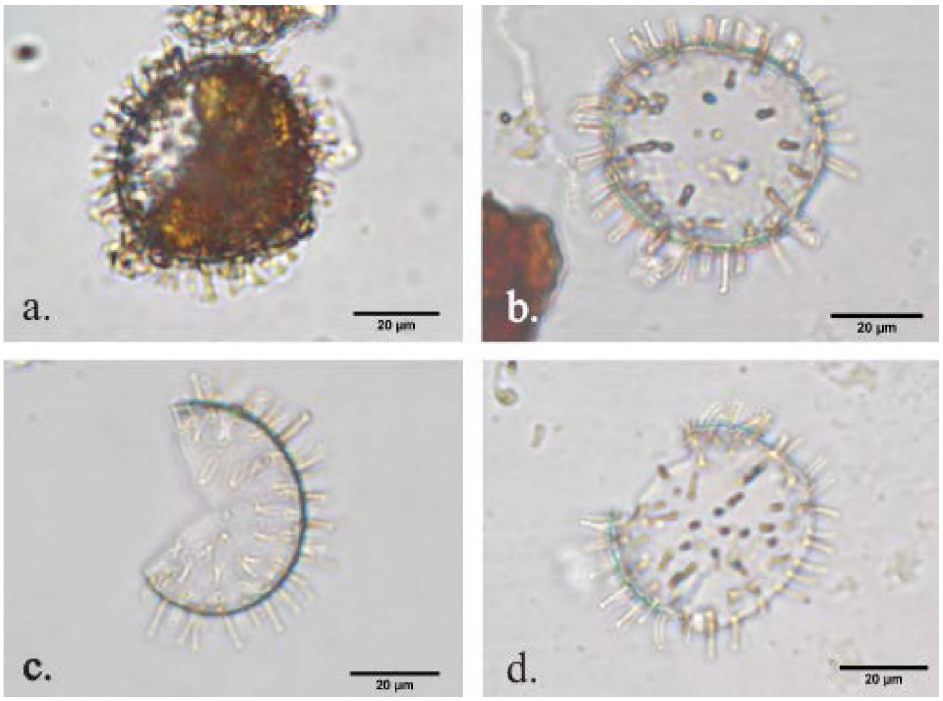

Fig 2. Pyrodinium bahamense cysts collected in 0-5 cm of the sampled surface sediments. a. Living cyst and b. Empty cyst from Ambon Bay, October 2014; c. Empty cyst from Jakarta Bay, May 2014; d. Empty cyst from Lampung Bay, May 201.

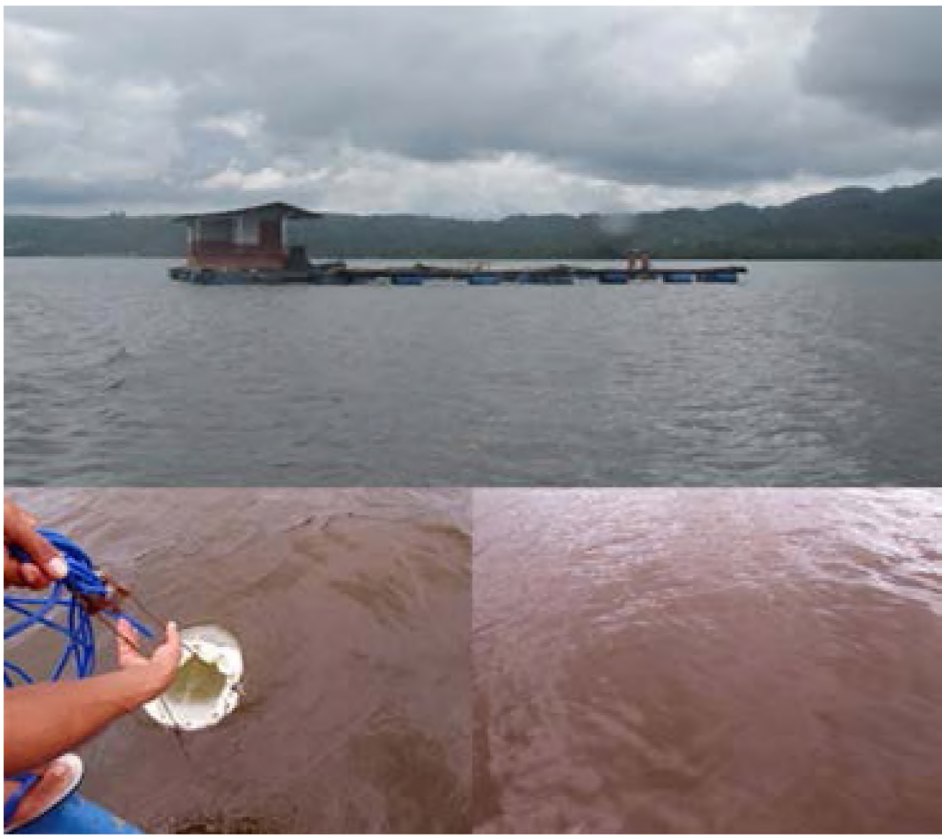

Fig 3. Pyrodinium bahamense var. compressum bloom forming red brown discoloration of sea waters close to fish cages at Passo in Ambon bay (12th July 2012)

event [7] (Fig. 3). From all the above it can be concluded that evaluation of the cysts beds would be important additional information to Pbc vegetative cell monitoring and shellfish toxicity surveys in any programme aimed at improving food safety of local people and establishing mitigation measures.

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Encystment and excystment of tropical strains of the toxic dinoflagellate *Alexandrium minutum* (Dinophyceae)

The marine dinoflagellate *Alexandrium minutum* is one of the harmful species responsible for paralytic shellfish poisoning (PSP) in the warm Western Pacific region. This highly toxic species was first known in Malaysian waters after the PSP incident in Tumpat, Kelantan (Fig. 1), in September, 2001 [1], when six people were hospitalized with one fatality [2]. Although there has been no recurrence of blooms since then, cells of *A. minutum* were commonly found in the plankton samples. Factors promoting bloom formation of this species remain unclear, in particular the role of encystment-excystment in bloom initiation.

Dormant resting cysts play an important role in the survival, dispersion, bloom initiation and recurrence of dinoflagellate populations [3]. Like many toxic dinoflagellates, *A. minutum* produces resting cysts in its life cycle. Little is known about the bloom dynamics and life cycle of the tropical toxic *A. minutum* and it is suspected that the mechanisms differ from those of temperate waters as the environmental regimes of tropical waters are so different.

In an attempt to understand the encystment and excystment of *A. minutum*, 15 strains of *A. minutum* were used in cross-mating experiments with a pairwise combination. Sexual fusion of gametes, formation of planozygotes and hypnozygotes (resting cysts) were investigated and confirmed under light microscope daily (Fig. 2). Hypnozygotes were found in nine out of 15 combinations of strains, with the earliest found on the third day after cross-mating of culture strains (3–6 days). Resting cysts of *A. minutum* were spherical. Some were ellipsoidal, with a thick layer of transparent cyst walls [4]. The appearance of the cyst content was granular and a condensed yellow-orange accumulation body was present.

In the excystment experiment, *A. minutum* cysts were isolated individually and transferred into a 96-wells culture plate containing seawater or seawater with enrichment medium. Cysts were monitored daily to determine the dormant period of individual cyst. They were observed to germinate 3–5 days after encystment. In the enriched seawater medium, ten cysts out of 35 were successfully germinated during the first 25 d of observation. A 60-d observation period revealed an excystment rate of 62.9%.

The results of this preliminary study showed that the dormant period of *A. minutum* cysts was relatively short when compared to its temperate counterparts. Dormancy periods of temperate strains of *A. tamarense* and *A. fundyense* were six and 11 months, respectively, where cyst germination was commonly reported in spring and autumn [4,5,6]. In contrast, encystment/excystment of tropical *A. minutum* was estimated less than two weeks. We hypothesized that the low cyst abundances observed in the tropical region are due to the extremely short dormancy period. This information is pivotal in understanding the initiation and development of blooms of the tropical dinoflagellate *A. minutum*.



Fig. 1. A semi-enclosed lagoon in Tumpat, Kelantan, Malaysia where blooms of Alexandrium minutum and PSP were first encountered.

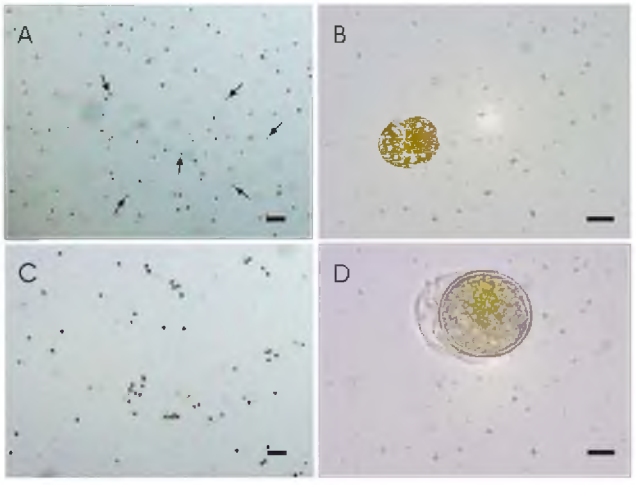

Fig. 2. (A) Cross-mating experiments of Alexandrium minutum, arrows indicate gametes, bar = 100 µm. (B) Gamete, bar = 10 µm. (C) Cysts formed at the bottom of plate, bar = 100 µm. (D) Hypnozygotes or Resting cysts, bar = 10 µm.

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Extensive outbreaks of heterotrophic dinoflagellate *Noctiluca scintillans* blooms along coastal waters of the South Eastern Arabian Sea

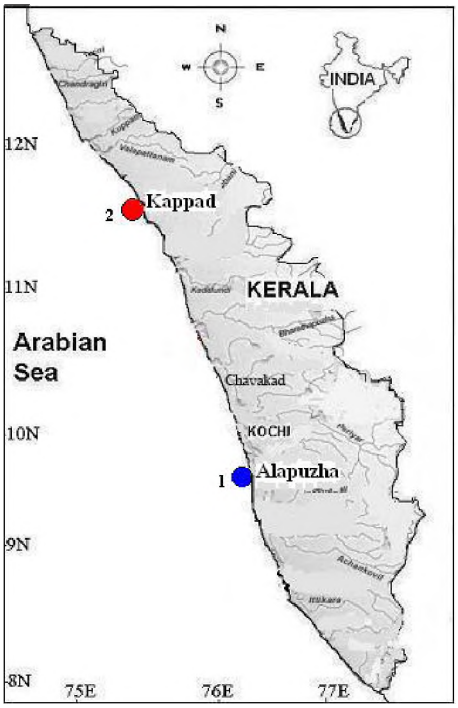

Fig. 1. Map showing Noctiluca bloom locations (1) Bioluminescent bloom off Alapuzha (2) red surface water discolouration off Kappad- Calicut



Fig. 3. Red surface water discolouration by Noctiluca scintillans bloom along the coastal waters off Kappad- Calicut (Photo courtesy: www.malayalivartha.com)

The heterotrophic dinoflagellate *Noctiluca scintillans* has long been present in the eastern Arabian Sea. It forms recurrent blooms during summer and winter monsoons along the south and northeastern Arabian Sea shores respectively. Since the first reported incident in 1908 [1] the southeastern Arabian Sea (southwest coast of India) (Fig 1) has been experiencing *Noctiluca* blooms annually for the past two decades [2-5]. Seasonal blooms of *Noctiluca* in the area were rarely associated with mortality of marine fauna, odour events etc [6, 7].

During summer monsoon of 2015 two massive outbreaks of red *Noctiluca* occurred along the south eastern Arabian Sea (Fig. 1). In the second week of July 2015, a 'glowing sea' was observed during the night time along the inshore waters off Alleppey (Lat. 09°49 N; Long. 76°31 E) and nearby areas (Fig. 2). The coastal inhabitants of these areas had concerns about this phenomenon and the 'glowing sea' condition was the main topic of conversation amongst them. Following newspaper reports, water samples were collected during the day and in the evening time when it was dark. Analysis of water samples confirmed that the 'glowing sea' was due to a bloom of *Noctiluca scintillans* (3.1×10^5 cells L^{-1}). The *Noctiluca* cells were between 600 and 1000 µm in diameter and were "red *Noctiluca*", i.e. lacking the prasinophyte endosymbiont *Pedinomonas noctilucae. Noctiluca* (latin meaning: night shiner) is commonly known as 'sea sparkles' because of its ability to produce blue bioluminescence at night. This bioluminescence is produced by a luciferin-luciferase system located in thousands of spherically shaped organelles, or 'microsources', distributed throughout the cytoplasm of this single-celled protist [8].

On 5th August 2015 the coastal waters off Kappad- Calicut (Lat. 11°38 N; Long. 75°72 E) became red and appeared to have a tomato soup-like consistency (Fig. 3) creating panic among the coastal dwellers. The very next day water samples were collected from the



Fig. 2. Dense bloom of bioluminescent Noctiluca scintillans along Alapuzha coast, here the algal cells sparkle as waves splash on the shore (Photo courtesy: www.metrovaartha.com)

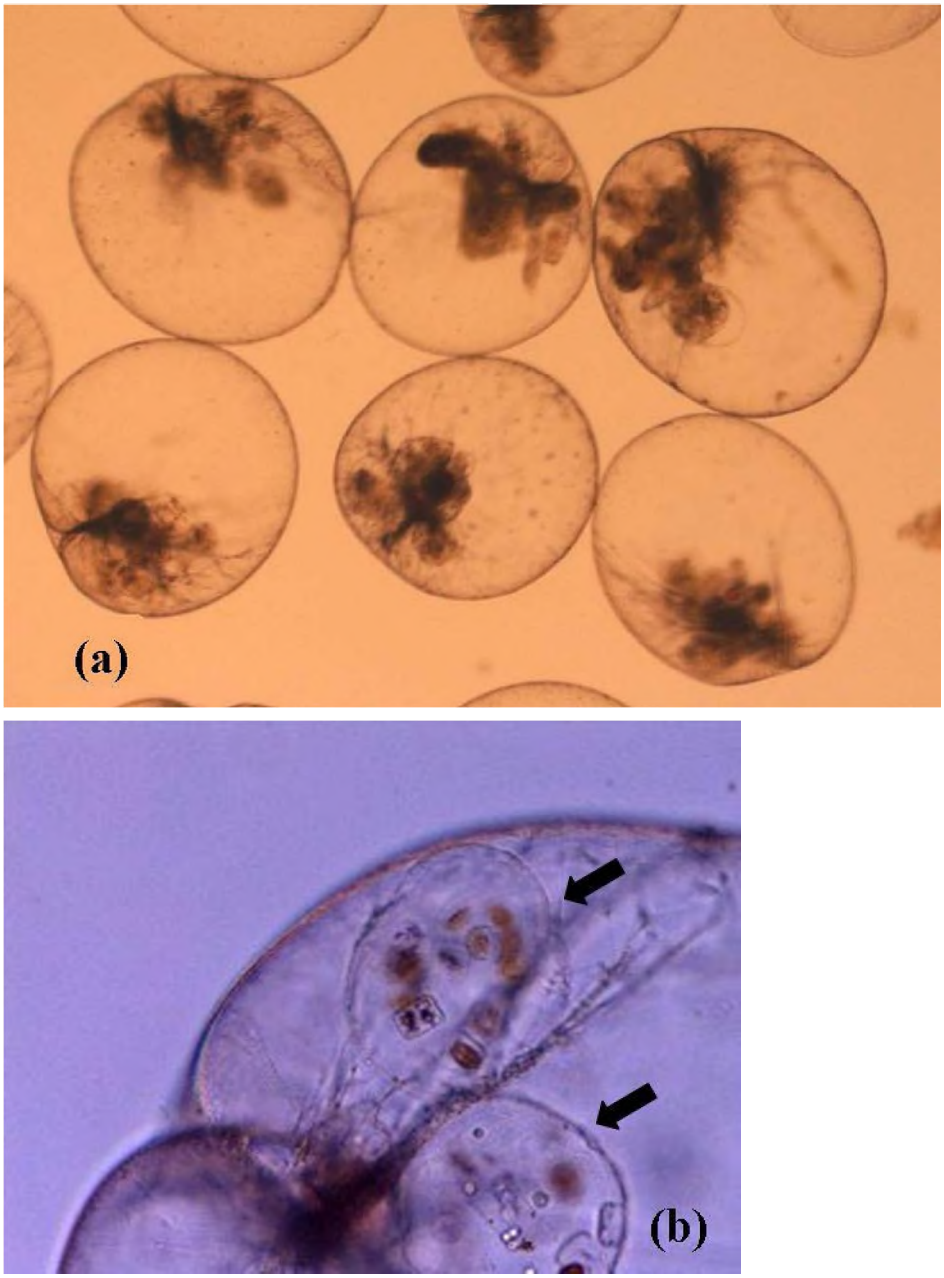

Fig. 4. Microphotographs (a) Noctiluca scintillans (x 40) (b) Food vacuole with diatoms cells inside (x 200)

incident area and analysed. Microscopic observations showed that the red surface water discolouration was due to high densities (5.3x 10^6 cells L^{-1}) of red *Noctiluca*. During this and the previous event *Noctiluca* cells had food vacuoles containing cells (Fig. 4) from co-occurring diatoms. The percentage composition of *N. scintillans* and mainly chain forming centric diatoms (*Thalassiosira* spp., *Rhizosolenia* spp. *Chaetoceros socialis, C. curvisetus, Fragilariopsis cylindrus*) in the plankton community in the bloom waters were 60% and 40% respectively. Although the blooms were dense mortality of marine fauna was not reported during the two incidents. Local fishermen reported that fish catches were considerably lower during the bloom period and shoreward drifts of moribund crabs were observed in these regions.

Data from the regular HAB monitoring, that is part of the MLR programme (MoES, Govt. of India), indicate clearly that the incidence of *N. scintillans* blooms has increased in the last decade during summer monsoons along the coastal waters of south eastern Arabian sea. The frequent occurrence of these blooms poses a threat to coastal ecosystem health. Summer monsoon upwelling and associated high primary production are one of the major contributors of the biological production of eastern Arabian Sea. With increased occurrences of *Noctiluca*, a voracious heterotroph [9] unpalatable to other animals, disruptions in the coastal marine food chains such as shorter food chains and decreased diversity of coastal ecosystems are the most probable outcome. The recent incidence of jellyfish blooms [10] along the coastal waters of the south eastern Arabian Sea can be considered as an outcome of such changes. Since jellyfish are the primary grazers of *Noctiluca*, their abundance can increase following these dinoflagellate blooms. The increased frequency of *Noctiluca scintillans* blooms can be considered as an indication of a species shift in the primary producers in this region.

Acknowledgments

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Predicting the unknown from the unknown: Can we model our way out of ecological ignorance?

Charles Darwin´s passionate belief that *"Ours is but to observe the ways of Nature"* helped lay the foundation for what we now call ecology – but his approach would not stand a chance in today´s funding system for science. Is this because science has honestly developed beyond the "primitive" concepts he and his colleagues gave us two centuries ago, or have we as a society lost the plot? I was provoked into thinking about this recently by a powerful emerging trend in research on harmful algal blooms (HABs or "red-tides"). Here, as in many other fields of research, scientists are trying to predict future events, but the available background ecological data is inadequate, and scientific modelling is being proposed to somehow compensate for this. In my opinion, this is unrealistic for HAB-research or the many other fields of science facing the same dilemma. At its best, ecology involves observing which organisms live where and when, in order to ask the important questions of why and how they do so. This might eventually offer the possibility of predicting ecological events such as HABs, but can we ignore these first steps? Can we really model our way out of ecological ignorance?

The early ecologist´s world was very different, but they were at least as highly motivated as present-day scientists. These were a few, independently wealthy, privileged gentry, able to pursue basic science as a full-time "hobby". For them, it was sufficient to observe and record the ways of Nature for its own sake – revealing the wonderful "miracles" in the details of the natural world as an intellectual exercise for the few. It took another hundred years before David Attenborough could bring such delights into the homes of the masses through television, but in so doing, he too, always shows the greatest respect for these early pioneers – he certainly has not lost the plot! So what *has* changed?

Society´s basic attitude to science has definitely not changed since Darwin´s time; society still expects a "payback". Already back then, there were huge socio-economic benefits from "observing the ways of Nature" – for example, greatly improved agriculture and increased food production. But Nature paid a dreadful price; and today, what started as "observing the ways of Nature" has become an urgent, headlong rush into "*understanding* the ways of Nature", as the ecological damage caused by our "progress" threatens our very existence. "The gentlemen´s hobby" has rapidly developed into crisis management of the planet.

Today, basic scientific research is a "public service industry" – the public pay for research, and in return, they expect practical solutions for serious environmental problems: how damaged is the biosphere, and how will it respond to future global change? By now, the initial concept of observing the ways of Nature could, and should have led to a long-term monitoring effort that would give us at least one hundred years of ground-truth data on what has been happening in the natural world. Sadly, however, we have seriously neglected these first steps, and scientists now feel pressured into making ecological predictions, even in the absence of adequate data. This can be likened to a person trying to plan where to go, without knowing where he is coming from, or where he is. This is where the plot was lost, as illustrated by the example from HAB-research. HABs threaten public health, wealth and welfare worldwide, and hundreds of researchers are working on them. Some HAB-species in the phytoplankton (microscopic algae in the sea and lakes) produce toxins causing sickness, or even death in humans, other species kill fish, while others interfere with the workings of fishing gear or desalination plants. More than forty years of research has seen great progress in identifying the organisms and toxins for short-term public warnings of up to a few weeks prior to any danger. However, this has not led to a general ecological understanding of why these organisms cause problems where and when they do, and both scientists and the public should be asking: why? This is not just to point the finger of blame at science – scientists alone have not caused society´s problems with the natural environment, and they do not control funding priorities for addressing them – we are all in this together!

Nobody should be surprised at the lack of ecological answers, here, or elsewhere in science. Generally, there is no long-term phytoplankton monitoring prior to HAB-events that would provide ground-truth data for asking "why here and now?", and researchers have to devise their own investigative strategy "after the fact". It is hard to understand why it has proved so difficult to establish long-term environmental monitoring programs, but it probably involves political reluctance to commit long-term funding. Still, such monitor-





ing is the crucial first step towards understanding changing environments and biotic responses, and scientists and funding agencies together bear responsibility for not having pressed more for this at the highest levels of decision-making.

The alternative strategies have usually identified "the target species", and attempted to study their ecological requirements with respect to a few abiotic factors such as temperature and salinity, often in laboratory cultures, and with little or no attention to the complex biotic interactions with other organisms within the plankton. Basic ecology teaches us that organisms live where they do in response to *both* biotic and abiotic factors, and it is therefore not surprising that the resulting ecological data is inadequate. However, if the data is inadequate for understanding past and present events, what possibility is there for plausible predictions of future events? Surely, we need to get back to basics and concentrate efforts more on "good old fashioned" environmental monitoring to find out what is really happening out there.

In Darwin´s time, customers could consult a fortune-teller gazing into a crystal ball to predict their looming fate. Today, science should be seen as doing better than re-inventing the crystal ball!

The following article was first published in Sherkin Comment [1], the environmental quarterly of Sherkin Island Marine Station. It expresses my personal belief that HAB research should reconsider the need for more long-term monitoring of the plankton. Recent meetings and workshops show that our science is seriously addressing possible effects of climate change. Lack of funding prevented my participation, but I presume those attending did consider the role of long-term monitoring, well aware of the seemingly impossibility of funding such efforts even if they were willing to tackle the issue. Bradburry [2] criticized HAB science as expressed in Anderson and Garrison [3] for disregarding the ecological principle that organisms respond to a combination of physical and biological factors in their environment ("physics is not biology"). I agree with this criticism, while at the same time acknowledging the awesome implications of facing up to it. I sometimes include in presentations a slide stating that this (plankton ecology) "is not rocket science", followed by another stating "it is much more complex". The one builds on well-known basic principles, while ours is still largely "work in progress" – we can land instruments on a small extraterrestrial body in motion out there, but we are generally unable to give long-term warnings of HAB down here. The threat of HAB to public health and welfare adds urgency to our need to probe the enormous complexity of plankton ecology – why, when and how HAB occur? As HAB research progresses, with projects aimed at "predicting" how HAB may be affected by an ever-changing world, I hope we will raise the effort along the way to find out what is actually happening out there in the plankton.

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IOC and IAEA together in a Regional Science Planning Workshop on Harmful Algae in the Caribbean and Adjacent Regions

The VI IOC Regional Science Planning Workshop on Harmful Algal Blooms in the IOCARIBE region was held at the Universidad Nacional Pedro Henríquez Ureña in Santo Domingo, the Dominican Republic, from 26 to 30 October, 2015. The workshop was part of an International Atomic Energy Agency (IAEA)-IOC "Regional Training Course on Ciguatera Fish Poisoning (CFP): Field Monitoring and toxin data management".

The workshop was sponsored by the Intergovernmental Oceanographic Commission (IOC) of UNESCO, and the IAEA project RLA7020: "Establishing the Caribbean Observing Network for Ocean Acidification and its Impact on Harmful Algal Blooms, using Nuclear and Isotopic Techniques".

The aim of the meeting was to:

i. Train participants to compile and share data and metadata on harmful algal events and occurrences of toxic microalgae in the IOCARIBE region, using the IOC/IODE datasystems HAEDAT and OBIS. This information is required to include the IOCARIBE region in the Global HAB Status Report in progress with the financial support of Flanders (Belgium).
ii. Strengthen the IOCARIBE/ANCA network through inclusion of IAEA/RLA 7020 project participants, and the planning of future joint regional activities.

Participants came from Colombia (2), Costa Rica (2), Cuba (4), Dominican Republic (4), El Salvador (2), Guatemala (1), Jamaica (1), México (2), Panamá (2), and Venezuela (1). Henrik Enevoldsen (IOC UNESCO) and Mary Kennedy assisted as HAEDAT and OBIS database trainers respectively; Patricia Tester and Santiago Fraga as international experts on Ciguatera, and Carlos Alonso as IAEA designated team member.





The participants actively exchanged knowledge on HABs from their respective countries; the full group assessed the gaps, constraints, and bottle-necks in each participating Member State; identified responsible individuals in ANCA and IAEA/RLA in each country for compiling data and uploading them to HAEDAT and OBIS. The responsible people in each country defined HAEDAT Area Codes for reporting HAB events, each country representative dividing his coast into areas that are meaningful in the context of how sampling is made. A timeline was agreed to for completing a first phase of regional data compilation.

For the next two years, the group agreed to:

i. Organize a HAB-Caribbean Symposium in the frame of the XXIII Congress of Science and Technology of the Sea to be held in Tapachula, Chiapas- México.
ii. Develop the "IV Theoretical and practical course on IOCARIBE - HABs: Emphasis on Public Health".
iii. Publish a special issue of harmful algae in the Caribbean in the Cuban Journal of Fisheries Research (RCIP).
iv. Generate a risk map of HAB vectors in the Caribbean.
v. Develop a proposal for ANCA participation in the implementation of a global Ciguatera strategy.

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Algae for the future: from harm to benefits – Highlights on the HABSCS-ICBUAB 2015, Hong Kong

The recent *6th International Conference on Prevention and Management of Harmful Algal Blooms in the South China Sea and the 2nd International Conference on Beneficial Uses of Algal Biomass 2015* (HABSCS-ICBUAB 2015) was attended by more than 100 participants. They gathered at the Open University of Hong Kong (OUHK) on 20–23 December to discuss the management of harmful algal blooms as well as the beneficial uses of algae. There were key note presentations, oral and poster contributions as well as a lively session of speed-talks by some of the numerous young students participating in the conference; some of them as participating in the student exchange programme between University of Xiamen and OUHK.

Management of HABs

Key speeches focused on HAB monitoring and prediction, investigations of HABs in Northeast Atlantic Europe (Beatriz Reguera), the biodiversity of harmful benthic dinoflagellates in Hainan Island, south China (Songhui Lu), control and mitigation of HABs, environmental-friendly prevention strategies for HABs using diatoms and algicidal bacteria (Ichiro Imai) and rapid treatment of ship's ballast water using hydroxyl radicals (Min-dong Bai).

Other studies concentrated on the genomic and proteomic analysis of harmful algae, including a review on the current status and future perspectives of marine dinofagellate proteomics (Dazhi Wang) and the molecular response of diatoms to nutrient stresses in the ocean. Studies presenting the toxicology results of the shellfish killing species *Karenia mikimotoi* (Tian Yan) and the effects of dietary composition and nutritional quality on the feeding and growth of *Noctiluca scintillans* (Hongbin Liu) were presented.

Beneficial uses of algal biomass

Microalgae are widely used in wastewater treatment, e.g. in the removal of polybrominated diphenyl ethers in wastewater by microalgal isolates (Nora Fung-Yee). Microalgal biofuel production was thoroughly discussed in the conference. Michael Borowitzka shared the cultivation experience, from strain selection to optimizing productivity in open ponds and Rainer Buchholz introduced the cultivation systems for high-value products derived from photoautotrophic microorganisms. Hongye Li talked about the metabolic engineering of microalgae for boosting neutral lipid accumulation.

Prof. Kin-Chung Ho, Chairman of the Conference Organizing Committee, gave concluding remarks at the end of the conference to summarize the current research status and future perspectives of algal studies. During the final day participants had the opportunity to visit the *Water Resources Education Centre* in Kowloon.

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Fig. 1. Group photo in the Opening Ceremony, HABSCS-ICBUAB 2015



Fig. 2. Fruitful discussion during Q&A and Poster Presentation Sections

Colombia organized its first national workshop on HABs



Harmful algal blooms (HABS) have been recognized as a public health issue in several countries for decades. However, Colombia is just starting to accept it as an issue that may affect public health as well as tourism. As a result of this late awareness, the Colombian Oceanographic Commission (CCO), the IOCARIBE-HAB-ANCA group and the Universidad Nacional de Colombia, working as members of the National Technical Committee on Marine Pollution, organized the first national workshop on HABs.

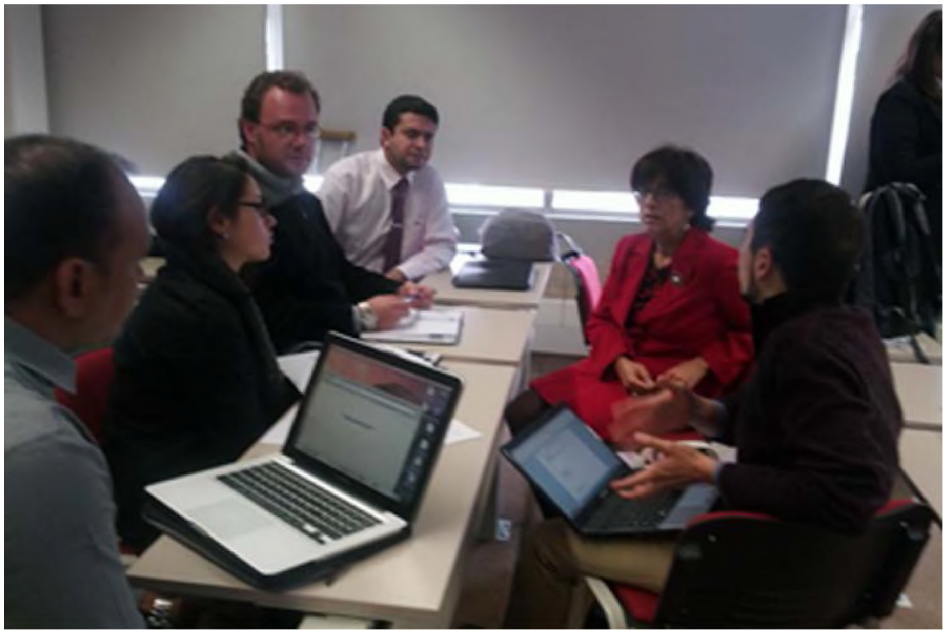
The meeting titled: "I Seminario-Taller Sobre Floraciones Algales Nocivas: Impactos en el Desarrollo Local, Regional y Nacional" (Seminar-Workshop on HABs: Impacts on local, regional and national development), was held on 5th June 2015 at the Universidad Jorge Tadeo Lozano, Bogotá and was part of the Colombian World Oceans Day celebration (Fig. 1). Representatives of 11 Colombian institutions and one from Spain (Ministry of Health and Social Protection, Ministry of Environment and Social Development, Chancellery, Institute of Marine and Coastal Research – INVEMAR, National Authority of Aquaculture and Fishery- AUNAP, Navy of Colombia, Gobernación de César, Executive Secretary of the Colombian Oceanographic Commission, Universidad Militar Nueva Granada, Universidad Tadeo Lozano, Instituto Español de Oceanografía, Universidad Nacional de Colombia) belonging to different sectors such as environment, public health, fishery, aquaculture, education, research, defense and international affairs attended the meeting.

The main goal of the workshop was to answer the question: *Which elements must be included in a Colombian HAB risk management plan?*

To answer this question a wide-perspective overview including international experiences of environmental and public health issues and the requirement for economic assessment was presented. Beatriz Reguera showed the Spanish approach as well as other European cases. J. Ernesto Mancera, current chair of IOCARIBE-HAB-ANCA and Colombian HAB focal point, presented general aspects of HAB and the Colombian situation, stressing the importance of including HAB issues as a part of the coastal development plans. Mónica Puyana introduced a case study of Cyanobacteria blooms in the SEAFLOWER International Biosphere Reservation. Milena Borbón emphasized the health aspects of HABs in Colombia, based on medical reports from San Andrés island. Finally, Julián Prato showed the importance and need to assess the economic impacts of HABs in the country.

For the second section of the workshop a prospective methodology was used which facilitated dialogue between experts of different topics and integrated their knowledge to plan future scenarios. During this section, the experts worked in small groups and identified priorities to be included in a future management plan. These activities were related to three main aspects: 1) Sociocultural factors of the Colombian population; 2) Public policy requirements and regulations, in areas such as public health, fishery resources and environment; 3) Needs for scientific research and environmental monitoring systems, including economic and health aspects.

Later, the participant discussed the results in a plenary session and drew a general list of activities. Using two criteria, Importance (I) and Governance (G), the experts assessed each activity, and results were plotted in an IGO-graphic, which grouped the activities according to four components: a) Strategic Actions: High importance and high governance; b) Challenges: High importance but low governance; c) Minor Actions: Low importance and low



governance; and c) Short Term Actions: Low importance and high governance (Fig. 2).

The results allowed us to identify elements that should be prioritized in future national risk management plans, highlighting the importance of inter-agency work to carry out the proposed actions.

A total of 35 actions were identified. The main strategic actions are: (i) map the annual cycle in the coastal regions of Colombia of the main artisanal and semi-industrial fishery products; (ii) determine the treatment given to the fishery products in terms of: cleaning, loading, transportation, storage and marketing; (iii) identification, monitoring, and evaluation of seafood habits of the Colombian population; (iv) educational programs and information campaigns on HAB impacts, addressing coastal and island communities in the Caribbean and Pacific coasts; (v) implementation of HAB training programs for administrators and decision makers; (vi) strengthen the national group of HAB experts to provide ongoing advice in monitoring, identification of toxic species and toxin analysis; (vii) establish a national network for monitoring, research, and communication on HAB events; (viii) make a survey of HAB events in Colombia and incorporate this information into the HAEDAT system of the IOC HAB Programme; (ix) strengthen environmental monitoring programs to improve the knowledge on harmful and toxic algae; (x) update the list of potential toxic species present in Colombian ecosystems.



The workshop stimulated interest in HABs in Colombia. It was an opportunity to introduce an ongoing problem in a comprehensive way. The representatives of different institutions expressed a high level of commitment. Based on these results, the National Technical Committee on Marine Pollution will work on a proposed HAB risk management plan for the next five years.

To complement to the workshop, a theoretical and practical course on HABs took place from 8th – 12th June 2015. Beatriz Reguera (IEO, Spain), Brigitte Gavio, Luis Carlos Montenegro, J. Ernesto Mancera (Universidad Nacional de Colombia) and Howard Junca (Universidad Militar Nueva Granada) gave lectures and laboratory practicals on the biology, taxonomy, and ecology of HABs, including Ciguatera Fish Poisoning (Fig. 3). A total of 20 participants from different universities and institutions from Colombia and Perú attended the course. As a result, knowledge and dissemination of information about HABs in Colombia was improved; the network of researchers was strengthened and the list of potentially toxic species present in the country was updated (Fig.4).

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Phytoplankton taxonomists at work!

The Marine Institute, Ireland, in conjunction with the IOC Science and Communications Centre on Harmful Algae in Copenhagen, Denmark organised the *2015 BEQUALM Marine Phytoplankton Intercomparison Exercise* three day workshop in the Danhostel, Hillerød, Denmark, 9th-11th November 2015. There were approximately 20 attendees from different laboratories from across Europe and Asia at the workshop this year (a full house). Lectures on the genera *Prorocentrum* and *Protoperidinium* were presented by Jacob Larsen as well as updates on nomenclatural changes, including the name change from *Ceratium* to *Tripos* and the latest changes under discussion about the *Alexandrium* genus. Nina Lundholm also gave two lectures focussing on the genus *Pseudo-nitzschia* reviewing all the new toxic species recently described and also taxonomic developments in the genus *Chaetoceros*. We are very grateful to have such an excellent quality lecturers at the workshop. Thanks also to Eileen Bresnan from Marine Scotland for her talk on carbonate chemistry and ocean acidification and Paula Hynes, Marine Institute for her talk on harmful algal bloom forecasting through the ASIMUTH project and its HAB bulletins.



Fig. 1. Jacob Larsen lecturing at the BEQUALM workshop

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IOC-SCOR GlobalHAB Steering Meeting, Oban, UK 8-10 March 2016

GlobalHAB, the new joint IOC-SCOR programme, will be launched in 2016, with financial support from the U.S. National Science Foundation (through SCOR) and from IOC. The overall goal of the new programme is to foster activities to stimulate the scientific advance for a better understanding of harmful algal blooms and to contribute to mitigate their impacts. GlobalHAB will address and build upon the most relevant objectives identified by the preceding GEOHAB programme and will incorporate new pressing issues that face the international harmful algal bloom (HAB) science community. GlobalHAB will also take into consideration recommendations posed by the international community to make the new programme relevant to contemporary and future research, funding, and management priorities, continuing to provide an international approach based on science.

The members of the GlobalHAB SSC are Elisa Berdalet (Chair), Neil Banas, Michele Burford, Chris Gobler, Bengt Karlson, Raphael Kudela, Po Teen Lim, Lincoln Mackenzie, Marina Montresor and Kedong Yin. The first meeting of the Scientific Steering Committee of GlobalHAB will be host by Keith Davidson at the Scottish Association for Marine Science (SAMS) in Oban (UK) on 8-10 March, 2016. In this first meeting, the SSC will design the general plan of the programme over the next decade, and map out specific activities for the next three years. The worldwide HAB research community is invited to actively engage in this project.

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Theme session about phytoplankton trends to be held at the ICES ASC 2016



The International Council for the Exploration of the Sea (ICES) Annual Science Conference will take place in Riga, Latvia from 19 – 23 September 2016. The ICES science committee has approved a joint proposal from the ICES Working Groups on Phytoplankton and Microbial Ecology (ICES WGPME) and Harmful Algal Bloom Dynamics (IOC-ICES-WGHABD) for a theme session on "Long-term phytoplankton trends: regional distribution, bloom dynamics and response to environmental drivers". This session aims to bring together phytoplankton and microbial ecologists, HAB scientists and statisticians to discuss observed long-term changes in the phytoplankton/microbial communities and associated impacts on fisheries, aquaculture and the marine environment. Presentations are welcome on a range of topics relating to phytoplankton and HAB ecology including dynamics of algal bloom development, long term changes in phytoplankton communities, large-scale comparisons (meta analyses) of long-term trends, phytoplankton/HAB responses to environmental drivers, ecosystem impacts/effects on ecosystem services, monitoring methods and assessment of ecological/environmental status. The call for papers will be released in January 2016. Further information about the theme sessions at the 2016 Annual Science Conference will be posted on the ICES website towards the end of this year (*http://www.ices.dk/news-and-events/asc/ASC2016/Pages/default.aspx*).

We look forward to seeing you there.

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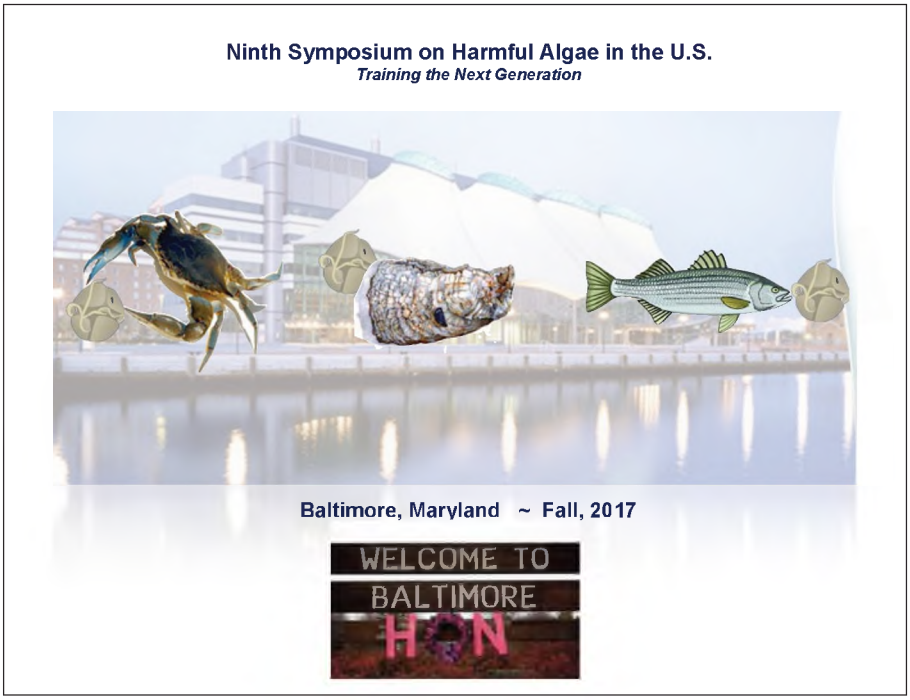
The US 9th Symposium on Harmful Algae

The National HAB Committee (NHC) announces that the US Ninth Symposium on Harmful Algae will be held in Baltimore, Maryland from October 28 to November 3, 2017. The theme of the Ninth Symposium will be "Training the Next Generation" and will include several hands-on workshops for the students and postdoctoral fellows. A presentation by the local DELMARVA organizing committee will be made at the Eight Symposium in Long Beach, California to provide additional details.



The SETAC Europe 26th Annual Meeting

The SETAC Europe 26th Annual Meeting, which will be held in Nantes, France, from 22-26 May 2016. Under the general theme *Environmental contaminants from land to sea: continuities and interface in environmental toxicology and chemistry*, experts from academia, government and industry will share the most recent advanced knowledge in environmental sciences in order to improve chemical risk assessment and support current and future policies.

A session on *Consequences and Solutions of toxins produced by microorganisms: monitoring, management, risk assessment, and future challenges* will be held.

For more information and updates on the meeting, please check website *http://nantes.setac.eu*

Tribute to Yves Collos (1949-2015)

We are extremely sad to inform you that Dr. Yves Collos (CNRS, Montpellier, France), who was head of the "Harmful Algal Blooms and Diversity" group for 8 years, passed away on Sunday 25 October 2015. Although Yves retired last year, he was still very active in writing scientific papers and giving us advice. We have lost a friend and a great scientist who made major contributions in the knowledge of the processes involved in the nitrogenous nutrition of the phytoplankton. More generally, he made a great contribution to the understanding of the marine phytoplankton ecology in the Mediterranean Sea with an emphasis on HAB species developing in the Thau Lagoon. We wholeheartedly express our sympathy to Yves' family.

Yves Collos completed his Bachelor of Science in the Old Dominion University, Norfolk, USA (Sept 1969-Aug 1970) and obtained his Master of Science in Biological Oceanography from University of Washington (Sep 1970-June 1973) Seattle, USA. He prepared his PhD (1982) in Biological Oceanography at Aix-Marseille University. Yves joined CNRS in the Oceanology Center in Marseille in 1976. He moved to l'Houmeau CREMA in 1982 for a 7-year period, after which he joined the Hydrobiology Laboratory in Montpellier where he finished his career as Director of Research. Yves has authored more than 110 papers, which included 91 peer-reviewed publications in the most relevant journals in marine science. He is also author of chapters in several books and made major contributions to nutritional studies in micro-phytoplankton. Over the last fifteen years, he published many relevant papers on the use of different forms of nitrogen by the neurotoxic *Alexandrium catenella* blooming in Thau lagoon and demonstrated that urea together with particulate organic nitrogen trigger *Alexandrium* development in this ecosystem and could explain the high growth rate (up to 1.3 d^{-1}) observed for this species *in situ*. In May 2013, Yves Collos received the Hutchison Medal named in memory of past President, Sir Kenneth Hutchison, which he was awarded for both practical and wide-ranging, philosophical



or thought-provoking published papers; among others for a joined paper with colleagues on "Ocean fertilization for geo-engineering: a review of effectiveness, environmental impacts and emerging governance", published in "Process Safety & Environmental Protection", November 2012. Dr. Collos was a member of the Association for the Sciences of Limnology and Oceanography, International Society for the Study of Harmful Algae and Phycological Society of America.

Only one year ago Yves wrote the following beautiful text on the eve of his retirement. It traces the events that have marked his career. We think this is the best way to discern the man and the scientist he was.

"Having started my career at the CNRS in Marseille Oceanology Centre in 1976, I worked in the team Pelagic Primary Production and participated in several missions at sea (off the West African coast mainly, but also in the North Atlantic and in the Antarctic Ocean). If these missions were sometimes quite exotic (14 m waves to attack the Marion Dufresne 66 ° S and the loss of the Jean Charcot anchor in the port of Nouadhibou in Mauritania after a very festive stop with our American colleagues Atlantis II were the most vivid memories), the extreme diversity of the sites brought some difficulties when writing my PhD dissertation (1982). This led me to change the laboratory in order to focus on a single site where one can easily return regularly to test working hypotheses. Thus, I was one of the gang of eight "CNRS mercenaries" led by Serge Maestrini in 1982 to found the new Laboratory at L'Houmeau, near La Rochelle. If the benefits (to draw ourselves the lab plans and work in brand new premises) were evident, in the longer term, the isolation of the laboratory would quickly become a disadvantage (University of La Rochelle had not yet been founded). After 7 years at CREMA (with a stay of one year at the University of British Columbia in Vancouver), I joined the Hydrobiology Laboratory of Montpellier 2 University in 1991. The Thau lagoon (West Mediterranean, France) had obviously caught my attention as a remarkable site and was easily accessible. Our first goal was basically to decipher the functioning of the ecosystem, particularly at the level of the nitrogen cycle (ah, fabulous 24 hours experiments at the edge of the pond with a barbecue and a night under the stars on the terrace of oyster mas Ifremer in Bouzigues). But it is the appearance of toxic algae blooms in 1995 which enabled the initiation of work of greater scope, because we could not only devote our study to a single site, (especially at the Crique de l'Angle which is a kind of giant continuous culture pond), but mostly studying a unique species among the phytoplankton communities. The combination of in situ and laboratory studies of Alexandrium catenella/tamarense was certainly the highlight of my career. So if we had to leave a message for those who will follow, I would continue to study the Mediterranean lagoons that are fabulous incubators of phytoplankton where time and space are no longer inextricably linked, and at a time when modeling seems to outweigh all else, remember that nothing can replace field observations."

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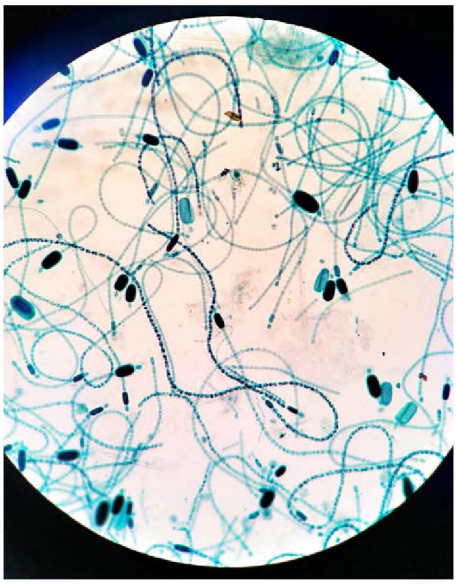
10th International Conference on Toxic Cyanobacteria



October 23–28, 2016 in Wuhan, China. Conference theme: *Research to Risk Management*

Scheduled sessions include: Cyanobacterial detection; Cyanotoxin analysis; Toxicology and toxicity assessment; Secondary metabolites production and functions: biosynthesis, regulation, biological functions; Ecology and Cyanobacterial Bloom Dynamics: the nutrient control of cyanobacterial blooms; Cyanotoxin compartmentation and persistence; Interactions between bacteria and cyanobacteria; Risk management of cyanobacterial blooms and cyanotoxins at scales of catchment, in-lake and water treatment; Remote sensing of blooms.

Abstracts of poster and oral presentations, deadline June 23, 2016.

For more information see: *http://www.ictc10.org/dct/page/1*

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Exceptional Summer Conditions and HABs of *Pseudochattonella* in Southern Chile Create Record Impacts on Salmon Farms

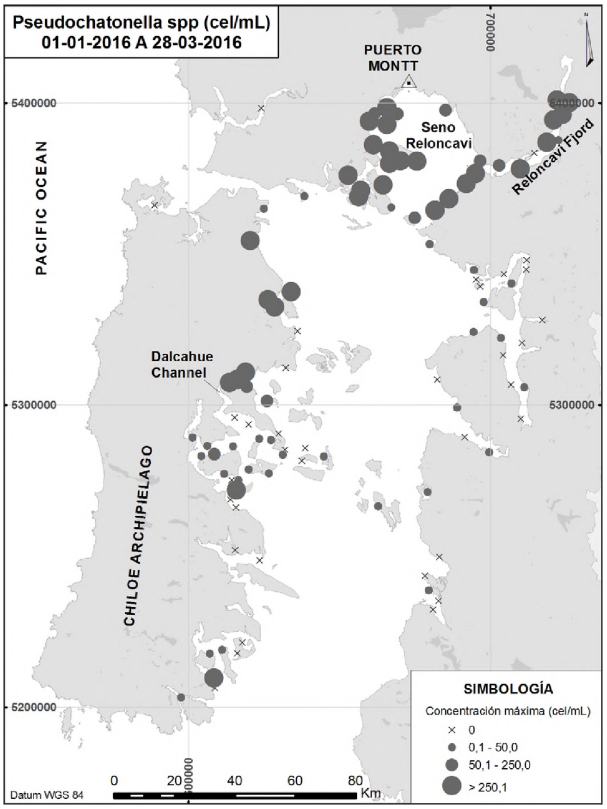

Fig. 1. Geographic distribution of Pseudochattonella *sp. in the Inland Sea of the Xth Region (ISEX) during summer 2016.*

The summer of 2016 was a great season for tourism in Southern Chile – however the fish farming industry was negatively impacted by exceptional harmful algal blooms of *Pseudochattonella* sp., along with minor blooms of *Alexandrium catenella* and *Leptocylindrus danicus*. This report focuses on the *Pseudochattonella* (=*Chattonella*) blooms at the Inland Sea of Region X in Chile (ISEX, Fig. 1). On 20th January 2016, phytoplankton samples were received at the Chiloé laboratory along with comments from a fish farmer, stating that Atlantic salmon had gill problems, low appetite and an increase in mortality rate. Results from the POAS (Programa Oceanográfico y Ambiental en Salmónidos) programme of Plancton Andino showed a mean of 64 x10^3 cell L^{-1} (CV 26 %) of *Pseudochattonella* sp. (Fig. 2) between 0 and 15 m in a relatively well mixed water column. Surface water temperature, salinity and dissolved oxygen were 15.1 °C, 32.5 psu and 8.3 mg L^{-1}, respectively. This was not a typical mono-specific flagellate bloom for this time or place as *Pseudochattonella* sp. cells represented approximately 15 % of the total microphytoplankton community. The diatom *L. danicus* had an important ecological role afterwards (data not shown). The *Pseudochattonella* sp. bloom (maximum of 7.7x10^6 cells L^{-1} on March 13) developed from the end of February to March 15th 2016, and resulted in massive fish kills in the northern Chiloé Archipelago, Calbuco, and Reloncaví Sound and Fjord.

Blooms of *Pseudochattonella* sp were previously observed in the region, in 2004 (≤ 4x10^4 cells L^{-1}) and 2009

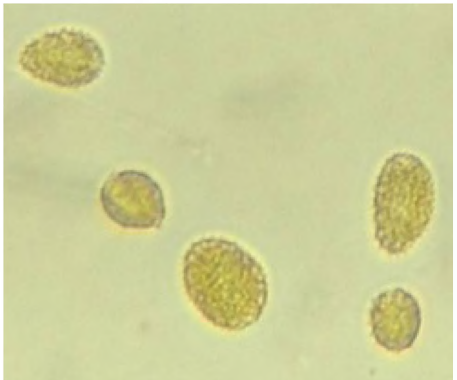

Fig. 2. Light microscope images (x400) of different forms of Pseudochattonella *sp.*

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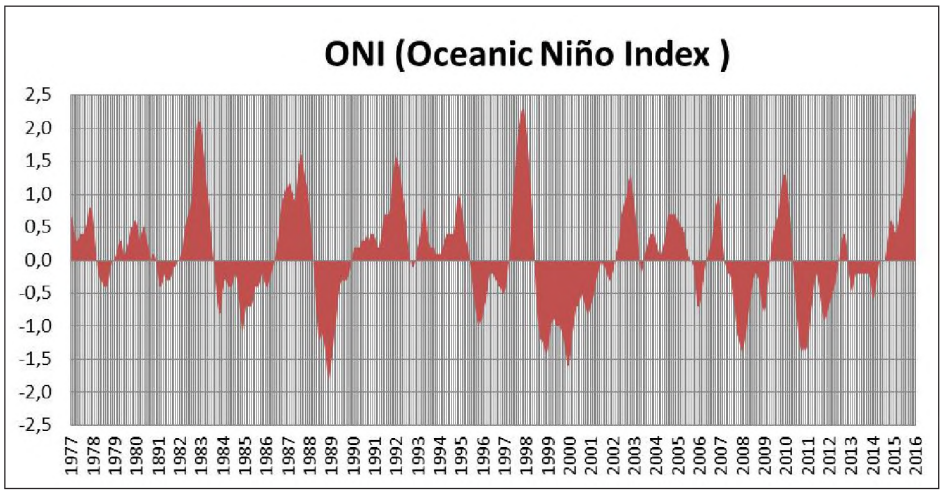

Fig. 3. Note the signal in late 2015 and 2016. Warm (+) and cold (-) periods based on a threshold of +/- 0.5°C for the ONI [SST anomalies in the Niño 3.4 region (5°N-5°S, 120°-170°W)]. Visit web page for details. ONI NOAA, Climate Prediction Center [4].

(≤ 4 x10^5 cells L^{-1}), but from an economic point of view, this 2016 *Pseudochattonella* bloom has been the most damaging event in South America, with losses up to US $ 500 M. The magnitude of the event was intensified by a climatic anomaly, among other features. This event, type of photoautotrophic flagellate, magnitude and economic impact is the first in Chilean HAB history [1-3].

El Niño and Meteorological Conditions: The recent "El Niño" signal in the South Eastern Pacific Ocean has been one of the strongest in the last decades [4]. Fig. 3 shows the inter-annual variability of the Oceanic Niño Index (ONI), with a large increase during the second semester of 2015. Additionally and as consequence, the oceanographic and meteorological anomalies during summer 2016 in southern Chile have also been remarkable: Chile had one of the driest summers on record with a -70 % rainfall deficit until March 23th [5], with more solar radiation than normal, and positive sea surface temperature anomalies of 1-2 °C. Fig. 4 shows the Osorno Volcano, located close to the marine area under study on March 15, 2016. Lack of snow and increased ice melt on the upper part of the Osorno cone is unusual during the second half of the 20th century, but has been more common in recent decades.

Biological features of the bloom: During Summer 2016 the ISEX area received warmer ocean waters and less freshwater influxes. Despite the lower run-off, thermohaline stratification developed (Fig. 5). It is known that autotrophic flagellates can be more competitive with high solar radiation, less wind stress and lack of upwelling. These flagellate cells can grow and/or aggregate in the pycnocline wheres density gradients are marked [6-8].

One of the main features of the event was its extremely high ichthyotoxicity, based on observations of extensive damage in salmon farms in a very short period of time (30 million fish killed in a few days). From an ecological point of view, the quasi-monoalgal thin layer formation of *Pseudochattonella* cells was the main feature. This feature was studied for more than 15 days. During the bloom, vertical CTD-O, *in situ* chlorophyll *a* and backscattering casts, and phytoplankton counts were made in the "hot spot" at the Seno Reloncaví area. We found surface temperatures above normal and sub-surface peaks of dissolved oxygen close to the 7-10 m layer. In most cases, phytoplankton assemblages and *in situ* vertical profiles of chl *a* fluorescence showed a sub-surface maximum in the pycnocline. This thin layer was dominated (93%) by *Pseudochattonella* cells (Fig. 6-7) which were photosynthetically very active (Fo > 10 and Fm > 20).



Fig. 4. Osorno Volcano, March 2016, showing the lack of snow and glacial melt at the upper part of the cone (Photo by Cristóbal Clément).

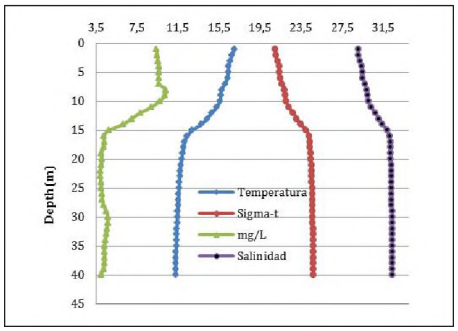

Fig. 5. Profiles of temperature, salinity, sigma-t and dissolved oxygen (mg/l) in Seno Reloncaví during the peak of the bloom (March 7, 2016).

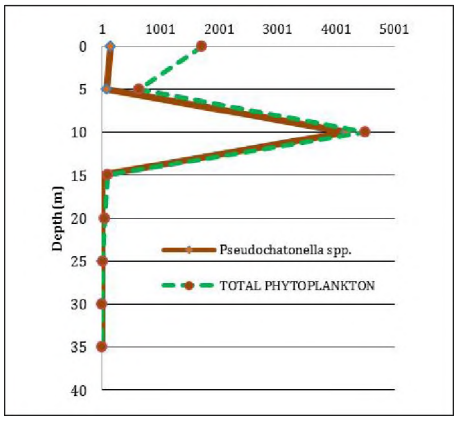

Fig. 6. Vertical distribution of phytoplankton and Pseudochattonella *(cell mL^{-1}), during the peak of the fish-killing event (March 7, 2016 at 1451).*

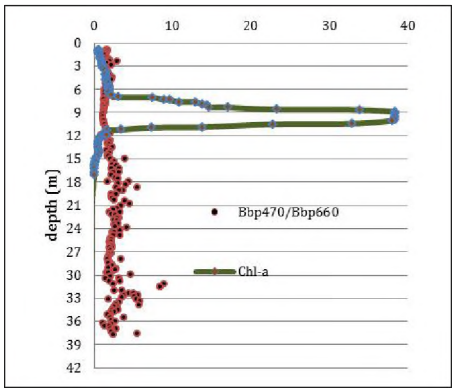

Fig. 7. Vertical profile of in situ chl a (mg m-3) and backscattering ratio (470 nm/ 660 nm) of the thin layer of Pseudochattonella *(same day and time as in Fig. 6).*

Final Remarks: The southern Chile summer 2016 HABs and consequent massive salmon mortality reduced the actual and potential supply, creating a related increase in the international salmon price, and also variability in stock markets prices. Information from the fish farmers suggested that Coho Salmon is more resistant to this HAB than Atlantic salmon during this event. The climatic anomalies and thin layer formation, among other features, are key issues in the dynamic of the different stages of *Pseudochattonella* bloom at the ISEX area.

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Forthcoming: Guide to the Identification of Harmful Microalgae in the Gulf of Mexico

A manual entitled *"Guide to the Identification of Harmful Microalgae in the Gulf of Mexico"* is nearing completion and the English version will be available August 2016. The Guide has chapters on *Pseudo-nitzschia* and other diatoms (Meave and Zamudio), identification of dinoflagellates (Steidinger), and raphidophytes (Tomas) as well as chapters on methods for benthic dinoflagellate sampling (Tester and Kibler), dinoflagellate cyst sampling (Williams), water column sampling strategies (Christman and Steidinger), sample preparation and enumeration (Steidinger and Christman), the use of remote sensing in the study of HABs (Cannizzaro, Soto, and Hu). Also included are several other introductory chapters, e.g., history of the HAB binational program (Allen) and public outreach (Brown), and appendices on sources of information. Production of the guide was supported by the Environmental Protection Agency Gulf of Mexico Program through a binational program with Mexico, the Florida Fish and Wildlife Conservation Commission, the Florida Institute of Oceanography, and other sources. This binational effort is edited by Karen Steidinger and Maria Esther Meave del Castillo and will be available for free over the internet as a download.

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Karenia mikimotoi, a rare species in Hong Kong waters, associated with a recent massive fish kill

Potentially toxic microalgae belonging to the genus *Karenia* occur all over the world. Although they can be detected occasionally in Hong Kong coastal waters, they had never bloomed before causing massive fish kills. From late-December 2015 to mid-February 2016, fish killing incidents were reported from several fish cultivation zones in Tolo Harbour and Long Harbour, Hong Kong (Fig. 1). At the very beginning (December 14, 2015), algal blooms mainly formed by *Karenia mikimotoi* (Fig. 2A) with a lower density of *Karenia* cf *papilionacea* (Fig. 2B) were found in fish cultivation zones and nearby waters in Yim Tin Tsai (Tolo Harbour) (Fig. 3). New affected cultivation areas were reported to the east in Lo Fu Wat and off Tolo Channel to Long Harbour (Hoi Ha Wan, Kau Lau Wan and Sham Wan), and offshore (Tap Mun, and O Pui Tong further north). By mid-February 2016, accumulated fish loss was nearly 221 t, mainly including Sabah grouper, green grouper and giant grouper. The only thing the mariculturists could do was just to collect and dispose of the dead fish. Mariculturists from culture zones that were not yet affected were advised to consider early harvesting to minimize the associated risks and loss. [1]

Karenia mikimotoi can produce hemolysins and icthyotoxins, while *Karenia papilionacea* can produce neurotoxic brevetoxins which cause neurotoxic shellfish poisoning (NSP) in humans and other mammals. [2, 3] However, the exact fish killing mechanism of these algal species is still under investigation.

In order to explore the probable cause of the fish killing incident, four sampling points including Tai Po Waterfront Park (TPWP), Lam Tsuen River estuary (LTR), Shing Mun River estuary (SMR) and Yim Tin Tsai (YTT) were chosen for further investigation (Fig. 3). Seawater samples were taken from these four sampling points on 4 January 2016 and a variety of parameters measured. The seawater qualities from four sampling points mostly achieved the Water Quality Objectives (WQOs) standard, except that the ammonia levels of TPWP and LTR were slightly higher than that of the standard (0.021 mg/L) [4]. In addition, the fish kill was not due to virus contamination. Viral nervous necrosis (VNN) and red sea bream iri-

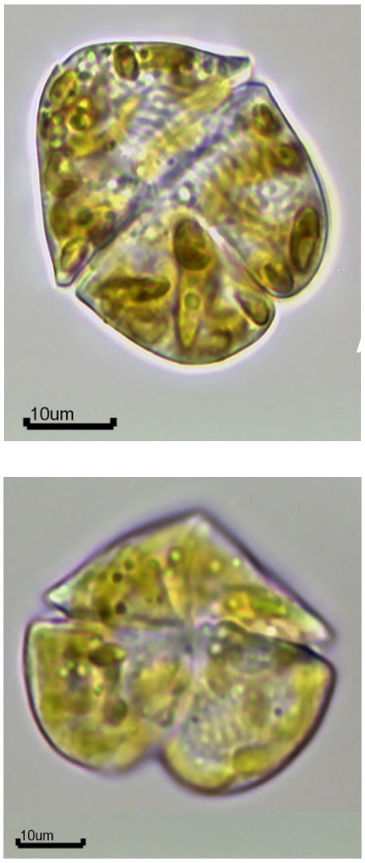

Fig. 2. Micrographs (X1000) of Karenia *specimens from Yim Tin Tsai, (Tolo Harbor, Hong Kong, collected in December 2015. A.* Karenia mikimotoi*; B.* Karenia *cf* papilionacea.



Fig. 1 Dead fishes found near Yam Mun Tsai fish cultivation zone, Hong Kong December 23, 2015.



Fig. 3. Cultivation areas in Hong Kong affected by fish kills and location of sampling stations (▲) in January 4, 2016: Tai Po Waterfront Park (TPWP), Lam Tsuen River (LTR), Shing Mun River (SMR) and Yim Tin Tsai (YTT).

doviral (RSIV) viral tests were done by Hong Kong Agriculture, Fisheries and Conservation Department (AFCD), and both of them showed negative results (AFCD, pers.com.).

Both potential toxin producers, *Karenia mikimotoi* and *Karenia* cf *papilionacea*, were found in seawater samples from the four sampling points. The cell densities of the two species were within the range of 10 to 40 cells mL^{-1}. Hence, an acute fish-toxicity test, with *Oryzias melastigma* (marine medaka) used as target species, was carried out for 96 hours with seawater collected from the four points. No mortality or abnormal behavior was observed either in fishes exposed to the seawater samples or in fish exposed to the artificial seawater (control).

From all the results above, the fish kills should not be attributed either to changes in environmental conditions (e.g. drastic change of pH or dissolved oxygen) or to virus contamination. Although *Karenia mikimotoi* and *Karenia* cf *papilionacea* were found in the seawater samples from all sampling points, their cell densities did not reach bloom levels. Therefore, they cannot be excluded as the causative species of the fish kills barely based on the fish exposure experiments conducted.

Karenia species seldom appear in Hong Kong coastal area. To investigate the relationship between air temperature and microalgal blooms, the temperature records from December 2015 to February 2016 were obtained from the Hong Kong Observatory (HKO) [5]. A relationship was found between increasing cell densities of *Karenia* species and the falling followed by sudden rising of air temperature values. For example, the air temperature dropped 11.2 °C between January 21, 2016 (16.2 °C) and January 24, 2016 (4.0 °C), and suddenly raised 12.3 °C on January 28, 2016 (16.3 °C). During this time, according to seawater analysis results disclosed by AFCD, cell densites of *Karenia mikimotoi* increased to 4,680 cells mL^{-1} and 5,267 cells mL^{-1} on January 29, 2016 and January 30, 2016 respectively. This trend (sudden falling followed by rising of air temperature coinciding with blooming of *Karenia* species) was also found in the periods December 2, 2015 to January 6, 2016 and December 24, 2016 to January 3, 2016. In the case of *Karenia* cf *papilionacea*, this trend was observed between December 24, 2015 and January 3, 2016. Several blooming species of *Karenia* have been reported from Hong Kong waters in the last twenty years. However, they were never associated with fish mortalities. Abrupt (falling and quick rising) changes of air temperature maybe the critical factor that triggered the blooms and fish killing events.

In summary, two potentially icthyotoxic species, *Karenia mikimotoi* and *Karenia* cf *papilionaceae*, bloomed in Hong Kong coastal from December 2015 to February 2016 and were associated with mass mortalities of fish. The fish kills could not be attributed either to changes in physical-chemical parameters or to virus contamination. However, sudden and drastic temperature changes may have been related to the microalgal blooming and toxin production mechanism. For further investigation, monocultures of *Karenia mikimotoi* and *Karenia* cf *papilionacea* will be established, and subsequent fish exposure to blooming levels of the algal species will be conducted. Studies of environmental factors, especially on temperature change, will also be needed to investigate the triggers for algal blooming and toxin production.

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Low dissolved oxygen concentration as the triggering factor of fish mass mortalities during algal bloom incidents in Jakarta Bay, Indonesia

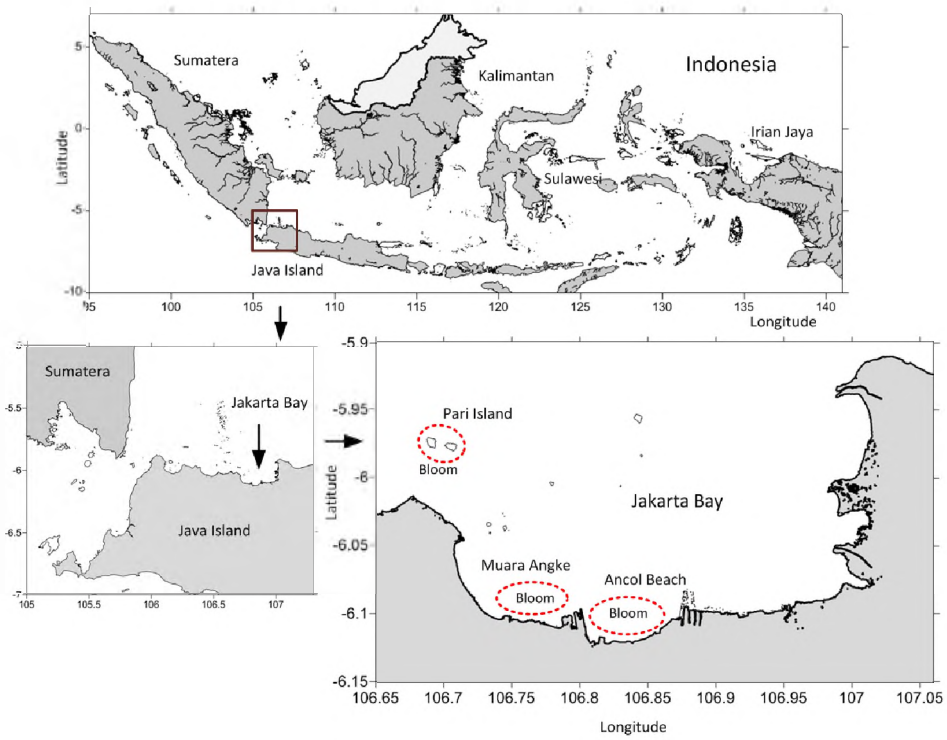

Fig. 1. Map of Jakarta Bay and the location of the algal bloom and fish kill events (red circle).

Recently, the recurrence of algal blooms has had a negative impact on fisheries in Jakarta bay and resulted in the mass mortality of wild fish in three locations (Fig. 1). These algal blooms developed in the transition period from the dry to the rainy season (October to December). The fish kills occurred on October 28, 2015 in Pari Island, on November 28, 2015 in Ancol Beach and on December 13, 2015 in Muara Angke. A large number of dead fish appeared spreading along the shore of the three locations after the bloom incident (Fig. 2). Most of the fish seemed impacted by oxygen depletion and were observed floating in surface waters in the early morning before sunrise. According to local people and fishermen, there were water discolorations a few days before the fish kill incident. They noted that the color of the surface waters become red-brownish around Ancol beach and Muara Angke, while in Pari Island, they changed to red-green.

The collection of water samples and measurements of environmental factors were conducted after the bloom incident. These data showed that the phytoplankton community in Pari Island was dominated by *Trichodesmium*, in Ancol by *Alexandrium* while samples from Muara Angke were dominated by *Skeletonema* (Fig. 3). It is a surprise that *Alexandrium* was found as the dominant species in samples collected from Ancol beach. This is the first record of a bloom of this genus in these waters. However, it is still unknown whether this species was the only bloom species involved in the event in Ancol beach as the samples were collected a few days after these fish kills. *Skeletonema* and *Chaetoceros* regularly form blooms in Jakarta Bay and are considered to have an important role in the ecology of these waters.

During these events, the seawa-



Fig 2. Some photographs of dead fish caused by algal blooms in Jakarta Bay.

ter temperature ranged from 28.30 to 29.90 °C, and salinity from 29 to 33 psu. The concentration of phosphate and nitrate in Ancol beach ranged from 0.004 to 0.032 mg L^{-1} and 0.139 to 0.374 mg L^{-1} respectively, and of phosphate and nitrate in Muara Angke from 0.05 to 0.19 mg L^{-1} and 0.09 to 0.78 mg L^{-1}, respectively. These concentrations exceeded the standard limits based on regulations implemented by the Indonesian Ministry of Environment (2004), i.e., 0.008 mg L^{-1} for nitrate and 0,015 mg L^{-1} for phosphate. There was no measurement of environmental parameters in Pari Island during the event.

The concentration of dissolved oxygen (DO) in Ancol beach ranged from 0.01 to 1.47 mg L^{-1}, and in Muara Angke from 1.0 to 2.0 ppm, respectively. Generally, the concentration of dissolved oxygen in ocean waters is typically between 7 and 8 mg L^{-1}. If concentrations fall below 4 mg L^{-1}, the organisms will begin to react, with mobile organisms either avoiding or migrating out of the area. Waters with less than 2 mg L^{-1} of dissolved oxygen is termed hypoxic and unable to support most forms of life. So it is most likely that the cause of fish kills in Jakarta Bay was due to the very low concentration of dissolved oxygen in the waters.

The intensity and distribution of algal blooms in Jakarta Bay has increased recently and a solution is required to address this natural phenomenon. One of the causes can be anthropogenic pollution which causes eutrophication of the bay [4]. Nevertheless, it is important to note that these blooms are not always accompanied by fish mortalities, except if their occurrence coincides with the period of *lowest mean sea level* (LMSL) during night time.

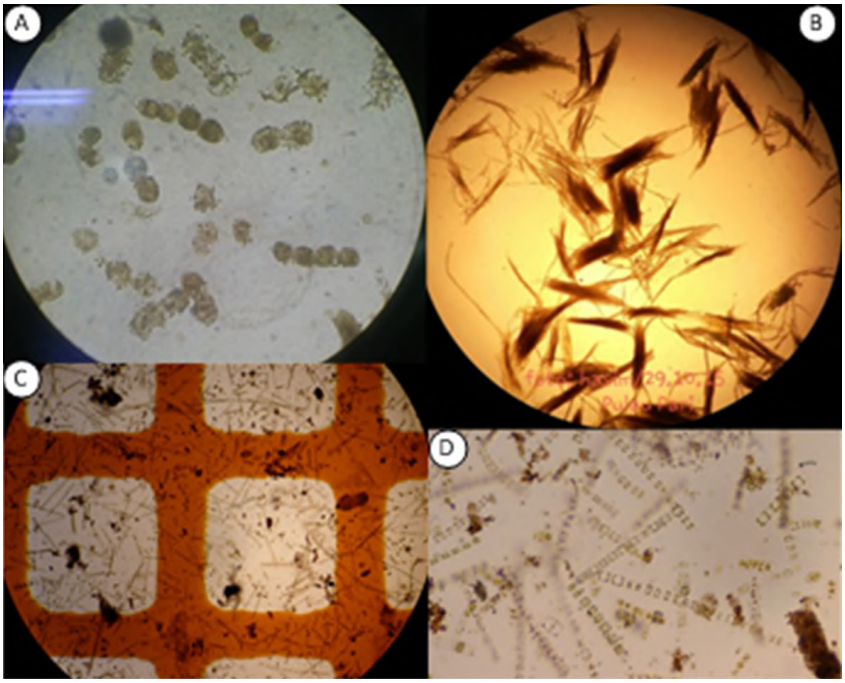

Fig 3. Photographs of the dominant genera after bloom incidents in Ancol beach: Alexandrium *(A); Pari Island:* Trichodesmium *(B) and Muara Angke:* Skeletonema *(C) and (D).*

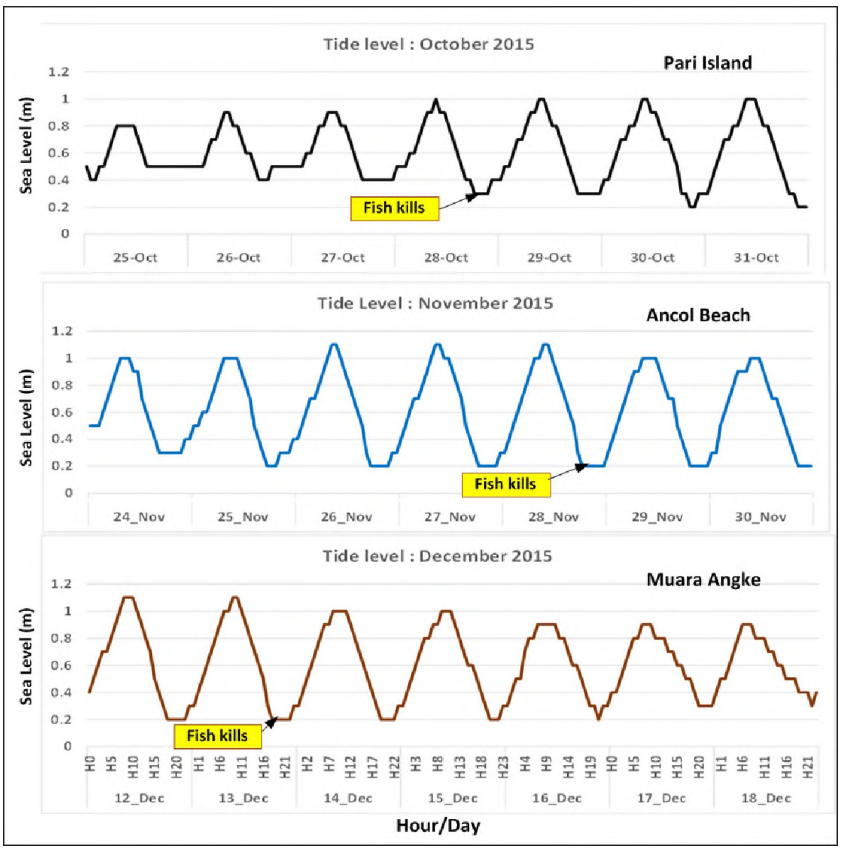

Fig 4. Tide levels during bloom events in Jakarta Bay. Tide level in Pari Island: October 25 (blue), Ancol Beach: November 24 (red) and Muara Angke: December 12 (grey). Fish kills occurred in October 28, November 28 and December 13, 2015 coinciding with periods of lowest sea level.

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A *Cochlodinium polykrikoides* Margalef bloom in the channels of Marina Hemingway, La Habana, Cuba

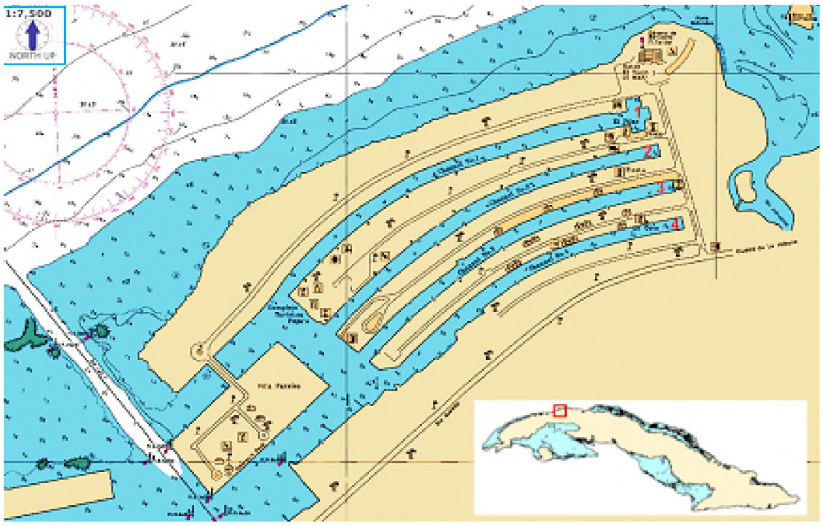

Fig. 1. Location of Marina Hemingway in Cuba

Cochlodinium polykrikoides Margalef [1] formed a conspicuous bloom in Marina Hemingway, west of La Habana city, Cuba. Marina Hemingway is formed by four interconnected artificial channels of 30 x 800 m parallel to the open coast, a mean depth of 5m, with a common connection to the sea (Fig. 1). No freshwater inputs exist in the marina and tidal water exchange is very limited due to the length of the channels. Water discolorations (Fig. 2), first detected on September 5, 2015 (1.8 x 10^6 cells L^{-1}) persisted for more than a week and 18 days later densities still were as high as 7.5 x 10^5 cell L^{-1}. Marina Hemingway is an important tourist (yachting) resource. There were no apparent deleterious effects in marine life, and there are no cultivation resources to be affected in the area, but the bloom caused was social alarm.

The bloom appeared and was maintained under sunny and calmed weather conditions, with seawater temperatures of 30-32 °C, salinity 32-36 and pH 7.2-7.8, and declined following two days of torrential rain.

Samples were taken to the plankton laboratory at the Centro de Investigaciones Pesqueras and observed under a MOTIC BA210 microscope, coupled to a digital camera, at 100x and 400x magnification. Images were processed with the software MOTIC Images-2000, version 1.2

The cells appeared mainly in chains of two to four cells. The cingulum encircled the cell almost twice, and the sulcus could be observed as a very thin line parallel to the cingulum and just beneath it (Fig. 3). Each cell had a reddish eyespot on the dorsal side, and rod-shaped chloroplasts. These morphological characteristics permitted the differentiation from the close species *Cochlodinium fulvescens* Iwataki, Kawami et Matsuoka [2].

C. polykrikoides was first described from samples collected in Bahía Fosforescente, Puerto Rico [1]. This bay became famous for having spectacular bioluminescent red tides caused by *Pyrodinium bahamense* which bloomed together with *C. polykrikoides*. The bay is formed by three branches in a mangrove ecosystem with a small connection to the sea. Like in Marina Hemingway, its restricted circulation caused limited dilution of the blooms. Blooms of *C. polykrikoides* have also been recorded in Bahía de Cienfuegos, southwest coast of Cuba, where they were associated with mass mortalities of marine organisms, such as oysters, crabs and fishes [3].



Fig 2. Water discoloration due to Cochlodinium polykrikoides *in one of the channels of Marina Hemingway.*

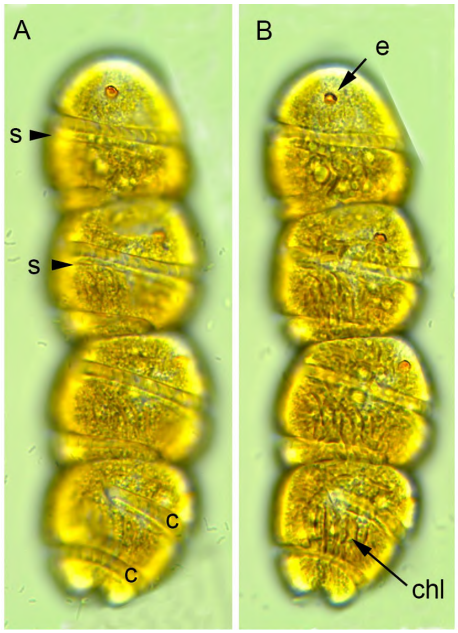

Fig 3. Cochlodinium polykrikoides. *A-B Light microscopy micrographs of a four-celled chain at different focus: s, sulcus; c cingulum; e, eyespot; chl, rod-shaped chloroplasts.*

Acknowledgements

We thank Oriel Rico and Daylin Pérez their reporting of the occurrence of the red tide.

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Prorocentrum lima and *Prorocentrum balticum* blooms in Cartagena de Indias, Colombian Caribbean

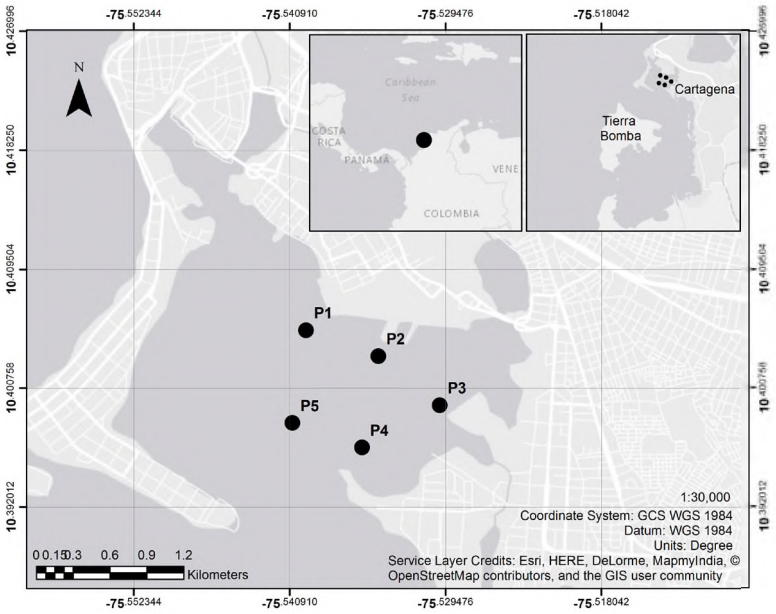

Fig. 1. Cartagena de Indias sampling sites during the algal bloom on December 2015.

Cartagena de Indias, declared as "Heritage of Humanity" by UNESCO in November 1984, is situated in the Caribbean, and represents the most important tourist and historic district of Colombia. Due to its geographical location, different industrial and port activities take place there. However, its architectural, cultural and gastronomic richness positions it as a preferred national and international tourist destination.

Cartagena Bay (10° 26'- 10° 16' N and 75° 30 '- 75° 35' W) is part of a semi-closed estuarine system of 82 km^2 and 16 m average depth [1]. Tierra Bomba island separates the bay from the Caribbean Sea, leaving two communication channels: "Bocagrande" in the northeast and "Bocachica" to the southeast. The northern end, called internal bay, has no direct communication with the Caribbean Sea. Cartagena Bay receives water rich in nutrients and sediments through the Dique Canal, an artificial branch of the Magdalena River [2].

During late December 2015, a red water discoloration occurred in the northern part of Cartagena bay (internal bay, Fig.1). On December 21 and 22, five surface phytoplankton samples were collected each day and fixed with neutral formaldehyde (4%, with sodium tetraborate) solution. Samples (10 mL) were examined using an inverted microscope (X600) according to the Utermöhl quantitative method.

No unpleasant odors or dead organisms were observed or reported during the algal bloom. On December 21, the total phytoplankton cell density ranged from 1.67 x 10^6 to 7.56 x 10^6 cells L^{-1} (Fig. 2). Blooms of *Prorocentrum lima* and *Prorocentrum balticum*, with population densities of 0.72 - 3.56 x 10^6 cells L^{-1} and 0.70 - 3.58 x 10^6 cells L^{-1} respectively, were identified as the cause of the discoloration. Other accompanying species were *Tripos furca, Prorocentrum gracile, Protoperidinium pellucidum, Skeletonema costatum, Thalassionema nitzschioides, Chaetoceros curvisetus, Ch. compressus* and *Guinardia striata*; which presented a total population density between 0.41 x 10^6 and 0.1 x 10^6 cells L^{-1} (Fig. 2).

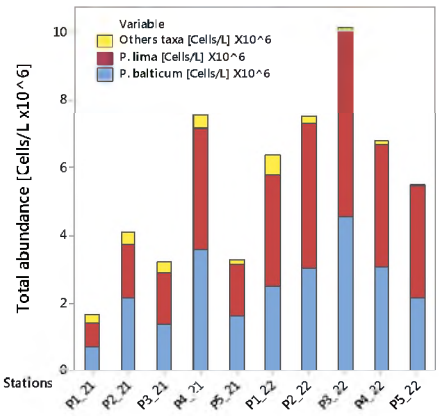

Fig. 2. Phytoplankton population densities during the algal bloom on December 2015. Each bar corresponds to an observation. Samples collected on December 21 and 22.

On December 22, the bloom remained in the same area but the cell density was greater than before, ranging from 10.16 x 10^6 to 5.51 x 10^6 cells L^{-1}. *P. lima* and *P. balticum* population densities, 2.1 - 4.5 x 10^6 cells L^{-1} and 3.2 - 5.4 x 10^6 cells L^{-1} respectively, were also higher. Of the species found, only *P. lima*, usually a benthic species, has been reported as a potentially toxic dinoflagellate, causing diarrhea in humans. This algal bloom should serve as a warning to the environmental authorities and the tourist industry of Cartagena de Indias. Only in the last tourist season of 2015, it is estimated that the city hosted about 450,000 tourists, 11% of which were international visitors coming from Europe, the United States, the Caribbean and South America.

The causes of the algal bloom in Cartagena are unclear, but dredging activities in Bocachica and the main channel during December 2015, along with high water temperatures (30,2 °C ±0,12 SD), may have been the factors promoting such a *P. lima* bloom. It is important to increase the local understanding of the dynamics of algal blooms in the region in order to improve the ability to cope with its impacts which could be highly negative for the population and industry.

Acknowledgments

We are grateful to CIOH, Dirección General Maritima – DIMAR, for their support during sampling and laboratory analyses.

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Blooms of *Cylindrospermopsis cuspis* in oyster beds of Términos Lagoon, southeastern Gulf of Mexico

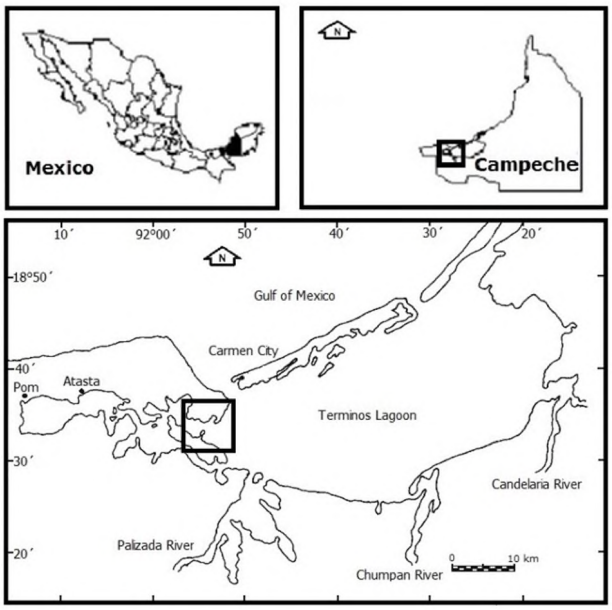

Fig. 1. Study area and location of sampling stations

Términos Lagoon is one of the key ecosystems in the southern Gulf of Mexico and has been the focus of both national and international attention because of its ecological and economic importance and due to the potential impact exerted on this ecosystem by human activities [1].

As part of a the project to describe the phytoplankton community with an emphasis on harmful species, six stations were monitored monthly in oyster beds of Términos Lagoon (Fig. 1), from August 2012 to September 2013. These are the areas of natural oyster harvesting to supply the local market for human consumption. In October 2012, a bloom of the cyanobacterium *Cylindrospermopsis cuspis* Komárek et Kling, reaching $1.3x10^6$ cells L^{-1}, caused the temporary closure of the extraction activity of *Crassostrea virginica* Gmelin for 15 days. This bloom lasted until November 2012 (Fig. 2). Salinity was 7.3 and temperature 27 °C. No discoloration of water was observed. Cyanobacterial abundance increased to 10^6 cells L^{-1} in October, and small peaks were also observed during the rest of the year. Among cyanobacteria, the genus *Anabaena* Bory de Saint-Vincent occurred throughout the study period, with a maximal density of $1.9x10^6$ cells L^{-1} and a minimum of $3.6x10^4$ cells L^{-1}, without any apparent harmful effect.

Both taxa have been previously recorded in the fluvial-lagoons Pom-Atasta and Palizada-East adjacent to the study area, with densities up to 10^3 cells L^{-1} [2]. It should be noted that densities were lower than those reported in this study because they were found in winter. *Cylindrospermopsis cuspis* was reported in the region of Tuxtlas, in the southern part of the state of Veracruz [3-4], without apparent toxic events.

Cyanobacterial blooms have been recurrent in some lagoons of the Gulf of Mexico. In Catemaco Lake in Veracruz, a bloom of *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya et Subba Raju occurred, causing a high bioaccumulation of toxins (cylindrospermopsin and paralytic shellfish toxins) throughout the food chain [5-6]. This species contributed more than 90% of the total abundance of cyanobacteria, and it was also dominant in two other lakes from Los Tuxtlas region in the state of Veracruz, Nixtamalapan and Encantada [7]. In October 2013, in the Alvarado Lagoon System, Veracruz, a bloom of *Dolichospermum flos-aquae* (Bréb. ex Bornet et Flahault) P. Wacklin, L. Hoffmann et J. Komárek was observed, with a maximum abundance of $9.1x10^7$ cells L^{-1}, but in that case there were no harmful effects reported [8].

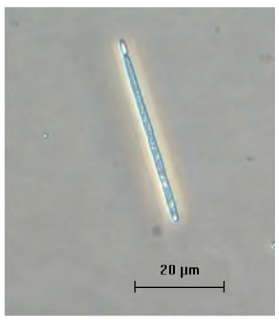

Fig. 2. Cylindrospermopsis cuspis *from Términos Lagoon, southeastern Gulf of Mexico*

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HABs in Paradise



Fig. 1. Sampling sites at Raoul Island, Kermadec Islands (images by Charlie Bedford)

Ciguatera fish poisoning (CFP) is caused by species in the dinoflagellate genus *Gambierdiscus*. CFP has been called a neglected tropical disease, but recently research funds have become available and new research projects are being planned. Cawthron Institute has a small project on 'Climate change and HABs' within its government-funded Safe New Zealand Seafood programme. The main driver for this research is the potential for CFP risk to increase in New Zealand's northern sub-tropical waters as our oceans warm up. *Gambierdiscus* and the related *Fukuyoa* have already been reported in Northland, New Zealand, although in low concentrations [1]. Research has already been carried out in the Cook Islands, in collaboration with the University of Technology Sydney, Australia (contact: Prof Shauna Murray) and with Cook Islands researchers (contact: Dr Teina Rongo, Ministry for Climate Change). CFP occurs regularly in the Southern Cooks and intensive sampling there, using new molecular tools (for example, metabarcoding) and new chemical detection methods [2], has determined that the main causative organism there is the ciguatoxin producer, *G. polynesiensis* [3]. Two new *Gambierdiscus* species from the Cook Islands, which produce uncharacterised toxins, are currently being described.

The Kermadec Island group lies between the Cook Islands and New Zealand and falls within New Zealand's economic zone. A recent announcement by the New Zealand government has promised that 620,000 km^2 in the Kermadec region is to become a fully protected, 'no take' ocean sanctuary. It will become one of the world's largest and most significant protected areas. Previous samples received at Cawthron Institute from Raoul Island, Kermadec Islands, contained *Gambierdicus* cells, but these failed to survive in culture (Fig. 1) [4]. A recent expedition (November 2015) to the Kermadec Islands, led by Dr Tom Trnski, Auckland Museum, resulted in sea water samples from Meyer Island, which lies to the north east of the larger Raoul Island. The concentrations of *Gambierdiscus* in these samples, collected from macroalgae and coralline turfs, suggests blooms of this genus were occurring at depths of 12 to 17 m. Preliminary investigations showed the presence of *G. australes* (which was dominant), *G. carpenteri* and *G. polynesiensis*. More research into the Kermadec Island samples is being carried out and toxin production will be determined once cultures are fully established.

The risk of CFP occurring in New Zealand is increased by these findings, not just through the presence of blooms in New Zealand's territorial waters, but through fish passaging between the Cook Islands via the Kermadec Island group to New Zealand. Further sampling will be carried out in the future to assess changes in the occurrence of *Gambierdiscus* in New Zealand. Comparisons with 'base-line' samples from Northland (2014) will be made based on metabarcoding data analyses. This will be of particular interest with the current El Niño climate event, expected to be one of the strongest on record.

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A first study of the diversity of the *Tripos* genus in the marine waters of Belize and the Gulf of Honduras

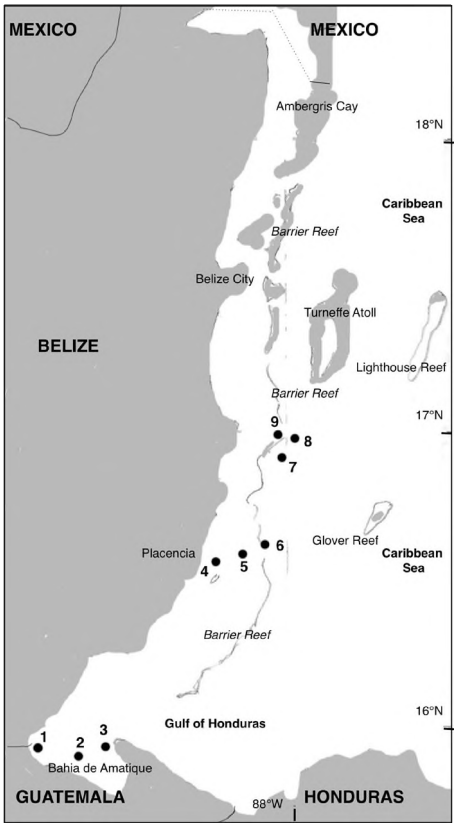

Fig. 1. Sampling sites (filled circles) off Belize and the Gulf of Honduras (Bahia de Amatique). 1. Off Livingston Sea Buoy; 2. Ox Tongue Bight; 3. Off Tres Puntas 4. Off Big Creek; 5. South Long Coco Cay; 6. North Long Coco Cay; 7. South Water Cay 8. Outside Barrier Reef 9. Tobacco Range

Dinoflagellates are common components of the tropical phytoplankton and some can form harmful algal blooms. A first study of the biodiversity of the dinoflagellate genus *Tripos* (Bory de Saint-Vincent) from Belize's barrier reef and the nearby Gulf of Honduras was performed between 2010 and 2013. The reef is an endangered UNESCO world heritage site and the region has extremely important and diverse ecosystems [1]. Species within the *Tripos* genus can cause serious ecosystem stress when they form dense blooms [2,3] and can also be considered as valuable monitors of ocean currents and ocean warming [4,5]. Prior to this study there was little information about the diversity of the *Tripos* genus (recently re-named from *Neoceratium* /*Ceratium* [6]) in these waters.

A sub-surface, 53-µm mesh, plankton net was towed at nine sampling sites (Fig. 1) inside and outside Belize's barrier reef and the nearby Gulf of Honduras (Bahia de Amatique) during the months of March and April from 2010 to 2013 inclusive. Approximately 5 m^3 of water was filtered at each site. Twenty seven species and sub species were found which were similar to those reported for other tropical seas and in the nearby southern Gulf of Mexico [7] with the additional records of *T. contortus* var. *robustus, T. vultur* f. *japonicus,* and *T. reflexus* [6,8]. Some species were rarely observed so only one record is given.

Un-stained light microscope photographs of the species listed in Table 1 are shown in Fig. 2 and Fig. 3. The bracketed numbers in the figures are individual cell width and cell length (width x length) and the antapical horn span (span was measured only in cells where the antapical horns curved towards the apical horn). Cell width is the most reliable of these measurements as broken horns, undulating focal planes and cell debris can all compromise measurements of cell span and length.

In conclusion the study will act as a baseline for the zonal biodiversity and distribution of the *Tripos* species inside and outside Belize's barrier reef and the adjacent Gulf of Honduras (Bahia de Amatique). This is important as some cosmopolitan species of the genus reported here – *T. furca, T. fusus* and *T. muellerii* (Table 1) are known to cause harmful (discolouration and hypoxia) algal blooms. The tropical species of the genus in Table 1 could also be used as biological indicators of global warming [5].

Species	Width (µm)	Length (µm)	Span (µm)
1. *T. arcuatus* f. *karstenii*	80.00	420.00	220.00
2. *T. bigelowii*	25.00		
3. *T. contortus* var. *subcontortus** and	66.67 ± 4.29	365.00 ±68.74	176.8 ±9.94
4. *T. contortus* var. *saltans* (pooled data)	(n=12)	(n=12)	(n=10)
5. *T. contortus* var. *robustus*	100.00	404.00	320.00
6. *T. contrarius* (could be morphotype of *T. trichoceros*)	48.00±12.81 (n=4)	335.00±68.62 (n=4)	362.00±71.14 (n=3)
7. *T. dens*	67±3.83 (n=4)	253.00±73.64 (n=4)	275.00±25.17 (n=4)
8. *T. extensus*	25.25±2.76 (n=8)	1387.38±368.65 (n=8)	
9. *T. furca* var. *eugrammus*	27.53 5.33 (n=21)	166.14±45.11 (n=21)	
10. *T. furca* var. *hircus*	42.73±4.48 (n=9)	236.67±50.46 (n=9)	
11. *T. fusus*	20.63±2.75 (n=6)	532.91 ±104.20 (n=6)	
12. *T. gibberus* f. *dispar*	60.00		112.00
13. *T. hexacanthus*	70.43±7.07 (n=2)	474.67±239.00 (n=2)	
14. *T. horridus* var. *buceros*	36.00	172.00	120.00
15. *T. inflatus*	24.80±3.35 (n=5)	614.00±100.90 (n=5)	
16. *T. kofoidii*	23.60±2.19 (n=5)	135.40±10.43 (n=5)	
17. *T. longirostrus*	24.00	610.00	
18. *T. macroceros* var. *gallicus*	40.00		
19. *T. massiliense* var. *armatus*	63.71±9.51 (n=28)	421.00±101.98 (n=24)	350.29±69.13 (n=21)
20. *T. muellerii* f. *atlanticus* **	67.00±6.86 (n=36)	222.30±25.39 (n=33)	172.21±11.60 (n=19)
21. *T. muellerii* var. *brevis*	68.44±6.15 (n=9)	231.75±26.28 (n=8)	154.25±15.54 (n=8)
22. *T. muellerii* var. *tripodioides*	60.83±5.53 (n=11)	254.91±36.31 (n=11)	154.90±23.48 (n=10)
23. *T. reflexus*	70.00	240.00	200.00
24. *T. symmetricus*	62.80±3.35 (n=5)	138.40±14.31 (n=5)	128.00
25. *T. teres*	38.40±2.19 (n=5)	134.00±25.14 (n=5)	
26. *T. trichoceros*	44.11±13.49 (n=18)	304±47.46 (n=17)	234.22±57.14 (n=9)
27. *T. vultur* f. *japonicus*	66.00±2.83 (n=2)	368±45.25 (n=2)	118.00±25.46 (n=2)

* *C. contortum* var. *contortum* [7] ** *C. tripos* var. *tripos* [7]

Table 1. Means ± standard deviation (SD) of cell width, length and span of the various Tripos *species obtained from the tropical marine waters of Belize and the Gulf of Honduras. Observation numbers are given in parenthesis (n). (The* T. contortus *mean value is the pooled data. Span measurements taken from the right antapical horn elbow to the left horn tip.).*

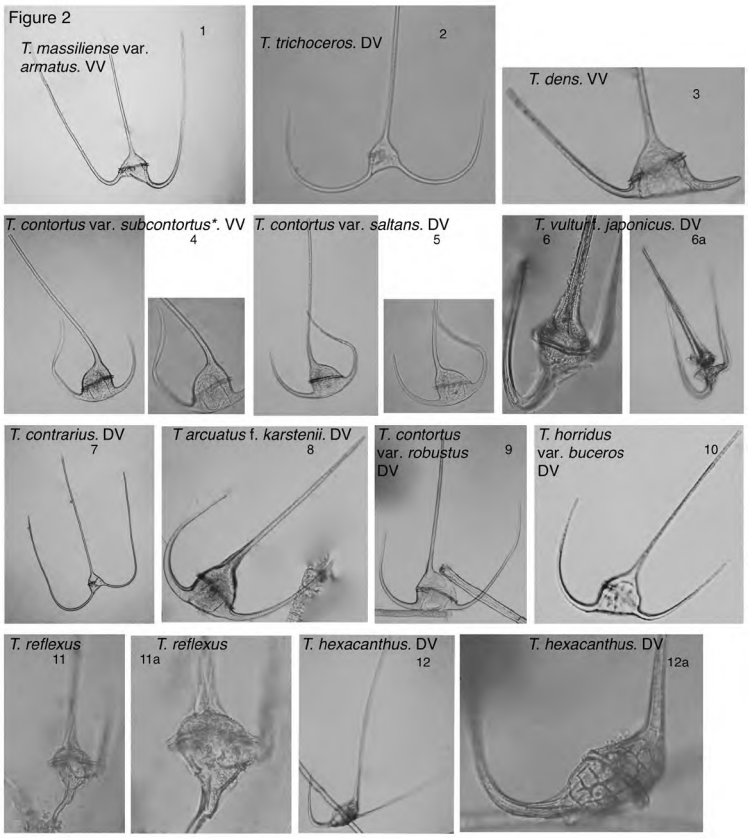

Fig. 2. 1. T. massiliense *var.* armatus *(70 x 460 µm, span 315 µm). 2.* T. trichoceros *(40 x 248 µm, span 312 µm). 3.* T. dens *(64 x 260 µm, span, left antapical horn tip to right horn tip, 280 µm). VV. 4.* T. contortus *var.* subcontortus *(64 x 400 µm, span, left horn tip to right horn elbow, 168 µm).(*C.* contortum *var.* contortum *[7]). VV. 5.* T. contortus *var.* saltans *(68 x 380 µm, span, left horn tip to right horn elbow, 180 µm). DV. 6.* T. vultur *f.* japonicus *(60 x 400 µm). DV. 6a.* T. vultur *f.* japonicus *(64 x 336 µm, span 136 µm). DV. 7.* T. contrarius *(40 x 320 µm). DV. Note:* T. contrarius *could be a morphotype of* T. trichoceros. *8.* T. arcuatus *f.* karstenii *(80 x 420 µm, span 420 µm). DV. 9.* T. contortus *var.* robustus *([8], Fig. 67), (100 x 404 µm, span 320 µm). DV. 10.* T. horridus *var.* buceros *(36 x 172 µm, span 120 µm). DV. 11. and 11a.* T. reflexus *(70 x 240 µm, span of antapical horns, 200 µm). 12.* T. hexacanthus *(70 x 240 µm, span 200 µm). DV. 12a.* T. hexacanthus *(60 x 332 µm). DV. VV (Ventral View). DV (Dorsal View).*

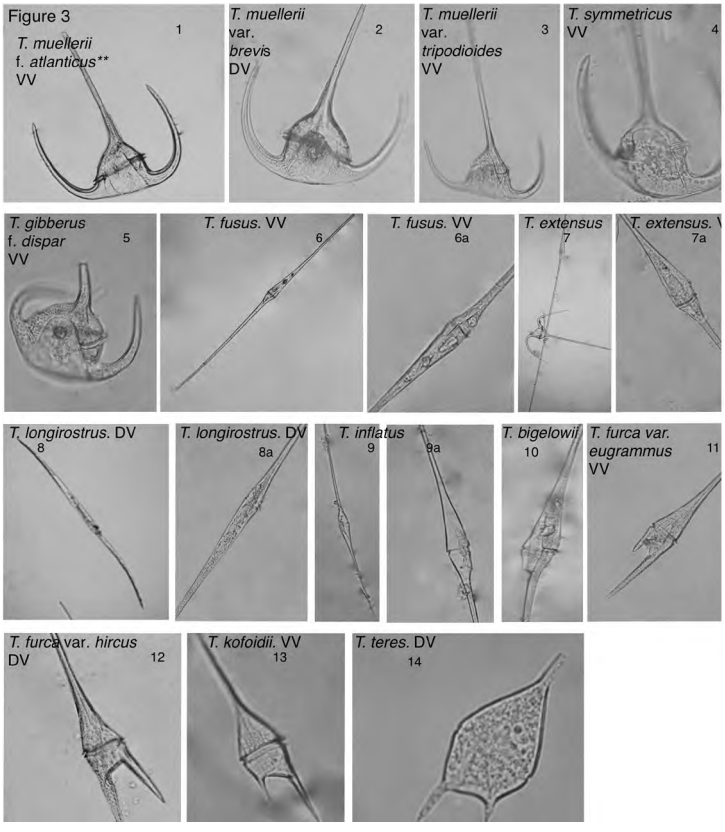

Fig. 3. 1. T. muellerii *f.* atlanticus *(62 x 240 µm). (**C.* tripos *var.* tripos *[7]). VV. 2.* T. muellerii *var.* brevis *(76 x 204 µm, span 164 µm). DV. 3.* T. muellerii *var.* tripodioides *(64 x 212 µm, span 120 µm). VV. 4.* T. symmetricus *(64 x 160 µm, span 128 µm). VV. 5.* T. gibberus *f.* dispar *(cell body width 60 µm, span 112 µm). VV. 6. and 6a.* T. fusus *(20 x 620 µm). VV. 7. and 7a.* T. extensus, *long horns, (28 x 1120 µm). VV. Also in 7. see* T. massiliense *(68 x 370 µm). VV. 8 and 8a.* T. longirostrus. *Left antapical horn curved to left, (24 x 610 µm). DV. 9 and 9a.* T. inflatus *(26 x 400 µm). DV. 10.* T. bigelowii *(cell width 25 µm). VV. 11.* T. furca *var.* eugrammus *(36 x 240 µm). VV. 12.* T. furca *var.* hircus *(44 x 216 µm). DV. 13.* T. kofoidii *(26 x 143 µm). VV. 14.* T. teres *(40 x 98 µm). DV. VV (Ventral View). DV (Dorsal View).*

Acknowledgements

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IOC-UNESCO Taxonomic Reference List of Harmful Microalgae

The Taxonomic Reference List of Harmful Microalgae has for a decade now been available on the internet (*www.marinespecies.org/hab*). The list presently comprises a total of 149 species: 20 diatom species, 8 haptophyte species, 84 dinoflagellate species, 4 raphidophytes, 3 dictyochophytes and 30 cyanobacteria. Most work on the list has recently centered on adding cyanobacteria. These were not previously included but we have, assisted by Gertrud Cronberg in Lund, Sweden, started updating this part of the list. The procedure is the same as with other species. The original literature on toxic events caused by a given species is checked and, if the evidence for a toxic effect is accepted and the species appears to be correctly identified and named, it is added to the list. In case of doubt about the causative species for a harmful event (for example when the plankton contains several species), the species is usually not accepted for inclusion in the list. Gertrud Cronberg and Helene Annadotter have kindly allowed us to include in the list the excellent light micrographs of harmful cyanobacteria from their book [1]

When fully updated, the list of cyanobacteria is expected to double. Before continuing with the list and the taxonomic problems associated with it, I would like to draw the attention to some information I learnt from working with the list and which, although not completely new, is probably not generally known. It relates to what is probably the most serious problem associated with cyanobacteria in the sea (in brackish water), the large blooms of the filamentous species *Nodularia spumigena*, not least in the Baltic. Such blooms are news on Danish TV every summer, and we usually warn people not to swim in the water during heavy blooms. However, during the work with the list I came across a number of articles containing much more disturbing information. It is well known that *Nodularia* produces the tumor-promoting pentapeptid nodularin, but these toxins have now been found to accumulate in both blue mussels (*Mytilus edulis*) and in the liver and muscles within the two fish species examined, flounder (*Platichthys flesus*) and round goby (*Neogobius melanostomus*). I refer the reader to recent studies from the Bay of Gdansk in Poland, cited in the IOC List.

The IOC List generally comprises species known to or suspected to produce toxins, which have harmful effect on humans and/or fish. For many fish killers the toxin has not been identified with certainty, but these species have been included in the list anyway due to their known toxic impact on the fish. Blooms of non-toxic algae, which may cause fish kills in fish pens and among bottom invertebrates due to oxygen depletion, are not included, nor are species that physically irritate fish gills because of spines (the setae of the diatom *Chaetoceros*) and cause loss of fish.

The IOC List was begun as a list of correct names for harmful species. However, there is no agreement among taxonomists as to what constitutes a species. We have taken a pragmatic view, as the list is being used for monitoring, and academic matters (no matter how interesting!) are kept to a minimum. Taxonomic changes are therefore treated in a very conservative way, name changes are made only when they are supported by strong evidence, which makes it unlikely that the change will have to be rolled back when more data become available. Changes in the names cannot be completely avoided. A case in point comes from two recent large monographs, not on algae, but on a different biological subject, the conifers. Both authors described and discussed all the conifers of the world. However, one author–the lumper–considers 545 species to be present, the other–the splitter–splits them into 615 species. The problem is how to define a subspecies, a variety, etc. The authors also differ in the number of genera present in the world, and in several generic names. These problems relate to very large plants, not to microscopical algae.

We cannot avoid name changes completely but we can aim at keeping the number down. Personally I have over the years developed skepticism when the number of species in a genus is concluded solely from single–or a few–gene phylogenetic trees, rather than from morphological characters, believed to be the results of numerous genes. Among recent changes in the list is that the two species of the raphidophyte *Chattonella*, *C. ovata* and *C. antique*, have now been reduced to variety level, and the species *C. marina* therefore now comprises three varieties, var. *antiqua*, var. *ovata* and var. *marina*. The varieties can usually be distinguished by cell shape or molecular signature, but overlaps occur. We recognize, however, that it is a matter of personal preference whether to treat the three taxa as species or varieties.

Merging of several species of *Microcystis*, advocated recently from studies of a few genes, is not accepted in the list. Several of the species selected for merging are morphologically distinct, casting doubt on the usefulness of the molecular data used for species identification.

Finally, a pledge: While the main purpose of the IOC List is to provide correct names, we also try in the list to provide information on other issues such as toxic effects. This is a field in which new findings are made regularly and we are grateful for any new information: new toxic species, new studies on toxic effects.

References

1. Cronberg G & H Annadotter 2006. Manual on Aquatic Cyanobacteria. ISSHA and IOC of UNESCO, 106 pp LINK

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Plankton Planet

Plankton, which includes all organisms drifting with the currents (from virus to animals), are the basis of the oceanic food chain and produce ~50% of the oxygen we breathe. Plankton dynamics are primarily influenced by oceans/atmosphere's physics and key environmental drivers such as temperature and CO_2 concentration. As a result, planktonic organisms are excellent indicator of the effect that stressors such as pollution and climate change have on our oceans.

Currently, all oceanographic models aimed at predicting global ecological changes lack good quality and high resolution biological data on oceanic plankton. There is a clear and urgent need for innovative approaches enabling collection and analysis of plankton biodiversity and distribution data over much larger spatio-temporal scales than is currently possible. These data would bring the knowledge of plankton ecosystems closer to the one we have from terrestrial and coastal benthic systems and thus provide an invaluable resource to detect, quantify, and predict global ecological changes in the Earth system.

Plankton Planet (P2) is an international project (France, New Zealand, USA) that promotes citizen-based sampling of marine plankton to measure the genetic biodiversity and biological health of the world oceans on relevant spatial and temporal scales. The team has developed very simple sampling tools and protocols allowing volunteer citizen-sailors (planktonauts) to isolate total DNA from plankton communities while sailing and without any specialised expertise. Samples are then sent to scientists who process them using massive sequencing of DNA barcodes (metabarcoding). Analyses of the metabarcodes allow then to detect and quantify all eukaryotic plankton biota in any given sample, providing critical data to measure biodiversity and species interaction networks over space and time. This is a revolutionary approach to biological oceanography, which capitalizes on the thousands of citizens sailing around the world every day. It forgoes the need to rely on high carbon-footprint and extremely costly research vessels, and allows rigorous surveys of global oceanic plankton over spatio-temporal scales that have not previously been possible.

See more at: *www.planktonplanet.org*

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A mandala of plankton diversity. Photo credit: Christian Sardet.
Plankton Chronicles From "Plankton - Wonders of the Drifting World" Univ. Chicago Press 2015.

GlobalHAB – a new initiative in the HAB community

GlobalHAB, the new international scientific programme on Harmful Algal Blooms (HABs) was launched this year. The first meeting of the newly formed GlobalHAB Scientific Steering Committee (SSC) was hosted at the Scottish Association for Marine Science (SAMS) in Oban, Scotland from 8 to 10 March 2016. GlobalHAB is an initiative under the auspices of the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission of UNESCO (IOC). GlobalHAB builds on the wealth of scientific results delivered through the recently completed Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) programme (www.geohab.info).

The steering committee has representatives from across the globe (Fig. 1): Elisa Berdalet (Chair, Spain), Neil Banas (UK), Michele Burford (Australia), Chris Gobler (USA), Bengt Karlson (Sweden), Raphael Kudela (USA), Po Teen Lim (Malaysia), Lincoln Mackenzie (New Zealand), Marina Montresor (Italy), and Kedong Yin (China). Liaisons from ICES (Eileen Bresnan), IPHAB (Gires Usup), PICES and ISSHA (Vera Trainer), and SAMS (Keith Davidson) participated in the meeting and are helping develop the GlobalHAB plans. Henrik Enevoldsen from IOC and Ed Urban from SCOR assisted the GlobalHAB SSC and provided input from sponsoring organizations.

In this first meeting, with a positive and active atmosphere, the participants defined the fundamental elements that will structure the GlobalHAB Science and Implementation Plan is expected to be completed by June 2016:

The **mission** of this cutting-edge programme is *to improve understanding, prediction, management and mitigation of HABs in aquatic ecosystems.*

The scientific **goals** of GlobalHAB include:

- To address the scientific and societal challenges of HABs, including the environmental, human health and economic impacts, in a rapidly changing world.
- To involve participants from related fields of natural and social science, and will link with other regional and international organizations, and initiatives relevant to HABs.
- To foster intercalibration among existing methods, as well as promote the development and adoption of new technologies.
- To promote training, capacity building and communication of knowledge about HABs to society.
- To serve as a liaison between the scientific community, stakeholders and policy makers, promoting science-based decision making.

A range of **implementation activities** were identified to be undertaken in the next three years and beyond. Namely, workshops and open science meetings will specifically address toxin-related challenges (detection methods, action mode, molecular basis), evaluate the impacts of aquaculture on HAB occurrences in different regions, and ascertain the potential climate change impacts on HABs occurrence in freshwater and marine ecosystems. Science/stakeholder forums will be organized to assess the potential socio-economic impacts of HAB occurrences and to engage the medical community to improve human health protection. GlobalHAB implementation will be conducted with new linkages with the existing international and regional initiatives including IPHAB, the ICES-IOC Working Group on HAB Dynamics, PICES Section on Ecology of Harmful Algal Blooms in the North Pacific, GOOS, the IOCCG/GEOHAB Ocean Colour & HAB working group, GEO and IAEA.

The GlobalHAB Science and Implementation Plan will be presented at the 17th International Conference on Harmful Algae in Florianopolis, Brazil in October 2016. This will be an extraordinary opportunity to involve the international community working on HABs with the new programme. As in its predecessor programme, GEOHAB, the international coordination approach to address the fundamental problem of HABs is the keystone of GlobalHAB.



GlobalHAB Scientific Steering Committee (SSC)
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First session of the IOCAFRICA Group of Experts on Harmful Algal Blooms

The third session of the UNESCO/IOC's Sub Commission for Africa and the Adjacent Island States, IOCAFRICA, (14-15 April 2015, Nairobi, Kenya) established a Group of Experts on Harmful Algal Blooms to develop a detailed proposal for an IOCAFRICA programme on HABs in consultation with the IOC HAB programme for consideration by the IOCAFRICA Officers and the fourth session of IOCAFRICA.

Examples were given of cyanobacteria in Egyptian waters, blooms experienced off Madagascar and the increasing incidence of *Sargassum* affecting Ghana, Cote d'Ivoire and Sierra Leone in West Africa. The rapid growth of economies in the region is likely to lead to increased incidences of HABs due to biological and chemical sources.

IOC has implemented regional activities on HABs in Africa, ranging from training in technical skills for monitoring algae and algal toxins to development of regional manuals and guides [1] and has worked with specific member states to establish national monitoring and sea food control programmes. IOC has also worked closely with IAEA on regional HAB training in Africa during the past 10 years and has a regional network on HABs in North Africa (HANA) that meets every second year depending on available funding. The IOC is in close cooperation with other UN and regional organizations globally compiling data on HAB species occurrences and HAB events. There is at present no network of reporters/editors in sub Saharan Africa although this would be highly desirable. The data compilations (in the IOC databases HAEDAT and OBIS) will provide an important basis for a Global HAB Status report which is in preparation.

The meeting of the IOCAFRICA group of HAB experts was hosted by the Kenya Marine and Fisheries Research Institute (KMFRI) from 22-26 February 2016 in Mombasa, Kenya, and attended by experts from 10 African coastal countries (Fig. 1). The meeting was officially opened by Director of the Kenya Marine and Fisheries Research Institute, Dr. Renison Ruwa, who appreciated the efforts of IOC/UNESCO and IAEA for creating a platform for marine scientists to share their ideas and experiences in conserving the ocean world's resources. He noted that HABs are a global threat to living resources and have had significant impacts on the livelihood and the health of coastal communities for decades in a number of countries. He stressed that the oceans and seas connect a number of world countries and economies and a regional/ global approach to predicting and monitoring HABs is needed to protect human health and marine resources. He informed the meeting that HAB occurrence and impacts are likely to increase in the face of emerging issues, such as climate change, increase in sea surface temperatures, eutrophication and ocean acidification, and challenged scientists that they have the responsibility to generate and provide accurate information to the decision-makers on HAB occurrences, their triggers and possible impacts.

The objectives of the meeting was to prepare an inventory of HABs related events and HAB related studies and publications in the region, as well as the development of a detailed proposal for an IOCAFRICA HABs programme. The terms of reference of the IOCAFRICA Group of Experts on HABs were:

(i) Prepare an inventory of availabile capacity for HAB related studies in the region (expertise, equipment and other facilities)
(ii) Prepare an inventory of HAB related publications from/about the region
(iii) Compile records of HAB events for the period 2010-2015 and thereby establish a database of HAB species identified in the region and prepare a report which would both contribute to HAEDAT and the IOC Global HAB Status Report.
(iv) Identify potential contributing factors for HAB events
(v) Prepare a review document quantifying the scale, nature and extent of the problems associated with fish killing algae in the region.



Fig 1. Participants at the I IOCAFRICA – HAB meeting, Mombasa, Kenya, 22-26 February 2016 (Photo from the Kenya Marine and Fisheries Research Institute)

(vi) Prepare a detailed proposal for an IOCAFRICA programme on HABs (including capacity development requirements and monitoring programme).

(vii) Formulate a strategy for communication to and engagment of scientists and managers in Africa working with different HAB aspects, including identification of relevant partners in regional IOCAFRICA HAB activities.

Country reports were presented for Cote d'Ivoire, Egypt, Ghana, Kenya, Madagascar, Morocco, Namibia, and Tanzania. Highlights of the reports included: HAB events responsible for toxin production, PSP, ASP, lipophilic toxins in shellfish as well as fish mortality, and cyanobacteria blooms.

The group of experts agreed that members would generate regional baseline data, develop an inventory of all HABs events from 2010-2015, assess capacities and facilities to manage HABs effects (human, equipment, laws/regulatory, programs, financial), and tabulate monitoring activities of HABs in African countries. They also agreed on the need for mapping of the occurrences of HABs with existing data, basic and advance training on phytoplankton and advanced microscopy capacity building, development of a regional HABs identification guide and the use of standardized nets for sampling benthic microalgae to fully understand HABs related issues in Africa.

The group of experts noted that HABs issues have not been prioritized despite the fact that commercial fisheries/aquaculture play a key role in food security and boosting the economy and as such there is need for documentation of possible HAB occurrences and impacts in Africa.

Other challenges facing adequate understanding of HABs related issues include the lack of adequate databases, insufficient funds, lack of awareness on HAB issues in some countries, insufficient technical capacities, poor coordination at national and regional levels, insufficient long term commitment of governments, insufficient expertise, lack of cooperation south-south and North-south and insufficient regulations.

The participants also had a session to develop a joint work plan with the national coordinators for the International Atomic Energy Agency's project on "Applying Nuclear Analytical Techniques to Support Harmful Algal Bloom Management in the Context of Climate and Environmental Change"

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Authors

Mika Odido, IOC Africa Coordinator, IOC Sub Commission for Africa and the Adjacent Island States, UNESCO Regional Office for Eastern Africa, UN Gigiri Complex Block C, P.O. Box 30592-00100, Nairobi, Kenya

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The 2016 meeting of the ICES – IOC Working Group on Harmful Algal Bloom Dynamics

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics was hosted by Dr. Raffaele Siano at the IFREMER laboratory, Brest, France from the 19th – 23rd April 2016. Fifteen delegates from ten countries attended with a further two participating by correspondence. Terms of reference addressed during the meeting included a review of the dynamics of *Alexandrium minutum* in the bay of Brest, production of a status report of harmful algal events in the ICES area using data held in the IOC-ICES-PICES HAE-DAT database (*http://haedat.iode.org/*), experiences using molecular methods during HAB monitoring and research, and the use of trigger levels in HAB monitoring programmes. New findings included the first detection of tetrodotoxin (TTX) in Dutch waters, mercury in phytoplankton from Puck Bay, Poland, *Ostreopsis* in Spanish waters, satellite monitoring of cyanobacterial blooms in the Baltic and SPATT monitoring of lipophilic shellfish toxins on the west coast of Scotland. National reports included reports of closures for paralytic, lipophilic and amnesic shellfish toxins in Europe and observations of *Ostreopsis* in France and Spain. Cyanobacterial blooms were observed in the Baltic. A range of HAB impacts were recorded in the USA during 2015 with the extensive *Pseudo-nitzschia* bloom resulting in problems from high concentrations of domoic acid recorded along the west coast of the USA and Canada. The next WGHABD will be hosted from 25th – 28th April 2017 by Dr. Anke Kremp, Finnish Environment Institute, Helsinki, Finland.



Fig. 1: Participants at the ICES-IOC WGHABD meeting, 19-23 April 2016, Brest, France.

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Regional Training Course on Ciguatera Fish Poisoning (CFP): Field Monitoring and Toxin Data Management. Santo Domingo, October 26 - November 6, 2015

This course was organized by IAEA as an activity of project RLA7020 *Establishing the Caribbean Observing Network for Ocean Acidification and its Impact on Harmful Algal Blooms, using Nuclear and Isotopic Techniques* in collaboration with the IOC of UNESCO, Universidad Nacional Pedro Henríquez Ureña and the Ministry of Environment and Natural Resources of the Dominican Republic. Participants were from Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Guatemala, Jamaica, Mexico, Nicaragua, Panama, and Venezuela (Fig. 1).

The objective of the course was to improve the skills of participants in field collection of benthic dinoflagellates (using artificial substrate and macrophytes) and sample processing prior to reviewing the taxonomy of benthic HAB (BHAB) genera. In addition data management using the Harmful Algae Event Database (HAEDAT) was addressed and an event reporting network with partners of IOC-ANCA was established.

During the first week, information was shared with the IOC working group on Benthic HABs and discussions were directed to the types of data sharing activities possible with the HAEDAT (*http://haedat.iode.org/*). Lectures on the physiology and biogeography of benthic HABs in the Caribbean region were provided. In addition, a lecture on the international effort via IOC-International Program on Harmful Algal Blooms with the aid of FAO to have ciguatera fish poisoning (CFP) declared a "neglected tropical disease".

The objective of the second week of the workshop was to improve the skills of the participants in benthic phytoplankton field collection using both macrophytes and a new artificial substrate method (Figs 2-3) and review the taxonomy of BHAB genera. Boca Chica, approximately 32 km East of Santo Domingo, was selected as field sampling location. A number of benthic HAB species were found there including



Fig. 1. Participants in the IAEA Regional Training Course on Ciguatera Fish Poisoning (CFP) Field Monitoring and Toxin Data Management.

some *Gambierdiscus* species, and the sampling site was accessible to all participants. The gently sloping beach, less than 1.5 m depth proved a safe site for all. Hands on training was provided so participants could make their own artificial sampling devices for deployment during the next two days of field work.

Lectures on taxonomy of toxic marine benthic dinoflagellates with emphasis on *Gambierdisucs* were provided. Practical identification with the microscope was performed with pure algal cultures of six species of *Gambierdiscus* provided by the two trainers (Pat Tester and Santiago Fraga), and of other benthic dinoflagellates from the genera *Ostreopsis, Prorocentrum* and *Coolia*. Practical training was provided on single cells isolation with a micropipette from field samples spiked with cultured cells to start new cultures.

Cell culture media and culturing techniques were discussed in lectures. The use of Sedgewick-Rafter counting chambers was demonstrated. Time was set aside to discuss monitoring strategies and country specific needs and limitations were considered in revised monitoring plans. In addition to generating regional technical capacity in data management using the Harmful Algae Event database (HAEDAT), the workshop was an opportunity to meet with partners of IOC-ANCA to establish a network for event reporting.

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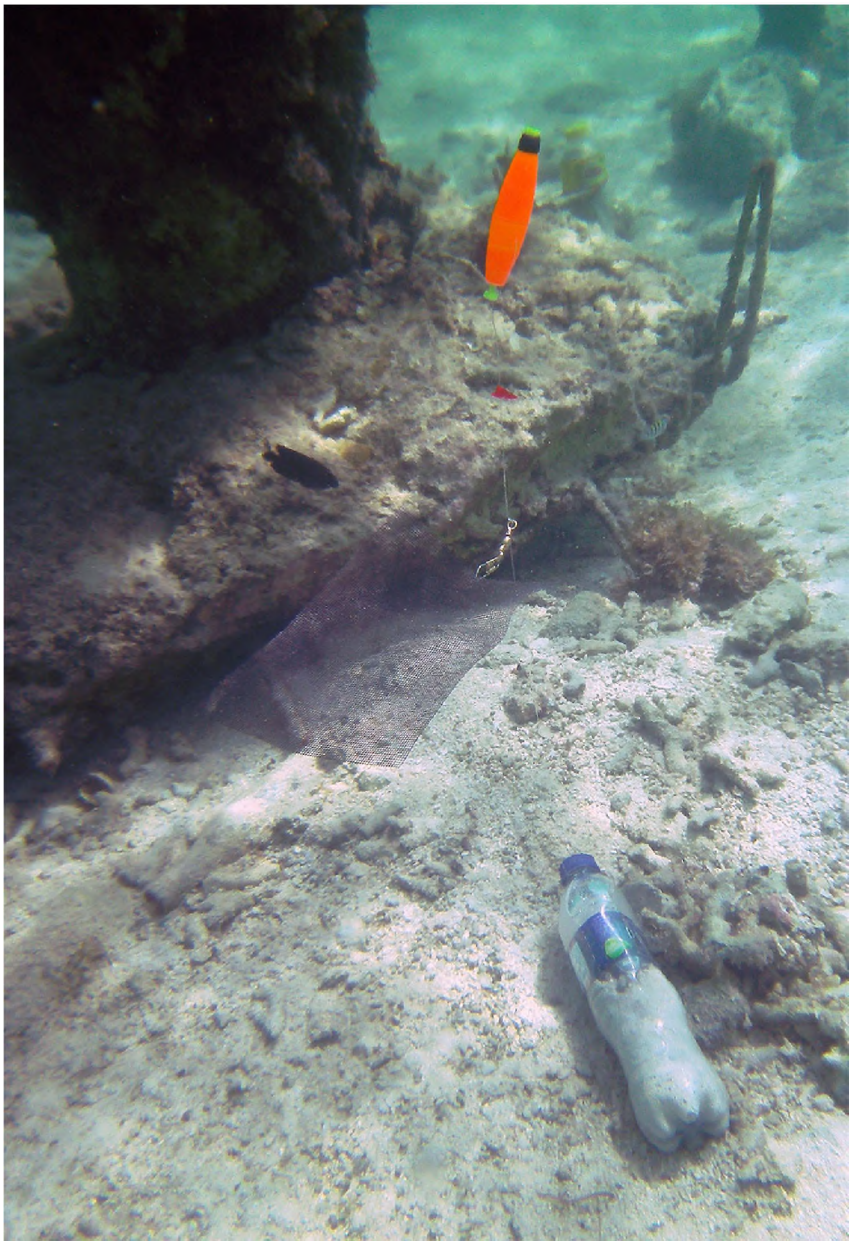


Fig. 2. A screen used as an artificial substrate, moored with a full of sand mineral-water bottle with a little buoy attached to it.

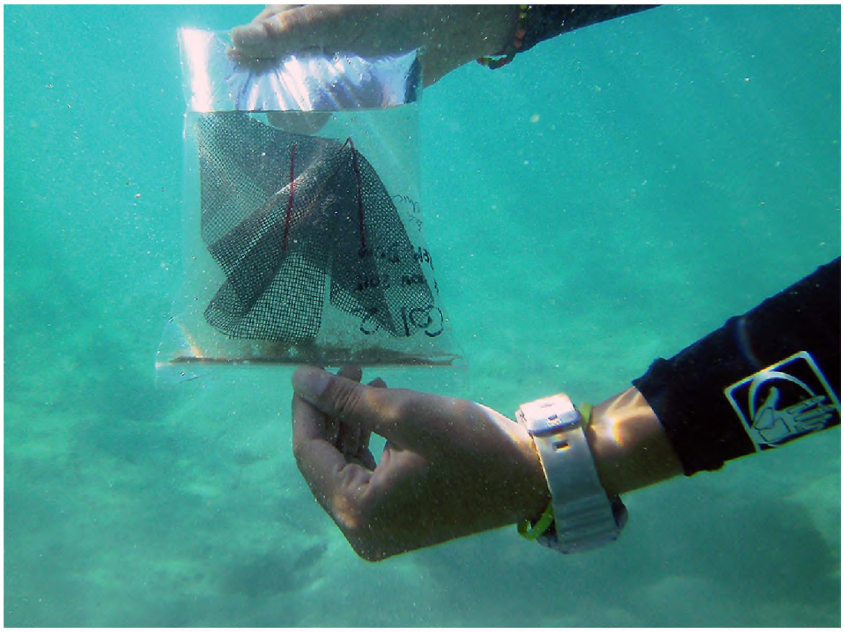
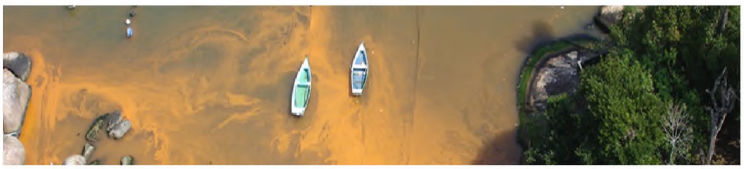


Fig 3. Sampling with the new artificial substrate method. Labeling and collection by a snorkeler.



ISSHA's Corner

Dear ISSHA members and other HAB experts and mangers: The time for the 17th International Conference on Harmful Algae (Florianópolis, Brazil, 9-14 October 2016) is approaching. The local organizers and ISSHA committees are doing their best to try to make this event more exciting than ever, in particular for young scientists attending this conference for the first time in their short career. This is the first time the HAB conference is held in South America, so it is a very special event for all our Latin American colleagues. We are pleased to invite you to participate in important decisions/activities, some of which have now become part of our traditions during the ICHA Conferences, and some others which are innovations to make the conference more proactive.

First of all, we invite you to become an ISSHA member if you are not still so. By being a member, you will get a 20% reduction in your registration fee, and most important, your contribution will help to facilitate the participation of young PhD students and/or experts from developing countries/economies in transition. The issues for which we need your help are:

1. **New! Scientific Speed Networking For Students**
2. Nominations for the Yasumoto Award and the Young Scientist Award (**Deadline May 15**)
3. Travel Awards to attend the 17th International Conference on Harmful Algae (**Deadline May 15**)
4. Bids to host the 19th ICHA Conference (**Deadline July 31**)
5. Donations for the ISSHA Auction 2016.
6. Nominations for ISSHA Officers and Council members.

1. Scientific Speed Networking For Students

Panelists and Mentors: Charles Trick, Vera Trainer, Lisa Campbell, Paulo Salomon, Silvia Nascimento

It can be daunting to try to introduce yourself to someone at a large scientific meeting, but given the right opportunity, a quality exchange can have a lasting impression. Scientific speed dating is a twist on the popular singles speed dating phenomenon. The goal here is to foster an interactive environment between small groups of advanced scientists and students in hopes of creating some short, high impact exchanges. It's amazing what can be accomplished in just a few minutes! Join us for this workshop to start building new connections.

Time: 13:00-16:00 on Sunday, October 9 in Florianopolis Brazil
Place: ICHA 2016 C onference Hotel
Who: Open to the first 50 students who sign up (please register at the ICHA2016 website)"

2. Nominations for the Yasumoto Award and the Patrick Gentien Young Scientist Award

ISSHA members are invited to submit nominations for the Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award. Further information on the appropriate profile of the nominees can be found at ISSHA website ACHIEVEMENT AWARDS

Any ISSHA member in good standing may submit nominations for either achievement award, which should include a description of the nominee's contribution (not more than one page). Please, make sure that you and your nominee have renewed your membership for the period 2016-2017. Nominations should be sent by e-mail to Drs. Marta Estrada (*marta@icm.csic.es*) and Pat Tester (*ocean.tester@gmail.com*), Co-chairs of the Committee on Achievement Awards, deadline: **May 15, 2016**. Please write "ISSHA Achievement Awards 2016" in the message subject. Nominations will be considered by the ISSHA Council, and eventual awards will be presented during the closing ceremony by the end of the 17th ICHA Conference in Florianópolis.

3. Travel Awards to attend the 17th International Conference on Harmful Algae

ISSHA will offer a limited number of travel awards for students and post-doctoral investigators to attend the 17th International Conference on Harmful Algae, 9-14 October 2016, in Florianópolis, Brazil. Awards will be limited to a maximum of $1,000 US. Successful applicants will need to demonstrate that other sources of funds are available to supplement their award. Criteria for selection and information to be included in the application letter can be found at ISSHA TRAVEL AWARDS.

To apply for funds, please send the **application letter**, as a PDF File, to Dr Christine Band-Schmidt, Chair of the Committee on Travel Awards (*cjband@yahoo.com*) following the format available on the ISSHA website. Please write "ISSHA Travel Awards 2016" in the message subject. Applications not submitted this way may be rejected.

The deadline for receipt of applications is **May 15, 2016**, with decisions announced approximately one month thereafter (depending on confirmation of available funds from different sponsors).

4. Bids to host the 19th ICHA Conference

During 2016, ISSHA members will be asked to vote for which site will host the 19th ICHA (2020) that will follow the 18th ICHA in Nantes, France (2018). The chosen site will be announced during the closing ceremony of the 16th ICHA (Florianópolis, October 2016). Now is the time to ask for bids to host the 19th ICHA, so that voters will have the time to examine the different options and make up their mind. ISSHA members in good standing who cannot attend the Conference will have the opportunity to vote on-line at the ISSHA website. Dead-

line for on-line voting (the only way to do it) will be **October 4, 2016 at noon (12.00) Brazil time.** The elected venue will be announced during the closing ceremony of the 17 ICHA.

Bids should be submitted before 31 July 2016, and should include the following information:

- Scientists and organizations who will serve as local hosts, including evidence of their prior expertise in the organization and financial management of similar-sized international conferences.
- Description of the meeting venue and capacity of meeting space for a 5-day meeting, including a plenary room, 2 rooms for simultaneous sessions and several small rooms for administrative use and other necessities.
- Estimated participation is between 400 and 600 people, but sometimes there are surprises, as it was the case during the 10th ICHA (St Pete Beach, Florida), with around 800 participants.
- Availability of accommodations at a range of costs, including the number of rooms, distance from the meeting location, and room costs for high and low seasons.
- If university residence or other inexpensive accommodation is available for students, this will be considered favourably.
- Distance of meeting and lodging facilities from major airport(s) and cost of transportation between airport and meeting and lodging facilities.
- Availability of local public transportation.
- Expected cost of lunches per day.
- Availability and distance to restaurants for lunches.
- Expected level of local support and associated funding, including ability to provide meeting space, audiovisual support, and staff support, at no cost to the international organizers.
- Dates when the facility would be available in 2020.
- Plans for mid-week excursions and accompanying persons' activities.
- HAB scientists who would be responsible for editing the ICHA proceedings[1], including evidence of their prior experience in producing English language conference proceedings[1].

Enquiries and bids should be e-mailed to the President of ISSHA (*vera.l.trainer@noaa.gov*) c/c to the Chair of the ISSHA Conference Committee (*beatriz.reguera@vi.ieo.es*).Bids should be limited to 2-3 pages, plus supplementary illustrations and web links. Information on the previous ICHA venues can be found at the ISSHA website Conferences. Bids will be examined by the ISSHA Council and posted on the ISSHA website.

1) Nowadays, the edited ICHA proceedings are published on-line on the ICHA website (no hard copies). Traditionally, ICHA proceedings have been edited by a group led by the head of the local organizing committee. If the local hosts are not willing to do it, the ISSHA Committee on Publications and Dissemination will take it over.

5. Donations for the ISSHA Auction 2016

ISSHA auctions during past International Conferences on Harmful Algae have been a success and are now a tradition!! It is an opportunity for the conference participants to bid on donated items. The range of items is broad and may include books, signed reprints, photos, jewellery, T-shirts, paintings, algae-related items, liquor, and scientific equipment. The auction items are donated by ISSHA members from around the world. Funds collected during the auction are an important income for ISSHA. ISSHA was able to offer partial travel support to 30 students to attend the 2014 conference in Wellington.

Please contact the responsible person for the auction, Dr Ester Garcés (*esther@icm.csic.es*) about your donations for the auction and the way you are going to transport it. Please, write "ISSHA Auction 2016" in the message subject. You may also include a picture of your item if you wish. A list of items will be presented on the ISSHA website. An address where items may be sent in advance of the meeting will be provided on the conference website.

6. Nominations for ISSHA Executive and Council (Elections 2016)

The composition of ISSHA Executive and Council following the 2014 elections is as follows:

Executive Officers

- President: Vera TRAINER (USA)
- 1st Vice-President: Gustaaf HALLEGRAEFF (Australia)
- 2nd Vice President: Robin RAINE (Ireland)
- Secretary: Anke KREMP (Finland)
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According to ISSHA's statutes, all the executive officers can be re-elected, after nomination, for another term (2 years) with the exception of Robin Raine, who has already served as Vice-President for two terms.

Nominations are invited for candidates to stand for election/re-election to the Executive Committee (Officers) and the Council of ISSHA. Nominations may be made by any current ISSHA member, including the nominee, and should specify the position concerned (President, Vice-President, Secretary, Treasurer or Ordinary Council Member). Written consent of the prospective candidate should always accompany the nomination. **Nominations from Asia are encouraged, because this part of the world has lots of ISSHA members but is poorly represented in the Council.**

Nominations should be sent to both the president, Vera Trainer (*vera.l.trainer@noaa.gov*) and the secretary Anke Kremp (anke.kremp@ymparisto.fi). The deadline for receipt of nominations is **15 June 2016**. Elections will be held before the General Assembly scheduled for 13 October 2016.

John W. Hurst, Jr. 1927 - 2015

For 53 years, John W. Hurst, Jr. was a fixture at the Maine Department of Marine Resources, and known throughout the world for his contributions to the field of harmful algae. John was born and raised in Bozeman, Montana and after graduating from Montana State University in 1949, he began his life-long career at the Maine Department of Sea and Shore Fisheries (now Department of Marine Resources - DMR) where he developed the monitoring system for PSP. Over the course of his career he was called many things, "Grandfather of Red Tide", "the Czar", and the "Red Tide Guru" among them. But to most who knew him, he was John - a very giving mentor and friend.

John started the Maine Paralytic Shellfish Poisoning (PSP) monitoring program in 1958 following a serious outbreak of toxicity in neighboring Canada. Over the ensuing 52 years, John continued to develop and perfect that program, and guarded and protected the State of Maine shellfisheries in the face of a highly unpredicatable public health issue. It is a testament to his knowledge and perseverance that his program has been held up to the rest of world as the 'gold standard' of monitoring programs.

One cannot think about shellfish safety or red tide anywhere in the world without hearing the name John Hurst. John understood the importance of long-term data sets before it was a 'term' and made his data available to many scientific collaborators over the years - indeed, many of the models being developed today are based on what John knew to be true 40 years ago! Perhaps of even more consequence was his very special ability to engage young kids and fuel their curiosity and enthusiasm.



His office was filled with testaments to his accomplishments. He and his several teams were recognized with the Commissioner's Special Citation from the U.S. Food and Drug Administration and the Governor's Award for Special Teamwork, the Canadian Food Inspection Agency, the Maine legislature, and Governor Angus King. In 2001, John was awarded the Wallace Award from the National Shellfisheries Association, and in 2002 he was presented with a Lifetime Achievement Award for his contributions to research and monitoring of marine biotoxins at the Xth International Conference on Harmful Algae. The International Society for the Study of Harmful Algae (ISSHA) designated John as a Trailblazer (see *http://www.issha.org/Welcome-to-ISSHA/HAB-Trail-Blazers/John-Hurst*). All of these recognitions were richly deserved, but John was most proud of the fact that no cases of PSP were ever reported in the State of Maine from commercially harvested shellfish while he was on the job. He listened to the industry and they listened to him and together they built a stellar monitoring program which was emulated globally. John was a constant source of data and experience and shared his knowledge freely with scientists and managers from all corners of the globe for over five decades.

On a more personal note, John was my introduction to the world of toxic algae in 1982 and we remained friends and colleagues for the decades that followed. He was encouraging early on when I suggested that the toxins might well be affecting the shellfish, always interested in research questions, and always willing to provide support where he could and lively discussions and wisdom on a daily basis to any who asked (and to some who didn't!).

He and his young son Dan (who predeceased him) named their bassett hound after me and I always smiled when I saw John walking the legs off that dog on their daily jaunt to town. After I left the DMR, John and I remained colleagues and friends and I always knew where to turn when I needed some common sense advice and information. He's missed.

John is survived by his wife Nancy, sons John and Peter, daughter Lucy, their spouses, three grandchildren, and a global cadre of people who are better for knowing him and for his contributions.

Sandra E. Shumway, Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Road, Groton, CT 06340

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The 10th International Conference on Toxic Cyanobacteria (ICTC10), *Cyanobacteria and Cyanotoxins: From Research to Risk Management*, organized by the Institute of Hydrobiology, Chinese Academy of Sciences, will be held in Wuhan, China, on October 23-28, 2016.

Topics of the conference include: (1) Cyanobacterial detection; (2) Cyanotoxin analysis; (3) Toxicology and toxicity assessment; (4) Cell physiology and molecular biology of cyanobacteria; (5) Secondary metabolites production and functions: biosynthesis, regulation, biological functions; (6) Cyanotoxin compartmentation and persistence; (7) Ecology and cyanobacterial bloom dynamics: environmental controls; (8) Interactions between cyanobacteria and other organisms; (9) Risk management of cyanobacterial blooms and cyanotoxins at scales of catchment, in-lake and water treatment; (10) Remote sensing of blooms; (11) The regulatory environment and compliance.

More information in: *HTTP://WWW.ICTC10.ORG*

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Deadline
Deadline to submit material for HAN 54: 21 July 2016

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Exceptional climate anomalies and northwards expansion of Paralytic Shellfish Poisoning outbreaks in Southern Chile

Harmful Algal Blooms (HAB), in particular recurrent events of the Paralytic Shellfish Poisoning (PSP) agent *Alexandrium catenella,* pose the main threat to aquaculture and public health in Southern Chile [1, 2]. The development of measures to control these events or, at the very least, to minimize their impact requires a detailed understanding of the triggers for bloom initiation [3]. A common factor underlying dinoflagellate bloom initiation, termination and the long-range dispersal of cells in several important HAB species—including *A. catenella*—is the encystment, germination and transport of highly resistant sexual cysts [4]. The absence of large "seedbeds" observed in several studies conducted in the Chilean Island Sea [5, 6] suggested that *A. catenella* blooms are not the result the massive in situ germination events. Therefore, the germination of dispersed cysts and transport of vegetative cells [7], are probably responsible for the intense proliferations observed. Recently, Díaz et al. [6] suggested the inflow of offshore planktonic populations or cysts as a possible mechanism for the development and recurrence of blooms in this area.

In 2016, an exceptional late summer bloom of *A. catenella*—in terms of intensity and geographic extension—was observed in Southern Chile. A remarkable northwards expansion of previously PSP-affected areas, that reached the Valdivian coasts (39°45'S), was observed in the first week of May (Fig. 1). There were 12 cases of human intoxication. This situation led the health authorities to enforce shellfish harvesting closures throughout extensive geographic areas (up to 500 km) causing dramatic socio-economical impacts. This exceptional *A. catenella* bloom was associated with extreme hydroclimatic anomalies, a consequence of the strong El Niño Southern Oscillation (ENSO) recorded in 2015/2016 (Fig. 2). Weakening of wind strength, high positive Sea Surface Temperature (SST, Fig. 3) and negative rainfall anomalies were calculated. The total rainfall in the summer-autumn months preceding the *A. catenella* bloom (January-May) was 253 mm, 60% below the historic mean of 56 years (1961-2016, data not show). A maximum of 2.5×10^5 cells L^{-1} in integrated (0-10 m) hose samples was found at the outermost station in Chacao Channel (41°45'S, see Fig. 1) during the last week of April (Fig. 4), coinciding with a peak of 5×10^3 µg STX eq. kg^{-1} in PSP toxin levels in Pacific clams (*Gari solida*). Likewise, maximum levels of PSP toxins around 9×10^4 µg STX eq. kg^{-1} were recorded at the same time in surf clams (*Mesodesma donacium*) from Cucao beach (Fig. 1), on the open coasts of Chiloé Island, 100 km southwards. From the second week of May onwards, a significant decrease of PSP toxin levels was record-

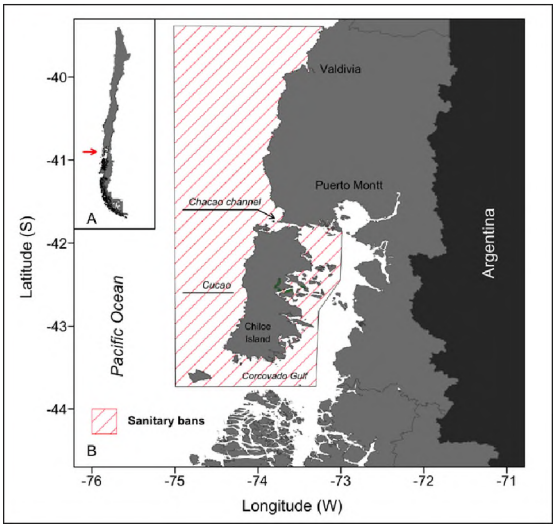

Fig. 1. Map of the study area showing the geographic extension of the shellfish harvesting closures (dashed area) during the PSP outbreak.

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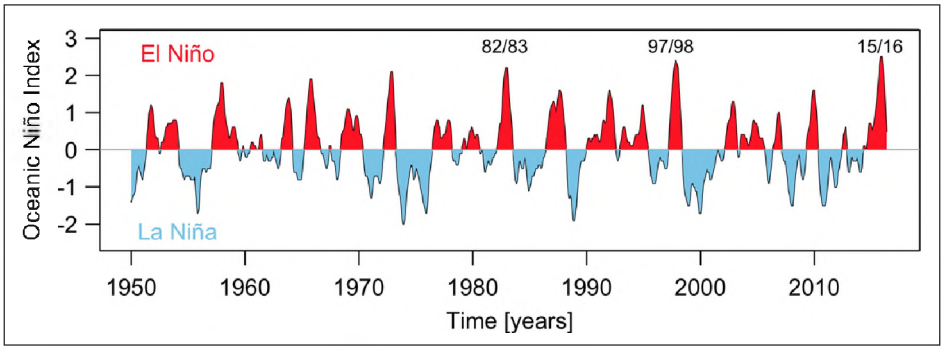

Fig. 2. Evolution of the Oceanic Niño Index (ONI) from 1950 to 2016.

ed, coinciding with the absence of vegetative cells in the water column.

Our results suggest that large-scale atmospheric and oceanographic processes (climatic anomalies) modulated the late summer *A. catenella* bloom observed in southern Chile. Considering that the most affected areas were observed on the open coasts of Los Lagos Region (Chacao channel and Cucao beach), the hypothesis of offshore planktonic populations advected in to the coastal region gains strength. Parameterization of the effect of these climatic anomalies—in terms of intensity and phenology—on the development of massive proliferation *A. catenella* is poorly understood in this area. Therefore, the identification of climatic signals and the development of predictive simulations on oceanographic conditions that might serve as early warning of future *A. catenella* events is a priority for the region.

Acknowledgements

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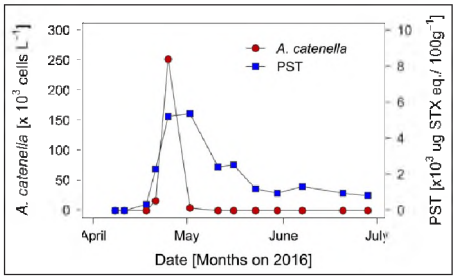

Fig. 4. Weekly changes in Alexandrium catenella *cell densities (red circles) and paralytic shellfish toxins (blue squares) in Chacao Channel, Los Lagos Region.*

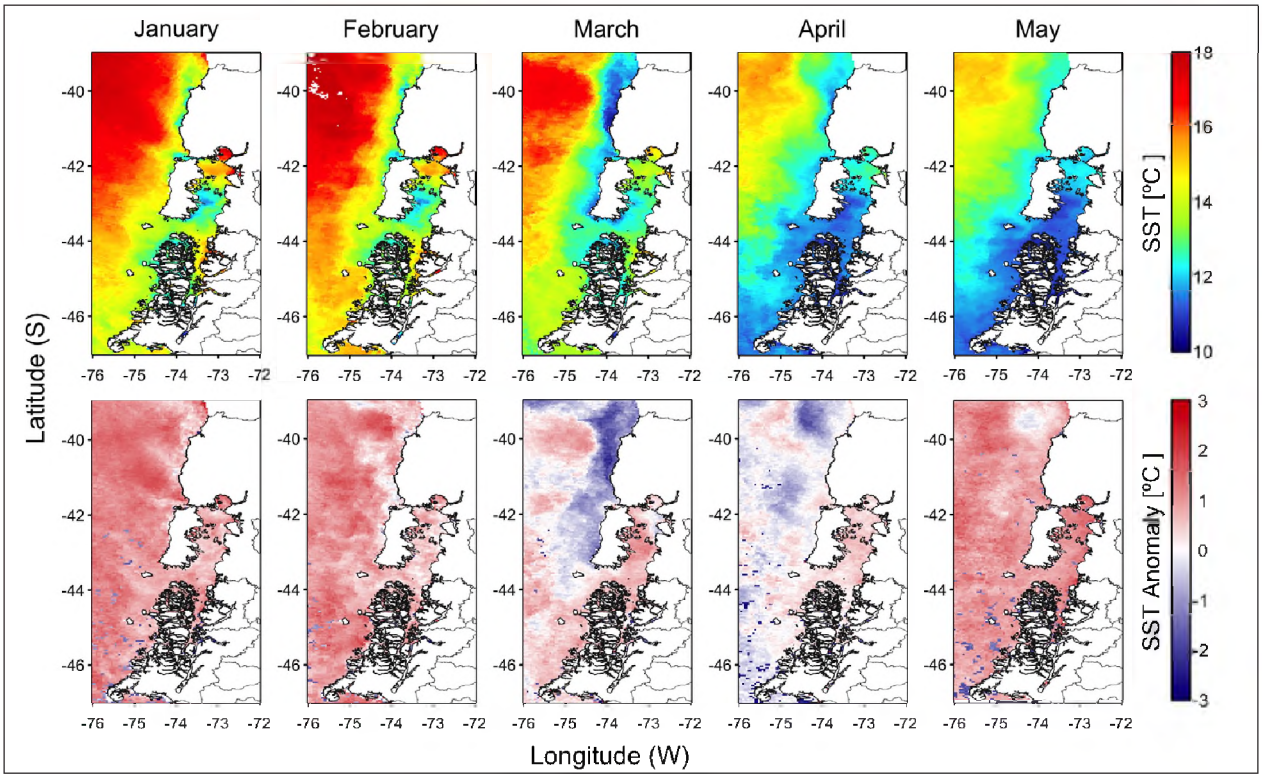

Fig. 4. Monthly Sea Surface Temperature (SST, upper panels) and SST anomalies (lower panels) from January to May. Anomalies were computed with the 2003-2015 monthly means from MODIS images.

Cochlodinium polykrikoides blooms in southern Cuba during anomalous hot dry seasons

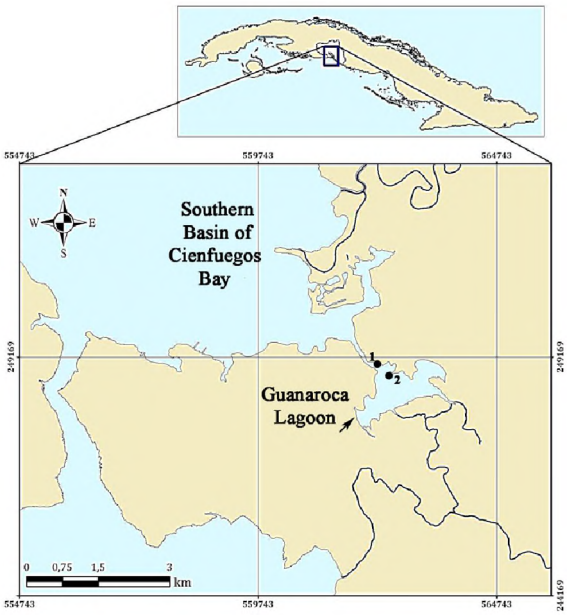

Fig. 1. Study area and sampling stations in Guanaroca Lagoon, southern Cuba (January 2014).

The athecate dinoflagellate *Cochlodinium polykrikoides* is a common marine invertebrate and fish killer in Japan and Korea, but in the last two decades has been reported to cause problems in many other countries including Canada, USA, Pacific Central America, China, Philippines and the Middle East [1].

A bloom of *C. polykrikoides* was identified in mid-January 2014 in Guanaroca Lagoon, southern-central Cuba (Fig. 1). Patches of red water were noted in the lagoon, extending into neighboring areas of the southern basin of Cienfuegos Bay. At the same time, hundreds of juvenile "bocón" fish (*Cetengraulis edentulus*), some oysters and blue crabs appeared dead in the shore areas (Fig. 2).

Guanaroca Lagoon (22°04'N, 80°22'E) is a small (2.2 km^2) shallow (average depth, 1.2 m) estuary fed by the Arimao River. This wetland is on the southwest of the semi-enclosed Bay of Cienfuegos (southern Cuba) and connected to it by a single channel. The area is subject to two main seasons: dry (November -April) and rainy (May-October). The annual mean air temperature is 24.7° C, with the highest (monthly mean 27.0° C) in the rainy season, in June, and the lowest (21.6° C) in January. The lagoon is part of a protected area for the conservation of biodiversity, in particular local and migratory birds, with a great variety of marine fauna including "blue crabs" (*Callinectes sapidus*), "ostión" (*Crassostrea virginica*), mussels and juvenile fish species.

During the bloom event, water samples were collected with a Niskin bottle for temperature, salinity and dissolved oxygen measurements and nutrient analysis (nitrite and phosphate). Phytoplankton samples were settled using 10 ml sedimentation chambers, and cells were counted under a Zeiss (Axiovert 40) inverted microscope.

Specimens collected at Guanaroca Lagoon had the features typical of *C. polykrikoides*, such as rod-like chloroplasts and a narrow sulcus located immediately below the cingulum. A large nucleus and reddish orange pigmented body were located at the epicone. Cells were more or less oval, with a girdle making 1.8–1.9 turns around the cell. The anterior part of the epicone was conical and the hypocone subspherical. The hypocone of the chain's terminal cell was bilobed. Cells within the chain were either spherical or slightly dorso-ventrally depressed, and smaller than single cells. Single cells were 35–45 µm long (41.23 ± 3.54, n=30) and 30–35 µm wide (33.83 ± 2.68, n=30). *C. polykrikoides* specimens in the present study were slightly smaller than the holotype (L: 50 µm) described by Margalef from the neighboring island of Puerto Rico [2]. During the bloom, chains of two (predominant), four and eight cells were observed and single cells were found occasionally.

	Collection sites inside the lagoon	
	1	2
Temperature	24,5°C	24,5°C
Salinity	31,3	31,4
pH	8,49	8,60
Dissolved O_2	13,7 mg L^{-1}	21,6 mg L^{-1}
PO_4^{3-}	1.59 µg L^{-1}	3.78 µg L^{-1}
Total P	31.6 µg L^{-1}	42.1 µg L^{-1}
NO_2^-	0.303 µg L^{-1}	2.88 µg L^{-1}

Table 1. Some abiotic parameters measured during the C. polykrikoides *bloom in Guanaroca Lagoon (January 2014)*

The *C. polykrikoides* bloom reached a maximal density of 7.4 x 10^7 cells L^{-1}. Environmental conditions (high temperature, salinity and irradiance) were consistent with those previously reported as favourable for this species in Cuba [3]. Nitrite was low; however moderate concentrations of inorganic phosphate and very high of total P, in particular in the more restricted area of the lagoon (Site 2) may have stimulated the bloom development. The high concentration of dissolved oxygen was a good indicator of microalgae overgrowth (Table 1).

The high residence time, salinity and transparency of Cienfuegos Bay waters during the dry season seem to be the key factors behind the development and maintenance of the blooms [4]. Coincidentally, *C. polykrikoides* populations have been observed only during the dry (March, November-December) and early rainy (June) seasons, and are generally restricted to the less eutrophic area (southern basin) of the bay. *Cochlodinium* blooms have not been observed during the rainy period (September), possibly affected by increased river inflow in that season [5]. Temperature was another important environmental factor associated to the *C. polykrikoides* event. The bloom observed in the Guanaroca Lagoon coincided with winter (December-February), with expected average air temperatures between 21 and 22° C. Higher temperatures were recorded during the first days of January and throughout the preceding month (December 2013). This was one of the warmest Decembers (average temperature of 24.4° C) in the Cienfuegos Province since records began with a record number of days (14) with maximum temperatures above 30° C [6].

There is recent evidence of increasing air temperature and drought periods in Cuba associated with the ENSO. 2015 was the warmest of the last 64 years in Cuba with the annual air tem-

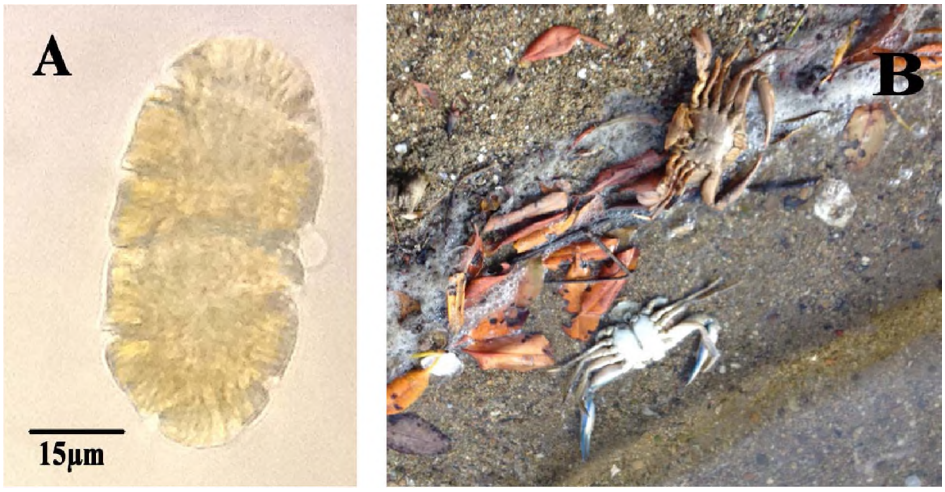

Fig. 2. C. polykrikoides *(A) and dead blue crabs (*Callinectes sapidus*) (B) during the bloom in Guanaroca Lagoon (January 2014)*



Fig. 3. A more recent C. polykrikoides *bloom from beaches in Cienfuegos Bay (June 2016). A.* Cochlodinium polykrikoides. *B. Discolorations and foam from* C. polykrikoides *blooms in the beach.*

perature exceeding the historical average of 25.49° C by 1.06° C and 68% of the Cuban territory suffered severe droughts from November 2014 to October 2015, affecting both the dry and rainy season [7]. These extreme weather conditions appeared to stimulate the growth of some dinoflagellates in embayments and restricted coastlines in Cuba. Within the same period (August 2015), a bloom of *Vulcanodinium rugosum* caused acute skin irritation in bathers from Cienfuegos Bay (in prep.), and more recently during the early rainy season (June 2016), another bloom of *C. polykrikoides* was observed in beaches of Cienfuegos Bay without apparent harmful effects (Fig. 3).

Fish-killing blooms of *C. polykrikoides* similar to those from Guanaroca Lagoon/Cienfuegos Bay have been recorded only in one other embayment of south-eastern Cuba (Santiago de Cuba Bay), and always during hot dry periods (April/May 2005 and January 2015) [8-9]. During the 2015, a bloom of *C. polykrikoides* was also recorded in channels of a small marina from Havana, north-western Cuba, in September, without apparent damage to marine life [10].

Acknowledgements

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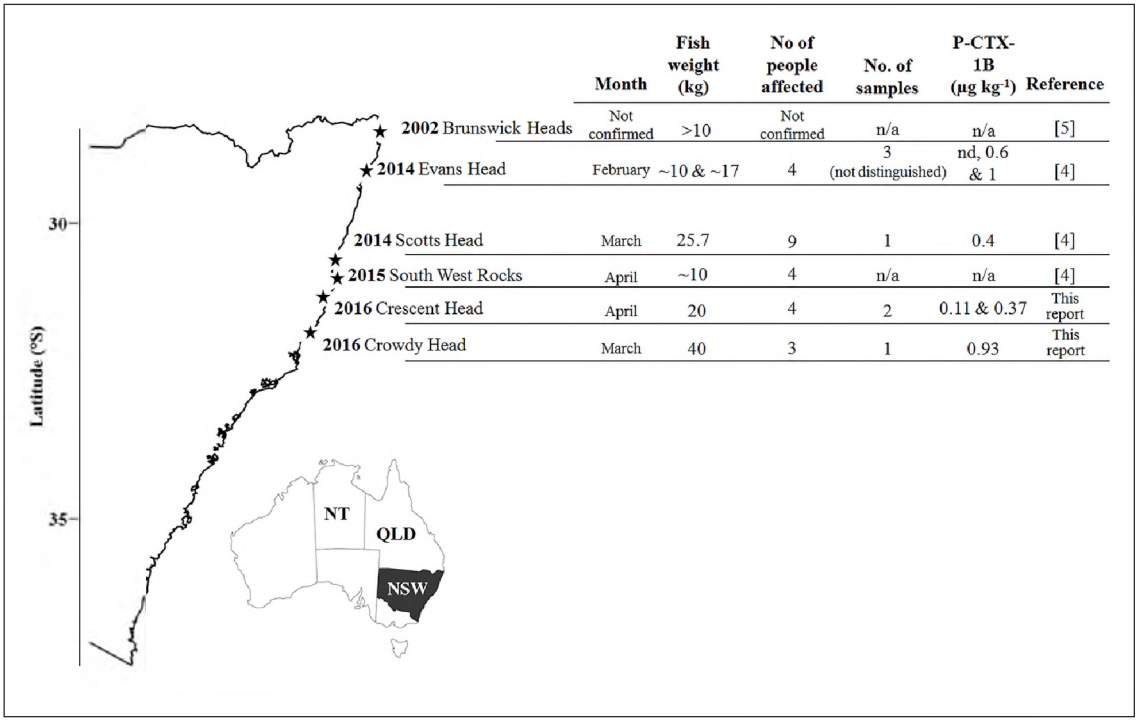
Is Ciguatera moving south in Australia?

Ciguatera Fish Poisoning (CFP) is well-known in tropical regions around the world, including the Pacific. The illness occurs through the consumption of fish that have accumulated naturally occurring ciguatoxins (CTXs) produced by the dinoflagellate genus *Gambierdiscus*. In Australia, there were more than 1,400 documented cases between 1965 and 2010 [1-3], including two fatalities, despite a significant rate of apparent under-reporting. Cases of CFP have occurred principally due to fish caught in tropical Queensland (QLD) and Northern Territory (NT) waters. Spanish mackerel (*Scomberomorus commerson*) is the fish species that has been most commonly involved in Australian CFP cases [4]. CFP is recognised as the most frequent cause of seafood-related illnesses in Australia [3].

During March and April 2016, two outbreaks of CFP were reported from consumption of Spanish mackerel caught in New South Wales (NSW) coastal waters. NSW is the state immediately south of QLD, along which the East Australian current runs in a southerly direction (Fig.1). This marks the third consecutive year since 2014 where CFP has been linked to Spanish mackerel caught in NSW waters. Twenty-four individuals were affected over five separate outbreaks between February 2014 and April 2016. These occurrences appear to be uncharacteristic for the region. A CFP outbreak following consumption of Spanish mackerel caught off Brunswick Heads (Fig. 1) in 2002 was the single recorded case from NSW prior to 2014 [4,5].

Pacific ciguatoxin-1B (P-CTX-1B) is regarded as a potent fish metabolite and is common to ciguatoxic fish sourced from the Pacific region [6]. Following the two CFP outbreaks in NSW during 2014, P-CTX-1B was reported at concentrations between 0.4 and 1 μg kg^{-1} in three of four fish samples (Fig. 1). These results were likely an underestimation of P-CTX-1B due to significant matrix suppression from fish tissue during the analyses [4]. At that time, testing was completed at the Cawthron Institute (New Zealand). Since then, capability to test for CTXs in fish samples has been established in NSW, and supported the 2016 illness investigations. Spanish mackerel fillet samples from the fish implicated in the Crescent Head (two samples, Fig. 1) and Crowdy Head (one sample, Fig. 1) cases were analysed. Following the illness reports, samples were kept frozen prior to analysis. A Thermo Orbitrap liquid chromatography mass spectrometry (LC-MS) system was used to detect P-CTX-1B in the fish samples. The performance of the method is the subject of a manuscript currently in preparation [7]. All samples from the 2016 cases were positive for P-CTX-1B with indicative concentrations of 0.11 and 0.37 μg kg^{-1} (20 kg Spanish mackerel, Crescent Head, Fig. 1) and 0.93 μg kg^{-1} (40 kg Spanish mackerel, Crowdy Head, Fig. 1). It is also highly likely that other CTXs were present in these fish samples, although at the time of analysis only P-CTX-1B was available as reference material.

While traditionally considered as a tropical or sub-tropical disease, CFP has become an emerging issue in locations previously thought to be outside its range of impact. The concentrations of P-CTX-1B in the fish that caused illness in NSW were up to two orders of magnitude higher than the US Food and Drug Administration's action level (0.01 μg kg^{-1} CTX equivalent for Pacif-



	Month	Fish weight (kg)	No of people affected	No. of samples	P-CTX-1B (μg kg^{-1})	Reference
2002 Brunswick Heads	Not confirmed	>10	Not confirmed	n/a	n/a	[5]
2014 Evans Head	February	~10 & ~17	4	3 (not distinguished)	nd, 0.6 & 1	[4]
2014 Scotts Head	March	25.7	9	1	0.4	[4]
2015 South West Rocks	April	~10	4	n/a	n/a	[4]
2016 Crescent Head	April	20	4	2	0.11 & 0.37	This report
2016 Crowdy Head	March	40	3	1	0.93	This report

Fig. 1. Map of coastline of New South Wales (NSW), Australia documenting catch locations of fish associated with ciguatera fish poisoning outbreaks. In all cases, the implicated fish was identified by the fisher as Spanish mackerel (S. commerson). The weight of the fish, number of affected individuals and indicative concentration of P-CTX-1B are reported where available. (Note: n/a = not available, nd = not detected, NT = Northern Territory, QLD = Queensland).

ic CTX) [8]. The illnesses occurred between February and April each year in NSW (Fig 1), coinciding with the peak Spanish mackerel fishing season. There is an apparent southern expansion of the geographic range of CFP along the east coast of Australia (Fig. 1). The catch locations of the fish from the 2014 and 2015 outbreaks were up to 300 km south of the QLD border. The fish from the 2016 cases were caught up to 120 km further south along the eastern seaboard. This highlights an urgent need to further understand the dynamics of CFP in NSW waters.

It has been thought that larger sized (>10 kg) Spanish mackerel specimens were more likely to have detectable levels of CTXs than smaller sized, and this information was conveyed to the fishing community following the outbreaks [9]. There are established commercial fishing bans, including Spanish mackerel of any size in designated QLD locations, due to the risk of CTX-contaminated fish [1,2]. While localised populations exist, Spanish mackerel can migrate several hundred kilometres [5]. Although increased reports of CFP may be a reflection of increased awareness of the disease in NSW, increasing ocean temperatures and an intensification of the East Australian Current [10] could influence the behaviour of Spanish mackerel and CTX-producing *Gambierdiscus* spp. Further research is essential to simultaneously mitigate the risks to consumer health and minimise the impact to the fishing community.

Acknowledgements

We are grateful to Dr Chowdhury Sarowar for his assistance with toxin analyses. The authors wish to thank NSW Food Authority officers for aiding with sample collection. The authors also wish to thank the individuals affected by CFP who participated in the investigations, and medical personal and NSW Health officers who provided notifications of the illness outbreaks.

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The CODIMAR launches a bilingual taxonomic guide of marine dinoflagellates

CODIMAR, the Marine Dinoflagellate Collection, Mexico, was established by Consejo Nacional de Ciencia y Tecnología in 2004. CODIMAR has launched a bilingual guide of the taxonomy of marine dinoflagellates. The guide uses file cards and original photographic sheets to illustrate the taxonomic and morphological characteristics of 26 dinoflagellate species that were isolated mainly from the southern Gulf of California and Cuban coastal waters. It also brings together a review of 26 scientific articles that contain information on some strains of CODIMAR. The genera described in the guide are: *Akashiwo*, *Alexandrium*, *Ceratium*, *Cochlodinium*, *Coolia*, *Gymnodinium*, *Lingulodinium*, *Ostreopsis*, *Pentapharsodinium*, *Prorocentrum*, *Protoceratium*, *Pyrodinium*, *Pyrophacus*, *Scrippsiella* and *Vulcanodinium*. Updated information on biological, ecological and geographical distribution aspects, as well as harmful effects and/or toxicity, are also included.

Revenue from the sale of this guide will be used entirely for maintenance and progress of CODIMAR. Contact details and information about how to order the guide are given below.

Scientific publication from the Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR) | MEXICO

Illustrated taxonomic guide of the Marine Dinoflagellate Collection (CODIMAR) | Guía taxonómica ilustrada de la Colección de Dinoflagelados Marinos (CODIMAR)

Lourdes Morquecho-Escamilla, Amada Reyes-Salinas, Yuri B. Okolodkov

ISBN 978-607-7634-20-1

Languages: English-Spanish

$27 USD + shipping costs

More information at: *lamorquecho@cibnor.mx*, *http://www.cibnor.mx* [Research > Biological Collections > CODIMAR]

A survey of BHAB assemblages utilizing the artificial substrate method in Rawa Island, Malaysia

Benthic dinoflagellates are marine tychoplanktonic inhabitants on the natural substrates of coral reefs in tropical and subtropical coastal waters. These dinoflagellates are associated with their prefered natural habitats [1,2]. Some of these species are known as bioactive compound producers. The compounds (e.g. ciguatoxin) may accumulate in reef fishes via the food web.

To assess benthic harmful algae (BHAB) assemblages in tropical coral reefs, we selected a fringing reef, Rawa Island, on the east coast of Peninsular Malaysia. The island is an ideal study site due to its abundance and diversity of coral reefs and other tropical marine resources. The fringing reef is covered with various types of hard corals, i.e. staghorn corals, table corals, cabbage corals (Fig. 1). At deeper water, large patches of soft corals (e.g. mushroom corals), sponge and giant clams are found. Nonetheless, some parts of the reefs appear severely disturbed, and coral rubbles there are covered with macrophytes. The co-occurrence of both pristine and disturbed reefs areas around the island makes it an ideal site for comparative studies to investigate the effect of anthropogenic activities on coral reefs and the associated BHAB communities.

Sampling of benthic dinoflagellates at Rawa Island was conducted monthly using the artificial substrate method (Fig. 2) [3,4]. This study focuses on five dinoflagellate genera: *Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, *Coolia*, and *Amphidinium* [2-4]. BHAB species were observed and identified under a light microscope (Fig. 3). *Ostreopsis* spp. were predominant in most samples, with a maximal cell density of 11,000 cells/100 cm^2. This study provides an important baseline data of BHABs in the western Pacific region, in particular on species composition and distribution in relation to their habitat preferences.



Fig.1. Map of Rawa Island and the common coral communities.

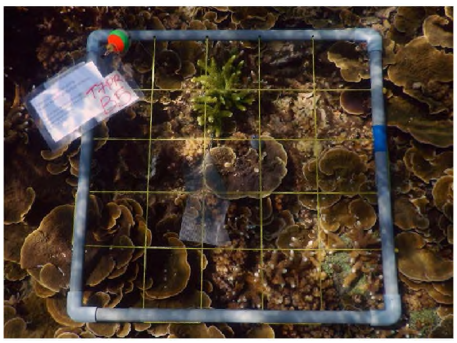

Fig. 2. Artificial substrate deployed underwater.

Acknowledgements

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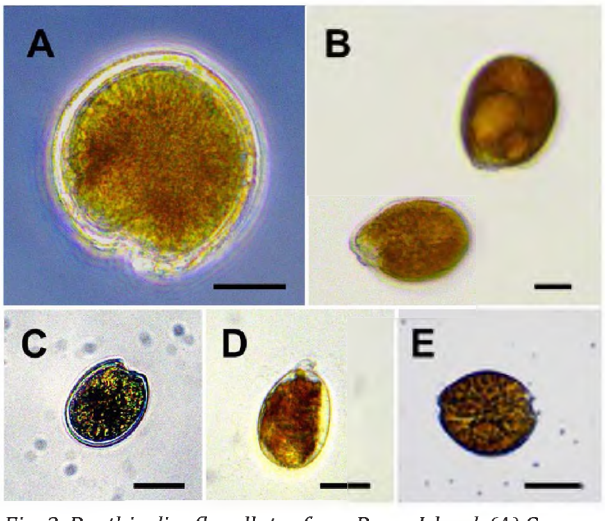

Fig. 3. Benthic dinoflagellates from Rawa Island. (A) Gambierdiscus *sp. (B)* Ostreopsis *sp. (C)* Prorocentrum *sp. (D)* Amphidinium *sp. (E)* Coolia *sp. Scale bars, 20 µm.*

Annual variability of *Prorocentrum lima* and abiotic factors in Chengue Bay, Tayrona National Park, Colombian Caribbean

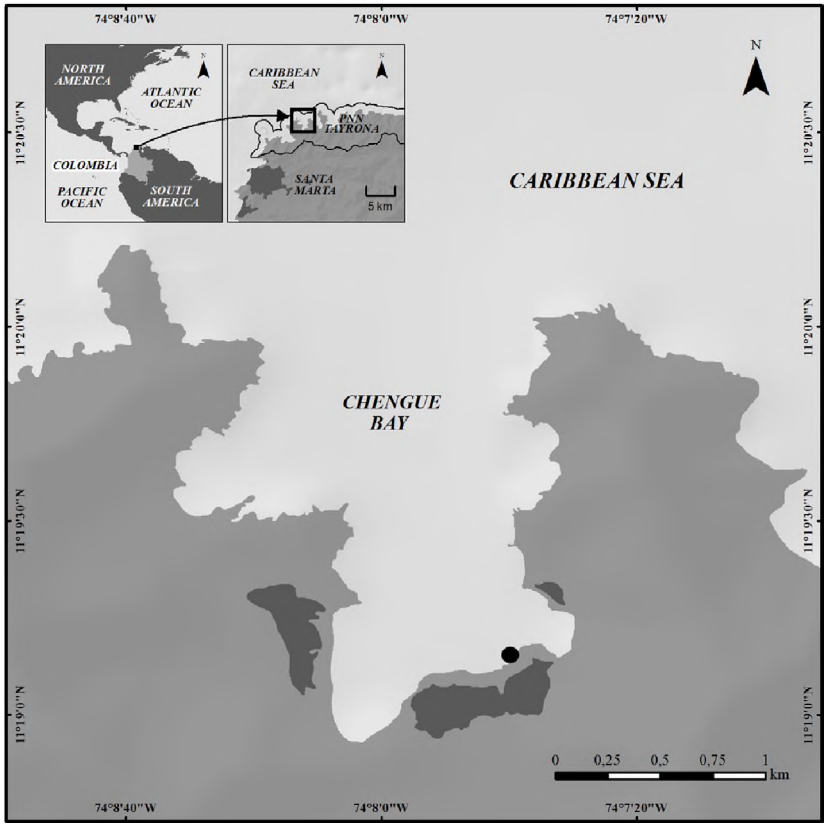

Fig. 1. Study area where samples of Thalassia testudinum *were collected. Chengue Bay (black star), Tayrona National Park, Colombian Caribbean*

Prorocentrum lima is a cosmopolitan species distributed in tropical and temperate seas. This dinoflagellate has been characterized in different regions as a toxin producer (okadaic acid and its analogs) and agent of diarrheic shellfish poisoning [1]. *P. lima* is one of the most abundant and frequent component of the epiphytic dinoflagellate community on the Turtle Grass (*Thalassia testudinium)* in the Caribbean. The objective of this study was to describe the annual variability in population abundance of *P. lima* in Tayrona National Park, Colombian Caribbean, to understand possible patterns related with climate.

Sampling was conducted in Chengue Bay. This bay, part of the Tayrona National Park (Fig. 1), is about 14 km east of the tourist city of Santa Marta (11 ° 18' - 11 ° 20' N and 74 ° 09' - 74 ° 07'W). Chengue Bay occupies an area of approximately 2.5 km^2 with a climate influenced by the two annual seasons: dry from December to April and rainy from May to November, although variations have been reported in recent years. The bay harbors a variety of tropical ecosystems such as mangroves, seagrass beds, coral reefs, soft bottoms and rocky shores [2].

Between January and December 2014, samples of *T. testudinium* were collected every month to analyze dinoflagellates present in the epiphytic community. In the laboratory, microalgae were detached and preserved in formalin. *P. lima* was identified in SEM micrographs. Cell counts were carried out with Sedgewick-Rafter chambers under a Zeiss Primo Star optical microscope. *P. lima* (Fig. 2) was the most frequent and abundant species of the 14 taxa observed. Cell densities ranged from 232 to 15,867 cells g^{-1} ww (wet weight) of *T. testudinum* (Fig. 3), with an average of 3,081 cells g^{-1} ww and maximum values in August and September. These values were lower than the maximal densities recorded by other authors during May 2005 (29,756 cells g^{-1} ww) in a semi-protected reef area of Veracruz, Gulf of Mexico [3], but higher than those observed in the coastal area of the Yucatán Peninsula (max. of approximately 1,100 cells g^{-1} ww of *T. testudinum*) in April 2013 [4].

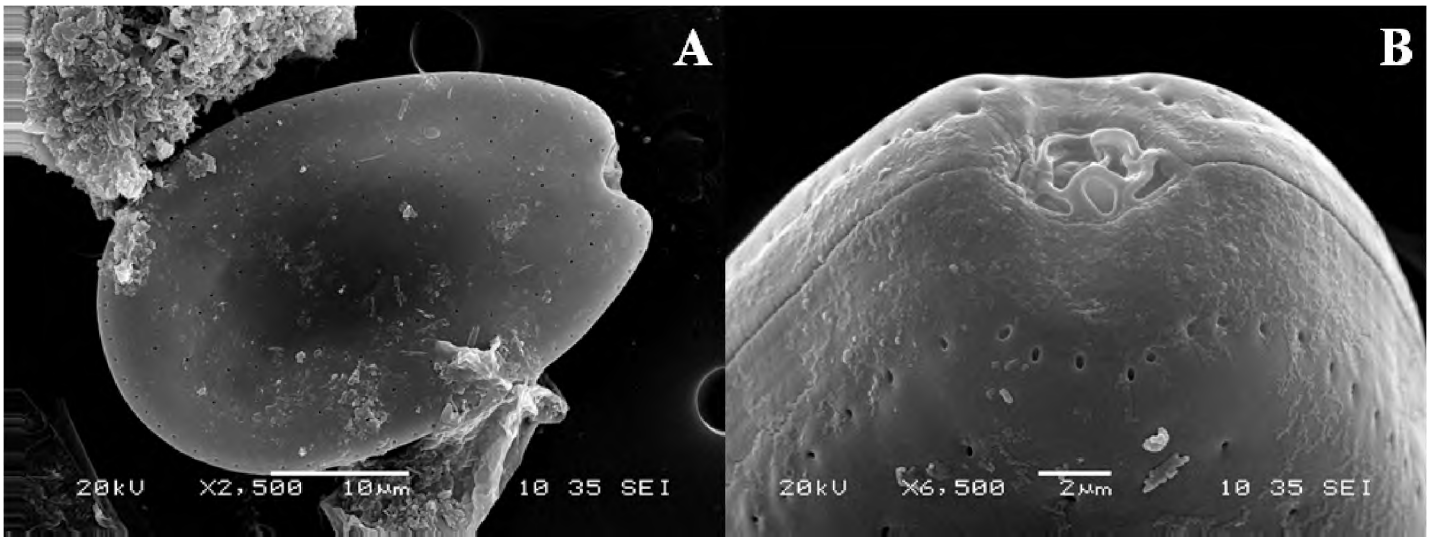

Fig. 2. SEM images of Prorocentrum lima. *A. Ventral view, with pore pattern and* B. Periflagellar *area with intercalary band and suture view*

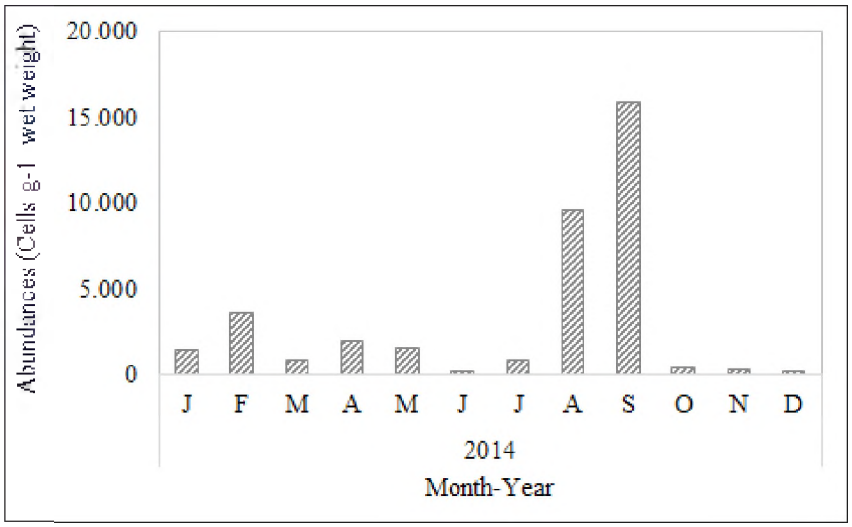

Fig. 3. Monthly changes in cell density of P. lima *on* Thalassia testudinum *in Chengue Bay, Colombian Caribbean, during 2014*

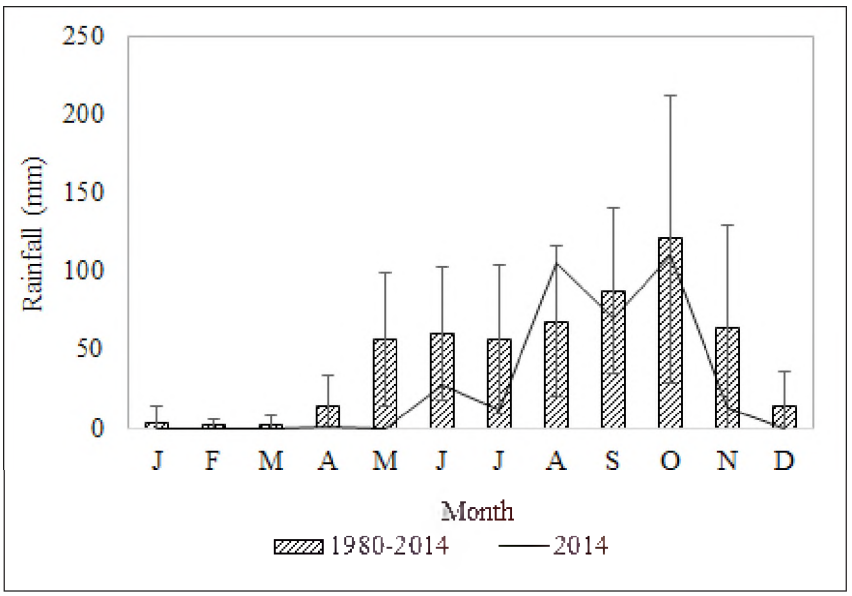

Fig. 4. Time series of rainfall (monthly average and standard deviation) from1980 to 2014 (bars), and total monthly rainfall during 2014 (black line), in the Magdalena region (airport station-IDEAM)

Maximal cell density of *P. lima* was found at salinities near 35, temperature above 29 °C, high N:P ratio (>16), nitrite 2.4 µg L^{-1}, nitrate, ammonia and phosphate concentrations below 2.7 µg L^{-1}, and high concentrations of total suspended solids. These environmental features are common when rainfall is low (70-110 mm monthly rainfall).

Rainfall did not follow the usual pattern during 2014 with values well below the historic mean (Fig. 4). These results suggest that *P. lima* population is likely to benefit during periods of low rain, small decline in salinity, high water temperature and lower inorganic nutrients in the environment.

For future risk management plans it will be important to consider toxin analyses to establish the risk involved with the presence of this species in the Colombian Caribbean. These results will help involve the public health sector, improve the diagnostic tools and reduce negative consequences of potentially toxic dinoflagellates in an important natural and tourist area.

Acknowledgements

This study was part of a technical cooperation project (RLA7/020) between the Instituto de Investigaciones Marinas y Costeras "Jose Benito Vives de Andreis" - INVEMAR in Colombia (CRTB-1153) and the International Atomic Energy Agency (IAEA). We are grateful to the Universidad Nacional de Colombia, Instituto de Estudios de Ciencias del Mar -CECIMAR (sede Caribe) y Programa de Postgrados en Biología - Línea Biología Marina (contribution 430). Thanks also to the Banco Español de Algas de Gran Canaria, in particular to Mr. E. Soler Onis, and to the Jardin Botánico Canario "Viera y Clavijo" for support with the SEM, and to the Instituto de Hidrología, Meteorología y Estudios Ambientales de Colombia -IDEM for providing rainfall data.

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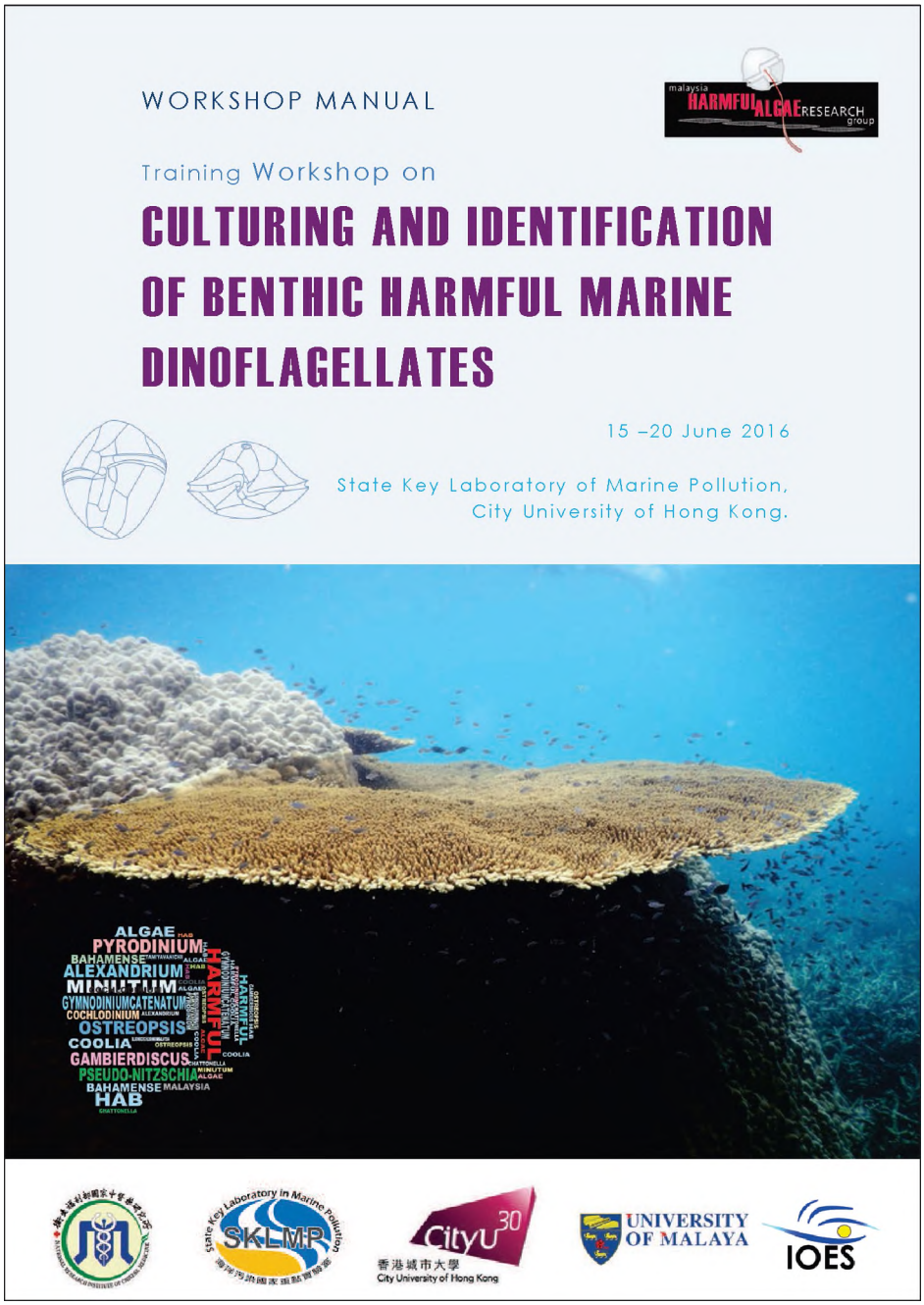
Training Workshop on the Culturing and Identification of Benthic Harmful Marine Dinoflagellates

A Training Workshop on the Culturing and Identification of Benthic Harmful Marine Dinoflagellates was held in the State Key Laboratory in Marine Pollution (SKLMP), City University of Hong Kong between 14 and 21 June, 2016. This training workshop was sponsored by the Collaborative Research Fund (CRF), of the Hong Kong Research Grant Council (grant # 8730040). It aimed to train the young scientists and government authorities in Hong Kong and mainland China in the standard protocols for sampling, isolation, culturing and identification of benthic harmful algal bloom (BHAB) species in the coastal coral ecosystems in the South China Sea, where the occurrence and distribution of these dinoflagellates are not known.

The workshop drew over 16 participants, including representatives from the Agriculture, Fisheries and Conservation Department (AFCD) of Hong Kong, research staff and postgraduate students of the SKLMP (Fig. 1). A series of lectures on the existing BHAB sampling methods by collecting natural substrates and deploying artificial substrates, as well as the most up-to-date knowledge about morphology-based taxonomy and molecular phylogenetic classification of BHAB species was delivered in the workshop by Dr Po Teen Lim (Associate Professor of the University of Malaya), Dr Chung-Kuang Lu (Associate Research Fellow of the National Research Institute of Chinese Medicine) and Dr Chui Pin Leaw (Senior Research Fellow of the University of Malaya). In the workshop, the participants were also given hand-on training on basic and advanced techniques on microalgal culturing and BHAB species identification including the epifluorescence microscopy technique (Fig. 2).



With the support of the Hong Kong Government, a collaborative research project has been initiated to study the effect of BHABs on marine ecosystems and to investigate the primary factors that regulate the distribution, growth and toxicity of BHAB species in Hong Kong waters and along the coast of south China, Taiwan and Malaysia. Toxins produced by BHABs can kill fish and disrupt food web structures and the functioning of coral ecosystems. One of the outcomes of the project will be a map that shows levels of BHAB-associated risks. The map should enable marine ecologists, coral and fish conservationists and governments to design strategies to monitor BHABs, to develop plans to conserve local coral communities and fisheries resources, and to protect consumers against BHAB-associated illness.

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This work was supported by the Hong Kong government through the Collaborative Research Fund (CRF) of the Research Grant Council (grant # 8730040).

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Fig. 1. Participants in the workshop at the State Key Laboratory of Marine Pollution, City University of Hong Kong, 14-21 June, 2016.



Fig. 2. Demonstrating basic techniques in BHAB culturing (Po Teen Lim, Chung-Kuang Lu, Chui Pin Leaw and the participants).

Regional Training Course on Identification of Harmful Algal Bloom Species in the ASEAN Region, Singapore, 18th-22nd July 2016

Harmful algal blooms (HABs) can cause major environmental disturbances, severe economic loss to industries, fisheries and tourism, and also pose significant health risks to humans. Considering the increase in HABs occurrence and the lack of taxonomy experts in Asia-Pacific region, a regional training course on identification of harmful algal bloom species in the ASEAN region was held at St. John's Island Marine Laboratory, Tropical Marine Science Institute, National University of Singapore (SJIML, TMSI NUS) from 18th-22nd July 2016.

This training course was organized by TMSI NUS, Southeast Asian Fisheries Development Center (SEAFDEC) and Agri-Food & Veterinary Authority of Singapore (AVA), sponsored by Japan Trust Fund, and in kind contributions by UNESCO Intergovernmental Oceanographic Commission Western Pacific-HAB (IOC WESTPAC-HAB), Japan Funds-in-Trust (JFiT), WESTPAC and Asian Natural Environmental Science Center (ANESC). Dr Yasuwo Fukuyo (University of Tokyo), Dr Lim Po Teen (University of Malaya), Dr Mitsunori Iwataki (ANESC, University of Tokyo), Dr Sandric Leong (National University of Singapore), Dr Kazumi Wakita (Tokai University) and Dr Lim Hong Chang (Tunku Abdul Rahman University College) were invited as trainers and to deliver lectures.

This training course was attended by twenty participants from ten countries (Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam) from Fisheries Agencies (Fig. 1). The main objective of this training course was to improve identification knowledge of harmful micro-algae through lectures and laboratory practicals. The course focused on the identification of harmful and red tide causative species including diatoms, dinoflagellates and raphidophytes that are commonly found in the ASEAN region. Moreover, another objective of the course was to encourage participants to establish an international network for communication and exchange of information in the case of HAB events.

The programme consisted of a series of lectures coupled with hands-on training sessions, field trips, demonstrations on sampling techniques, the use of Sedgewick-Rafter counting chambers, and observations of microalgae using light microscopy and field microscopy (Fig. 2). Accurate species identification is very crucial for the relevant authorities to prevent and mitigate HABs impacts. A short introduction to freshwater bloom-forming phytoplankton species, in particular cyanobacteria, for countries which have no marine HABs problems was provided. The training sessions also included discussions on current status, challenges and management of HABs in each country.



Fig. 1. Group photo of regional training course in Singapore, July 18-22, 2016.

Participants were encouraged to organize local or regional training courses to train more experts on harmful algae identification. The IOC WESTPAC-HAB working group shall assist local groups in training their local community by sending suitable trainers to the training workshop and by providing teaching references and materials. During the course a platform was created for participants and trainers to exchange HABs information and establish networks among scientists and managers. Posters on toxin-producing dinoflagellates and red tide species are expected to be produced by participants by end of this year. Subsequently, the posters will be distributed to local communities to enhance public awareness on HABs.

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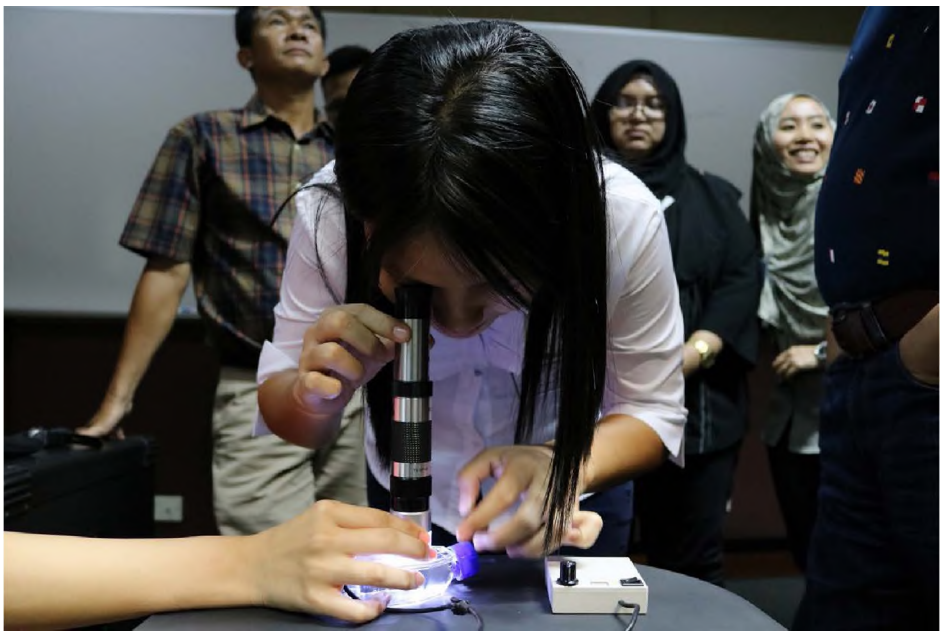
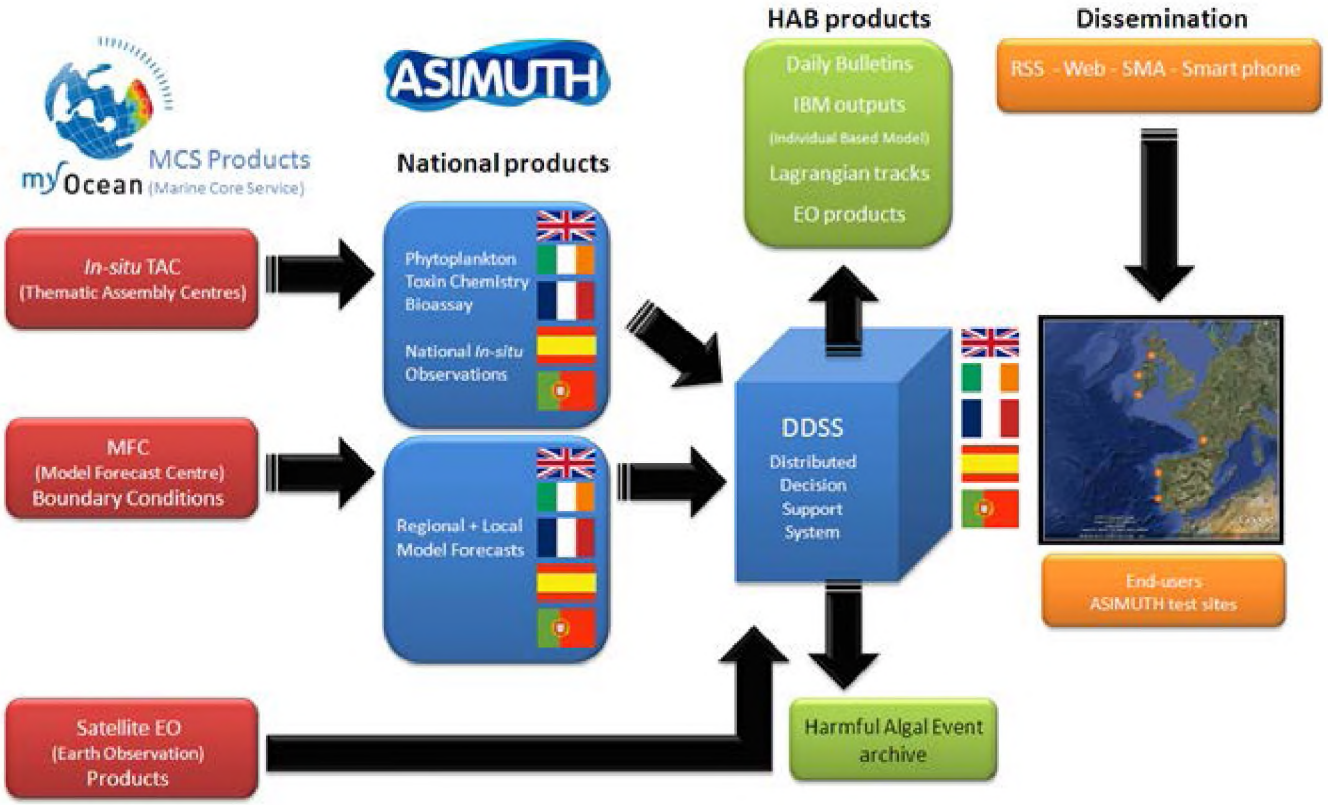


Fig. 2. Hands-on activities during the course: Light microscopic observation of specimens and field samples; Plankton net sampling at Singapore Straits on July 20, 2016; Loading of sample into Sedgewick-Rafter counting chambers; Observation of phytoplankton sample using portable field microscopy.



ASIMUTH special issue of Harmful Algae

The European Commission (EC) 7th Framework Programme (FP7) project **A**pplied **S**imulations and **I**ntegrated **M**odelling for the **U**nderstanding of **T**oxic and **H**armful Algal Blooms (ASIMUTH) ran from 2010-2013.

It involved 11 partners from Portugal, Spain, France, Ireland and Scotland from Europe's Atlantic coast, in regions that experience HAB problems with prolonged closures of aquaculture areas as a result of toxic HAB events and in some cases large losses of farmed fish.

The project had the aim of producing realistic HAB advisory and forecasting capability as a GMES (Global Monitoring for Environment and Security) downstream service to the European aquaculture industry, with a view to allowing fish and shellfish farmers to adapt their culture and harvesting practices in time, in order to reduce potential losses.

The project was the winner of the "best service challenge" at the Copernicus Masters Awards (Nov 2013)

Volume 53 of the Elsevier Journal 'Harmful Algae' (March 2016) edited by Keith Davidson, Marcos Mateus, Beatriz Reguera, Joe Silke and Marc Sourisseau is a special issue devoted to the ASIMUTH project: *www.sciencedirect.com/science/journal/15689883/53*

The special issue contains 14 papers that describe some of the modelling and early warning advances made during the project. It included work on a number of HAB genera including *Dinophysis, Alexandrium, Karenia, Pseudo-nitzschia* and *Gymnodinium*.

ASIMUTH has a legacy of continuing HAB risk alert systems in both Ireland and Scotland that can be accessed at *www.marine.ie* and *www.HABreports.org* respectively.

This project is supported by the EC FP7 Programme, Space Theme, Grant Agreement No.: 261860

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Last News on the 17th ICHA

The local and international organizing committees are busily planning the 17th International Conference on Harmful Algae (17th ICHA), in Florianopolis, Brazil from 9th-14th October 2016. For the first time in history, Latin America will host the conference and is looking forward to welcoming approximately 450 participants from many different countries from around the world.

REGULAR registration is open until August 31st. From September 1st, LATE onsite registrations will be accepted at the ISSHA rates of 1800 Brazilian Real R$ (non-ISSHA member, 2000R$) and 1000 R$ for students (non-ISSHA students, 1100 R$). So it's not too late to make your travel plans to attend the conference so that you can reconnect with your colleagues and hear many exciting talks about HABs around the world!

A new student **speed networking** workshop is scheduled for 13:00-16:00 on Sunday, October 9 at the Intercity Hotel (the conference hotel). This is an excellent opportunity for young scientists to meet and exchange ideas prior to the main conference. The networking workshop will foster an interactive environment between small groups of advanced scientists and students in hopes of creating short, high impact exchanges. There is still space, so sign up today!

The ICHA 2016 **photo competition** is still accepting photos until 30 September 2016 from registered participants. Photos can be submitted in the categories of microscopy, harmful algae and aquaculture, freshwater harmful algae, tropical harmful algae, harmful algae scientists at work and play. Please submit your best photos!

Several very reasonably-prices **tours** are planned for Wednesday, October 12, including a Florianopolis city walk, a trip to the stunning views and beaches of Lagoa de Conceição, an island adventure, an oyster experience, a sailing trip, a trekking experience and an Oktoberfest party. Sign up lists for these tours will be available at the conference.

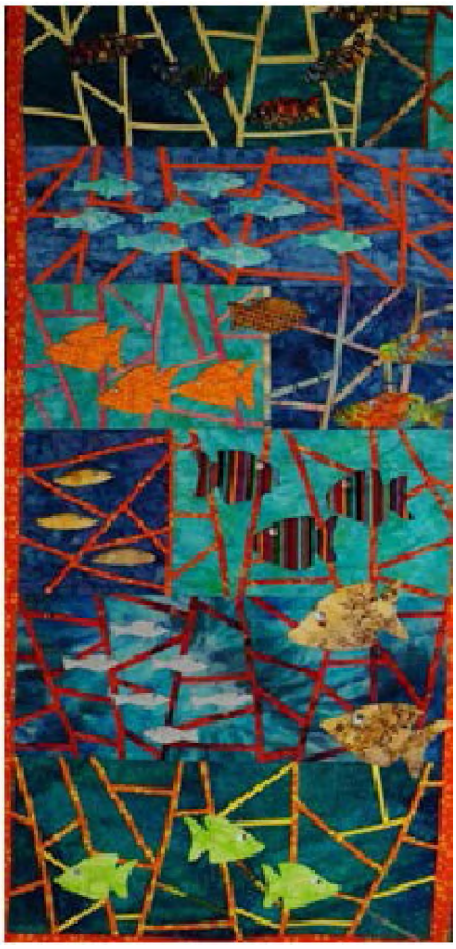

Fig. 1. A beautiful handmade tapestry for the ISSHA Auction 2016

Voting for ISSHA Executive and Council Members will take place from October 1 to October 12 midnight Brazilian time. Voting for the 2020 ICHA venue will be open until October 13 midnight Brazilian time. Bids will be published on the ISSHA website and the candidate countries will make their presentations at the ISSHA General Assembly lunchtime meeting on Thursday October 13. All ISSHA members, including those who cannot attend the ICHA 2016 in Florianópolis are encouraged to vote ON LINE.

And last but not least, don't forget our **biannual auction**. If you have items to donate or know of a business who might be interested in supporting our society, please contact Esther Garcés at *esther@icm.csic.es*. Or just bring your items with you and drop them off at the ISSHA booth at the conference. Remember that the auction is one of the funding sources for ISSHA that allows many deserving students to attend our international conferences. This is a very worthy cause!

For more information about the conference program and travel details, please see *www.icha2016.com.*

Vera Trainer, ISSHA President

Important Deadlines to vote on line (www.issha.org)

Voting for ISSHA Executive and Council Members: October 12 midnight Brazilian time

Voting for the 2020 ICHA venue: October 13 midnight Brazilian time

Forthcoming events

Seafood Safety: New Findings & Innovation Challenges
Brussels, 25-26 January 2017
Deadline for registration: 15 January 2017 (early bird 25 November 2016).
Abstract submission deadline: 15 October 2016.
www.ecsafeseafoodconference.com

11th International Conference on Modern and Fossil Dinoflagellates
Bordeaux (France), July 17-21, 2017
Latest developments in studies of living and fossil dinoflagellates.
laplf.org/dino11/news.htm

11th International Conference on Molluscan Shellfish Safety
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Deadline
Deadline to submit material for HAN 55:
31 October 2016

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AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

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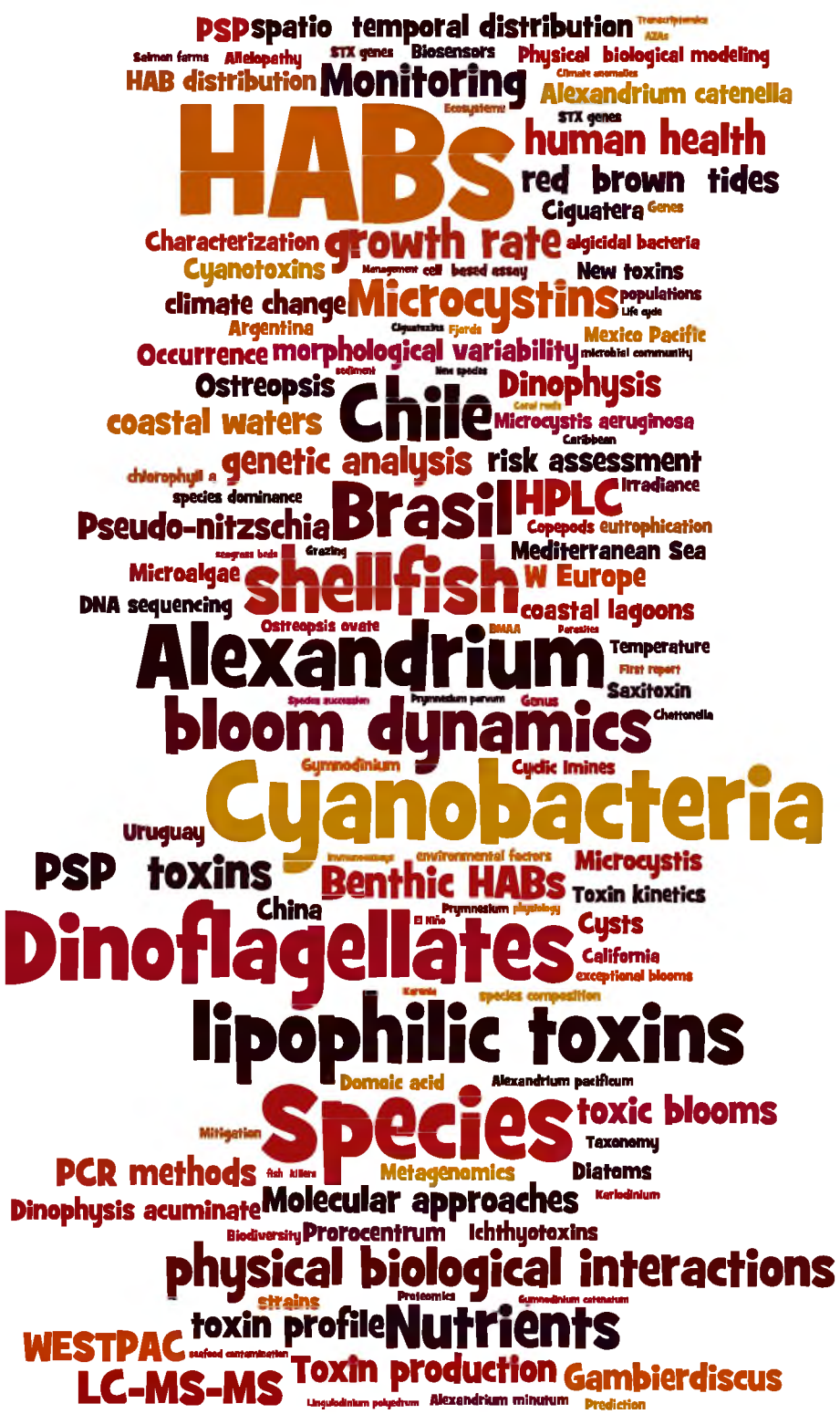
United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Highlights of the XVII International Conference on Harmful Algae



"Words Cloud" generated from the key words and titles of all contributions to 17 ICHA.

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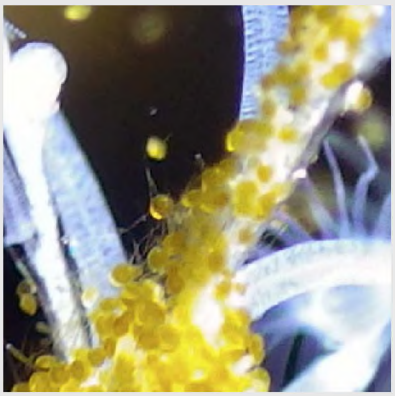

Gambierdiscus *cells on a cnidarian colony (see p 14)*

Florianópolis, "Floripa" to the locals, is the capital city of the state of Santa Catarina, in southern Brazil. Many small commercial fishermen populate the island, and the fishing boats, the folklore, the cuisine and the colonial architecture contribute to make it a very attractive tourist resource for many Brazilians and Argentineans escaping from overpopulated cities. But most important for us, the Florianópolis area is the major producer of cultured oysters and mussels in the country and suffers from periodic harmful algal bloom events caused by *Alexandrium*, *Dinophysis* and *Gymnodinium* species. All this contributed to make the Brazilian colleagues choose Floripa as the venue for the XVII International Conference on Harmful Algae (ICHA 17), 9-14 October 2016, the first one to be held in Latin America.

ICHA 17 was hosted by *The Federal Institute of Santa Catarina* (IF-SC). The local organizing committee was chaired by Dr Luis Antonio Proença. The theme of the conference was: "Harmful Algae, from cells to fisheries: species, toxins, ecology, management and new technologies"

The Conference was attended by 350 participants from 35 countries. There were 2 keynote speakers, 8 plenary speakers, 145 orals presentations, 20 fast-talks, 250 posters and a heavily attended round table to discuss about the recent events in Chile.

The outstanding topics of the Conference were:

- **Climate anomalies**, global change, record blooms, heavily influenced by the dramatic salmon-kills and PSP events suffered by Chilean producers in 2016.
- **Advanced 'omics** and in situ sensors, enabling tests of and answers to scientific questions that had been limited by technology
- **Cyanobacteria**: over 60 contributions. Not surprising considering that cyanobacterial toxins have been traditionally the main threat to public health of Brazilian freshwater bodies.

The "words cloud" generated from titles and keywords from oral and poster contributions illustrates well the dominant issues. As usual, *Alexandrium*, in particular the highly toxic *Alexandrium catenella* (=*Alexandrium fundyense* or *Alexandrium* Group 1) was the predominant genus, followed by an increasing number of contributions on benthic HABs (*Gambierdiscus* + *Ostreopsis*) and *Dinophysis,* now matching those of *Pseudo-nitzschia*. Morphological, toxinological and genetic descriptions of SPECIES were top of the list. Emerging (cyclic imines) toxins, cyanobacterial microcystins and the arduous identification of fish-killing substances attracted much attention. The location of the venue favoured a good list of contributions from Chile, Argentina, Uruguay and Mexico in addition to those from the host country.

Detailed scientific highlights of different conference topics were prepared by senior participants to summarize the main research and management advances for the HAB scientific community, especially for those who were not able to make it to Floripa.

Beatriz Reguera (IEO, Vigo, Spain) & Luis Proença (IF-SC, Santa Catarina, Brazil)





At the Public Market during the 17th National Oyster Festival in Florianópolis: Left, hanging baskets of oysters and scallops and right, mussel seed collectors (Photo Yolanda Pazos).

HABs in a Changing Climate

Much of the session focused on the extraordinary natural bloom events in 2015-2016, including in Chile, the Western USA, and the eastern coastline of Tasmania.

There is a long history of *Alexandrium* blooms along the Chilean coast, and the more recent blooms have progressed northwards up the coast over the past several decades (**Alejandro Clement**, **Leonardo Guzmán**). The extraordinary bloom of *Pseudochattonella verruculosa* and *Alexandrium catenella* in 2016 began in the outer coastal waters and was transported into the inland sea region, where it devastated fish farms leading to economic and social upheavals. There is a long history of *Alexandrium catenella* along the Chilean open coast, going back over 40 years of records, so the bloom stemmed from shifting environmental conditions rather than recent species introduction. The event coincided with an anomalous movement of the southeast Pacific high pressure region offshore linked to the Pacific Decadal Oscillation, suggesting there may be a climatic link that enhanced this bloom event.

Blooms of a more toxic *Alexandrium* species, *Alexandrium* Group 1 (*A. catenella* = *A. fundyense*), along the eastern coast of Tasmania appeared in 2012, for the first time in over 30 years of observations, and expanded greatly in 2015 and 2016. This coastal region is a climate change "hotspot", resulting from the southward movement of the East Australian Current, bringing more warm water further south. The working hypothesis is that the recent appearance of the more toxic *Alexandrium* strain is the consequence of a complex interaction of increased temperature, flood events and changes in the southward extension of the East Australian Current enhancing growth of the ende-

mic, but rare, more toxic strain. (**Gustaaf Hallegraeff**)

The massive *Pseudo-nitzschia* bloom event along the western USA also appears to be associated with an anomalous warm "blob" of surface waters that pushed onto, and retracted from, the entire coastal region. Data suggest that these anomalous warm conditions inshore led to the selection of *Pseudo-nitzschia* over other diatoms in the near shore waters, due to their competitive ability at low nutrient concentrations. As the warm waters retracted with the onset of upwelling, this seed population of *Pseudo-nitzschia* was stimulated and sustained to create the bloom conditions along the entire coastline. (**Vera Trainer**)

There is laboratory evidence of a potential link between increasing ocean acidification, nutrient limitation and toxicity of phytoplankton. Although dinoflagellates and cyanobacteria are anticipated to benefit from increasing pCO2, by reducing the need for energy-consuming carbon concentrating mechanisms, the expected benefits are small for *Alexandrium fundyense* and *Scrippsiella trochoidea*. However, decreased pH enhanced N assimilation under N limiting conditions, leading to increased toxin production. The findings suggest that ocean acidification may lessen the negative effects of N limitation and influence the composition and toxicity of phytoplankton assemblages. (**Dedmer Van de Waal**)

Warming of the surface ocean has coincided with the increasing presence of HABs globally over the last 3 decades, and modelling of the optimal growth conditions of *Cochlodinium*, *Dinophysis*, and *Alexandrium* shows expansion of the optimal growth conditions and bloom windows in the Atlantic and Pacific regions by more than a month between 1982 and 2015. While ocean warming is expanding the niche for HABs, coinciding with increasing HAB frequency, the mechanistic for this linkage is not understood. (**Chris Gobler**)

Studying anomalous HAB blooms, or HAB "weather", can provide insights to how climate pressures might affect HABs in the future, but forecasting climate driven changes in HABs will require new research approaches. Improved response planning for extraordinary HAB events, better focus Intergovernmental Panel on Climate Change (IPCC) projections for regional changing conditions, and unification of experimental methods will be necessary if we are to move forward quickly enough to forecast, rather than hindcast, the effect of climate on HABs. (**Mark Wells**)

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Taxonomy at the XVII ICHA

The word 'species' appears 550 times in the abstract book of the conference, pointing at the importance of giving names and assessing relationships between the entities around which harmful algae science accumulates. Yet still many taxa exist without our recognition. Indeed a steady number of new species are introduced at each ICHA as well as in the intersessional periods, which reminds us that our knowledge on the diversity of harmful algae is still work in progress. Luckily and despite the long blamed taxonomy crisis there are still scientists that are able to recognise the novelties and produce nice accounts of shapes and patterns that allow other scientist to recognize them too. This time also we were presented with new players in the game of HABs.

A new, non-toxic species of *Alexandrium*, *A. fragae* was desccribed from Brazilian waters, along with the first finding of *A. tamutum* in the same area (**Suema Branco**). The new species morphologically resembles some Mediterranean forms of *A. minutum* that have a smooth epitheca and heavy reticulated hypotheca, which were shown at the ICHA conference in Lund [1]. Two new *Gambierdiscus*, *G. balechii* and *G. lapillus* were identified, the former in Celebes Sea (**Santiago Fraga**) and the latter in the Great Barrier Reef (Australia) (**Anna Liza Kretzschmar),** further raising the number of species in a genus considered as monospecific until a few years ago. Another new microbenthic species, *Ostreopsis rhodesae* from East Australian Coast was introduced (**Arjun Verma**). Although no palytoxin-like molecules were produced in this species, transcripts related to polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs) multi-enzyme complexes were found, which are active in the synthesis of palytoxin-like substances. The recently described *Ostreopsis fattorussoi* [2] instead does produce ovatoxins.

Diversity knowledge is growing at a very high pace also in the plankton genus *Azadinium* (**Urban Tillmann**), where 11 species have been recognised in 7 years since its establishment, some of which (e.g. *A. poporum*) are quite widespread worldwide. We can blame the small size of these species (generally < 15 **µm**) for the late recognition of so much diversity, which may also explain why the discovery of the real culprit of azaspiracid production took some time. Interestingly, some of the 10 species in the related genus *Amphidoma* also produce AZAs. A new entry is registered also in naked dinoflagellates with a



Birds of a feather flock together. Prof Moestrup and other well known taxonomists solving nomenclature problems (Photo Mathias Schramm)

still unnamed woloszynskioid species showing characters intermediate between the families Borghiellaceae and Suessiaceae (**Kazuya Takahashi**).

The fast evolution of molecular approaches at times may show contrasting scenarios over time: despite distinct morphologies: three *Chattonella* species, *C. antiqua*, *C. marina*, *C. ovata* had been merged in a single species based on the identity of most molecular markers [3]. Now, using a more sensitive technique relying on microsatellites – ISSR genotyping by sequencing (MIG-seq) analysis–it is shown that the three can be at least identified molecularly, although this does not prove they are separate species (**Satoshi Nagai**). With all these novelty and due to some taxonomic changes, it is not surprising that to keep the IOC taxonomic list of Harmful Species updated is a hard job (**Øjvind Moestrup**).

Montresor M et al 1990. In: Granéli E et al (eds) Toxic marine phytoplankton (Elsevier, Amsterdam) pp 82-87
Accoroni S et al 2016. J Phycol: doi:10.1111/jpy.12464
Demura M et al 2009. Phycologia 48: 518-535

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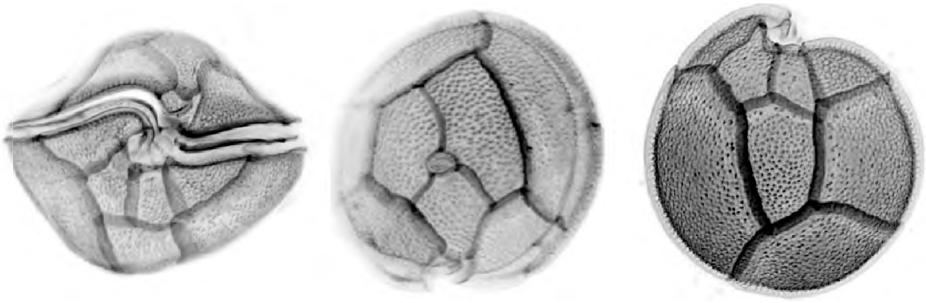
Benthic HABs

Benthic harmful algal blooms (BHABs) have an increasing interest based primarily in ciguatera but also due to problems caused by *Ostreopsis* in some coastal waters. The knowledge of the ecology of benthic microalgae is far from the well developed studies on plankton ecology, but some important progresses in the ecology of *Gambierdiscus* (**Chui Pin Leaw, Masao Adachi, Michaela Larsson, Ingrid Sassenhagen**) was presented as well as in modelling *Gambierdiscus* bloom dynamics (**Michael Parsons, Steven Kibler**). In parallel to the description of new *Gambierdiscus* species, the known distribution of the genus has increased considerably including some new reports: Cuba (**Lisbet Díaz Asencio, Angel Moreira**), El Salvador (**Rebeca Quintanilla**), Colombian Caribbean (**Anderson Ruiz Gómez**), Brazil (**Silvia Nascimento**), Philippines (**Rhodora Azanza**) Indonesia (**Santiago Fraga**) and Canary Islands (**Francisco Rodríguez**), and



SEM micrograph of Gambierdiscus balechii *(Photo Santiago Fraga).*

their expansion to new areas (**Michaela Larsson**). For the first time, the transfer of *Gambierdiscus* toxins to herbivorous fish in laboratory conditions was studied and clearly demostrated (**Rachel Clausing**).

Far from the planktonic HABs is the monitoring of BHABs. *Gambierdiscus* monitoring was only reported from the Philippines (**Ma Llorina Rañada**) but due to the efforts of IAEA on training scientist of many countries (**Yasmine Bottein**) the implementation of BHABs monitoring programs in the near future can be expected.

Although ciguatera is the most important harmful effect of benthic algal blooms, other genera like *Ostreopsis* may cause harm when some blooms of species of this genus were related to human respiratory problems. Reports of the presence of *Ostreopsis* were abundant from many countries and the presence of toxic and non toxic species was reported (**Olga Carnicer, Cristina Mendes, Silvia Nascimento, Mariana Santos**). Progress in the study of the *Ostreopsis* bloom conditions and/or associated macroalgae were reported (**Rodolphe Lemée, Daniela Catania, Stefano Accoroni, Chui Pin Leaw, Elisa Berdalet**).

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Cyanobacteria

Over 60 studies were presented and distributed in 5 main areas: i) Toxins analysis; Surveillance and management; iii) Ecology and bloom dynamics; iv) Toxicology; v) Cell physiology and vi) Others.

New reports about human oral exposure in different continents (South America and Africa) were presented including relevant case studies about recreational exposure to microcystins in Mozambique (**Olivia Pedro**) and Uruguay (**Daniela Sedan**). The later represented a serious health risk that included a case of a child suffering fulminant hepatitis requiring a liver transplant.

Advances on cyanobacterial cell physiology and intraspecific diversity analysis were given by **Iame Guedes** and **Elena Galvanese.** Other highlights included: Nodularin records in South America (**Luiza Costa**, **Savênia Silveira**); increasing surveillance and management data from different regions and continued exploration of clay and flocculants for removing cells and/or cyanotoxins (**Valeria Magalhães**, **Cinthia Bogarín**)

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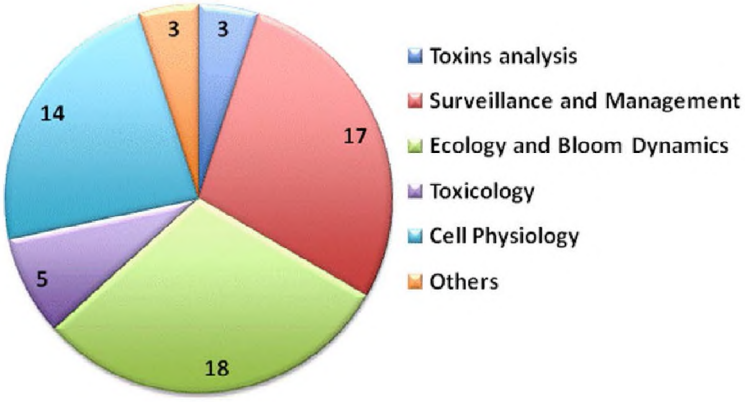


Percentage of contributions on different issues related to Cyanobacteria.

HABs Biology and Ecology

The recent advent of 'omics' technologies made possible the exploration of additional features to understand functional aspects of the biology and physiology of harmful species (reviewed in next section on molecular technologies), including the intricate sexual reproduction of *Pseudo-nitzschia* species (**Marina Montresor** keynote speech).

There was a good session about the diversity and role of parasites affecting various dinoflagellate (host) species with different susceptibility (**Esther Garcés**, **Albert Reñé**). In the field, recurrent blooms of *Alexandrium pacificum* in the Marlborough Sounds of South Island New Zealand were described. While hydrological modelling of the bloom environments were successful, biological modelling showed far less success. Bloom termination tended to be by encystment, but complications associated with parasitism may have accounted for the difficulty in the biological modelling (**Lincoln MacKenzie)**.

The role of β-N-methylamino-L-alanine (BMAA), the producer organism and their actual effects, continue to be a mystery (**Sandra Lage**).

Allelopathic interactions received considerable attention. Algicidal bacteria act on HABs, particularly those bacteria growing on macroalgae and seagrasses, which underlines the likely importance of conserving natural macrophyte vegetation in marine and freshwater habitats to stabilize the pelagic ecological system (**Ichiro Imai**). But there are at least class-specific relations. In this context, **Kathryn Coyne,** showed us the algicide IRI-160, produced by bacteria, and which is active against dinoflagellates, particularly *Pfiesteria*, but not against non-dinoflagellate algae. The harmful effects of toxin-producing *Dinophysis* cf *ovum* in culture on its ciliate prey *Mesodinium rubrum* were shown in videos of *Dinophysis* cells secreting mucus aggregates and strands that trapped the ciliates as they swam. We saw *Mesodinium* cells which appeared to be trying to escape from their elastic trap, much as a fly tries to escape from a sticky spider's web. However, perhaps weakened by some toxins (ROS, PUFAs?) different from okadaic acid and dinophysistoxins, they are eventually captured by the *Dinophysis* that suck out their contents with a feeding tube (myzocytosis) (**Luiz Mafra)**. **Yolanda Pazos** showed how blooms of *Dinophysis* in the real world generally follow blooms of their prey, *Mesodinium.* **Marc Long** (honorary mention for student oral presentation) treated the impact of *Alexandrium minutum* culture filtrate on *Chaetoceros neogracile*. An allelopathic action on the diatom's thylakoids and cell membranes, apparently involving ROS, was observed very quickly (~5 min) after exposure.

Turning to ecology, **Beatriz Reguera's** presentation on fine-scale physical-biological interactions in *Dinophysis acuta* dynamics showed the short-term impact of upwelling on net growth due to dispersion, but also decreased division rates due to the effect of increased shear stress and mixing on the cells. **Raphael Siano** showed us how *Alexandrium minutum* has now invaded the Bay of Brest, France, to give regular blooms now of up to 40 million cells/L^{-1}, whereas back in 1990, the cyst record (PCR detection) shows that it was totally absent. *Vulcanodinium rugosa*, a pinnotoxin producer from a Mediterranean lagoon, has increased in all NW Mediterranean lagoons in the last decade, but in this case introduction by shellfish transfer is suspected. **Alejandro Clément** showed how changes in climate and ocean circulation appear to be changing not only the timing of blooms in southern Chile, but also the synergistic effect of blooms of the diatom *Leptocylindrus danicus* and the flagellate *Pseudochattonella.*

Ian Jenkinson, Chinese Academy of Sciences, IOCAS, China & Agence de Conseil et de Recherche Océanographiques, France, ianjenkinson@qdio.ac.cn

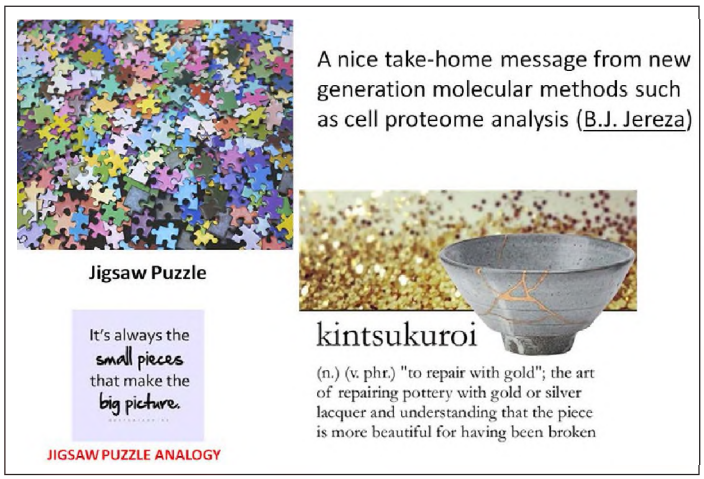
Application of Molecular Technologies in HAB Research and Monitoring

Molecular approaches for HAB studies covered a vast number of issues, with special emphasis on the use of "omic" technologies to understand functional aspects of the biology and physiology of harmful species, as well as their detection in the field for monitoring purposes.

Genomic and transcriptomic studies on the domoic acid producer *Pseudonitzschia multistriata* (**Mariela Ferrante**) provided new information on the structure and evolution of diatom genomes, as well as the identification of a series of genes involved in sexual reproduction. The complexity of dinoflagellates genomes and their unique features was addressed on *Amphidinium carterae* (**Allan Place**), regarding the characterization of mechanisms that regulate translational processes involved in the synthesis of proteins (novel mRNA caps and eIF4Es). Diversity of Poliketide Synthase genes was shown in dinoflagellate genera such as *Ostreopsis* (**Arjun Verma**) and *Gambierdiscus* (**Gurjeet Kohli**). Saxitoxin production and its variability are known in several dinoflagellate species and genera. Genes involved in their synthesis have been explored (**Armando Mendoza-Flores**) to gain new insights on the evolutionary pathways through which these genes have been transferred from cyanobacteria to dinoflagellates. The proteomes of dinoflagellate species provide another approach to understand specific metabolic pathways and a novel technique (shotgun proteomics) for mapping the proteome of *Pyrodinium bahamense* was presented (**Bryan Subong**).

The study of population genetics and plankton communities was also highlighted during the conference by metagenomic studies on Japanese coastal waters (**Satochi Nagai**) and population genetic studies using microsatellites in benthic dinoflagellates (*Gambierdiscus caribaeus*; **Ingrid Sassenhagen**), *Pseudonitzschia multistriata* (**Marina Montresor**), *Cochlodinium polikrikoides* (**Satochi Nagai**), as well as RAD-TAG studies in freshwater systems (*Gonyostum semen*; **Karin Rengefors**), to reveal geographical expansion and spatial differentiation among populations.

Studies of population genetic structure (SSU, V4rDNA) allowed the detection of entries of new species in the Gulf of Naples (**Adriana Zingone**). There are still some methodological issues (associated with DNA extraction, PCR efficiency and copy number per gene) to be improved for quantitation with metagenomics, but these are very helpful to unveil annual trends of plankton communities and their disturbances, as shown in Japanese coastal waters (**Nagai**) and in the Gulf of Naples (HTS metabarcoding; **Adriana Zingone**). Multiple studies also addressed the use



Cell proteome analysis, or how to use individual molecules, to make a beautiful picture about the nature of an organism, seen by the author as a jigsaw puzzle or "kintsukuroi".

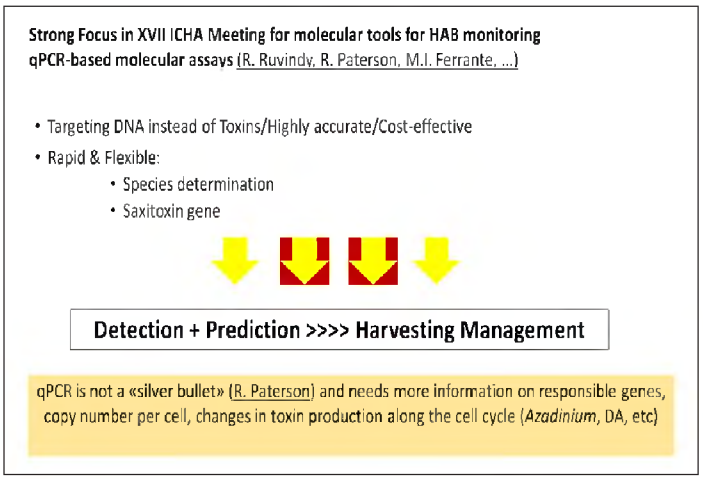


Diagram about the application of molecular tools (qPCR) for monitoring purposes.

of qPCR assays for HAB cells and cysts detection and enumeration (**Marcela Mora**, **Ruth Paterson**, **Raffaele Siano**), including the use of saxitoxin genes as a target (**Henna Savela**, **Claudia Piccini**, **Bruna Buch**, **Rendy Ruvindy**). Detection and prediction are two important issues for harvesting management that can be tackled by qPCR assays. In addition, we need more information on responsible genes, copy number per cell, changes in toxin production along the cell cycle, etc (**Ruth Paterson**).

Continuing a trend that started at least 20 years earlier, there was a strong set of talks on molecular assays for identifying and enumerating cells in monitoring programs. **Linda Medlin** updated participants on the status of phylochips now being used for multiple HAB species in five countries. Other highlights in this topic area were descriptions of the "Lab on a Chip", electrochemical detector (**Jahir Holguin**), and a fully automated, ship-based algal biosensor using electrochemical detection and phylochips (**Pim Sprong**). During a discussion at the end of the molecular detection session, it was clear that despite extensive development and testing by the research community and positive outcomes of that work, broad acceptance of molecular assays by monitoring and regulatory communities has not yet been achieved.

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New HAB Technologies

A noteworthy feature of the ICHA conference in Florianopolis was the clear advancement in the use of *in situ* biosensors and autonomous vehicles for HAB studies. **Lisa Campbell** gave a plenary presentation about the use of the Imaging FlowCytobot (IFCB), a submersible microscope capable of imaging thousands of cells every hour. She described how the IFCB is being used in Texas for early warning of HABs, resolution of phytoplankton community structure, and for long-term time series. Where the IFCB deployments in Texas were on dock or mobile (ship) platforms, **Mike Brosnahan** described an uncabled IFCB deployment with solar power, automated winch, and Wi-Fi for real-time profiling of the instrument and other contextual sensors. This yielded unprecedented insights of life cycle transitions underlying *Alexandrium* blooms. That talk also briefly described the co-deployment of an IFCB and ESP (Environmental Sample Processor), in which the high-frequency observations of the IFCB were used to guide the timing of the more limited sampling capabilities of the ESP – in that instance, used to measure toxin concentrations directly in the water.

Other instrument highlights included a plenary talk by **Raphe Kudela** on the use of multiple technologies for HAB studies (wave-powered profilers, AUVs, ESPs, IFCBs, and satellite remote sensing). Yet another talk in this context was by **Beatrix Siemering** on the use of a satellite-guided glider in mapping of hydrography and chlorophyll in HAB monitoring and forecasting.

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Solar powered raft platform for uncabled IFCB deployment for Alexandrium *and* Dinophysis *studies in Salt Pond, MA (USA). The submerged IFCB (and other instruments) is raised and lowered with an automated profiling winch. (Photo: M. Brosnahan)*

Fish Killing Algae

Fish-killing algae were well covered at ICHA17 with one key note address, 10 talks, 9 posters, and a dedicated round table event. Special attention focused on the massive February-March 2016 Chilean *Pseudochattonella* cf.*verruculosa/Alexandrium catenella* bloom (**Alejandro Clement**, **Ximena Rojas**, **Leonardo Guzmán**, **Alejandra Aguilera**, **Fabiola Villanueva**, **Rodrigo Montes**, *Óscar* **Espinoza-González**, **Daniel Varela**, **Ximena Vivanco**, **Javier Paredes**) that killed 40,000 tonnes of salmon and trout. This event impacted on global markets and triggered social unrest. **Gustaaf Hallegraeff** (keynote speech) proposed that the unrelated flagellates *Chattonella*, *Heterosigma*, *Karenia*, *Cochlodinium* cause irreversible fish gill damage through the release of free fatty acids (EPA, DHA, OPA) which in synergism with reactive oxygen species generate labile lipid peroxidation products. Microelectrode ion flux estimation (MIFE) techniques are pursuing a fuller understanding of this process (**Jorge Mardones**). The role of HAB cell lysis (*Alexandrium catenella*), ROS (*Chattonella*), and strain variation in ichthyotoxicity was emphasized. Aeration increased toxicity by *Prymnesium* (**Edna Granéli**). Critically, none of these "ichthyotoxins" (with exception perhaps of brevetoxins produced by *Karenia brevis* and relatives) are of human health significance. This means that recently killed fish are still fit for human consumption. We therefore need more investment in fish farm harvest techniques that allow fish to be kept alive while emergency harvesting operations can be instigated. In addition to airlift upwelling to dilute fish killing algal concentrations, the application of fine-ground clays which at environmentally acceptable concentrations can mop up ichthyotoxins offers considerable promise (**Andreas Seger**). Novel chemical structures were reported for metabolites from *Prymnesium* (B and C type prymnesins: **Per Juel Hansen**, **Aaron Andersen**, **Silas Rasmussen**) and *Karlodinium armiger* (karlotoxin congener termed karmitoxin: **Thomas Larsen**, **Sofie Binzer**). Karlotoxins disrupt lipid membranes via pore formation (**Allan Place**). Nighttime respiration by high biomass HAB events was demonstrated to drive anoxia in South Africa (**Grant Pitcher**).

Gustaaf Hallegraeff, University of Tasmania, Australia, Hallegraeff@utas.edu.au

Surveillance, management and mitigation

Two sessions considered the development of approaches for the role of surveillance, management and mitigation of HABs and their impacts.

In terms of surveillance many of the presented papers were related to the better understanding of how anthropogenic and/or climate drivers impacted HAB populations, with **Rajan Anbian** and **Kedong Yin** providing examples from Abu Dhabi and Hong Kong waters respectively.

The role of physical oceanography was highlighted by **Robin Raine**. In the culmination of many years of work on *Dinophysis* in Irish waters, he was able to demonstrate the role of a tidal front between the tidally mixed Irish Sea and the thermally stratified Celtic sea in developing populations of *Dinophysis acuta* that are subsequently transported to locations of aquaculture production.

The management of HABs is increasingly likely to require a diversity of approaches. An example of this was illustrated in the presentation of **Mercy Borbor-Cordova** who described a collaborative partnership between interdisciplinary researchers, public health staff, and key local stakeholders, to achieve HAB risk assessment and management in relation to climate-ocean drivers in Ecuador.

Alexis Fisher (best ISSHA student presentation) presented a novel paper that demonstrated the potential role of winter chilling in governing *Alexandrium* excystment and hence offering new opportunities to predict the time and location of bloom initiation.

A number of the papers considered the issue of mitigation of toxins present in the water column. Some techniques are well known to the HAB community such as using clay, but the technique continues to be optimised with **Andreas Seger** demonstrating refinement of the technique with different materials and low clay concentrations that reduces both cost and environmental impact.

Other novel approaches reported were the use of compounds secreted by algicidal bacteria (**Eva Ternon**) to control dinoflagellates

Keith Davidson, Scottish Association for Marine Science (SAMS), Oban, UK, kda@sams.ac.uk

Toxins and toxicity

The monograph by Lassus et al., 2016, presented at the conference, made it clear that the largest uncertainties in the toxicity of algal metabolites reside in the ichtyotoxicity of many algae. This was also a theme at this conference. Significant progress was reported on metabolites from *Prymnesium parvum*; the chemical diversity in several strains from US and Europe was clarified with novel Prymnesin classes identified and chemically characterised in European



Co-deployment of IFCB (left) and ESP (right) in Salt Pond (USA) during 2016 Alexandrium *bloom. (Photo: B. Keafer)*

strains (**Kristian Nielsen**, **Silas Rasmussen**, **Per Juel Hansen**).

Investigation of recently isolated strains of the genera *Azadinium* and Amphidoma led to the identification of more than ten novel analogues of Azaspiracids (**Bernd Krock**, **Urban Tillmann**).

STX analogues in *G. catenatum* were reviewed and several reports on allelopathic compounds of *G. catenatum*, *Alexandrium* spp. and several other species were presented (**Mike Quilliam**, **Elise van Meerssche**, **Hélène Hégaret**, **Chris Gobler**, **Stefanie Moorthi**, **Eva Ternon**, **Stefano Accaroni**, **Rui Wang**, **Mathias Chia**, **Kemal Ger**, **Lincoln MacKenzie**, **Renan Arruda**, **Anneke Purz**).

The combination of passive sampling and High Resolution Mass Spectrometry for algal and environmental metabolomics was presented as a break-through technology to investigate several aspects of toxin chemistry, including pinpointing towards novel toxins, chemogeography of toxins and spatio-temporal changes in the chemical profiles of coastal waters (**Phil Hess**).

Some progress was also reported in the evaluation of the toxicity of *Gambierdiscus* species, especially Atlantic strains (**Francesco Pisapia**).

Philipp Hess, IFREMER, Nantes, France, philipp.hess@ifremer.fr

Effects of HABs in shellfish and other higher trophic level organisms

Despite not causing an obvious harmful effect characteristic of the toxic syndrome, toxic harmful events can modify the susceptibility of bivalves to pathogens (**Celina Abi-Khalil**)

The HAB impacts observed on bivalves are not always due to the known toxins causing shellfish poisonings, such as PSP and DSP toxins, but can be associated to other un-characterized bioactive compounds produced by the toxin-producing species (**Elodie Borcier**; **Leila Basti**)

Intravenous Lipid Injection (ILE) increases the clearance of brevetoxins (PbTx) in turtle tissues and ameliorates symptoms of specimens exposed to toxic blooms of *Karenia brevis* (**Courtney Cocilova**)

Hélène Hégaret, LEMAR, Institut Universitaire Européen de la Mer, France, Helene.Hegaret@univ-brest.fr

Homage to Edna Granéli

Edna Granéli, Professor Emeritus at the Linnaeus University, Kalmar, Sweden, and Guest Professor at the University of Lund, Sweden, has devoted her life to the ecology and ecophysiology of harmful microalgae and their blooms. First in Lund and later in Kalmar, she had PhD-students and visiting post-docs from different countries. Nevertheless, Edna never forgot her Brazilian origins, and she was a key person in the promotion of scientific exchanges and international cooperation with her fellow countrymen. For these reasons, Brazilian HAB experts wanted to thank Edna for her strong support to PhD students, some of them now with good academic positions in Brazil or elsewhere. Homage to Edna was done in a very Brazilian way: a barbecue and good samba music and dancing open to all participants in the 17 ICHA.





Edna Granéli with Luis Proença (top, photo M Schramm) and in the barbecue party with Proença and Paulo Salomon (bottom, photo P Salomon)

DEADLINE submission of manuscripts for the 17 ICHA Proceedings

The proceedings of the 17 ICHA conference will be published under the auspices of the International Society for the Study of Harmful Algae (ISSHA). The complete proceedings will be published in digital format on the ISSHA web site, no later than 12 months after submission deadline, but reviewed, revised and accepted submissions will be uploaded prior to that as they are completed. Past conference proceedings can be accessed at www.issha.org

Only manuscripts relating to conference presentations will be considered.

Deadline for manuscript submission is 1st March 2017

The proceedings template and guidelines can be found at: *http://www.issha.org/News/ICHA17-Proceedings*















From left to right and from top to bottom: 1-3, registration and icebreaker; 4-5, poster sessions; 6-7, socializing at the first day reception, including a "Macarena" dance. All photos by M Schramm, except 4 (Yolanda Pazos) and 6 (Don Anderson)

Bloom of *Vulcanodinium rugosum* linked to skin lesions in Cienfuegos Bay, Cuba



Fig. 1. Map of Cienfuegos Bay, Cuba and location of sampling stations. The arrow indicates the area where V. rugosum *bloomed in summer 2015.*

Vulcanodinium. rugosum is a pinnatoxin-producing benthic dinoflagellate recently described from the Mediterranean [1, 2]. Pinnatoxins are included in the group of lipophilic "fast-acting toxins" (FAT) due to the strong toxicity observed in mouse bioassay via intraperitonal injection [3]. Extracts of *V. rugosum* showed cytotoxicity and genotoxicity activities against cell lines derived from the nervous system and intestine [4]. To date, there is no evidence for human intoxication by shellfish contaminated with pinnatoxins [5]. The presence of *V. rugosum* has also been documented from Australia, China, Japan, Hawaii, Mexican Pacific and New Zealand [6].

During summer 2015, between 23 and 26 July, around 60 cases (most of them children) of skin lesions were recorded from some popular beaches in Cienfuegos Bay, a semi-enclosed embayment with estuarine characteristics from central-southern Cuba (Fig. 1)[7]. The affected people showed symptoms quite different from those caused by coelenterates which are very frequent in summer and known in Cuba as "Caribe". The skin lesions eventually got infected and purulent and had to be treated with antibiotics. Analyses were carried out at the Cienfuegos Provincial Health, Epidemiology and Microbiology Centre and at the Centre for Environmental Studies (CEAC) and the possibility of bacterial infection or chemical pollution behind the skin lesions was discarded. Health authorities swiftly enforced beaches closures to the public. This caused social alarm and affected local tourism [7].

Microbiological analyses (for viruses and bacteria) carried out by the Epidemiological Center from Cienfuegos Province (CPHE) gave negative results. Subsurface water samples for phytoplankton analysis were collected from the beaches and fixed with Lugol's iodine. Live specimens were observed under a light microscope Laborlux, Leica-Leitz with phase contrast, and micrographs taken. For scanning electron microscopy, the Lugol's-fixed material was rinsed with distilled water, dehydrated in an ethanol series, critical-point dried, and finally coated with gold.

Reddish-brown long discontinuous patches, mainly 3-6 m nearshore, were observed at the beaches affected by the dermatitis outbreak. The extent of this intense water discoloration was about 1 km. Patches were located near the bottom during the morning and moved to the surface during mid- day, a behavioural pattern common to benthic species (Fig. 2). The bloom persisted for around two months, until 20 September 2015.

Microscopic observations revealed the presence of a *Vulcanodinium rugosum* bloom. Some typical features of this peridinoid dinoflagellate, such as the distinctive apical pore complex on the motile cells, through which mucilaginous material is extruded, were corroborated. All plates were ornamented with numerous circular tricho-cyst pores and prominent longitudinal ridges, the presence of a narrow and invaginated 1' plate and the production of abundant spherical cyst-like cells encased in a gelatinous matrix. (Figs. 3-4). Ongoing studies will be performed on their toxicology and genetic characterization.

V. rugosum formed an almost monospecific bloom during the first 20 days, when other dinoflagellates and diatoms, including several bloom-forming species, such as *Dinophysis caudata*, *Peridinium quinquecorne*, *Prorocentrum micans*, *Tripos furca* and *T. trichoceros*, co-occurred in the samples in very low concentrations.

For the Western Atlantic, *V. rugosum* has only been reported in ship's ballast water samples from Tampa, USA [8]. To our knowledge, the occurrence of *V. rugosum* in a natural environment from Cienfuegos Bay, Cuba, represents the first report of this species for the Caribbean region and for the Western Atlantic.

Environmental conditions during late July 2015 in Cienfuegos Bay – high salinity (34-36), water temperature above 30°C and strong irradiance – were favourable for dinoflagellate blooms. In addition, the semi-enclosed

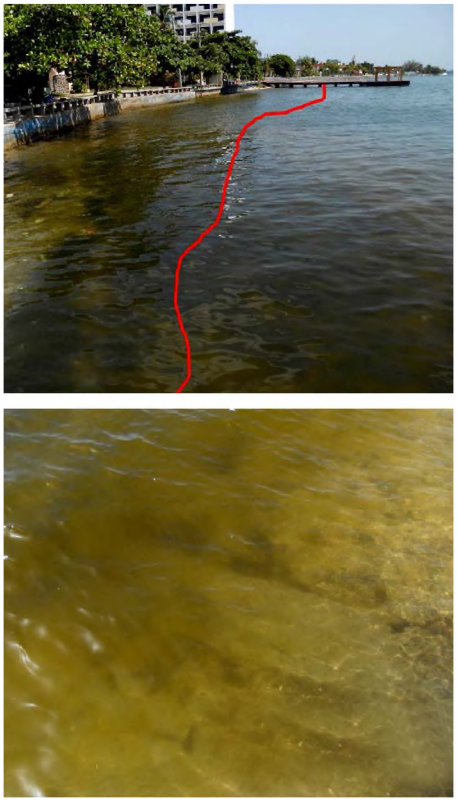

Fig. 2. Intense water discoloration due to the bloom of V. rugosum *from the beaches of Cienfuegos Bay, Cuba. The red line indicates the length of the patch.*

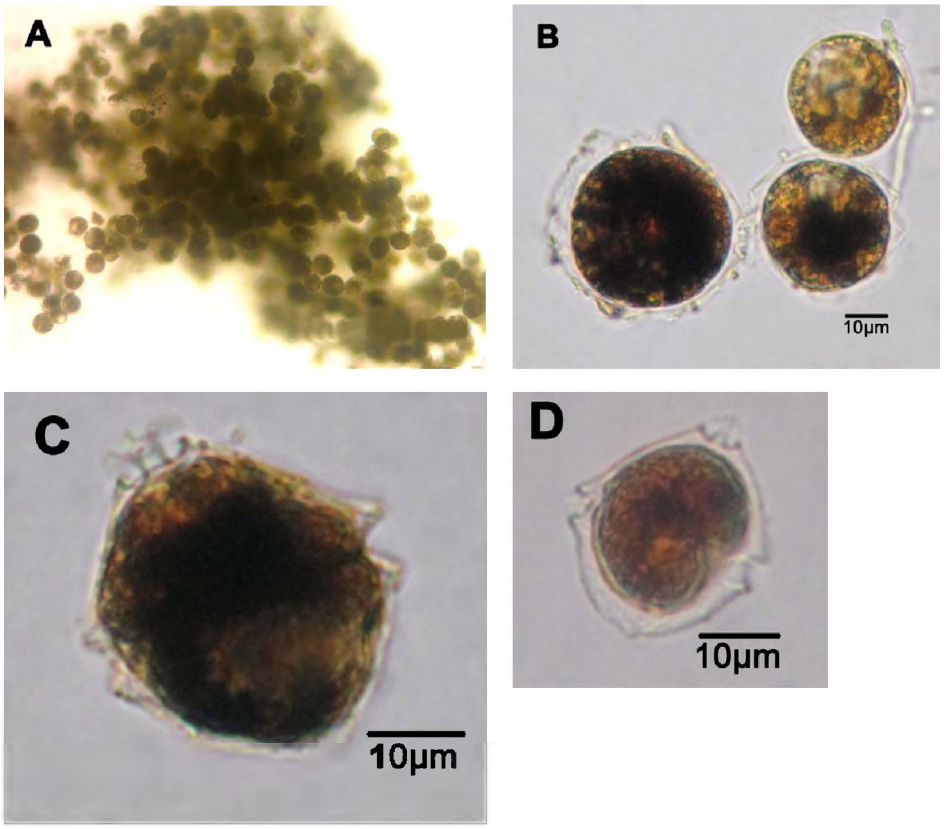

Fig. 3. Light microscopy micrographs of V. rugosum *from Cienfuegos Bay, Cuba. A. Nonmotile spherical cells encased in a gelatinous matrix. B. Nonmotile spherical cells. C-D. Motile cells showing the distinct apical pore complex of the species.*

nature of Cienfuegos Bay promotes the development and retention of blooms. The occurrence of *V. rugosum* coincided with the summer-rainy season. This season is characterized by decreasing salinity values and increased water turbulence, leading to resuspension of a high abundance of estuarine diatoms and chlorophyceans in the bay [9]. Nevertheless, 2015 was extremely dry and the hottest of the last 64 years in Cuba [10]. These extreme weather conditions (high salinity, temperature, irradiance and water residence times) appeared to be optimal for bloom-forming dinoflagellates.

Predictions indicate that the Cuban climate will be affected by warming and drought periods, which seems appropriate for the development of harmful/toxic blooms. This relation between weather conditions and HABs is valuable information to improve national capabilities to predict HABs in Cuban embayments. Recent harmful algal blooms, including fish-killing blooms of *Cochlodinium polykrikoides*, have been recorded in Cienfuegos and Santiago de Cuba bays, always during hot and dry periods. These blooms caused significant damage to the marine fauna. [11].

Acknowledgements

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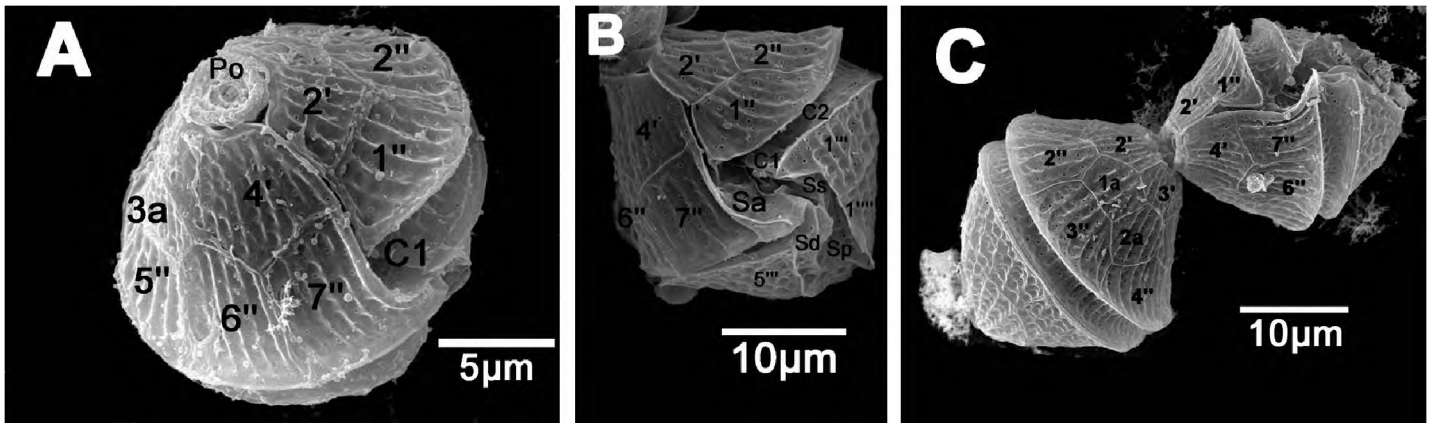

Fig. 4. SEM micrographs of V. rugosum *from Cienfuegos Bay, Cuba. A. Epitheca of a motile cell in ventral view showing the large pore plate (Po). B. Ventral view of a motile cell showing the narrow 1' plate and the girdle displacement. C. Two motile cells linked together by the mucus exudated through the apical pore complex.*

Ability of giant clams to bio-accumulate ciguatoxins from *Gambierdiscus* cells

Ciguatera Fish Poisoning (CFP) is a seafood poisoning classically related to the consumption of tropical coral reef fish contaminated with ciguatoxins (CTXs). These polyether neurotoxins are produced by dinoflagellates in the genus *Gambierdiscus* [1]. Pacific Island Countries and Territories (PICTs) communities, who are dependent on seafood for their subsistence but also for fishery and tourism industries, are among the populations most affected by CFP worldwide [2]. In these countries, marine invertebrates such as giant clams also constitute highly prized food resources. Unfortunately, in several of these PICTs such as French Polynesia, New-Caledonia, Cook Islands and Republic of Vanuatu, atypical ciguatera-like poisonings incidents following the consumption of giant clams *Tridacna maxima* (Fig. 1) have been reported in recent years [3,4]. As an example, in French Polynesia, official reports of these atypical forms of poisonings following the consumption of various marine invertebrates including *Tridacna max*ima (giant clam) [4], *Tripneustes gratilla* (sea urchin) [5], and *Tectus niloticus* (gastropod) [6] exist, although they represent less than 10 cases/year *versus* an average of 300 fish poisoning cases officially reported annually (*www.ciguatera-online.com*). These statistics about poisonings triggered by consumption of marine invertebrates may however be largely underestimated, as marine invertebrate meals are often omitted in clinical reports whereas fish meals are incriminated [5]. During these ciguatera-like intoxications, classical symptoms of CFP are observed (gastrointestinal disorders, reversal of hot and cold sensations, itching, paresthesia, asthenia, muscular pain, dizziness), in addition to atypical symptoms (alteration of the taste, burning sensation on the tongue and the throat, paralysis) [4], suggesting the involvement of several toxin families, including CTX-like toxins.

Giant clams are bivalve molluscs capable of filtering seawater and retaining particles that are in suspension in the water column such as microalgal cells. *Gambierdiscus* cells have a tychopelagic life style, meaning that this benthic dinoflagellate can temporarily become free-swimming in the water column, especially in high-energy environments. In the process, senescent *Gambierdiscus* cells can release dissolved CTXs in the surrounding water. It is thus likely that giant clams living in areas contaminated with toxic *Gambierdiscus* blooms can potentially accumulate CTXs in their tissues, either by direct ingestion of toxic *Gambierdiscus* cells dispersed in the water column, or by filtration of seawater containing dissolved CTXs. To test this hypothesis and to assess the ability of giant clams to bioaccumulate CTXs upon an episodic exposure to high densities of *Gambierdiscus* cells, *ex situ* contamination experiments of giant clams with *in vitro* cultures of *Gambierdiscus* strains were conducted (Fig. 2) [7].



*Fig. 1. Giant clam (*Tridacna maxima*). © M. Roué.*

Giant clams were exposed for 48 h to live cells or lyzed cells homogenates of *Gambierdiscus*, to mimic natural exposure to free-swimming *Gambierdiscus* cells and dissolved CTXs, respectively. Two distinct strains were used: (i) *G. polynesiensis* TB92, a highly toxic strain containing approximately 5.83 ± 0.85 pg P-CTX-3C equiv. $cell^{-1}$; and (ii) *G. toxicus* HIT0, a strain 2,850-fold less toxic than TB92, containing approximately 2.05 ± 1.16 x 10^{-3} pg P-CTX-3C equiv. $cell^{-1}$. Exposure experiments were conducted at an overall concentration of 150 cells mL^{-1}, equivalent to 0.86 µg P-CTX-3C equiv. L^{-1} and 0.31 x 10^{-3} µg P-CTX-3C equiv. L^{-1} in the case of TB92 and HIT0 strains respectively. The presence of CTXs congeners in giant clams tissues was further assessed using the mouse neuroblastoma cell-based assay (CBA-N2a). Results showed that giant clams exposed to either live cells or lyzed cells homogenates of TB92 were able to bio-accumulate similar levels of toxins, *i.e.* 2.92 ± 1.03 and 3.28 ± 1.37 ng P-CTX-3C equiv. g^{-1} flesh (wet weight) respectively (Table 1). These concentrations are well above the safety limit of 0.01 ppb P-CTX-1B (or 0.02 ppb P-CTX-3C) recommended for human consumption in the Pacific region [8]. In contrast, control animals (no exposition to *Gambierdiscus* cells) and giant clams exposed to cells of the weakly toxic strain HIT0 were found to be free of toxins (Table 1), suggesting that the nature, the risk of contamination of these bivalves is established only in the presence of highly toxic blooms of *Gambierdiscus*. Liquid chromatography mass spectrometry (LC-MS/MS) analyses confirmed CBA-N2a results and revealed that P-CTX-3B was the major CTX congener retained in the tissues of giant clams fed with TB92 cells.

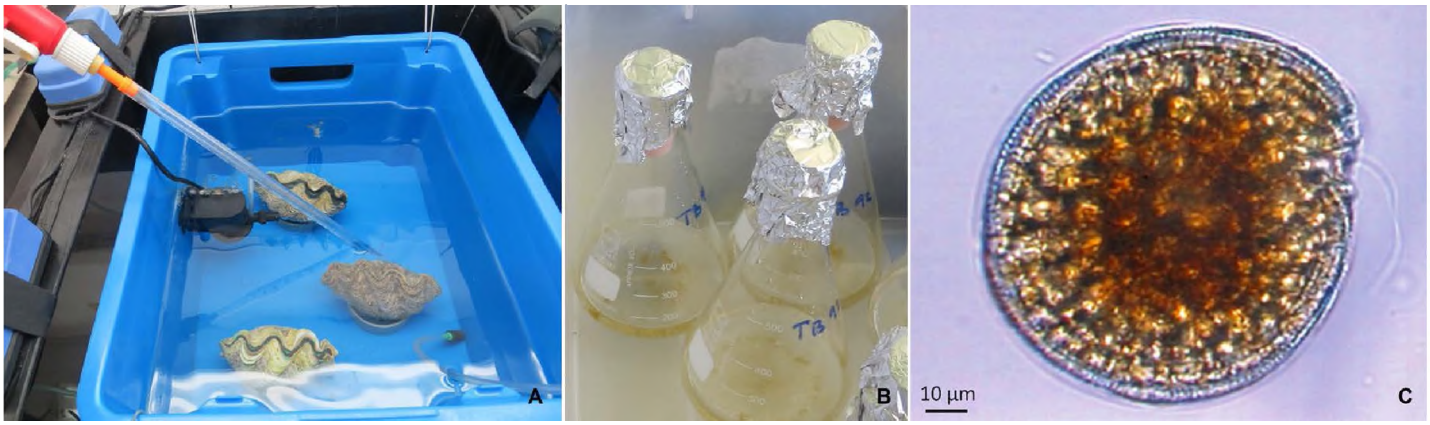

Fig. 2. Ex situ *contamination experiment: giant clams were first acclimated in experimental tanks containing 20 L of seawater (A) and further exposed to in vitrocultures of* Gambierdiscus *spp. (B-C). © M. Roué (A, B); © ILM (C).*

The results of this study [7] provide evidence of the bioaccumulation of CTXs from *Gambierdiscus* cells in giant clams and thus confirm that these molluscs, which are part of the diet of many populations in PICTs, can actually constitute another pathway in ciguatera poisonings in areas where toxic *Gambierdiscus* populations are endemic.

These findings highlight the need for an improved global strategy on ciguatera risk assessment and management programs currently on-going in PICTs. These programs, so far limited to the survey of lagoon fish, should also take into account all major seafood resources consumed within island communities, including those, such as giant clams, commonly regarded as being at low risk of ciguatera.

Acknowledgments

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Table 1. Estimation of toxin contents in giant clams exposed to live cells or lyzed cells homogenates of Gambierdiscus, *using CBA-N2a. ND: not detectable.*

Condition of exposure	Strain	Giant clams per tank	Giant clams total wet weight (g)	Toxin content ± SD (ng P-CTX-3C equiv g^{-1} wet weight of flesh)
Lyzed cells	control	3	213	ND
	HIT0	3	180	ND
	TB92, tank 1	3	176	3.74. ± 0.26
	TB92, tank 2	3	159	4.96 ± 0.58
	TB92, tank 3	3	137	2.02 ± 0.10
	TB92, average of tanks 1-3	**9**	**157**	**3.58 ± 1.32**
Live cells	control	2	95	ND
	HIT0	3	172	ND
	TB92, tank 1	3	170	3.94 ± 1.21
	TB92, tank 2	3	224	2.48 ± 0.29
	TB92, tank 3	3	197	2.34 ± 0.54
	TB92, average of tanks 1-3	**9**	**197**	**2.92 ± 1.03**

Bloom of *Gambierdiscus caribaeus* in the temperate-subtropical waters of El Hierro, Canary Islands (North East Atlantic)

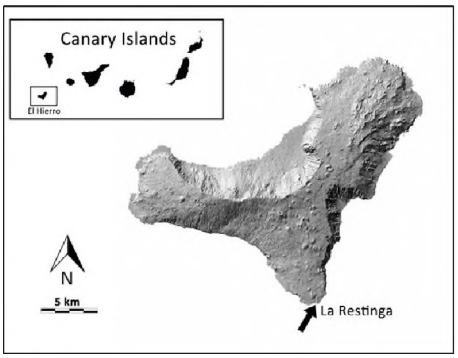

Fig. 1. Shaded relief image of El Hierro (GRAFCAN), Canary Islands. Location of the study area in La Restinga.

The Canary Archipelago (Fig. 1) is located in the southern limit of the temperate Northern Atlantic (28°06´N, 15°24´W) and together with the archipelagos of Azores, Madeira, Selvagens and Cape Verde, is part of a unique biogeographical region known as Macaronesia. El Hierro is the southernmost, smallest and most isolated island of the Canary archipelago and has been declared a UNESCO Biosphere Reserve.

The first record of a harmful algal bloom in the Canary Archipelago occurred quite recently, in August 2004, when a bloom of the planktonic cyanobacteria *Trichodesmium erythraeum* Ehrenberg ex Gomont developed along the coasts of Lanzarote, Fuerteventura, Gran Canaria and Tenerife [1]. In January, this same year, an outbreak of Ciguatera Fish Poisoning (CFP) was documented for the first time in the Canary Islands, after the ingestion of a locally captured amberjack (*Seriola rivoliana* Valenciennes 1833) [2]. This event was quite unexpected since, by then, the Canary Islands were considered outside the recognised endemic area for CFP and the causative species (*Gambierdiscus* spp.) had not been reported for the region.

The presence of *Gambierdiscus* in the archipelago was first reported in November 2004, at the XI International Conference on Harmful Algae (Cape Town, South Africa), from samples collected in March of the same year on the northern coast of Tenerife [3,4]. Since then, five toxic species of *Gambierdiscus* have been reported for the Canary Islands, including two newly erected species for science, *G. excentricus* S. Fraga and *G. silvae* S. Fraga & F. Rodríguez [4-6]. Until now, most work on *Gambierdiscus* has resulted from opportunistic samples and short sampling expeditions, from which cells have been isolated and cultured under laboratory conditions for further taxonomical and toxicological studies. High densities have never been reported.

In the third week of October 2016, the international subaquatic photography contest Open FotoSub took place in La Restinga – Mar de las Calmas, El Hierro, an ecosystem with a high biodiversity, declared as a protected marine reserve. When diving, some of the participants were intrigued by the presence of a weird-looking brownish matt covering the sea bottom (Fig. 2). To investigate these observations, samples of the macroalgae *Padina pavonica* (Linnaeus) Thivy, *Dictyota* spp. and *Halopteris scoparia* (Linnaeus) Sauvageau were collected, by snorkelling (ca. 2m depth), using plastic bags, from 5 sampling sites in the harbour of La Restinga (27º 37,5′ N 17º 59′ 5″ W). Samples were vigorously shaken and the water suspension was collected in stoppered tubes for further analysis in the laboratory. At the time of sampling water temperature was 25°C and salinity 36.8.

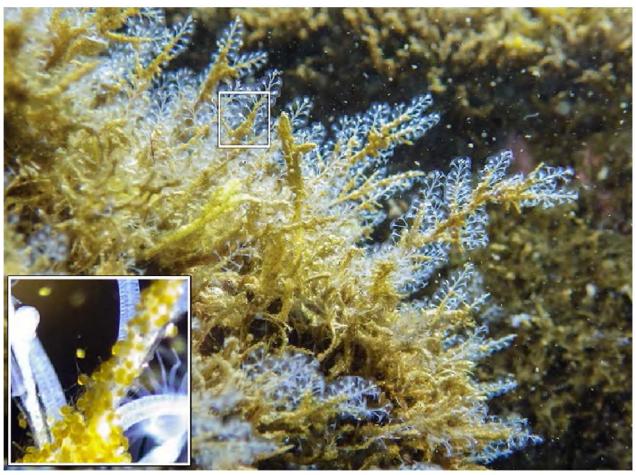

Fig. 2. Gambierdiscus *mats smothering a cnidarian* Halecium *sp. colony (courtesy L. Moro). Inset: detail showing cells of* Gambierdiscus *spp.*

The epiphytic assemblages were investigated by light microscopy (Leica DM6000B) and Scanning Electron Microscopy (SEM) (JEOL JSM-6380LV). For plate tabulation description of *Gambierdiscus* we followed the nomenclature according to [3]. Cells were measured from SEM images using SEM Control User Interface v.7.11A by JEOL. Only cells that were clearly in apical and antapical view, were measured.

Sample observation revealed *Gambierdiscus* spp. was the only dinoflagellate present in the recovered epiphytic community (Fig. 3). A diverse diatom flora co-occurred with *Gambierdiscus* spp., namely species of the genera *Actinocyclus*, *Achnanthes*, *Amphora*, *Nitzschia* and *Pleurosigma* (Fig. 3). In one sample of *H. scoparia* the concentration of *Gambierdiscus* spp. was estimated, reaching more than 10^4 cells g^{-1} wet weight. This value is close to some of the maximal concentrations referred for other species of *Gambierdiscus* in CFP affected areas [7].

All cells of *Gambierdiscus* analysed were antero-posteriorly compressed and round to slightly oval shaped in apical view. The thecal surface was smooth with evenly distributed round pores. Observation by calcofluor staining and SEM revealed an almost centrally located Po (slight ventral displacement) with a fish-hook like slit, surrounded by a row of pores, in some areas with 2 rows (Fig. 4). The chloroplasts were golden-brown. The dorso-ventral depth (DV) range was 71.4-82.8µm (n=7) and the width (W) range 62.2-82.9µm, with a mean DV/W ratio of 1.1±0.11. In the episome (Fig. 4), plate 2' was the biggest apical plate and varied from rectangular to hatched-shaped with the ratio between the suture length of 2'/1" and 2'/3" ranging between 0.63 and 1.0 (mean=0.84±0.14, n=9). Plate 3' was pentagonal and small relative to 2'. Plate 4' and plate 3" were symmetrical. Plate 1' was very narrow and small, not visible in apical view but clearly visible in ventral view (Fig. 5). Specimens observed



Fig. 3. SEM micrograph of epiphytic community (a. G. caribaeus*; b.* Pleurosigma *sp.;* Actinocyclus *sp.).*



Fig. 4. Gambierdiscus caribaeus*, apical view with plate tabulation (SEM).*

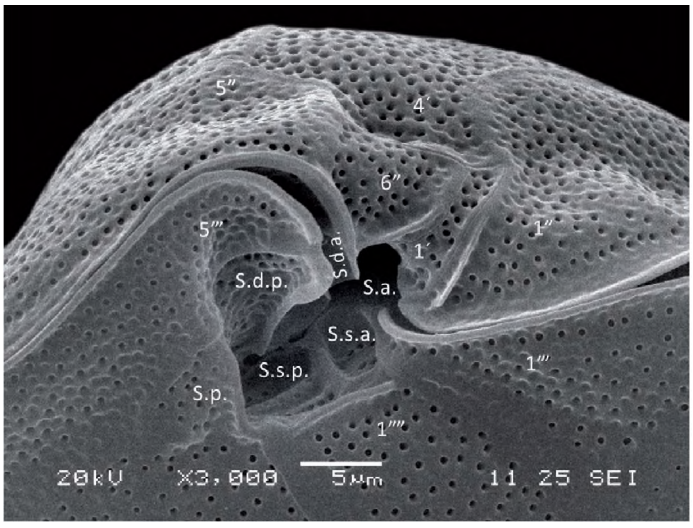

Fig. 5. Gambierdiscus caribaeus*, ventral view, with identification of sulcal plates (SEM).*

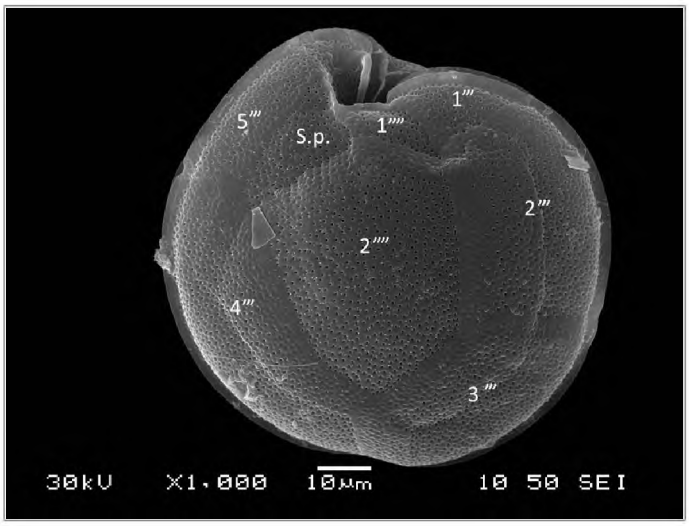

Fig. 6. Gambierdiscus caribaeus*, antapical view showing the broad 2⁗ plate (SEM).*

in antapical view were characterized by a broad 2⁗ plate, almost symmetrical, with a pointed dorsal end (Fig. 6). Length of plate 2⁗ ranged between 45.3 and 51.8µm (mean=48.9±2.62, n=5). Plate 1⁗ contacted plates 1‴, 2‴ and 2⁗. All specimens observed in antapical view showed the same tabulation. The hyposome tabulation, combined with surface characteristics and cell morphometry, is in good agreement with the original description of *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester [7], a species recently reported from the Canary Islands [6]. Although in some of the studied specimens the diagnostic criteria of the episome showed some variability, several specimens agreed well with the original description of *G. caribaeus* [8] (Figs 4-6). This, combined with the unambiguous tabulation of the hyposome, suggests *G. caribaeus* was the dominant species in the El Hierro bloom. The observed variability on the episome may either correspond to natural intra-specific variability or indicate that cryptic species of *Gambierdiscus* were present. Ongoing genetic characterization of the original bloom samples and of clonal isolates will allow us to clarify the mono- *versus* pluri-specific nature of the El Hierro bloom.

The high concentrations of *Gambierdiscus* spp., reported here from La Restinga, suggest that the Ciguatera outbreaks reported for the Canary Archipelago and other Macaronesia islands, in the last two decades, may result from locally toxified fish and not from long-distance migration of fish from other affected areas.

The frequency of HABs in the Canary Islands has been increasing since the first reported event in 2004. BEA (Banco Español de Algas), in cooperation with the regional environmental authorities (Viceconsejería de Medio Ambiente del Gobierno de Canarias), has been monitoring and studying these events since then. Blooms are quite diverse and include the planktonic species *Trichodesmium erythraeum* (August 2004 and 2011) and *Noctiluca scintillans* (Macartney) Kofoid & Swezy (March 2012 and May 2016) [9] and the benthic toxic species *Lyngbya majuscula* Harvey ex Gomont (2010-present) [10] which has a dramatic impact in regional seagrass meadows of *Cymodocea nodosa* (Ucria) Ascherson. Human mediated global changes, such as ocean warming, may be driving the regional increase in HABs. The herein reported bloom of the

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Sand-lance, a key PSP-toxins vector in the Aleutian Pribilof Islands

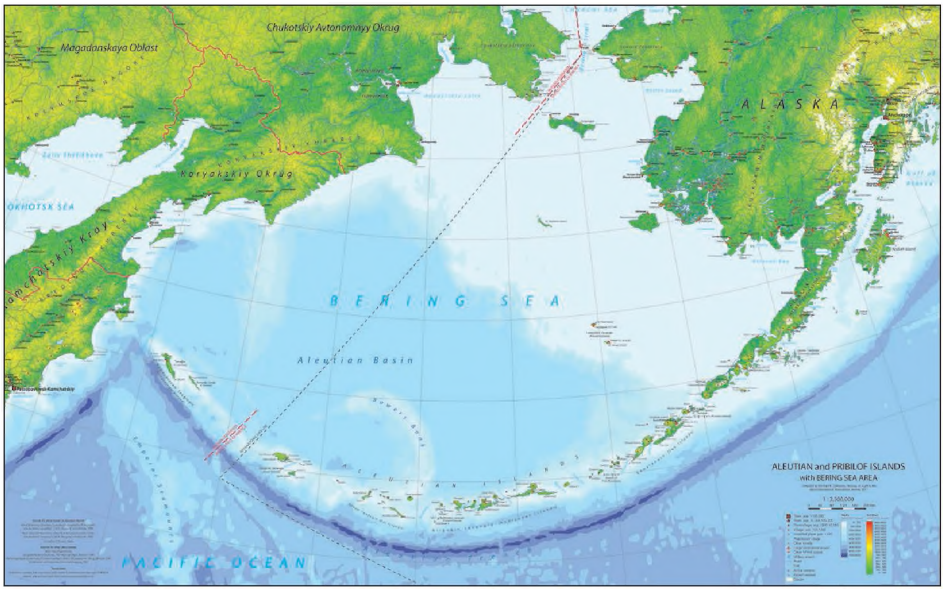

Fig. 1: Map of the Aleutian Pribilof Islands in the Bering Sea, off Alaska

The Aleutian Pribilof Islands Association, Inc. (APIA) is a tribal consortium serving Alaska's thirteen Aleut Tribes. APIA's purpose is: to promote the overall economic, social, and cultural development of the Aleutian and Pribilof Islands Region; to help Tribes to protect public health and the environment; and to encourage sustainable management of the natural resources upon which the Aleut people depend. Environmental programs are located within APIA's Health Department. The specific mission of this department is achieving safe, healthy and sustainable communities.

The APIA harmful algal bloom program goals and objectives meet evolving needs by the Tribes to understand the risks of illness and death and the associated shifts in the marine ecosystems from harmful algal blooms.

Scientists from the Aleutian Pribilof Islands Association (APIA), off Alaska, USA (Fig. 1), have been working with governmental institutions and university researchers since 2006 to study paralytic shellfish poisoning (PSP) events in the region. Their research may help to determine the causes of near extinction of some of Gulf of Alaska top predators, such as sea otters and sea lions, and the decline of other predator populations. Studies of food, predation and disease have not satisfactorily resolved the reasons for these declining populations and one hypothesis under investigation is that wide-spread PSP toxins (PST), transferred through the food web, may be partially responsible.

Every year coastal Alaska suffers from blooms of the PST producer *Alexandrium* spp. (Fig. 2a). PSP toxins can sicken wildlife and people and both can die if PST concentrations are high enough. The most common reason people get sick is from eating PST-contaminated shellfish (mussels, clams and scallops) which filter the toxic *Alexandrium* sp. from the water column. Zooplankton, including copepods, may also eat *Alexandrium* cells and concentrate their toxins.

Sand lance, also known as "sand eels" or "needle fish", gets its name from its slender body and pointed snout (Fig.2b). Sand lance primarily feeds on copepods and may become toxic with PST transferred by copepods. Many of the marine predators in the North Pacific Ocean depend upon sand lance as an important food source. This fish is a highly caloric food due to its high content in lipids (fats), and contain many important nutrients. Some of the predators that feed on sand lance are whales, sea lions, seals, sea otters, marine birds, and fish including salmon and halibut. PST affect the vertebrates central nervous system; when sand lance become contaminated with PST they may lose their ability to swim and may not be able to avoid predators. In Alaska's Taiyasanka Harbor, Dungeness crab became so contaminated from eating dead PST-contaminated sand lance and mussels that they were toxic enough to sicken people when consumed. Sea birds have also been reported to have died on Kodiak Island and in the Aleu-

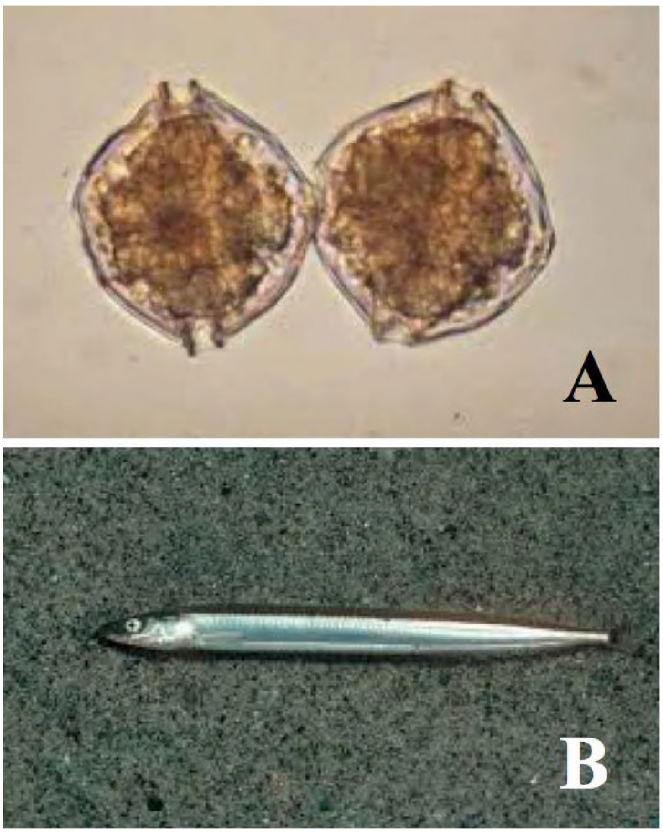

Fig. 2. Top: Alexandrium *paired cell; bottom: a sand lance.*

tian Islands from eating contaminated sand lance.

Yukon king salmon also eat PST-contaminated sand lance when reared in coastal waters. Dead seabirds washed up on the beach may have been killed by eating contaminated sand lance. The way to find it out is to check the bird's crop, to see if the bird ate sand lance, and test the toxicity in the fish.

WARNING: We need your help! Contact APIA senior scientist if you find sick acting or dead sand lance on the beach or in shallow water. You can handle the sand lance without risk of getting sick, but don't eat any dead or sick sand lance. If you find dead or dying sand lance, collect 5 specimens, put them in a Ziplock bag, label it with your name, location collected and date, freeze and contact: Bruce Wright at 907-222-4260 or *brucew@apiai.org* (Fig. 3).

More information at: *http://environmentalaska.us/psp-harmful-algal-blooms*

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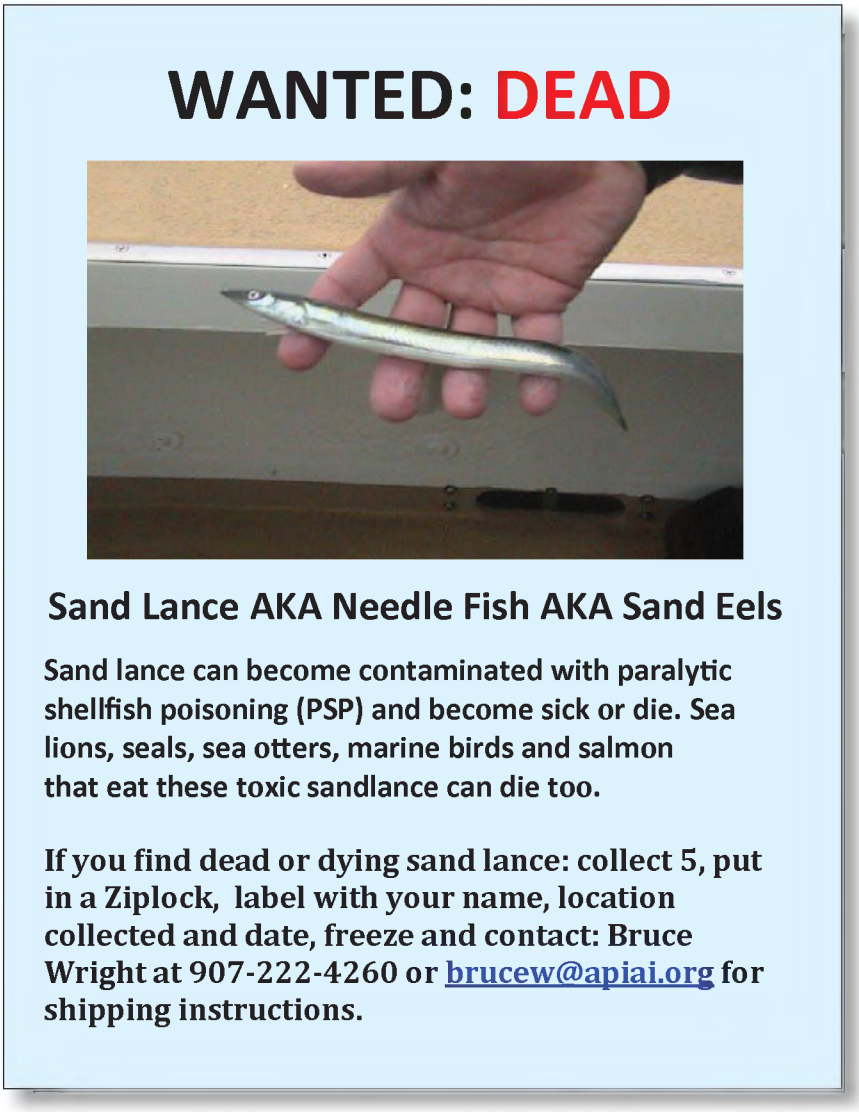

Fig. 3: Flyer asking for citizen's collaboration with the sand lance project.

Continued from page 15

tropical species *G. caribaeus* in El Hierro, may represent additional evidence for the poleward shift of environmental conditions favouring the proliferation of warm water species at higher latitudes [11].

We are grateful to Leopoldo Moro for the photo of *Halecium* sp. and to Julia Pérez de Paz from Departamento de Biología Reproductiva y Micro-Morfología, Jardín Botánico Canario "Viera y Clavijo" for access to SEM facilities. A. Amorim acknowledges Fundação para a Ciência e Tecnologia (FCT) for funding through grant SFRH/BSAB/127851/2016 and strategic project UID/MAR/04292/2013.

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Massive bloom of *Tripos furca* in Luanda Bay, Angola

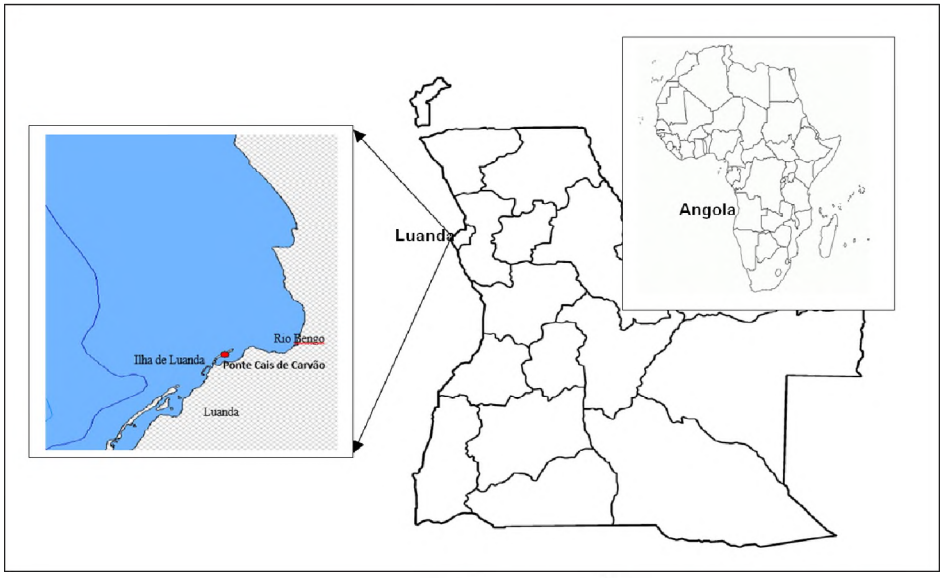

Fig. 1. Location of Ponte Cais (red dot) 8°46.74" S; 13°14.75" E, in Luanda Bay.

On August 12th, 2016, waters in the entry area of Baía de Luanda, Angola, were discoloured brown. Three days later this discolouration was also observed at Ponte Cais de Carvão (Fig 1), close to the National Fishery Research Institute (INIP). Staff from INIP, Department of Oceanography and Marine Health Ecosystem, promptly collected water samples and measured environmental parameters (temperature, salinity, dissolved oxygen) *in situ* to determine if the cause of the discolouration was due to a microalgal bloom.

Surface water samples were collected and fixed with a formalin (2%) solution. Microplankton identification and counts were conducted using a Zeiss Axiovert 200 inverted microscope using the Utermöhl method [1].

Oxygen levels (that in cold waters can reach values up to 10 mL L^{-1}) dropped from 7 to 2 mL L^{-1} during the bloom although high values were recorded at surface on the first day of bloom related to photosynthesis. Temperature (13°C) and salinity (35.5) remained constant and pH varied from 7.4 to 8.5 [2].

Sample analyses confirmed the occurrence of a massive dinoflagellate bloom. The dominant species was *Tripos furca* (Fig. 2) with a density of 6.7 x 10^5 cells L^{-1} (Fig. 3) Densities of this species >10^5 cells L^{-1} have been defined as a bloom in previous studies. Chlorophyll *a* reached values of 12.65 µg L^{-1} and dropped to 0.17 µg L^{-1} when the bloom was over.

Tripos furca has been classified as a potentially noxious microalgae species by the IOC of UNESCO. Even though is not classified as a toxin producer, the occurrence of massive blooms of this species may cause fish kills due to occlusion of the fish branchia, a situation that can also reduce the fish condition factor. This effect is more noticeable in plankton feeding *Sardinella* (Clupeidae family). Nevertheless, there was no visible fish mortality associated with this bloom in Luanda Bay. In contrast, in August-September 2010, a brownish discolouration was also observed in the water, caused by another dinoflagellate bloom where the dominant species was *Prorocentrum micans* (6 x 10^5 cells L^{-1}). In that bloom, *Tripos furca* (4.4 x 10^4 cells L^{-1}) was a co-occurring species and there was a mass mortality of fish at Luanda Bay. The cause of this mortality has not been identified.

Acknowledgements

To all the technicians involved in the investigation of this incident at the Department of Oceanography and Health of the Marine Ecosystem of the National Institute of Fisheries Research.

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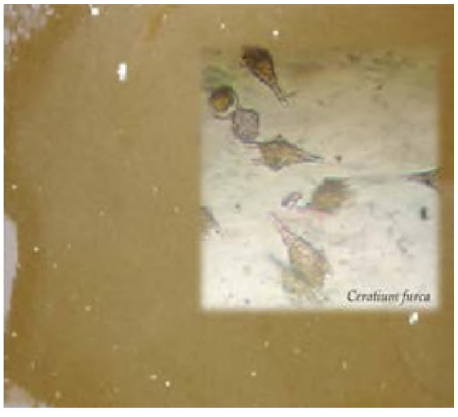

Fig. 2. Brownish water discoloration from a Tripos furca *bloom.*

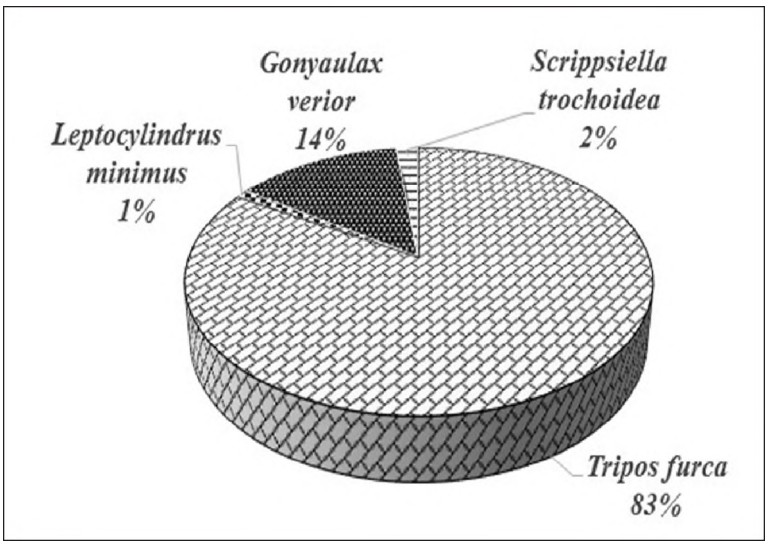

Fig. 3. Contribution of the dominant phytoplankton species during the bloom

Water discoloration, fish kills and the occurrence of a multispecific dinoflagellate bloom off Kodikkal, southwest coast of India

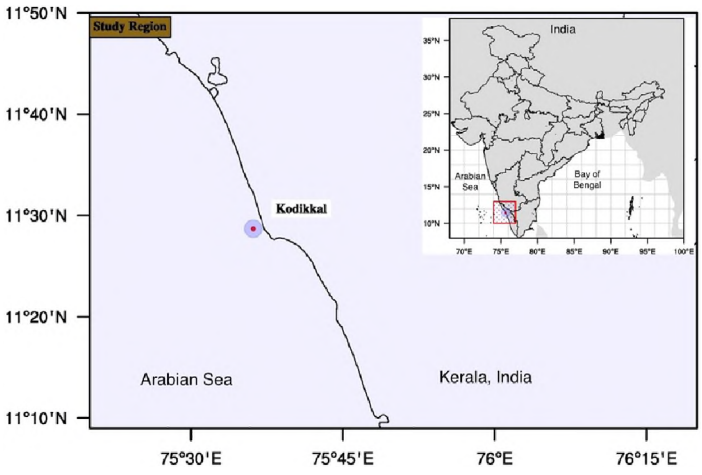

Fig. 1. Map of the study area in Southwestern Indian coastal waters.

Conspicuous surface water discoloration and fish mortality were reported by fishermen along the northern coast of Kerala, especially off Kodikkal (11°28'43"N & 75°36'10"E), southwest India, from 24 to 26 August 2015 (Fig. 1). Patches spread over an area of about 2 km off the coast. Surface water samples revealed they were dominated by dinoflagellates *Dinophysis caudata* (1.5 x 10^4 cells L^{-1}) and *Tripos massiliensis* (formerly *Ceratium massiliense*) (1.1 x 10^4 cells L^{-1}) (Fig. 2).

Other phytoplankton species that contributed to the bloom included *Tripos trichoceros* (formerly *Ceratium trichoceros*), *Nitzschia closterium, Proboscia alata, Noctiluca scintillans, Pseudo-nitzschia* cf *seriata* and *Thalassiosira* sp. [1-4]. Surface chlorophyll *a* concentration was 17.58 µg L^{-1}, dissolved oxygen 5.47 mg L^{-1} [5]. Nutrient concentrations recorded in the bloom area were: nitrate 0.148 µmol L^{-1} [6], nitrite 0.039 µmol L^{-1}, phosphate 0.245 µmol L^{-1} and silicate 1.617 µmol L^{-1}. Nevertheless, the fish killing mechanism was not clarified.

The potentially toxic dinoflagellate, *Dinophysis caudata*, has been previously observed in west Indian waters [7-8]. An exceptional bloom of this species was reported along the southeast coast in 1994 [9].

This is the first report of a bloom involving *Dinophysis* along the southwest coast of India. The species forming the bloom are usually present offshore and their presence in coastal waters might be due to the advection of oceanic water. The southwest coast of India, especially the Malabar coast, is highly vulnerable to algal blooms. Seasonal upwelling and monsoonal forcing, which result in nutrient–enriched waters, followed by advective processes, provide a competitive edge for the formation of phytoplankton blooms.

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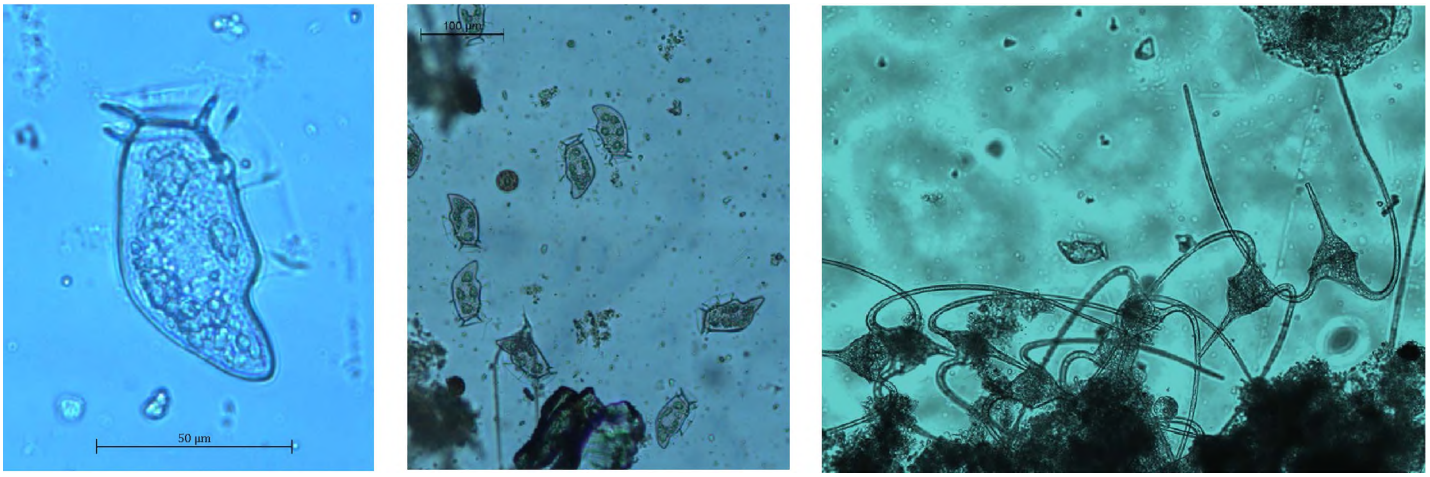

Fig. 2. Micrographs of Dinophysis caudata *field populations during the bloom event: a) Single cell of* D. caudata*; b) sample with dominance of* D. caudata*; c) isolated cells of* D. caudata *within a population dominated by* Tripos massiliense.

Azadinium Research Survey aboard the Celtic Voyager completed successfully

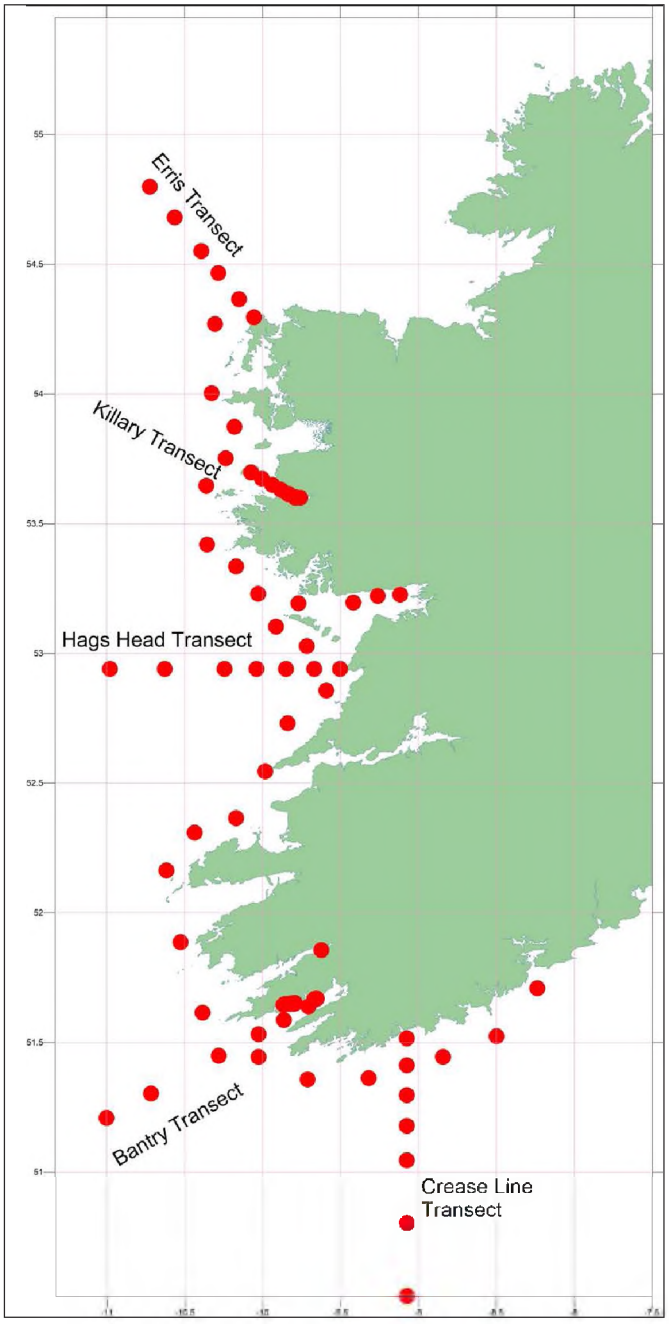

Fig. 1. Map of sampling stations visited during the Azadinium *Research Survey*

Ongoing research to understand the distribution and mechanism of *Azadinium* species and the production of Azaspiracid has been a major focus of research scientists at the Irish Marine Institute for several years. A survey aboard the Research Vessel *Celtic Voyager* was carried out in August along the south and western coasts to repeat a similar survey carried out in 2012 (Fig. 1). This recent survey generated samples from 56 stations along the track helping us to understand how the presence of *Azadinium* can result in shellfish toxicity that causes annual closures and economic hardship to many shellfish farmers and fishermen around the coast. In addition the survey took sediments for benthos analysis from 20 stations to add to the existing monitoring programmes in the MI and to look for the presence of interesting cyst stages. The survey completed 5 transects perpendicular to the coast far out onto the continental shelf making vertical profiles through the water to visualise the physical oceanography and collect depth samples. Underway sampling was carried out between transects to collect phytoplankton, and an ADCP (Acoustic Doppler Current Profiler) was deployed on the seabed in Killary Harbour which will continue to measure high resolution current speed and direction for the next couple of months.

Altogether, over 1000 samples were collected and returned to the lab for analysis of various types including toxin analysis and PCR analyses for the presence of the *Azadinium* genus and species within. Initial results of this PCR analysis indicate that 2016 was quite different to 2012. The presence of *Azadinium* was very low this year in contrast to 2012 when it was found at most stations. This ties in well with the observations of toxins in the shellfish, 2012 being a year where AZA caused lots of closures to shellfish production areas while AZA was not a problem in 2016, a year which was more dominated by *Dinophysis* and DSP toxicity in the South West of the country. The water profiles from both years indicated a broadly similar salinity structure but slightly warmer surface waters in 2016. There was a well-developed pycnocline present both years between approximately 20-30 metres depth. Analysis of the samples will continue to look in particular for the presence of cysts and to build up our knowledge of why some years are more dominated by Azaspiracid toxins than others. Further surveys are planned in 2017 and 2018 to continue to build up our data on the occurrence of this interesting and novel HAB.

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Azadinium spinosum *(photo Urban Tilmann)*

MARBioFEED – enhanced biorefining methods for the production of marine biotoxins and microalgae fish feed

MARBioFEED is a three year project supported by the First Call for Transnational Research Projects within the Marine Biotechnology ERA-NET, involving partners from Ireland (Marine Institute), Norway (Norwegian Veterinary Institute), Spain (Spanish Oceanographic Institute and Neoalgae) and Canada (National Research Council).

Project background and aims

The quality assurance of fish and shellfish for human consumption is of paramount importance in ensuring the marketability of products from this expanding and predominantly rural sector. The global demand for fish is increasing due to an expanding population and the awareness that fish is a healthy food source. With increased pressure on land-based agriculture due to climate change, the potential for sustainable marine sourced food is growing. The industry and regulators rely heavily on analytical reference materials to guarantee safety for human health. This project will involve large-scale algal biotechnology for the production of value added products (marine biotoxin reference materials and fish feed).

Large scale purification of marine biotoxins using enhanced biorefining methods for low cost reference materials and research

Most shellfish production sites in the EU are prone to closures due to the accumulation of biotoxins, produced by certain phytoplankton. Over 26 EU-regulated toxins currently require statutory monitoring and many more are being discovered. This places pressure on monitoring laboratories and researchers to develop and produce reference materials that allow for their analysis in a cheap, timely and effective way, while still maintaining the highest standards. The importance of aquaculture industries to peripheral European regions cannot be overstated. Most shellfish producing countries, including Ireland, Norway and Spain have implemented programmes to monitor both toxic algae and shellfish, potentially exposed to these microalgae, as a result of legislative requirements.

One element of the project will focus on isolation of large quantities of biotoxins to produce reference materials for a range of toxins to safeguard the sustainability and cost effectiveness of monitoring programmes, thereby supporting the long term expansion and viability of the shellfish industry. Further supplies of purified toxins will be commercially available at low cost for researchers in areas such as marine environment, toxicology, pharmacology, assay development, mode of action, mitigation, etc. Sourcing of naturally contaminated shellfish, bulk algal culturing, harvesting of algal blooms *in situ* and enzymatic conversions will be performed to source toxins of importance. The development and use of novel immunoaffinity and polymeric columns will be investigated to enhance purification efficiencies to reduce cost and increase economic viability.

Nutritious and sustainable microalgal biomass and shellfish for fish feed

Manufactured feeds are an important part of modern commercial aquaculture, providing the balanced nutrition needed by farmed fish. Fish feed is made, in part, from fishmeal and fish-concentrated products requiring much larger volumes of small ocean fish (such as anchovies, herring and sardines). Efforts are now being made to shift fish feed away from marine sources due to rising costs, reduced availability and environmental/sustainability issues. Research in this area is currently in its infancy and there is huge potential for innovative technologies and development in this field.

The project aims to support the efforts being made to produce more sustainable, highly nutritious but low cost feed for fish. Microalgae provide an excellent alternative to fishmeal, having exceptional nutritional value with high contents of essential amino acids and lipids. There is strong need to exploit the great potential offered by this diverse group of organisms. Medium/small scale culturing of microalgae will be performed to determine optimum growth and nutrition with subsequent transfer of technologies developed to large scale culturing using 1,200 L photobioreactors. Harvested materials can then be formulated to produce fish feed.

Mussels unsuitable for human consumption also offer another environmentally friendly alternative to fishmeal, but require research to evaluate the potential effects of the algal toxins they sometimes contain, both on farmed fish and on fish consumers. Work on farmed salmon in Norway has highlighted the need for studies to be performed on the chronic effects of algal toxins on fish health, in addition to possible exposure to humans through the food chain. Such studies require substantial amounts of purified toxins which currently are too costly to purchase at current market prices and are unavailable in the required amounts.

For further information please contact the project co-ordinator Jane Kilcoyne.

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ISSHA'S CORNER

Conference News

Bonjir (Bom dia), *Floripa*! (Hello, Florianopolis!)

This was the greeting in Portuguese for the attendees of the 17th International Conference on Harmful Algae (ICHA2016) in Florianopolis, Brazil, the first ever conference to be held in South America. The conference, from 9-14 October 2016, featured 350 participants from 35 countries who presented 145 talks and 210 posters. A series of "ignite" talks (2 sessions of 10 presentations each) were a great way for poster presenters to give a 5-minute introduction to their topics. Among the submitted papers, 355 were selected for presentation in 14 different topic areas. Considering all the works submitted, the total number of authors participating in the event reached about 1500, representing 438 institutions around the world. We extend our sincere thanks to Luis Proença, the local and international organizing committees, and the staff at the *Federal Institute of Santa Catarina*, IFSC for making the conference a great success. We thoroughly enjoyed the excellent scientific program, the conference locale including lovely historical Floripa and its fantastic seafood, and the Wednesday afternoon tours that gave us a "taste" of the surrounding region. At the conference banquet, after enjoying the world-class local food and drink, we were treated to a samba dancing demonstration complete with accompanying troupe of drummers from the local *Escola de Samba*. It was hard to sit still and many of our colleagues joined together on the dance floor for a lively international party.

About 110 participants from 35 countries responded to a pool about the 17 ICHA and their satisfaction level. The score for the overall subjective evaluation was 8.25 over 10 and for the objective evaluation (26 questions), 4.25 over 5.

There were 36 applicants for the first ever *Student Speed Networking* mini-course, held on Sunday, October 9, led by mentors Vera Trainer and Lisa Campbell, Adriana Zingone and Marina Montresor and Mohammad Abdul Baki. Highlights were our discussion on the importance of networking and how to give an interesting and captivating *elevator speech* to colleagues. It was emphasized that it should take about 30 seconds to introduce yourself and your work by including a *hook* or something that captures your listener, and ending by summarizing where you are in your career and whether you are looking for a position. Students were encouraged to tell their listener when their presentation (poster or talk) would be given at the meeting. They rotated among 4 tables and while practicing their speeches, had the opportunity also to get to know one another. These students commented that the speed networking session was a nice *ice breaker* that enabled them to meet one another and more senior members of the HAB community.

There were 37 applications for travel awards from 19 countries to attend ICHA2016. The International Society for the Study of Harmful Algae (ISSHA), through its travel award funds, was able to support 27 students from 13 different countries. There were 71 candidates for the Maureen Keller student award, given to outstanding graduate student oral and poster communications at the ICHA conferences.

Takayama's mini-carvings, generously donated to ISSHA, reached very high prices in the auction bidding. Only Senior scientists could afford them. (Photos M. Iwatataki)

Two *Yasumoto Lifetime Achievement Awards* were given this year, to Mike Quilliam and Allan Cembella. The *Patrick Gentien Young Scientist Award* was given to Dedmer Van de Waal.

The 2016 auction was a success as more than 3000 € were raised. A new feature of the auction considered a success was a student lunch with Gustaaf Hallegraeff, enabling a student to obtain valuable career advice from a senior scientist (see p. 27). Stay tuned for such innovative opportunities at future auctions!

Other highlights of the 17th ICHA (2016) included a *Sea Dance,* which was available during coffee breaks. This was a motion-sensing dance designed to serve as an outreach project to explain the marine food web and blooms of algae. It is an interactive game in which the dancers get to know species and organisms that comprise our marine ecosystem while dancing to the music. It was great fun to watch our colleagues whirl around the dance floor!

Lovely textiles were available at the conference, designed by *Crubag* and sponsored by ISSHA, to represent harmful algae in artist ways. Scarves, bow ties and bags were sold; a portion of the proceeds will benefit ISSHA. The new book, *Toxic and Harmful Microalgae of the World,* by Nicolas Chomérat, Philipp Hess, Elisabeth Nézan and Patrick Lassus, was revealed at the conference and is now available at cost on the ISSHA website (see http://www.issha.org/News/Book-release).

The ISSHA members were busy during the meeting and elected the following new council members to serve as your representatives: Wayne Litaker (USA), Ichiro Imai (Japan) and Po Teen Lim (Malaysia). The other Executive and Council members were re-elected for another 2 years (see new Council composition below). We thank both Mexico and Japan for submitting excellent bids for the 19th International Conference on Harmful Algae. The vote was close, and the 2020 conference will be held in Baja California, Mexico, hosted by IPN-CICIMAR in La Paz. In two years from now (2018), the 18th International Conference on Harmful Algae will be in Nantes, France, hosted by IFREMER. Stay tuned for more details about the next conferences and other news worthy items, such as job postings, workshops and courses, and HAB publications at the ISSHA website.

The deadline for submission of the conference proceedings is 1 March 2017, so please don't delay writing your papers. Manuscripts are a maximum of 4 pages long, with a fixed format. For more information, please go to the ISSHA website at *www.issha.org* for conference photos, conference highlights and information on the format for the conference proceedings.

Vera Trainer, ISSHA President
Beatriz Reguera, ISSHA Past President
Luis Proença, Chair Local Organizing Committee for the 17th ICHA

ISSHA Officers and Council members 2016

Officers
President: Vera Trainer (USA)
1st Vice-President: Gustaaf Hallegraeff (Australia)
2nd Vice-President: Wayne Litaker (USA)
Secretary: Anke Kremp (Finland)
Treasurer: Henrik Enevoldsen (Denmark)
Past-President: Beatriz Reguera (Spain)

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Amorin, Ana (Portugal)
Band-Schmidt, Christine (Mexico)
Davidson, Keith (United Kingdom)
Estrada, Marta (Spain)
Garcés, Esther (Spain)
Godhe, Anna (Sweden)
Hess, Philipp (France)
Ichiro Imai (Japan)
Jenkinson, Ian (China, France)
Lebret, Catherine (Sweden)
Lim, Po Teen (Malaysia)
MacKenzie, Lincoln (New Zealand)
Proença, Luis (Brazil)
Tubaro, Aurelia (Italy)

The profiles of the ISSHA officers and council members can be found at *http://www.issha.org/Society/ISSHA-Council-Members*

2016 Achievement Awards

The ***Yasumoto Lifetime Achievement Award* 2016** was given to Profs. **Mike Quilliam** and **Allan Cembella**. Short biographies of these two eminent scientists and community activists can be found at the *HAB Trail Blazers* section on the ISSHA website (*www.issha.org*).

Michael A. Quilliam was recognized for outstanding contributions to the field of algal toxin chemistry and analysis. A leading analytical chemist in the field, following in the footsteps of Prof Yasumoto himself. His life-time work covers amnesic, diarrhetic, paralytic, azaspiracid, cyclic imine and cyanobacterial toxins, including the identification of many new toxin structural analogues from both marine and freshwater environments. The 'toolbox' of novel analytical methods and Certified Reference Materials (CRMs) developed by his group are now used routinely in research and regulatory laboratories worldwide and have resulted in a paradigm shift in the way algal toxins are monitored.

Mike graduated in 1977 with a PhD in Chemistry from the University of Manitoba based on research into new organosilicon reagents for synthesis, chromatography and mass spectrometry of nucleic acids. During post-doctoral research at Univ. Montréal and a subsequent appointment with the Ontario Ministry of the Environment he developed further experience in mass spectrometry and delved into the analytical chemistry of environmental contaminants. From 1978 to 1987, he



Mike Quilliam, Yasumoto Award 2016 (Photo M. Iwataki)

served as assistant and later Associate Professor of Chemistry at McMaster University focusing on detection and identification of carcinogens and their metabolites. During this time, he did some of the first work in Canada on the now standard interfacing of liquid chromatography with mass spectrometry (LC-MS). In 1987, he joined the National Research Council of Canada (NRCC), where he is now a Principal Research Officer for Biotoxin Measurement Science. He also holds an Adjunct Professorship in Chemistry at Dalhousie University. His research at NRCC has focused on the analytical chemistry of toxic substances and the production of CRMs. He has worked on various environmental and food contaminants, including polycyclic aromatic compounds and veterinary drug residues, but his main focus has been on marine and freshwater biotoxins.

Mike has published more than 240 refereed papers and book chapters and over 90 CRM certification reports. He was from 1994 to 2002 General Referee for Phycotoxins in AOAC International and from 1997 to 2001 Chairman of the "APEC Task Team on Analytical Methods and Standards for Marine Algal Toxins". He served on numerous national and international committees on shellfish toxins, such as Joint FAO/IOC/WHO Expert Group on Biotoxins in Molluscan Bivalves reporting to the *CODEX Alimentarius Commission's Committee on Fish and Fishery Products* in 2004/5. He co-organized many symposia and conferences such as toxin sessions at AOAC and the *HABTech2003 Workshop on Technologies for Monitoring of Harmful Algal Blooms and Marine Biotoxins* in New Zealand. Mike has collaborated with over 150 young and established scientists, many of whom spent time training in his laboratory. Many of these scientists now play key roles in their countries in diverse fields of toxin research and monitoring. In 2013 and 2015, Mike was included in the "Analytical Scientist's 'Power List' of 100 most influential people in the field of analytical chemistry internationally.

Allan Cembella was recognized for a long and productive scientific career entirely devoted to HABs, starting with his graduate work with Max Taylor in the 1980s. In his early studies of *Alexandrium*, he was one of the first to use toxin composition as a characteristic to distinguish among different strains or isolates, and was also the first to use enzyme electrophoresis to provide yet another perspective on intra-species heterogeneity. It may seem obvious that there is extensive heterogeneity with a species in these times of powerful molecular tools, but in those days, the methods for that type of investigation were far more limited and experimental, and Allan was at the forefront of that phase of discovery.

Allan continued his studies of *Alexandrium* after he moved to eastern Canada. At that time, he co-authored a paper with Christophe Destombe on the sexuality and mating dynamics of *Alexandrium* that remains a classic resource to this day. He and Destombe also published the first sequences for *Alexandrium,* opening the door to decades of work on phylogeny and molecular detection methods in that genus and many others. That was also the time he discovered the spirolide toxins and linked them to the source organism, *Alexandrium ostenfeldii* – a very nice piece of biological and chemical detective work. He remains the world's authority on spirolides. Some years later, Allan repeated this accomplishment by assembling a team at the Alfred Wegener Institute that solved the mystery of the source of azaspiracids, which until then had incorrectly been linked to a *Protoperidinium* species. Many of us consider that body of work to be a scientific tour de force, as in a very short period of time, the causative organism was identified, its taxonomic and phylogenetic affinities revealed, its toxin diversity elucidated, and its global biogeography defined. Allan Cembella thus has the unique distinction of being the only person in the HAB field who has been the first to identify and fully characterize the source organisms for two major toxin families.



Allan Cembella, Yasumoto Award 2016 (Photo M. Iwataki)

Allan has also been heavily involved in studies of allelopathy and in genetic studies of a variety of toxic HAB species, in collaboration with colleagues in many parts of the world. His scientific productivity is exemplary, with nearly 200 peer-reviewed papers on a wide array of topics in the HAB field.

In addition to his many scientific accomplishments, Allan has been heavily involved in HAB community activities, including serving on the *Scientific Steering Committee of GEOHAB* and chairing one of the Core Research Projects – on HABs in Fjords and Embayments. He served as Vice President of ISSHA, and has been a longstanding member of the I*CES/IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)*, and has also been the German representative at many meetings of the *Intergovernmental Panel for HABs (IPHAB)*. He has been an enthusiastic and effective mentor to many students and young scientists worldwide. In addition to his educational activities in Germany, he is heavily involved and committed to HAB projects and education in Latin America, with students and projects in Chile, Argentina and Mexico.

In summary, Allan Cembella is a creative thinker with has has positively impacted all of our lives and careers with broad and significant impacts in HAB research, as well as major contributions to the policy and educational aspects of our field.

The ***Patrick Gentien Young Scientist Award*** was given to Dedmer Van de Waal (age 34) who received his PhD in June 2010, and currently has a tenure-track position at the Netherlands Institute of Ecology. Dedmer has substantially contributed to the conceptualization of harmful algae research. His most outstanding work couples the pro-



Dedmer Van de Waal, Patrick Gentien Award 2016 (Photo M. Iwataki)

duction of stoichiometrically distinct toxins to the elemental composition of its producer, and thus linking toxin production to the general ecological framework of Ecological Stoichiometry. Doing so, he has worked across salinity boundaries and his experimental work includes various marine dinoflagellate as well as freshwater cyanobacteria species.

Dedmer not only has a cumulative record related to this work, but also two 'breakthrough' papers in Ecology Letters, the second highest ecological journal for which he recently also became member of the Editorial Board. Beyond this topic, his publication record (currently 29 publications), impressive for his age, is rapidly expanding.

Dedmer contributed substantially to the studies on the effects of climate change on harmful algae, notably the impacts of elevated pCO2. His work crosses a range of organizational scales, from the regulation of genes, to eco-physiological responses (carbon and nutrient acquisition), and resource competition. Besides, his recent work includes the role of genetic diversity and phenotypic traits in the success of toxic algal blooms. Finally, Dedmer is an exceptionally creative thinker, ambitious, and active in the field of harmful algal research. He does not only bring harmful algal research to a broader scientific audience, but is also actively communicating HAB topics and respective research to the public, with various recent appearances on the Dutch national radio. Moreover, he is determined to connect HAB scientists and water managers of the Netherlands by organizing annual meetings.

Maureen Keller Student Presentation Awards

Best student oral presentation was awarded to **Alexis Fischer**, from the Massachusetts Institute of Technology – Woods Hole Oceanographic Institution Joint Program, for her contribution on "*Quantifying the chilling requirement for germinability of natural Alexandrium fundyense resting cysts*", co-authored with Michael Brosnahan and her advisor, Donald Anderson. A California-native, Alexis moved to New England to attend Wellesley College, where she graduated with a B.A. in Biology in 2010. After a year working on *Vibrio cholerae* ecology and evolution as a research technician at the University of Alberta, she returned to New England to begin her PhD on another toxic marine microbe: *Alexandrium fundyense*. She quickly became immersed in the Anderson laboratory's intensive field program in the Nauset Marsh system (Cape Cod, MA), where *A. fundyense* form recurrent blooms. Fascinated by the seasonality of the life cycle of *A. fundyense*, Alexis began to investigate how environmental and internal factors regulated excystment. Almost 30 years since her advisor discovered the endogenous annual clock in Gulf of Maine cysts, her research now addresses many of the lingering mysteries of cyst dormancy. The work presented quantifies how *A. fundyense* cysts use the environmental cue of winter chilling to restrict their germination until spring when favorable growth conditions are more likely to be sustained. This chilling response is a mechanism through which *A. fundyense*, and perhaps many other dinoflagellates, match the timing of germination to their environment, thereby maximizing bloom potential in a variety of temperature regimes and habitats. Currently, she is writing up her dissertation research, which includes experimental, observational, and modeling approaches, and considers how *A. fundyense* bloom phenology in Nauset may be affected by a changing climate.

Honorable mention for oral presentation to **Marc Long** from Université de Bretagne Occidentale (UBO, France) and University of Wollongong (UOW, Australia) for his contribution to "*Allelochemicals released by the toxic dinoflagellate Alexandrium minutum impact on Chaetoceros neogracile photosystem and viability*". Marc undertook his studies in Brest, France, where he obtained a Bachelor in Biology of Organisms and Populations in 2013 and a Master in Marine Biology in 2015. In parallel with his university course, Marc has been working on phytoplankton physiology as a part-time research as-



Alexis Fischer, Maureen Keller Award for Best student oral presentation



Marc Long, Honorable mention for oral presentation

sistant for nearly 6 years in the laboratory of marine environmental studies (LEMAR, France) under the supervision of Dr Hélène Hégaret and Dr Philippe Soudant. During his course, he also undertook overseas internships at the University of Wollongong (Australia) under the supervision of Prof D. Jolley and at the National Oceanic and Atmospheric Administration (NOAA, Northeast Fisheries Science Center, United States) in Milford Connecticut, always working with phytoplankton physiology, with Dr Gary H. Wikfors. The work presented at ICHA2016 was obtained from his first year of PhD and highlighted the impact of the unknown allelochemicals exudated by *A. minutum* on the diatom *Chaetoceros neogracile* physiology (primarily investigating at photosystem II and cell membranes) to better understand allelochemicals mode of action. During his PhD Marc aims to characterize the allelopathic activity from the dinoflagellate *Alexandrium*. To do so, he has developed a standardized bioassay with algal cells. For the next two year of his PhD project, his main objective will be to further investigate allelochemicals mode of action and to chemically characterize the nature of allelochemicals that may also have other cytotoxic activities.

Best student poster presentation to **Suema Branco** for her contribution: "*Morphology and molecular description of two species of Alexandrium from a tropical estuary in southeastern Brazil: Alexandrium fragae sp. nov. and Alexandrium tamutum".* Suema was born and raised in Rio de Janeiro, Brazil. She joined the Federal University of Rio de Janeiro in 2006 to do her bachelor of biology. Dr. Mariângela Menezes supervised her academic studies since internship to master and Ph.D. projects. In 2012, Branco obtained her master´s degree in Botany from UFRJ, whose subject of study was the taxonomy, ecology, and toxicity of marine species of Raphidophytes isolated from the Brazilian coast. In 2016 she concluded her doctorate at the UFRJ. Her thesis project was on taxonomy and phylogeny of various strains of planktonic marine dinoflagellates isolated from a tropical Brazilian estuary, mainly focusing on the potentially harmful species. In her thesis, she described a new species of



Suema Branco, Best student poster presentation



Jacqueline Jerney, Honorable mention for poster presentation

Alexandrium, *Alexandrium fragae*. She is currently a collaborating researcher in the Phycology Laboratory at the National Museum of Rio de Janeiro / UFRJ. Her primary interest is the taxonomy of harmful microalgae and the application of molecular tools in these studies.

Honorable mention for poster presentation to **Jacqueline Jerney**, University of Helsinki working at the Finnish Environment Institute – Marine Research Center for her poster on: "*Genetic diversity of seed banks and seasonal genotype dynamics of Alexandrium ostenfeldii (Dinophyceae) in shallow waters of the Baltic Sea*", co-authored by Conny Sjöqvist, Sanna Suikkanen, Satoshi Nagai and Anke Kremp. Jacqueline was born in Austria, where she grew up and started her academic career. She studied ecology at the University of Vienna, focused on Limnology during her studies and found her passion for phycology on the way. Jacqueline wrote her Master's thesis about algae turf scrubbers used for restoration of eutrophic waters and graduated in 2012. Afterwards she gained experience in the applied field of algal research at the University of Vienna, the University of Natural Resources and Life Sciences, and the company Erber Future Business in Austria. Doing so, she started missing ecology and basic research, which prompted her to start her PhD in Finland. Currently she is working on her PhD studies, supervised by Anke Kremp and Sanna Suikkanen at the Finnish Environment Institute. The main topic is ecological and evolutionary significance of seed banks for the expansion of phytoplankton blooms in the Baltic Sea.



Luis Proença (Chair) and members of his Local Organizing Committee receive a Gymnodinium catenatum carving as an ISSHA recognition for their strong commitment to make the 17 ICHA a success.

New Auction Item: Lunch with a famous HAB scientist



Lunch with a famous HAB scientist: PhD student Sara Harðardóttir and Prof Gustaaf Hallegraeff.

A new experiment during the ISSHA auction was the opportunity for students to bid for a **lunch opportunity with a famous HAB scientist**. During a delightful lunch at the Florianopolis food market Prof Gustaaf Hallegraeff thus offered his career counseling services to Copenhagen PhD student Sara Harðardóttir (pictured). At his home university in Tasmania Gustaaf offers annual workshops on what it takes to become a successful researcher (passion, creativity, traveling, thinking global, communication skills, building teams, daring to take risks etc). Needless to say Sara already ticked many of those boxes, and Gustaaf was pleased to note that promising postdoc career options and contacts he suggested had already been approached by the end of the conference week. For a student it can be a challenge to network at the busy ICHA conference and for Sara the opportunity to meet and receive counselling from Gustaaf was much welcomed and rewarding. Altogether, a successful experience for both, and worth repeating.

10th International Conference on Toxic Cyanobacteria

The 10th International Conference on Toxic Cyanobacteria was held in Wuhan, China from 23 to 28th October 2016. The conference is held every three years and brings together around 300 researchers from around the world to present their research findings. Toxic cyanobacterial blooms continue to be a major concern in freshwater systems worldwide and the recent blooms in Lake Erie, USA were highlighted, as well as the ongoing blooms in Lake Taihu and others in China. The dominant toxic species continues to be *Microcystis aeruginosa* but there were also a number of talks about *Cylindrospermopsis raciborskii, Dolicospermum* (previously *Anabaena*) *circinalis* and the benthic genus *Phormidium*. There was a strong emphasis on mitigation strategies, including the use of hydrogen peroxide and clay products, as well as new and faster detection methods. Molecular methods to understand bloom dynamics was a strong focus of the conference, and the integration of these methods with traditional ecological and physiological approaches.

One evening was devoted to a discussion of cyanobacterial management strategies and during this session, Michele Burford, a committee member on the newly formed IOC and SCOR funded GlobalHAB consortium, gave a talk on the aims of GlobalHAB and invited conference participants to be engaged. There was considerable interest, particularly from Chinese participants, and further information on the GlobalHAB on the new website is eagerly awaited.

Michele Burford, Australia Rivers Institute, Griffith University, Nathan, QLD, Australia

e-mail: m.burford@griffith.edu.au



Fig. 1. At the conference dinner. Left to right: Cheryl Orr, Tim Davis, Phil Orr, Michele Burford and Anusuya Willis



From left to right and from top to bottom: 1-5, happy faces at the 17 ICHA; 6-8, frantic activity and fun at the ISSHA auction; 9, ISSHA table; 10, dancer from the local "Escola de Samba" teaching the Brazilian rhythms to the participants after the conference banquet (all photos by Mathias Schramm except nº 6, from Mitsunori Iwataki)

New book

NEW! Toxic and Harmful Microalgae of the World Ocean

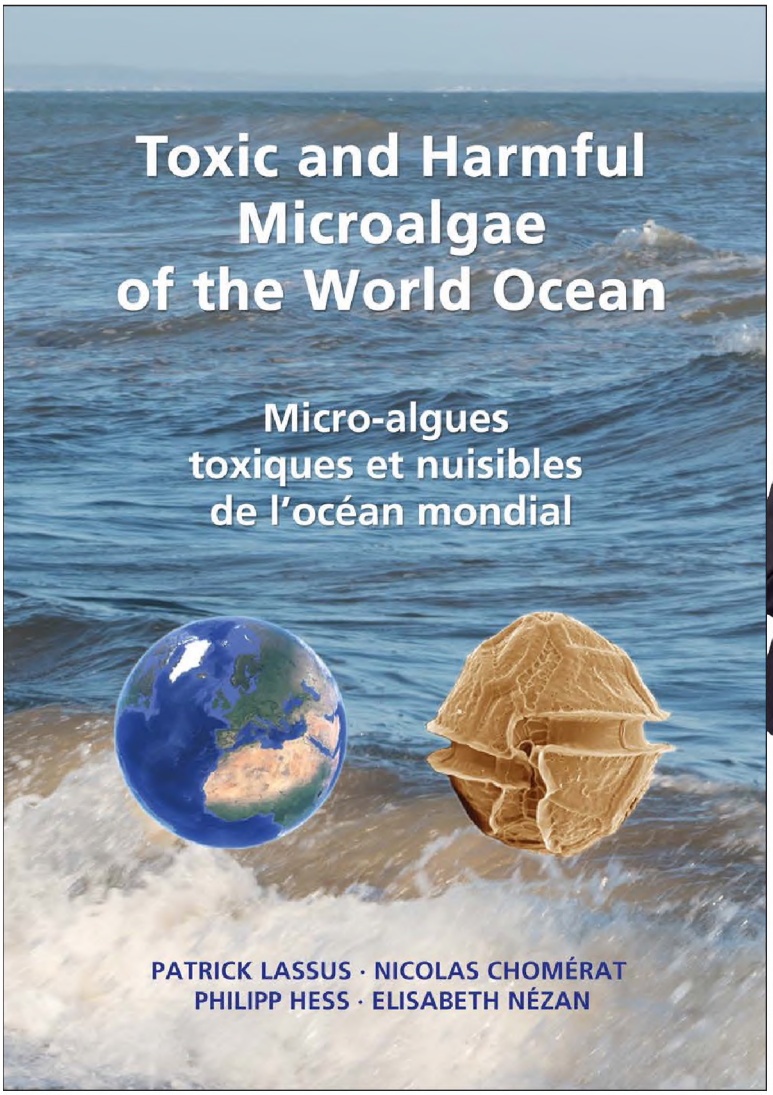
This ISSHA and IOC UNESCO publication, written by Patrick Lassus, Nicolas Chomérat, Philipp Hess, and Elisabeth Nézan constitutes the first ever global compendium on harmful algal blooms (HAB), microorganisms that deplete fish stocks, destroy fish farms and bring disease and death both to humans and to large sea animals.

In a case-by-case review of data on major harmful algal bloom incidents, the publication sounds the alert on increasing trends, as observed, for example, along the coasts of Florida (USA), where data has been compiled since the mid-19th century, India and Oman. In the Adriatic and Baltic seas, harmful algal incidents are on the increase despite governmental awareness and decisions to restrict the production and discharge of phosphate and other harmful chemicals.

This book updates available information from international databases on toxic and harmful species of phytoplankton and micro-phytobenthos. It lists regional occurrences at world-wide scale: localities, cell densities, toxicity levels in contaminated animals, human and animal poisoning, isolation of identified active molecules and toxins, documentary sources. Finally, this book addresses the geographic distributions of phycotoxin-producers from recent data. The objective is to analyze the evolution of toxic and harmful episodes over the past 30 years to identify trends, and to introduce a discussion on the reality of a time-dependent increase in the number of known species and toxins.

Order at the ISSHA website *www.issha.org* web shop.

Price (postage included) is 50 €



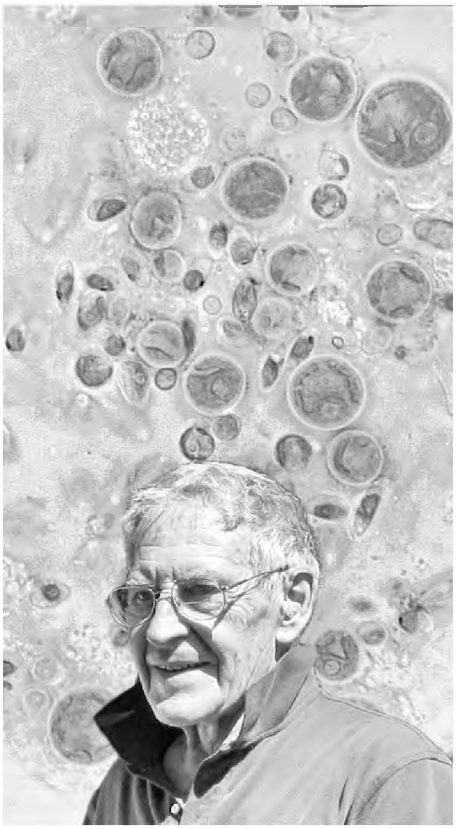


Philipp Hess, one of the book authors and Vera Trainer, President of ISSHA, present the new book at the 17 ICHA in Florianópolis, Brazil

Robert RL Guillard (1921-2016) In Memoriam[1]

Robert 'Bob' Guillard was an icon in all senses of the word, and many tributes have and will be written [1, 2] This is but a brief overview of a life very well-lived and shared.

Born in New York City on February 5th, 1921, Bob's love for the natural environment was firmly established during his time with his grandparents in Stonington, Connecticut. An early scholar, Bob completed grammar school in 7 years, graduated from Townsend Harris High School in New York City in 3 years, and earned a B.S. in Physics (City College New York, CCNY) at the age of 20. Bob was hired by the U.S. Navy to install and maintain antimagnetic mine equipment. He continued to pursue course work in the evenings and taught at New York University and CCNY. During this time he was smitten by a botany professor and decided that he wanted to become a 'naturalist'. He went to Yale in 1949 and received a Master's degree (1951) on route to his Ph.D. (1954) under the tutelage of G. Evelyn Hutchinson. Soon after, he was awarded a summer fellowship to the Woods Hole Oceanographic Institute - he changed fields from botany to oceanography. After a short period in Hawaii, Bob was hired by the U.S. Bureau of Commercial Fisheries Marine Laboratory in Milford, Connecticut where he was challenged by Victor Loosanoff with the task of 'figuring out how to grow oysters'. As Bob recounted it, his first thought was that the little oysters would need to be fed and he reasoned that they were partial to what was in the water column. That was when he began isolating phytoplankton and developing the techniques to culture them in volume. His first publication was in the *Proceedings of the National Shellfisheries Association* and it launched shellfish aquaculture as we know it today - the path to f/2 was being laid. That first paper demonstrated that individual phytoplankton isolates differed in their nutritional value to oysters, thereby establishing the foundation for "the Milford Method" of feeding hatchery seed of shellfish selected strains of microalgae later further developed by Ravenna Ukeles and others. Without Bob's pioneering work



in phytoplankton culture and nutritional value, the rearing of shellfish in captivity would not be possible.

In 1958, John Ryther recruited Bob to Woods Hole where he was a senior scientist and in 1981, he moved to the recently established Bigelow Laboratory for Ocean Sciences and established the collection of algae and other microscopic marine organisms that was designated by Congress as the *Provasoli-Guillard National Center for Culture of Marine Phytoplankton* - to honor two men whose research and mentoring had provided the world with a first-class source of algae for research and aquaculture. Anybody in the field of algal culture knows 'f/2' - but few realize that this was but a sideline for Bob. He was a global leader in the physiological ecology of phytoplankton. Bob reduced the curiosity that drove his science to this question: "Why do they live where they do?' Considering that phytoplankton cannot control their position, the question becomes almost metaphysical and leads one into the "n-dimensional hyperspace (Bob's phrase)" of physics, chemistry, and biology combined. Bob excelled at creating an environment in which ephemeral microbes could thrive and be sustained, and in 1962, he published a paper with John Ryther that became one of the most cited papers in marine science (over 4700 citations at last note) as it described in detail the most successful algal-culture medium ever developed [3].

Bob was anything but a one-dimensional scientist. He had a wide array of interests and talents outside the laboratory, including fencing (he was an instructor), Morris dancing (one of the best in the country), target shooting (his keenness for guns was legendary), and Transcendental Meditation, an activity that he shared with his wife Ruth, a TM teacher. His penchant for collecting all manner of what most would deem junk was well known - and those same doubters always knew where to go when they needed some esoteric item. Bob was recycling long before it was a word!

Bob's publication record was impressive by any standard and his advice was in constant demand. Ever the teacher, one frequently got far more than they bargained for when they asked what they initially deemed a 'simple question'. Out-of-the-blue phone calls that began with the words, "I've been thinking..." could lead to lifetimes worth of questions needing answers. There are countless 'Bob stories' and, while some are suitable for publication, these will be saved for later celebrations. He was generous with his time and expertise and never lost his curiosity and questioning mind. In 1995 Bob was named an *Honored Life Member of the National Shellfisheries Association* [4], and in 1998 he was presented with the *Honorary Life Member Award from the World Aquaculture Society*.

Bob passed away in his sleep on September 25, 2016 in Boothbay Harbor, Maine, leaving behind his wife of 53 years, Ruth, 3 stepsons and their families, and a barn full of decades of accumulated treasures - "just in case I need them someday" (including, one presumes, all those boxes of little blue paper circles that separate Millipore filters).

Bob would scoff at all the attention



Bob proudly showing his cultures to a visitor (photo from the Bigelow Laboratory website)

being paid to his accomplishments. His curiosity was at the core of his existence and this, his keen intellect, and sharp wit were evident after even the shortest discussion with him. Scholar, old Yankee, friend - it was an honor, privilege, and most entertaining to have known him.

Sandra E. Shumway, Groton, Connecticut
Gary H. Wikfors, Milford, Connecticut
sandra.shumway@uconn.edu

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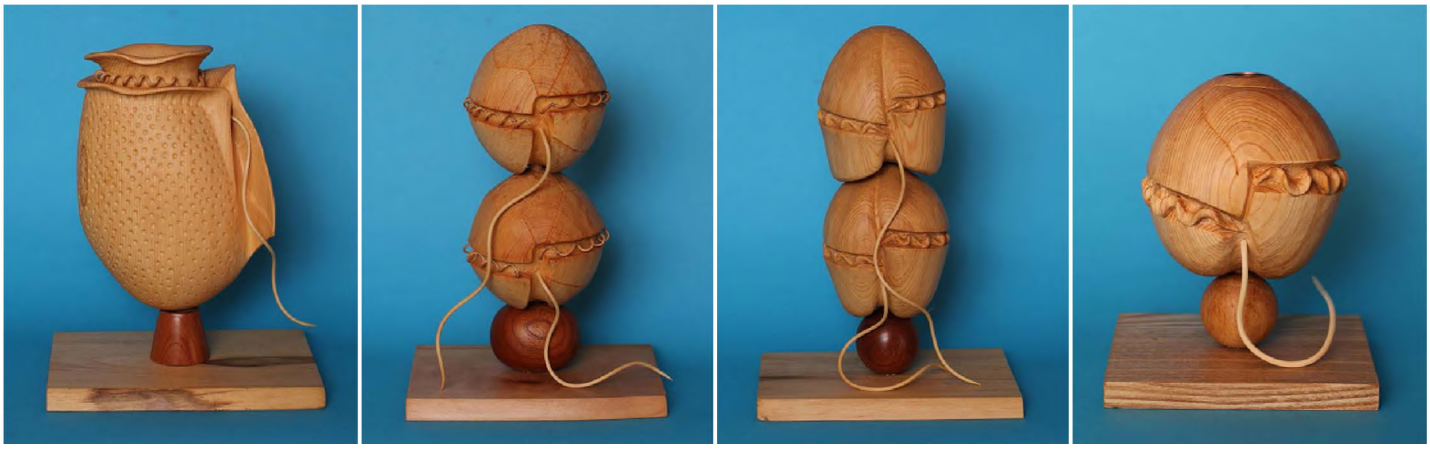
[1]Text and figure reproduced from the World Aquaculture Magazine 47(4), December 2016 with permission from the authors.

More information about Bob Guillard, the father of the f/2 medium, at the Bigelow Laboratory website

https://www.bigelow.org/news/articles/2016-10-04-2.html



Beautiful wooden carvings of harmful dinoflagellates, from Prof Takayama, made for the ISSHA 2016 awardees.
From left to right: Dinophysis fortii, Alexandrium tamarense, Gymnodinium catenatum *and* Takayama pulchella.

Forthcoming events

Crossing disciplinary boundaries across the freshwater-marine continuum to advance the understanding of harmful algal blooms (HABs)



This session (012), to be held during the next ASLO meeting (Honolulu, Hawaii, 26 Feb – 3 March), seeks to bring together HABs scientists who study multiple aspects of these events, including their biogeochemistry, chemistry, biogeography, toxicology, ecology, and epidemiology, as well as approaches to monitor HAB distribution, genetic diversity, and toxicity. All types of HABs studies are encouraged incl uding descriptive, correlative, empirical, and/or theoretical. Given the environmental and health threat that HABs pose to society, we encourage a focus on management solutions

NOTE:
Abstract submission deadline was October 14.

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ICMSS 2017: Protecting consumers, assuring supply, growing confidence

Now that the excitement of the Brazilian ICHA conference is over it is time to start looking towards 2017 and we are delighted to inform you that the **International Conference on Molluscan Shellfish Safety, ICMSS 2017**, will be hosted by The Marine Institute, Galway Ireland from May 14 - 18, 2017. This is the 11th conference in this series and offers an important multidisciplinary interface between regulatory, scientific and industrial representatives of the international molluscan food safety community. It is a biannual forum where unusual, emerging and novel shellfish risk factors are discussed, offering new information and solutions. The Marine Institute in association with *Bord Iascaigh Mhara*, The Food Safety Authority of Ireland, The Sea Fisheries Protection Authority and The Irish Shellfish Association will host the conference in the wonderful state of the art conference centre of the National University in Galway.

Ireland has a distinguished track record in the area of molluscan shellfish safety, in developing state of the art monitoring protocols, forecasting tools and a variety of cutting edge research activities. This is the second time the conference has been in Galway and we look forward to welcoming old friends and new to our conference on the theme of *"Protecting consumers, assuring supply, growing confidence"*. **ICMSS 2017** will include several keynote presentations from acclaimed international experts in the area. A series of workshops will be held in conjunction with the event on the 19th and 20th May. We encourage attendance from shellfish safety professionals and students including, microbiologists, toxin chemists, toxicologists, marine scientists, regulators, policy makers, food safety specialists, environmental health officials, engineers, environmental managers, academics and undergraduate and postgraduate students.

We invite you to join us for a stimulating conference to be held in a collaborative atmosphere in the vibrant and welcoming city of Galway on Europe's western margin. We promise a very exciting international event, providing an opportunity to bring together experts from all over the world to exchange ideas, information and the most up to date findings, allowing us to review current state of the art and future prospects in molluscan shellfish safety. So, don't hesitate to come and get involved. The abstract call is open at *www.icmss2017.com* and register for what promises to be a fantastic conference!

For more information see *icmss2017.*

com, follow twitter (#ICMSS17) or contact the conference convener Joe Silke (*joe.silke@marine.ie*)

Important Dates:
Call for Abstracts Opens - 09/2016
Abstract Submission Close - 12/2016
Early Bird Registration Close - 02/2017
Conference Opening - 05/2017

18th ICHA in Nantes, France, to be held 21-26 October 2018 – save the date!

The 18th International Conference on Harmful Algae will be held in Nantes, France. The conference venue has been booked for the dates from 21 to 26 October 2018 by IFREMER, the institutional host of the conference. Philipp Hess (IFREMER) and the French delegation (Research Network PHYCOTOX) gave a presentation of the project, the city and progress made in the organisation. A professional conference organiser will assist the local and scientific organising committees, and is about to be appointed. The website will operate from early 2017 and separate announcements will be posted through the conference mailing list and the ISSHA website – watch this space!



Philipp Hess (IFREMER, France) takes up the torch during the closing ceremony of the 17th ICHA to organize the 18 ICHA in 2018.

11th International Conference on Modern and Fossil Dinoflagellates (DINO 11)

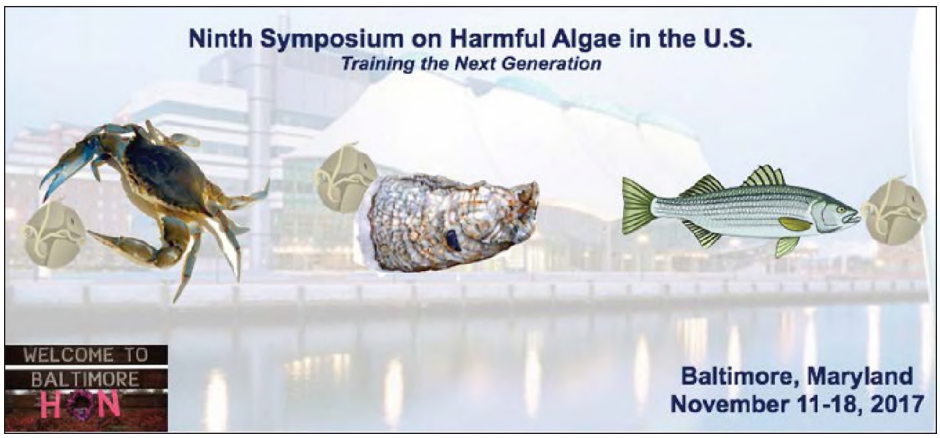


DINO11, the 11th International Conference on *Modern and Fossil Dinoflagellates* will be held at Bordeaux University, France, during July 17-21, 2017.

The scientific program will be devoted to the latest developments in studies of living and fossil dinoflagellates, this group being one of the most important among planktonic and benthic marine microalgae, and as such gathering both biological and geological interests.

All information about the conference can be found at the conference website *http://laplf.org/dino11/news.htm*

Ninth Symposium on Harmful Algae in the US (Maryland, 11-17 November 2017)



The National HAB Committee (NHC) is proud to announce the Ninth Symposium on Harmful Algae will be held in Baltimore, Maryland from November 11-17, 2017.

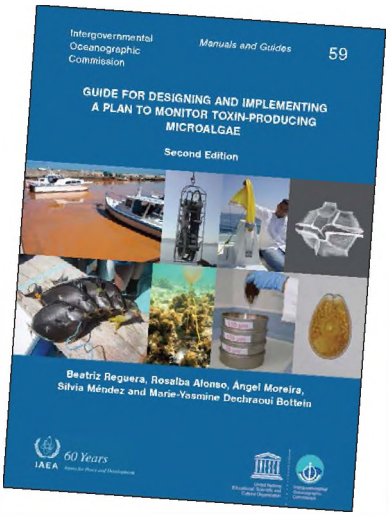
The theme of the Ninth Symposium will be *Training the Next Generation* and include several hands on workshops for the students and postdoctoral fellows. *http://www.whoi.edu/habsymposia/*

Soon coming: Guide for designing and implementing a plan to monitor toxin-producing microalgae

This Manual is a joint product of the IAEA and the IOC of UNESCO. The first edition was published in Spanish in 2011 and is available on line at the IOC-HAB website.

With the initiation of additional IAEA national and regional projects on HABs, the organization of regular IOC global and regional training courses, and the strengthened cooperation between the IOC and the IAEA on board capacity development, the need developed to translate this HAB manual into English, and expand it with the addition of chapters related to benthic HAB species. That was a natural next step to assist Member States to implement monitoring programmes to detect potentially toxic HABs species in their respective coastal waters.

This manual is intended as an introduction to basic analytical techniques that can be applied when designing a standard sampling protocol for both planktonic and benthic microalgae (and associated environmental conditions) and vectors of biotoxins (shellfish and fish). This standardization of methods will enable more robust data comparisons between countries and will yield improved risk assessments of potentially toxic HABs events.



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Deadline
Deadline to submit material for HAN 56:
28 February 2017

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AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Is *Gambierdiscus* expanding its geographic range in the Pacific region?



Fig. 1. Top, macroalgae in lagoonal waters, Rarotonga, Cook Islands, from where Gambierdiscus sp. CAWD233 was isolated, and bottom, Phoebe Argyle sampling from eel grass in Tongatapu, Kingdom of Tonga.

The genus *Gambierdiscus* is known from tropical regions world-wide [1-4]. Species in this genus produce ciguatoxins (CTXs) and/or maitotoxins (MTX), which are regarded as the causative agents of ciguatera fish poisoning (CFP) [1-4]. At the beginning of 2017, fourteen species of *Gambierdiscus* had been described [5-6] with three of these being described in 2016, an increase of nearly 30% in one year. The newly described species were *G. balechii, G. cheloniae* and *G. lapillus* [7-9]. Over the past few years, species which were previously only known in the Caribbean have been recorded in the Pacific [3], although this could be due to the greater research effort in recent years rather than due to introductions. Currently only *G. carolinianus* [6], *G. excentricus* and *G. silvae* [10] have not been detected in the Pacific region (Table 1).

Recently, genetically and morphologically identical isolates from as far apart as the Kermadec Islands (a New Zealand territory), the Cook Islands (Fig. 1) and the Kingdom of Tonga were determined to be unique. These isolates are maintained in the *Cawthron Institute Culture Collection of Microalgae* (CAWD242 (Fig. 2), CAWD233 and CAWD250 respectively) and the description is currently in review. Identical DNA sequence data to this currently undescribed species have also been recorded for an isolate from eastern Australia, although the isolate did not survive [2]. If accepted as a new species

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Table 1: Geographic occurrence and toxin production (as determined by LCMS/MS) of Gambierdiscus species in the Pacific region.

Species reported in the Pacific region	Countries where reported	Toxins produced (LCMS/MS*)	References
G. australes	Cook Islands; French Polynesia; Hawaii (USA); Japan; Tonga	MTX MTX-3	Chinain et al. 1999 [26]; Kohli et al. 2015 [3]
G. balechii	Celebes Sea, Indonesia	(positive MBA)	Fraga et al. 2016 [7]
G. belizeanus	Australia (Queensland); Malaysia	(positive RBA)	Kohli et al. 2015 [3]; Leaw et al. 2011 [28]; Tester 2015 [22]
G. caribaeus	French Polynesia; Hawaii (USA); Korea; Republic of Palau; Thailand	(positive MBA)	Kohli et al. 2015 [3]; Litaker et al. 2009 [6] Tawong et al. 2016 [29]
G. carpenteri	Australia (New South Wales); Fiji Islands; French Polynesia; Guam (US territory); Hawaii; Mariana Islands	MTX-3*	Kohli et al. 2015 [3] Litaker et al. 2009 [6];
G. cheloniae	Cook Islands	MTX-3	Smith 2016 [8]
G. lapillus			Kretzschmar et al. 2016 [9]
G. pacificus	French Polynesia; Cook Islands; Republic of the Marshall Islands; Republic of Tonga; Vietnam	MTX-3	Chinain et al. 1999 [26]; Kohli et al. 2015 [3]
G. polynesiensis	Cook Islands ; French Polynesia; Vietnam	**CTX** MTX-3	Chinain et al. 1999 [26]; Chinain et al. 2010 [27]; Kohli et al. 2015 [3]
G. scabrosus	Japan	(positive MBA)	Nishimura 2014 [30]
G. toxicus	Australia (Queensland); Fiji Islands; French Polynesia; Guam (US territory); Hawaii; New Caledonia; Republic of Palau; Republic of the Marshall Islands; Vietnam	(positive RBA)	Kohli et al. 2015 [3]; Richlen et al.2008 [4]
***Gambierdiscus* genus†**	New Zealand	NT	Chang 1996 [15]
***Gambierdiscus* sp. (isolates CAWD242, 233 and 250)**	Cook Islands; Kermadec Islands (NZ territory); Republic of Tonga	MTX-3	Richlen et al. 2008 [4]; Smith et al. 2016 [8]
Species not yet reported in the Pacific region: *G. carolinianus, G. excentricus, G. silvae* [6,10].			

MTX: maitotoxin; MTX-3: putative maitotoxin-3; CTX: ciguatoxin; MBA: mouse bioassay; RBA: receptor binding assay; NT: not tested; †: Reported as G. toxicus but species unconfirmed.
**Where LCMS/MS results are either negative or unavailable results of bioassays are presented in brackets; **Australian isolates of G. carpenteri were negative for MTX by LC-MS/MS but positive by MBA [31].*

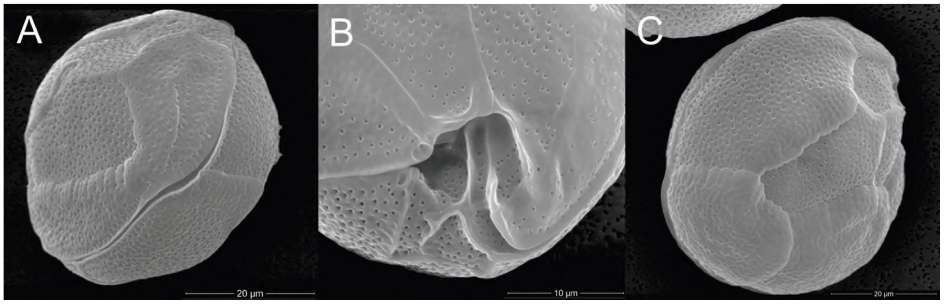

Fig. 2. Scanning electron micrographs of cells of Gambierdiscus sp., isolate CAWD242, from the Kermadec Islands: (A) view of cingulum and apical pore, (B) apical pore complex, (C) antapical hypotheca.

this will bring the number of *Gambierdiscus* species to fifteen.

A related species, originally described as *G. yasumotoi,* was reported in coastal waters of Northland, New Zealand, and in tropical eastern Australia [11-13]. It was later assigned to a new genus, *Fukuyoa* [14], and was reclassified as *Fukuyoa paulensis.* The three described species in the genus, *F. paulensis, F. ruetzleri* and *F. yasumotoi*, are not included in this discussion. *Gambierdiscus* was also reported as *G. toxicus* in northern New Zealand in 1995 [14], but without scanning electron microscopy or DNA sequencing data the species identification is uncertain. However, the presence of *Gambierdiscus* [15] and *Fukuyoa* [11] in New Zealand indicates that Northland's coastal waters can support those genera with the risk that cells concentrations could increase to form blooms under favourable environmental conditions.

Climate change, in particular rising sea temperatures and changing currents, means that an expansion in the geographic range of *Gambierdiscus* is likely to continue and possibly accelerate [16-17]. Certainly average annual increases in sea water temperatures have been reported for both south-eastern Australia and for New Zealand's coastal waters [18-19]. The appearance of *G. carpenteri* in the more temperate waters south of Sydney, New South Wales (NSW), Australia [16] may be explained by currents bringing cells from tropical Queensland to the warming NSW seas, but the adaptation of *G. carpenteri* to cooler temperatures may also contribute to the NSW records. The risk of CFP occurring in New Zealand's northern sub-tropical waters continues to increase and may impact on recreational fishing in the future and, potentially, the development of the commercial fishery.

Not all *Gambierdiscus* species produce CTXs or MTXs (Table 1). LCMS/MS allows specific and sensitive analysis of the targeted toxin groups and, based on our results, *G. polynesiensis* produces CTXs and *G. australes* MTX-1 [3,20]. However, all described species produce putative MTX-3. The toxicity of CTX is high by both intraperitoneal (ip) injection and by oral administration in mice, whereas MTX-1 is highly toxic by ip injection of mice, but much less so orally [20] and MTX-3 has relatively low ip and oral toxicity (R. Munday, unpub-

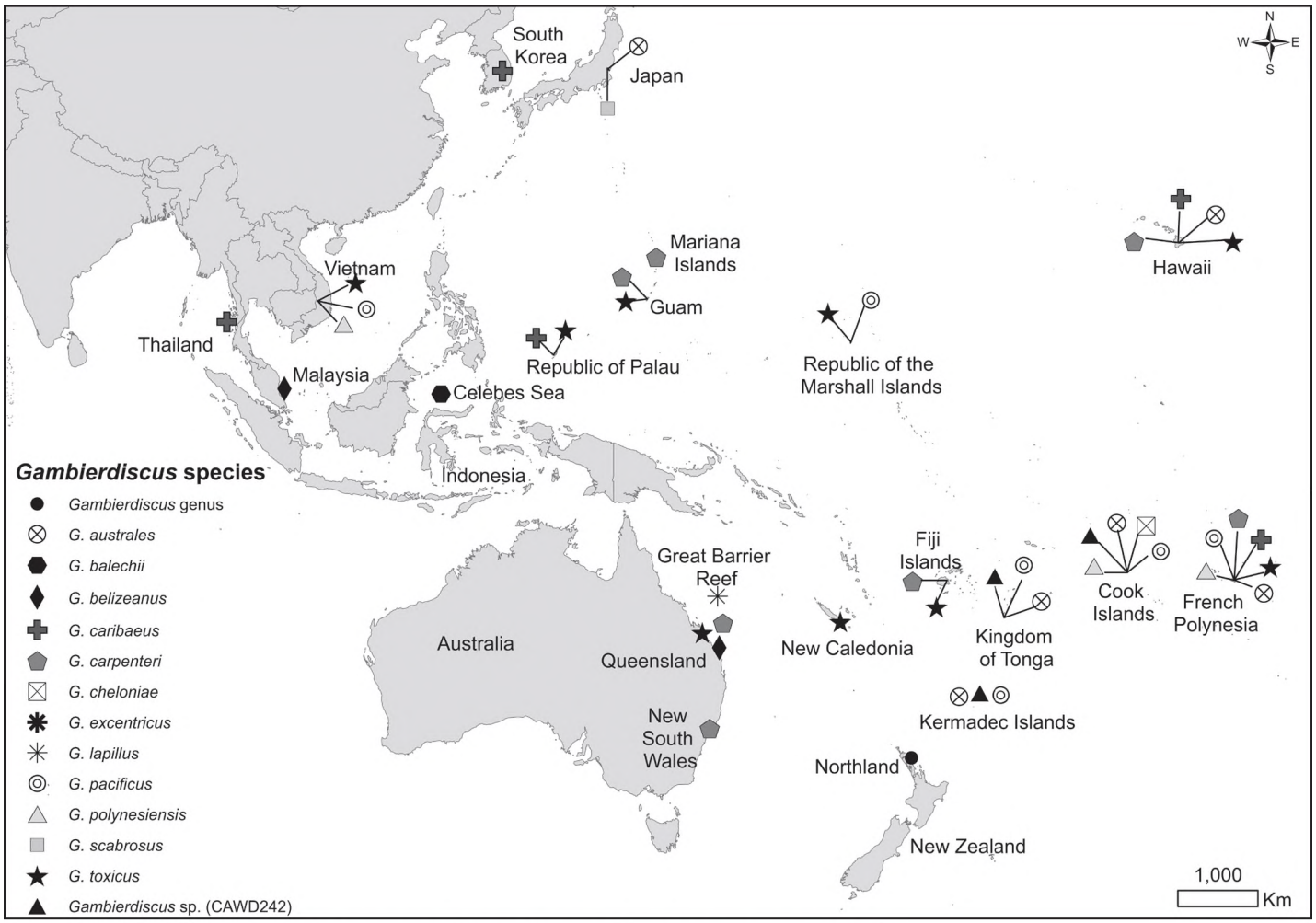

Fig. 3. Map of Pacific region showing the geographic distribution of Gambierdiscus species (2017).

lished data). The role of MTXs in ciguatera fish poisoning is uncertain and due to its low oral toxicity may only play a small part in toxic events [21].

A range of cell-based assays is available to detect CFP-related toxins, including receptor binding (RBA), Ca^{2+} flux, N2A and haemolytic assays [21] as well as whole animal assays (for example, mouse and larval bioassays [22-23]). Results of all these assays are not presented in detail here, but extracts of isolates of all species tested so far have shown a response in at least some of these bioassays, as can be seen in Table 1. These results suggest that compounds other than MTX-1 and CTXs may be produced by the species listed in Table 1. Other toxins known to be produced by *Gambierdiscus* species include gambieric acids, gambieroxide, gambierol and gambierone [20]. Despite the raft of methods available, the toxicity of isolates of *Gambierdiscus* still requires resolution to improve the knowledge of the functional biogeography of the genus.

The risk of ciguatera poisoning is clearly Pacific-wide and in some islands has caused many illnesses and even deaths [24]. The poisoning can have serious economic impacts, particularly on smaller Pacific Island communities [25]. The key producer of CTXs, *G. polynesiensis*, has been found from the far western edges of the Pacific (Vietnam) to eastern French Polynesia (Fig. 3) and is likely to be found further south with warming seas. Of course, the greater sampling efforts being undertaken are likely to add to the increasing detections.

Species such as *Ostreopsis ovata* and *Coolia malayensis* occur in the Cook Islands and French Polynesia, where *G. polynesiensis* has been reported. These co-occurring species with *Gambierdiscus* are also found in Melanesia, for example, Tongatapu, Kingdom of Tonga (Phoebe Argyle, unpublished data; Fig. 1) and the Fiji Islands. They also occur in Micronesia, for example, the Republic of the Marshall Islands, the US territory of Guam, and Tol-truk, Federated States of Micronesia [3]. This suggests that the environmental conditions may be suitable for the expansion of *G. polynesiensis* to these areas.

State-of-the-art molecular tools are likely to result in increased reports of all the known species of *Gambierdiscus* globally and assist in the discovery of new species. DNA sequencing data is critical for descriptions of new species as conservative morphological differences can be difficult to identify. Quantitative PCR assays and metabarcoding are already generating considerably more data than can be generated by traditional methods. New species records are being made at new sites, for example, in Northland, New Zealand, and the Cook Islands (K. Smith, unpubl. data).

Ciguatera fish poisoning has been known in the tropical Pacific region for centuries, but the increase in species numbers and the expansion of the geographic range of *Gambierdiscus* species over the last few years (for example, *G. carpenteri* in NSW, Australia [31]; Fig. 3) suggests that CFP will also become of greater concern for more temperate regions in the Pacific in the future.

Acknowledgements

We would like to acknowledge the team work, in particular with the University of Technology, Sydney, Australia (led by Prof Shauna Murray), the Ministry of Marine Resources, Cook Islands (Dorothy Solomona) and the Office of the Prime Minister, Climate Change Cook Islands (Dr Teina Rongo). Much of the Northland and Kermadec Islands, NZ, research has been funded by the NZ Ministry for Business, Innovation and Employment, Contract CAWX1317.

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Toxin production in lab-rat-diatoms (e.g. *Pseudo-nitzschia*) in the presence of copepods

From only 15 species in 1965, currently 49 *Pseudo-nitzschia* species have been described, of which 22 have the ability to produce the toxin domoic acid (DA), along with two *Nitzschia* species [1]. Both numbers are steadily increasing, illustrating an unexpectedly high diversity within the genus. Many of the species are morphologically similar, e.g. species in the *P. pseudodelicatissima* complex, making identification of *Pseudo-nitzschia* species difficult. The high and steadily increasing number of toxic species emphasizes the importance of considering *Pseudo-nitzschia* species to be toxic for monitoring purposes, unless proven otherwise. One may in fact ask whether all *Pseudo-nitzschia* species are toxic - given the right circumstances as several factors such as physics and chemistry as well as biological interactions are known to affect toxin production [reviewed in 2, 3].

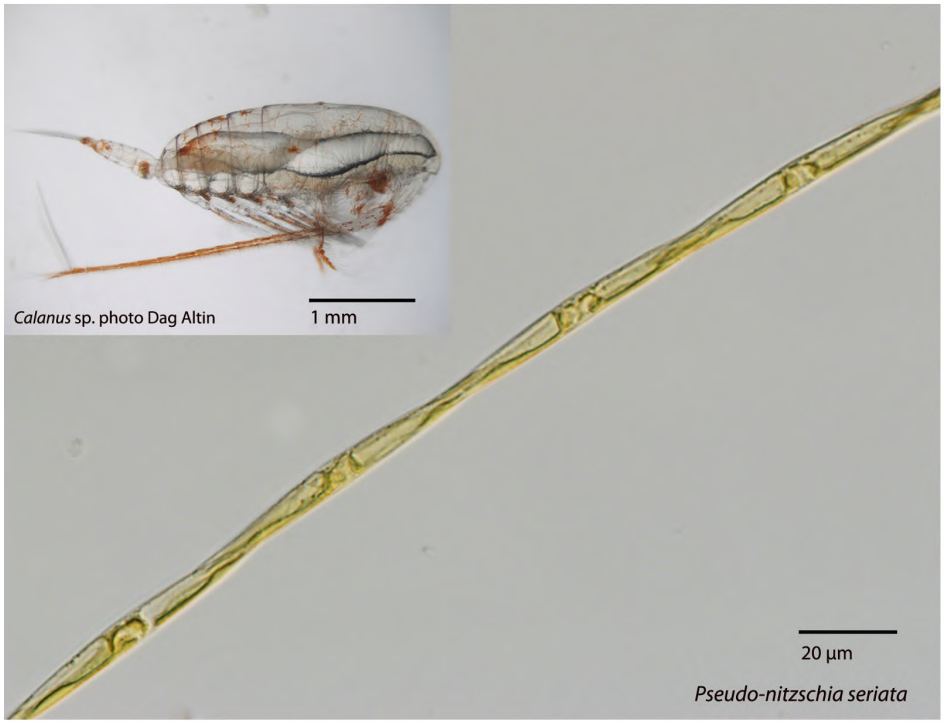
Toxicity in the laboratory and in the field – and copepods

It has been observed that *Pseudo-nitzschia* cultures tend to lose toxicity (toxin content per cell) over time in cul-

Table 1. Factors affecting production of DA in diatoms

Factor	Initial DA cell^{-1}	Final DA cell^{-1}	Times increase in DA content	Species	Ref
Copepod[1]	0.1	13.1	130.0	*P. seriata*	9
Copepod[2]	0.1	4.15	40.5	*P. seriata*	9
Copepod[3]	0.46	2.34	4.1	*P. seriata*	11
Copepod[2]	0.36	1.98	4.5	*P. seriata*	11
Copepod[4]	0.4	13.1	31.8	*P. seriata*	3
Copepod[4]	0.1	2.6	25.0	*P. seriata*	3
Copepod[5]	0.2	2.4	11.0	*P. seriata*	3
Copepod[4]	0.0*	0-2	24.0	*P. obtusa*	3
Copepod[2]	1.27	2.52	1.0	*P. multiseries*	10
↓pH (8.4→7.9)	2[6]	10[6]	4.0	*P. multiseries*	12
↓pH (8.4→7.9)	0.04[6]	0.07[6]	0.8	*P. multiseries*	12
↑pH (7.9→8.4)	1.9	4.2	1.2	*P. multiseries*	13
↑pH (7.9→8.4)	4.2	140	32.3	*P. multiseries*	13
↑pH (7.9→8.4)	158	213	0.4	*Nitzschia navis-varingica*	13
P depletion	0.04[6]	2[6]	49.0	*P. multiseries*	12
P depletion	0.07[6]	10[6]	141.0	*P. multiseries*	12
P depletion	1.0	5.9	5.9	*P.seriata*	14
Si depletion	BD**	14.7		*P.seriata*	14
Si depletion	0.002	0.3	125.3	*P.multiseries*	15
Si depletion	3.8[6]	11.9	3.1	*P.multiseries*	16
↑temperature	0.17[6]	17[6]	99.0	*P. multiseries*	17
↑temperature	0.12[6]	6[6]	49.0	*P. multiseries*	17
↑salinity	4.3	20.4	3.7	*P. multiseries*	18
Photoperiod short to long	1.4	2.4	0.7	*P. seriata*	19
Bacteria[7]	0.03	0.52	16.3	*P. multiseries*	20
Bacteria[7]	0.17	3.27	18.2	*P. multiseries*	20
Bacteria[7]	0.04	0.32	7.0	*P. multiseries*	20
Bacteria[7]	0.01	0.38	37.0	*P. multiseries*	20
Bacteria[7]	0.88	8.23	8.35	*P. multiseries*	20
↓Cell size	3.6	0.4	-0.9	*P. multiseries*	4
↓Cell size	2.4	0.05	-1.0	*P. multiseries*	4

**For calculation purposes set at the detection limit, **Below detection limits; [1]Calanus hyperboreus; [2]Calanus finmarchicus; [3]Calanus glacialis; [4]Calanus copepodites; [5]Calanus copepodamides; [6]Estimated from the publication; [7]Comparing axenic with non-axenic.*



ture and that these cells may end up as being non-toxic (see refs in [1]). This decrease in toxin content over time has been related to a simultaneous decrease in cell size [4], as *Pseudo-nitzschia* cells, like other vegetative cells of diatoms, decrease in cell size with time and thus contain less toxin per cell. This hypothesis has been supported by observations of *P. cuspidata* of which some (but not all) of the large cells after sexual reproduction are more toxic than the smaller parent cells [5]. Another explanation for the decrease in toxin content over time is related to bacteria. A correlation between decreases in *Pseudo-nitzschia* toxin content and simultaneous changes over time in bacterial communities in *Pseudo-nitzschia* cultures has been hypothesized. Because some bacteria have been shown to promote DA production whereas others degrade DA, changes in bacterial communities might potentially be an explanation [6]. Changes in bacterial communities over time have been observed in some studies, but not others [7, 8].

In recent studies on interactions between copepods and *Pseudo-nitzschia*, grazing copepods (like *Calanus* species) have been shown to trigger an increase in toxin production [3, 9-11]. We hypothesize that the reason for a decrease in DA production over time in laboratory cultures might be related to a lack of grazers in laboratory cultures. When exposed to grazing copepods, toxin production has been shown to increase up to 130 times, depending on exposure time (12 hours to 10 days) as well as concentration and species of copepod (Table 1, 2-5). One can easily imagine that the lack of such a potent toxin-trigger will have an effect. When establishing clonal cultures in the laboratory, single cells are removed from the natural environment and kept in pure cultures without exposure to grazers. The lack of the triggering factor that grazers exercise thus results in a continuous decrease in toxin production.

Toxin-inducing factors

A lack of copepod grazers may also explain why field data on cellular toxin content shows higher toxin levels than laboratory cultures of the same species from the same area [5]. But several other factors also influence production of DA. A preliminary comparison of some of the factors affecting DA production shows that toxin production may increase more than 100 times due to copepod grazing or depletion of phosphate and silicate, whereas presence of bacteria, salinity increase or pH increase showed a smaller degree of induction (See Table 1). This is not an exhaustive comparison and does not include the rate of toxin induction as the time of incubation was not available for many of the experiments, but may nonetheless give an indication of the importance of some of the factors affecting DA-production.

Apart from increasing toxin production in already toxic species/strains, presence of grazers may even induce toxin production in species previously considered non-toxic, as shown for *P. obtusa* [3]. It is therefore relevant to consider that all *Pseudo-nitzschia* species might have the potential to produce DA, given the appropriate triggering conditions. The results mentioned above illustrate the importance of considering different chemical, physical as well as biological factors when predicting and modelling toxicity of *Pseudo-nitzschia* in the field.

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Ecology of *Alexandrium* spp. in the Strait of Georgia, British Columbia, Canada 2015

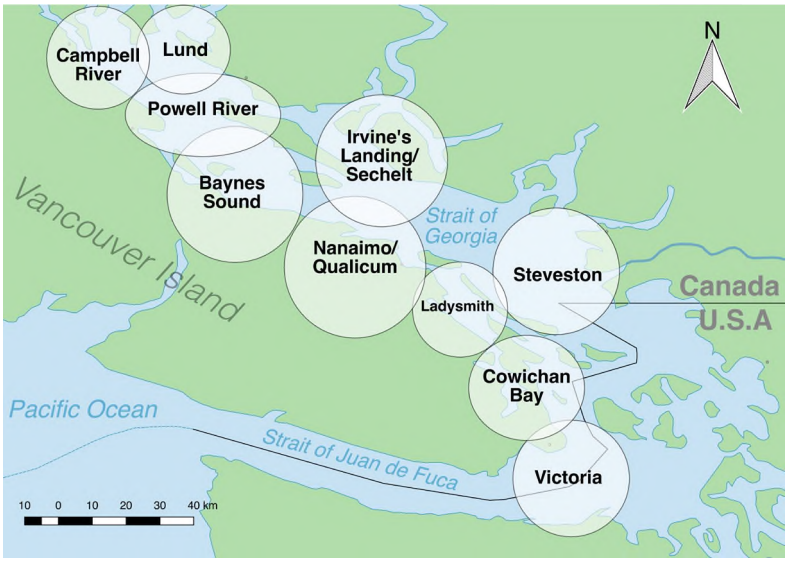

Fig. 1 Citizen Science sampling stations in the Strait of Georgia, Canada in 2015.

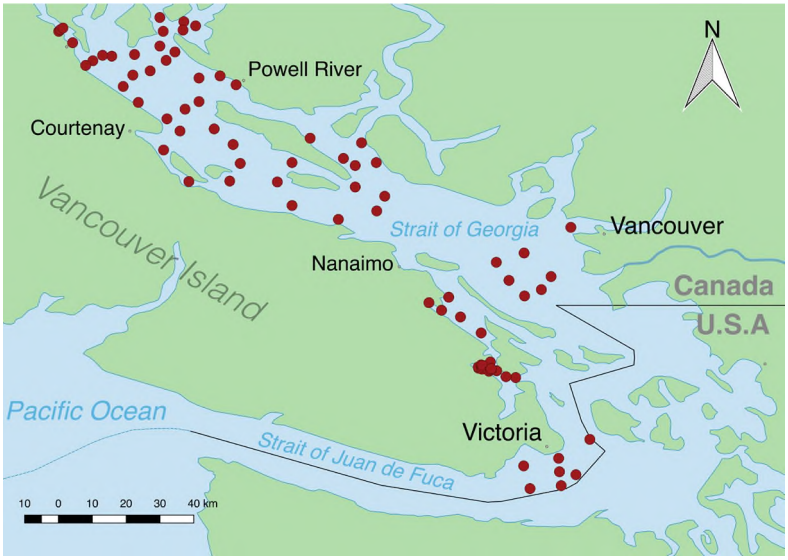

Fig. 2. Citizen Science sampling areas in the Strait of Georgia, Canada in 2015.

Coastal waters of British Columbia (BC), Canada have one of the longest documented histories of Paralytic Shellfish Poisoning (PSP) in the world [1, 2]. A monitoring program for the presence of toxins in shellfish was established in the 1940s [3]. Since then, PSP closures have been occurring every year. In the Strait of Georgia, PSP is primarily caused by the genus *Alexandrium*. Little is known about *Alexandrium* spp. distribution and its environmental preferences in the Strait of Georgia. Currently, the government does not have a harmful algae monitoring program in BC.

The Citizen Science Program was initiated in 2015 by the Pacific Salmon Foundation, the Department of Fisheries and Oceans Canada, and Ocean Networks Canada. The purpose of the program is to monitor the Strait of Georgia and provide data at a spatial and temporal scale not possible before. Properties/samples that are being measured/collected include phytoplankton, zooplankton, temperature, salinity, density, dissolved nutrients, fluorescence, oxygen, and turbidity. Sampling for phytoplankton is performed at approximately 80 sites (Figs. 1, 2) throughout the Strait every two weeks from February to October. At each site phytoplankton samples are collected from the surface (0 m). Among these 80 sites, there are ~10 stations where additional samples at 5, 10, and 20 m are collected. Nutrients and environmental data are collected at ~30 sites. The majority of the data can be found at the Ocean Networks Canada and Strait of Georgia Data Centre websites – *http://www.oceannetworks.ca* and *http://www.sogdatacentre.ca*. Here we share observations on distribution, abundance, and environmental preferences of *Alexandrium* spp. in the Strait of Georgia.

In 2015, 1037 sea surface water samples were collected in the Strait of Georgia. Of these samples, 107 (10.3%) contained cells of *Alexandrium* spp. *Alexandrium* spp. were observed most frequently in Ladysmith and Cowichan Bay samples, while least frequently in Campbell River, Nanaimo, and Steveston (Table1). It has to be noted, that the total number of samples collected in Ladysmith and Steveston in 2015 were very low (Ladysmith was an opportunistic area sampled by the Stz'uminus First Nations when possible and Steveston area was added to the Citizen Science Program half way through the sampling season). Thus, the frequency of occurrence in Ladysmith and Steveston is not as representative as in the rest of the areas, where sampling was done more systematically. As for the temporal distribution, cells of *Alexandrium* spp. were the most abundant in May – August samples (Table 1).

Cell densities. The majority of samples containing *Alexandrium* spp. showed low densities with only a few cells per mL (approximately 50% of the samples had ~ 1 cell mL^{-1} and 25% had ~2 cells mL^{-1}). The remaining 25% of the samples had densities of 3-18 cells mL^{-1} with all samples with densities > 8 cells mL^{-1} (N=15) occurring in Ladysmith and Cowichan Bay samples. Temporal patterns in these two areas (with averaged per area *Alexandrium* spp. densities > 1 cell mL^{-1}) are illustrated in Fig. 3 and Fig. 4.

Temperature and salinity. There were 48 cases when temperature and salinity were recorded (at 0.5 m) along with phytoplankton samples containing at least 1 cell mL^{-1} of *Alexandrium* spp. (at 0 m). *Alexandrium* spp. cells were observed in a wide range of temperatures from 9 to 22 °C and salinities from 20 to 31 ppt (Fig. 5). The highest densities of *Alexandrium* spp. (10-13 cells mL^{-1}, N=4) were observed in

Table 1. Number of water samples (N) collected by the Citizen Science Program, frequency of samples with Alexandrium spp. presence (Alex. +), and number of water samples with Alexandrium spp. cells present by month, Strait of Georgia 2015 (n/a - no samples were collected).

Area	Samples (N)	(%) *Alex.* +	Mar	Apr	May	Jun	Jul	Aug	Sep
Ladysmith	27	63	n/a	n/a	1	10	4	n/a	2
Cowichan Bay	149	26	1		10	n/a	18	6	4
Lund	128	13			3	6	5	1	1
Irvine Sechel	83	11	1		1		3	4	
Baynes sound	98	9		2	1	4		1	
Victoria	99	6				2	3	1	
Powell River	162	4	1		1	2		3	
Campbell River	144	2			1	2			
Nanaimo	107	2	1			1			
Steveston	40	0							

Table 2. Descriptive statistics of dissolved nutrients values (µmol/L) at 2 and 20 m when Alexandrium spp. cells were present at 0 m in 1 mL of the sample (N=20 samples)

	Nutrients at 0 m			Nutrients at 20 m		
	$NO_3 + NO_2$	SiO_3	PO_4	$NO_3 + NO_2$	SiO_3	PO_4
Min	0,03	11,30	0,24	10,70	8,97	1,31
Max	19,31	45,98	1,81	24,97	47,87	2,27
Mean	4,22	24,17	0,84	16,97	34,15	1,77
SE	1,18	2,25	0,09	1,13	2,16	0,07
Var	27,67	101,33	0,17	25,65	93,08	0,10

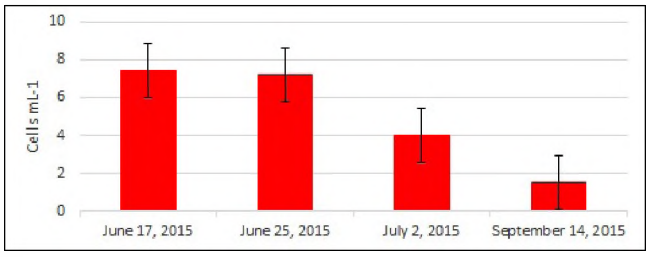

Fig. 3. Average density of Alexandrium spp. at the Ladysmith sites (N=5) from June to September, 2015

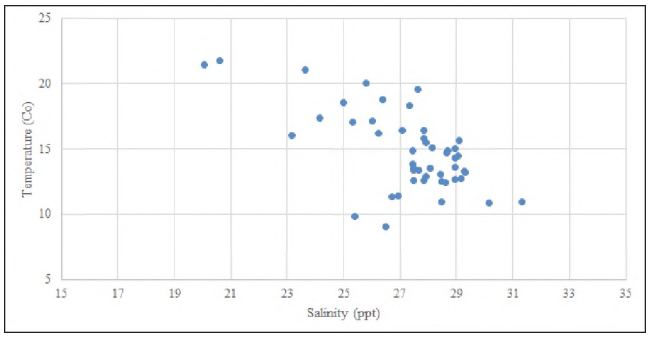

Fig. 4. Average density of Alexandrium spp. at the Cowichan Bay sites (N=13) from July to September, 2015

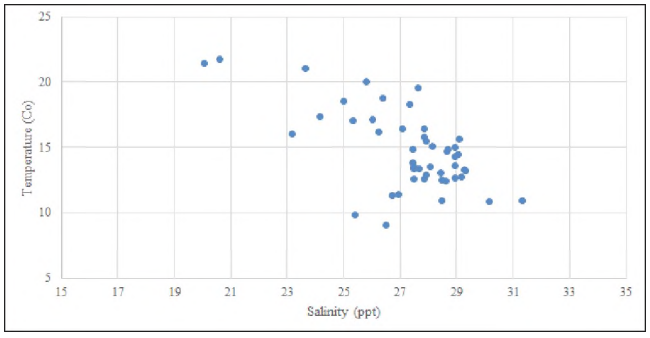

Fig. 5. Temperature and salinity values at 0.5 m when Alexandrium spp. cells were present at 0 m in 1 mL of the sample (N=48)

a range of 16-19 °C and 26-28 ppt.

Nutrients. There were only 20 cases when dissolved nutrient samples were collected (at 2 and 20 m) along with phytoplankton samples containing at least 1 cell mL^{-1} of *Alexandrium* spp. (at 0 m) and where nutrient values were above zero within analytical uncertainty. Nutrient values varied dramatically (Table 3) and due to the small sample size, no clear pattern between nutrient values and *Alexandrium* spp. presence was established. An interesting observation was that out of these 20 cases, there were only 2 with high *Alexandrium* spp. density (~8 and ~10 cells mL^{-1}; the remaining 18 cases had 1-3 cells mL^{-1}) and in both of these cases, nitrate and nitrite values (at 2 m) were relatively high (7.44 and 6.7 µmoles L^{-1}, respectively).

The year of 2015 was unusually warm due to El Niño. In the Strait of Georgia the majority of the total phytoplankton biomass was comprised of diatoms, while the dinoflagellate contribution was very low and silicoflagellates and raphidophytes were almost absent (Pacific Salmon Foundation, internal data). Hence, data on *Alexandrium* spp. distribution, abundance and environmental preferences reported here are limited and represent the behaviour of such harmful algae in an unusual year. The most important observation of the *Alexandrium* spp. distribution in the Strait of Georgia in 2015 was that location and season were the most important factors in *Alexandrium* spp. presence and absence. The Citizen Science Program supported by PSF is continuing the collection and analysis of samples through 2016 and 2017. This anticipated 3 year continuation of data collection will be highly beneficial in understanding the local ecology of PSP-causing species.

Acknowledgments

We are greatly appreciative to the numerous Pacific Salmon Foundation donors whose contributions fund the Salish Sea Marine Survival Project www.marinesurvivalproject.com and the Sitka Foundation for their generous support of the Strait of Georgia Data Centre www.sogdatacentre.ca. We thank all the Citizen Scientist crews and the Stz'uminus First Nations for diligently collecting samples across the Strait of Georgia. Thank you to Benjamin Skinner for producing maps for this report. Furthermore, we would like to acknowledge that the idea of preparing this report was conceived at the Marine Biotoxin Workshop 2016 organized by the BC Centre for Disease Control.

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A clarification of three unnamed raphidophytes previously described from British Columbia

An algal bloom implicated in the mass deaths of farmed salmon in Simoom Sound, BC, in early October 1990, was partly described in 1992 [1] and largely misdescribed in 2010 [2]. It was dominated by cold temperate raphidophytes, recorded, respectively as 'Raphidophytes 1 & 2', and *Chattonella* sp. and described in Waters et al [3] and Forbes & Waters [4]. The water temperature during the bloom was 13°C and the salinity 26ppt.

The *Chattonella* sp. grouping was a combination of two unrelated species, chiefly an unnamed dictyochophyte whose rapid change of form (see below) brings it into the genus *Vicicitus* as characterised by Chang et al [5] p. 409. The motile cells of *Vicicitus* sp. from this bloom were examined in a settling chamber using an inverted microscope. Most of the cells were globular, ~30-50 µm long and brownish to greenish gold in appearance. The chloroplasts were discoid, diameter 2.0-3.4 µm. Numerous minute osmiophilic particles in the cell periphery were interspersed with small vesicles. The cell recreated in Fig. 1 glided forward slowly, with the leading flagellum held straight, vibrating at high speed, while the other flagellum lashed around from time to time. The flagella arose together from a circular, conical, anterior depression that came into view when the cell turned downwards. After a while the cell flattened and suddenly became amoeboid, with several pseudopodia of different widths (see description in ([5] p. 408). These spread outwards fast, a long way, stopped briefly and returned slowly, all within 30 seconds (see [5] p. 408). This activity was repeated for at least 5 minutes most probably in an apparent attempt to find other cells (see [5] p. 408), but they were too far away and the amoeboid one eventually became inactive. The chloroplasts had single pyrenoids that showed up in the Lugol-fixed amoeboid cell in Fig. 2 #3. An elongated motile cell with very similar chloroplasts and the oval Lugol-fixed cell in Fig. 2 #3 were probably equivalent to the long cells formed by *V. globosus* in stationary growth (see [5] Figs. 7 & 8).

Raphidophyte 2 [3] (Fig. 2 #2), a bloom-forming species [4], may belong to the same genus as Raphidophyte 1 (below). A basic ventral view of a motile cell in Waters et al [3] is reproduced here as Fig. 3. Its jagged outline is due to its protruding extrusomes. The cells are dorsoventrally narrow, concave ventrally, convex dorsally, 8-13 µm long, 6-10 µm wide, with 4-6 yellow-brown discoid chloroplasts. The normal swimming action is a swivelling glide directed by the curving of the anterior flagellum.

Raphidophyte 1 [2]/F.J.R. Taylor's Undescribed chloromonad [6] is M.R. Droop's fragile, golden-brown Flagellate X [7]. Droop's species germinated at a water temperature of 6°C and formed a bloom at 7°C in upper Loch Striven, Argyll, Scotland, in early May 1979 [7]. It is described as follows: "Examination of live material (figure 5) suggested it to be a chloromonad related to *Vacuolaria* and *Gonyostomum*. It seemed especially similar to *Olisthodiscus*" (Droop et al [7]). The drawings in Droop's Fig. 5 are

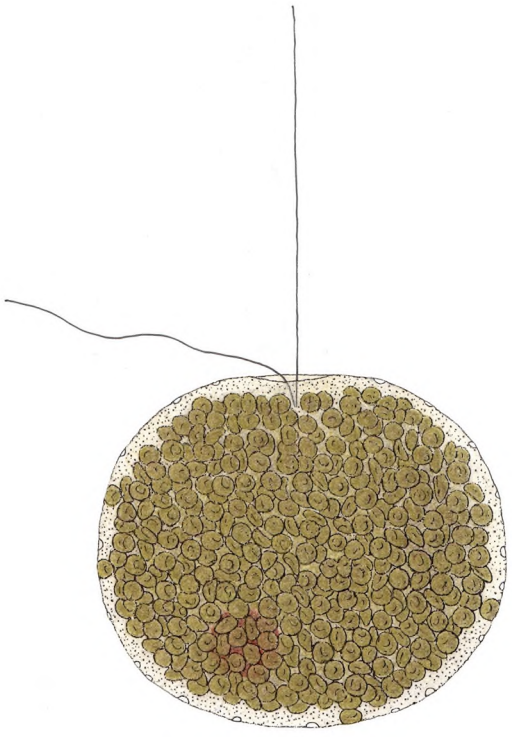

Fig. 1. Vicicitus sp. Motile cell, 47 µm long, 55 µm wide.

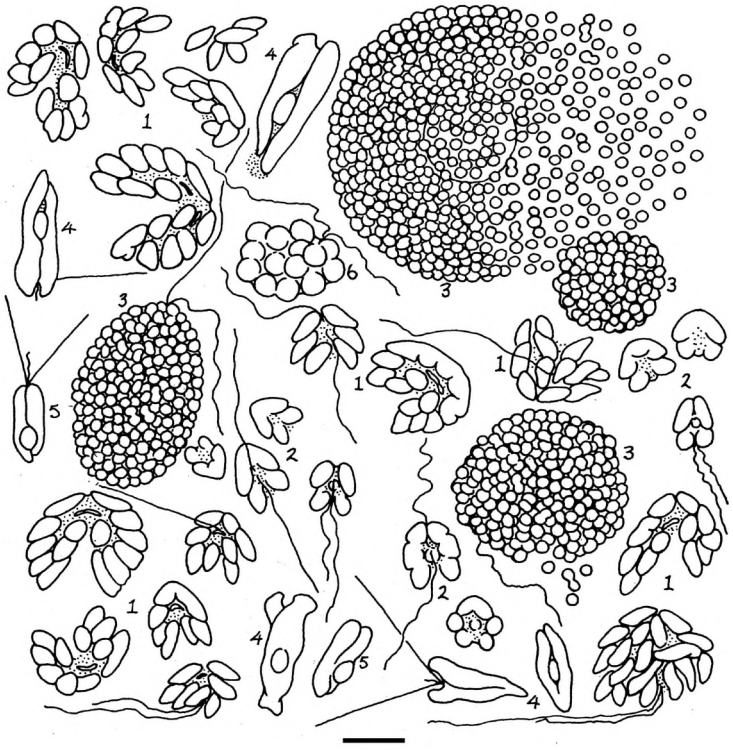

Fig. 2. Simplified depictions of six marine flagellates fixed with acid Lugol's solution. Scale bar 10 µm. #1: Raphidophyte 1 from the 1990 bloom; #2: Raphidophyte 2 from the 1990 bloom and ocean surface waters; #3: Vicicitus sp. from the 1990 bloom; #6 H. akashiwo: from the 1990 bloom.



Fig. 3. Raphidophyte 2 from a culture in 1974. Motile cell, ventral view 11.5 µm long.

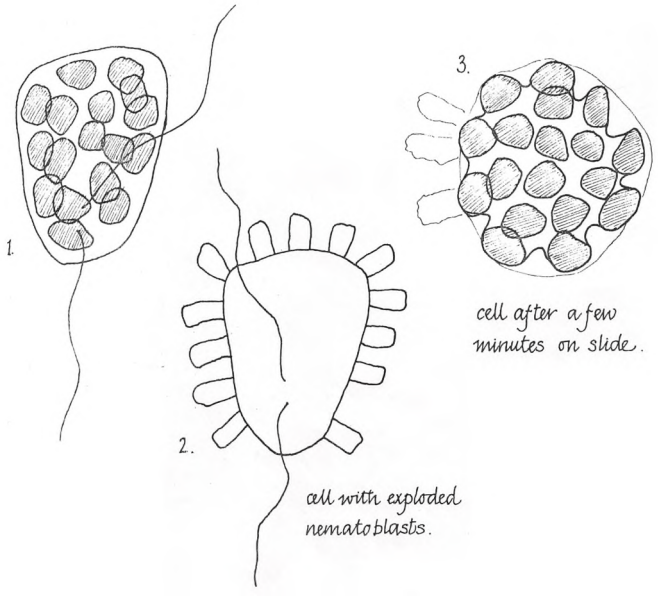

Fig. 4. Flagellate X from Droop 1979. Cell size about 20 µm. #1 Ventral face, anterior end down; note the thin and overlapping chloroplasts. #2 The same cell under stress, its ejected extrusomes apparently unfilled. #3 A cell shrunk by the apparent transfer of mucilage to the extrusomes, which have released it and dispersed (#1-3 captions are interpretations from the author).

reproduced here at a smaller size (Fig. 4) and are interpreted (in the figure caption) by the author.

Photographs of motile cells under stress received from Nicky Haigh (Microthalassia) (not shown here) reveal that the extrusomes are flexible and not the impediment to motility that they appear to be in Fig. 4 #2. They are more evenly distributed and more uniform in width except for constricted distal ends. Nicky's cells have a tubular posterior invagination on their ventral side and a corresponding shallow recess on their dorsal, which may explain why the cells in the 1990 bloom split open in the fixative (Fig. 2 #1). Those cells were growing fast when they were collected. Slow-growing cells have round chloroplasts in Lugol's-fixed material, where they can resemble *Heterosigma akashiwo* (Fig. 2 #6). Although the two species have different water temperature requirements, they can both be present at high concentrations in the same bloom in BC coastal waters [2,4].

Acknowledgements

I thank Patricia Smith for carrying out the OsO4 test on the *Vicicitus* and Nicky Haigh for the missing information.

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IOC-IAEA guide for designing and implementing a plan to monitor toxin-producing microalgae

The IOC and IAEA are proud to announce the publication of IOC Manuals & Guides no 59

This manual is intended as an introduction to basic analytical techniques that can be applied when designing a standard sampling protocol for both planktonic and benthic microalgae (and associated environmental conditions) and vectors of biotoxins (shellfish and fish). This standardization of methods will enable more robust data comparisons between countries and will yield improved risk assessments of potentially toxic HABs events. *http://unesdoc.unesco.org/images/0021/002145/214510e.pdf*



Autumnal algal bloom succession in northern coasts of Gulf of Oman

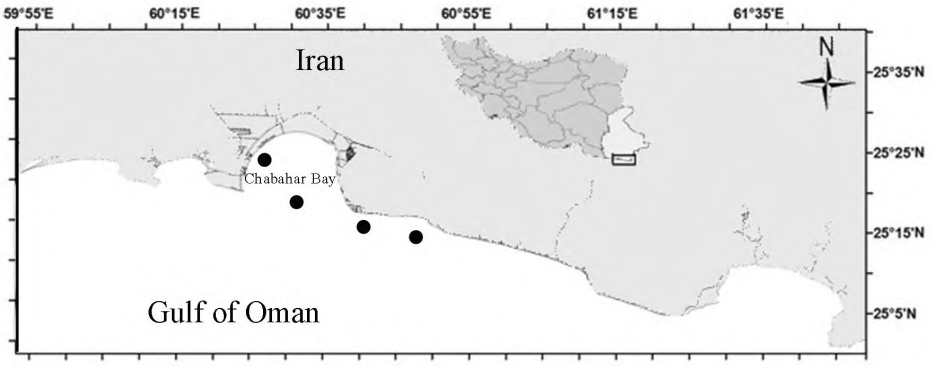

Fig. 1. Map of the study area

The composition of harmful algal blooms is determined by the diversity and abundance of different species present. Temporal fluctuations in phytoplankton populations can lead to a succession of different species dominating the phytoplankton community. Typically, phytoplankton blooms dominated by small and fast growing diatoms is followed by slow-growing larger diatoms which in turn is followed by the occurrence of large dinoflagellates [1]. Bloom succession is mainly controlled by physical factors, nutrient availability, mixotrophy and competition/predation [2]. The diversity within temporal phytoplankton bloom succession is discussed on the basis of decadal [3], annual [4], or seasonal [5] changes in species composition. Finer-scale changes (e.g. diurnal) may also occur [6]. The first phytoplankton bloom report in Gulf of Oman dates back to 1970s [7]. Since then, there have been regular reports of red tide incidents within the Gulf area which peaked in 2009 with more than 12 events in a single year [7]. To assess the autumnal succession of algal blooms on the northern coasts of Gulf of Oman, phytoplankton sampling was conducted irregularly (i.e. based on incidence of water discoloration) along Chabahar coasts (Fig. 1). Bloom species were identified and counted using light microscopy. The first detectable discoloration occurred on 26 October 2016 with a dominance of *Alexandrium* sp. (Table 1). This event was followed by a extensive bloom of *Prorocentrum* sp that lasted three days. This bloom resulted in the failure of a desalination plant in the city as a result of clogging. Next, an outbreak of *Cochlodinium polykrikoides* covered nearly the whole Chabahar Bay (i.e. from Ramin coast to Chabahar Bay). Cell densities at the time of the bloom exceeded 1.5 x 10^6 cells L^{-1} (Table 1). Approximately 20 days later, a green tide reported by local fisherman, occurred along almost the whole coast.

Fine examination of water samples indicated predominance of green *Noctiluca scintillans* (Fig. 2) in surface waters (Table 1). Finally, a dense bloom of *Mesodinium rubrum* (Fig. 2) occurred on 4th December 2016. To our knowledge this is the first report of *Mesodinium rubrum* blooms in the Gulf of Oman. During the course of these algal blooms, the maximum pH, temperature and dissolved oxygen coincided with the *Cochlodinium polykrikoides* bloom, while maximum salinity was recorded at the time of the *Noctiluca scintillans* bloom (Table 1). Recent evidence suggests that dinoflagellates are replacing diatoms (e.g. *Lauderia punctata*, *Chaetoceros* spp., *Guinardia striata* and *Thalassiosira* spp.) in the Gulf of Oman and becoming dominant the HAB species [8]. In this case, controlling future algal blooms may become more difficult in the region since dinoflagellate blooms are less predictable and more ephemeral compared to diatom blooms [9].



Fig. 2. Top, green tide of Noctiluca and bottom, bloom of Mesodinium rubrum in Chakbahar, Gulf of Oman, autumn 2016.

Table 1. Maximal mean density of the observed bloom species and physical-chemical properties of the near-shore waters during autumnal algal blooms in the northern Gulf of Oman

Date	Species/ Genus	Mean density maxima (×10⁶)	Temperature	Salinity	pH	$DO_{(mg/L)}$
26 October 2016	*Alexandrium* sp.	1.5	24.4	36.3	8.10	8.30
04 November 2016	*Prorocentrum sp.*	1.53	25.5	37.8	8.00	5.10
09 November 2016	*Cochlodinium polykrikoides*	1.7	27.0	36.4	8.40	11.20
29 November 2016	*Noctiluca scintillans*	0.007	25.1	36.6	8.14	6.58
03 December 2016	*Mesodinium sp.*	3.1	25.5	36.6	8.25	9.50

Acknowledgements

We are grateful to Dr. Malcolm Baptie, Dr. Andrey A. Kovalchuk, and Dr. Jeroen Van Wichelen for their assistance in species identification.

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6th Congress of the International Society for Applied Phycology (ISAP)



Over 400 scientists and producers of micro- and macro-algal products are expected to meet during the *6th Congress of the International Society for Applied Phycology* in Nantes from the 18-23 June 2017. To join them see the website for details: *https://isap2017.sciencesconf.org/*

Nodularin accumulation in New Zealand shortfin eel from Lake Forsyth/Te Wairewa

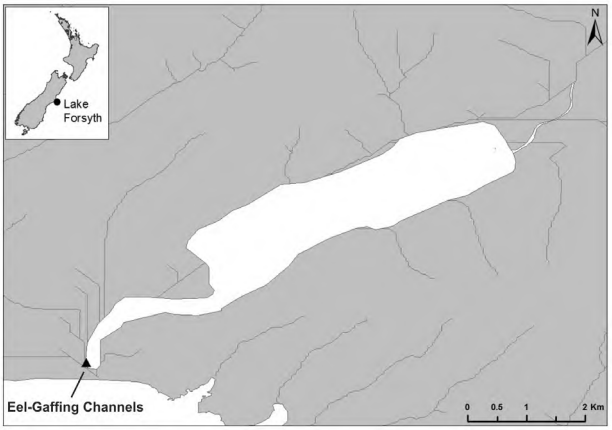

Fig. 1. Map showing Lake Forsyth/Te Wairewa, the eel gaffing site and the location of the lake in New Zealand (inset).

Lake Forsyth is a small coastal lake on the Banks Peninsula in the Canterbury region of New Zealand; 7.8 km long and 1.3 km at its widest point (Fig. 1). The Māori name for the lake, *Te Roto o Wairewa*, means 'water lifted up' and in Māori legends Te Wairewa was the last lake to be dug out by Rākaihautū as he sculpted the lakes and mountains of the South Island of New Zealand. The lake is very important to the local Rūnanga (tribe) and has provided an important food source, both historically and at present. In early historic times Lake Forsyth/Te Wairewa was an estuarine environment, however persistent deposition of gravel from nearby rivers eventually led to the closing of the estuary mouth. The closure and a significant increase in sediment flow into the lake over the last 150 years due to deforestation has, in part led to a decline in the lake's water quality [1] and since the early 1900s it has been described as eutrophic, suffering from persistent blooms of the cyanobacterium *Nodularia spumigena* (Fig. 2).

The water levels of Lake Forsyth/Te Wairewa must be artificially controlled to mitigate the risk from land inundation. To do this, a channel was cut through the gravel to link the lake to the sea. Typically the shortest route was taken. The channels are temporary; quickly filled in due to the high-energy waves moving gravel along the shore plus the dominant drift of sediment down the coast from the braided rivers located to the north. The earliest recorded artificial opening of the lake was in 1866 [2] and, from the 1940's onwards, openings have been carried out regularly. In 2008, the *rūnanga* attempted to create a permanent opening by constructing a 900 m canal and 300 m groyne at the eastern end of the beach against the cliffs. Although the end of this canal can become blocked, it is a more straightforward process to re-open it than creating a beach channel. The flow rates of the canal are also considerably lower than that generated from a beach channel. This has an advantage of being able to control lake levels more effectively and the less turbulent flow-rate may assist in the recruitment of elvers (glass eels) during spring openings. There has been some improvement in water quality and lake ecology over this time (e.g., growth of macrophyte beds and resulting clear water within the beds), which may be attributed to better control of lake levels. However, the lake has had a recent downturn in 2016, with some of the most severe and prolonged cyanobacteria blooms reported to-date. As *Nodularia spumigena* commonly produces the hepatotoxin nodularin, farm stock deaths have been encountered along the shores of Lake Forsyth/Te Wairewa. In 2016, 30 sheep were reported to have died after drinking from the lake, as well as several dogs belonging to nearby residents.

Lake Forsyth/Te Wairewa is a valuable wetland ecosystem, providing homes for a variety of waterfowl and aquatic animals such as flounder, bullies, whitebait, banded kōkopu and eel (or tuna in Māori). Introduced fish species such as perch and brown trout can also be found in the lake. As nodularin has been shown to accumulate in aquatic animals [3-7], we investigated the accumulation of the toxin in shortfin eel (*Anguilla australis*) from the lake [8] . Shortfin eel are native to New Zealand, but are also found in south-eastern Australia and some Pacific islands. In many of New Zealand's freshwater food webs they are the apex predator, and as opportunistic scavengers, they play an important role in recycling nutrients. They are primarily found in lowland lakes, estuaries and the lower reaches of rivers, and can reach a maximum size of approximately 3 kg and 1.1 m in length [9]. They are catadromous, and on reaching maturity (6-24 yrs for males, 10-34 yrs for females), the eels stop feeding and migrate up to three or four thousand kilometres over approximately four months to a deep water spawning ground somewhere off New Caledonia [10].

In New Zealand, tuna (eels) have huge customary and cultural significance to the indigenous Māori population. They are integral to their whakapapa (genealogies or stories which create a foundation of meaning for the people) and are an important mahinga kai (traditional food source). In recognition of the importance of eels to Māori, the New Zealand government set aside a number of waterways for eel fishing by only the local Māori people, including Lake Forsyth/Te Wairewa. In autumn, migrating eels gather at the gravel bar separating the lake from the sea where small channels are dug into the gravel bar at the bottom of the lake (Fig.3A; Site indicated on Fig.1). As the eels gather in the channels, they are gaffed (speared) using a pole with a sharp hook on the end (Fig. 3B). Historically, up to several hundred eels were harvested per night when conditions were suitable. More recent studies suggest that shortfin eel populations in the lake are in a state of decline [11].

Because of the persistent cyanobacterial blooms and the human consumption of these eels, we assessed if nodularin accumulated in shortfin eels from Lake Forsyth/Te Wairewa, and whether the concentrations would pose a risk to the health of humans who consume them [8]. Nodularin is heat-stable and thus not destroyed by cooking. With the help of the local rūnanga and others we collected migratory and feeding (non-migratory) shortfin eels, and analysed the liver and muscle tissue for nodularin content. Surface water samples were also collected to assess the concentration of *N. spumigena* and nodularin in the water. The samples were collected opportunistically between 2004 and 2014.

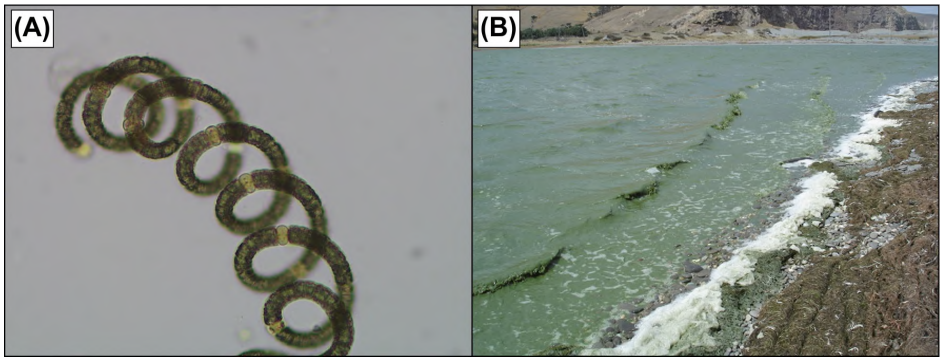

Fig. 2. Nodularia spumigena from Lake Forsyth/Te Wairewa at 400× magnification (A) and blooms of the cyanobacterium at the lake in 2006 (B).

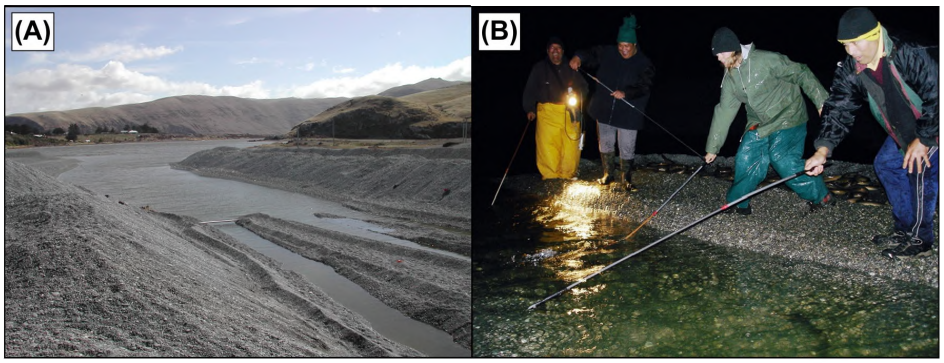

Fig. 3. Eel fishing channels at Lake Forsyth/Te Wairewa (A) and members of the rūnanga of Wairewa catching eels in the channels by gaffing (B; photo provided by Jack Jacob).

The data from the water samples indicated that there was seasonal variation in concentrations of *N. spumigena* and nodularin, but that the severity of the blooms were decreasing over the study period [8]. Being able to maintain higher lake levels via the new canal may have contributed to these decreases in *N. spumigena* blooms. However, high concentrations of other phytoplankton (including cyanobacteria) remain persistent in the lake and it is still considered hypertrophic [12]. In December 2016, a statutory plan for reducing sediment and phosphorus inputs into Lake Forsyth/Te Wairewa was announced and this may lead to further reductions in phytoplankton blooms in the lake.

In the eel samples, higher nodularin concentrations were observed in the liver of the eels than in the muscle tissue of the corresponding specimen. This was consistent with similar studies on nodularin accumulation in aquatic animals [4,13,14]. The highest nodularin concentrations detected in the eels from Lake Forsyth/Te Wairewa (29 µg kg^{-1} wet-weight in muscle tissue and 147 µg kg^{-1} wet-weight in liver) [8] were within the same order of magnitude for those reported in aquatic organisms gathered from other ecosystems experiencing *N. spumigena* blooms (473 µg kg^{-1} dry-weight in flounder muscle tissue, 139 µg kg^{-1} dry-weight in mussels and 399 µg kg^{-1} dry-weight in flounder liver) [4,7,13]. Mulvenna et al. [15] suggested a guideline for safe human consumption of microcystin and/or nodularin in fish of 24 µg toxin kg^{-1} (for a 2-16 year age group). The concentrations in the muscle of shortfin eels from Lake Forsyth/Te Wairewa exceeded this suggested safe human consumption guideline only on one occasion (29 µg kg^{-1} wet weight). Concentrations detected in the liver exceeded the suggested value in 36% of samples analysed. Fortunately, local Rūnanga (who are the main consumers of the eels) currently remove the liver prior to consumption, and removal of the gut/organs would be recommended for anyone consuming aquatic foodstuffs from bloom-affected waters.

Shortfin eels from Lake Forsyth/Te Wairewa are an important mahinga kai source for the local rūnanga of Wairewa and are a taonga species (treasured species) around New Zealand. Our study suggested that, provided the liver (and other intestinal organs) are removed, the risk of adverse effects to human health from consumption of the eels was low [8]. The data from our study also indicated that the concentrations of nodularin in the water column impacted on the levels detected in the eels. Therefore, the assumption of low human health risk may need to be revisited now that intense and prolonged blooms have returned to Lake Forsyth/Te Wairewa. While the risk to humans may be considered low, the consequences of nodularin exposure on the eels remains unknown. We suggest that better understanding of these effects and improving knowledge on the transport of nodularin through trophic levels should be a priority for future research in this area.

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The Scottish Coastal Observatory

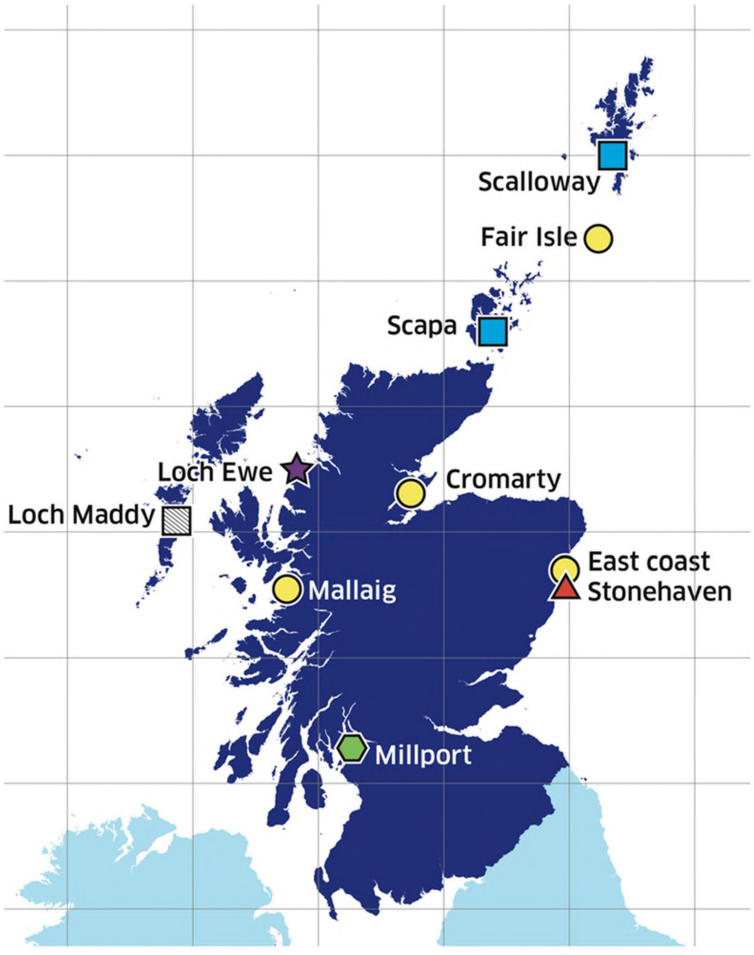
Scotland's coastal environment is subject to inherent variability resulting from short term processes such as tides and weather, the seasonal cycle, multi-annual cycles such as the North Atlantic Oscillation and larger scale influences such as climate change and ocean acidification. In order to understand how these processes impact the ecosystem goods and services produced in Scottish coastal waters, long term time series of data are needed.

Scientists in Marine Scotland Science (MSS), along with a small group of voluntary citizen-scientists, have been monitoring the physics, chemistry and biology at multiple sites in Scotland's coastal waters since 1997. Sustained time series of ecological data such as these are scarce and interest in this data set is growing at both a national and international level, both for assessments of the status and health of our marine ecosystems as well as for input to blue-skies research, such as project proposals and studentships.

MSS has recently re-badged this monitoring programme as the Scottish Coastal Observatory (SCObs). As a first step to improve information and the availability of data from this programme, a three part report [1] has been compiled to provide a basic description of the seasonality and variability of the main parameters examined between 1997 and 2013. These include temperature, salinity, nutrients, carbonate chemistry, chlorophyll *a*, algal toxins measured using passive sampling, phytoplankton and zooplankton. A chapter summarising supportive meteorological (air temperature, frost, sunshine) and river flow data has also been included. The data used in this report are available on request with a summary of monthly means of the parameters described in the report available for download [2].

HAB highlights include a description of the regional and interannual variation of shellfish toxin producing phytoplankton genera at the SCObs monitoring sites. A slight increase in the abundance of *Alexandrium* was observed at monitoring sites on the west coast towards the end of the period covered by the report. The diatom *Pseudo-nitzschia* was present in nearly every phytoplankton sample analysed but in contrast to the other SCObs monitoring sites is not a component of the spring bloom diatom community at the monitoring site at Scalloway in the Shetland Islands. *Dinophysis* cell densities showed the most variability between sites and years with cell densities increasing in at the monitoring site at Millport in the Clyde, and decreasing at Scapa Bay in Orkney during the monitoring period covered by [1]. Passive sampling methods revealed the increased detection of the algal toxin, azaspiracid, in the water column during winter months.

Monitoring at the SCObs sites is ongoing. Data from SCObs is a national resource and its value increases the more it is used. If you would like to find out more about SCObs or use these data, please email *Scobs@gov.scot.*



Map of monitoring stations in the Scottish Coastal Observatory. The different symbols represent the different combination of parameters measured; temperature only, temperature and phytoplankton, temperature, salinity, phytoplankton, temperature, salinity, nutrients, algal toxins, phytoplankton, temperature, salinity, secchi depth, nutrients, pigments, algal toxins, phytoplankton, zooplankton, temperature, salinity, secchi depth, nutrients, carbonate chemistry, pigments, phytoplankton, zooplankton.

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The "RedFAN"network: a working group to deal with HABs in Mexico

In October 2014 the network RedFAN (from the acronym in Spanish: "**Red** temática sobre **F**lorecimientos **A**lgales **N**ocivos) was created with the financial support of the Mexican National Council of Science and Technology (CONACyT). This network integrates the academic community (researchers and students), the public health, production and government sectors interested in problems related to harmful algal blooms in the coastal and offshore marine environments. The goal of RedFAN is to contribute to the understanding of HABs, their impact on the public health and ecosystems and, under an interdisciplinary approach, to propose mitigation actions and management plans for HABs in Mexico. This network looks for the integration of different social sectors affected by HAB phenomena to consolidate collaboration and to elaborate and implement a National Plan to respond to the increasing problem of the presence of HABs and their impacts in Mexico.

The organization of RedFAN started in 2014 with a small group of colleagues working on diverse aspects of harmful blooms in Mexico. In one of the working sessions, Juan Blanco (CIMA, Spain) shared his experience and made recommendations. A working plan was developed and five programs were created focused on the study of HABs: ecology, autecology, toxinology, taxonomy, mitigation, and socioeconomic impacts. An academic technical committee leads the actions and operation of the network.

Currently RedFAN has 120 members: 98% belong to the academic sector, i.e. researchers and students from Universities and research centers. Only 2% of the membership is from public health departments and the private sector. Most members represent institutions situated in Mexican coastal states. Participants of interior states are practically missing and only members from Mexico City (capital of the Country) form part of the network.

Activities began formally in June 2015 with a workshop on HAB in shelf waters, held in Cuernavaca, Morelos. It was followed by a workshop on emerging toxins held in La Paz, Baja California Sur in November, with Andrew Turner (CEFAS, UK) as a special guest. A last workshop on science dissemination was held in Ensenada, Baja California in December of the same year. In addition to these workshops, an introductory course on the study of HABs was created and has been offered to pre and post graduate students. This course provides an introductory overview of different aspects of HABs and presents the state of knowledge about them in Mexico. The course has been already held in Ensenada, Baja California in September 2015 and in La Paz, Baja California Sur in 2016. Professors from different institutes and expertise, all of them RedFAN members, participated in the course. Students (around 20), most of them supported by RedFAN, were from different regions of Mexico and from Central and South America (Panama and Peru).

In 2016 different actions for the internationalization of RedFAN were undertaken. The RedFAN network supported a research stay of Allan Cembella AWI, Germany) in La Paz, B.C.S., Mexico, where he collaborated with colleagues and students of two research centers: IPN-CICIMAR and CIBNOR.

Continued at page 21

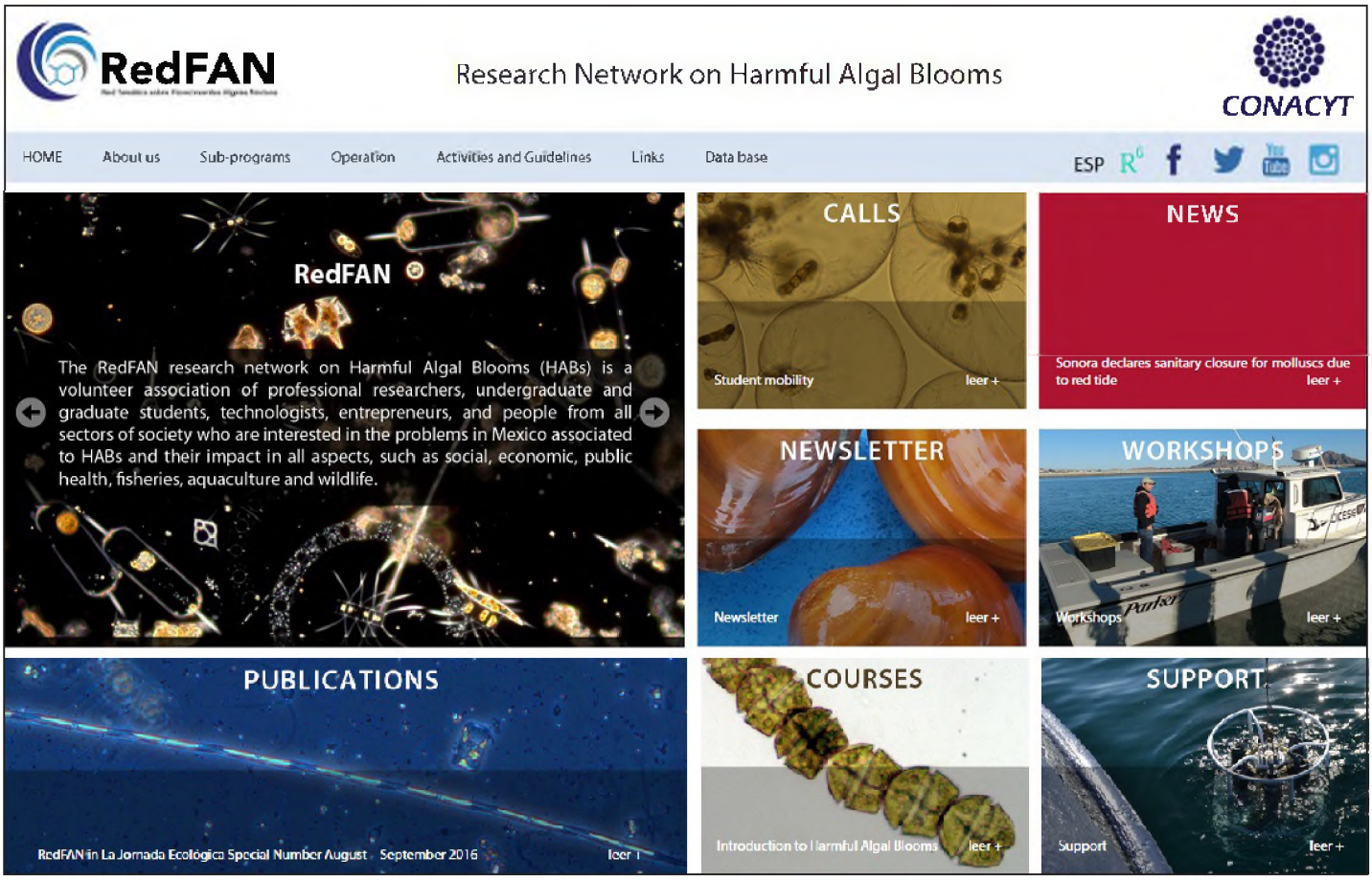

Fig. 1. Web page of the Mexican Network RedFAN (http://redfan.cicese.mx)

Caribbean Ciguatera Experts Discuss a Risk Management System for the IOCARIBE Region

Over the past three decades the incidence of ciguatera has increased in the Caribbean countries by about 32% [1]. Despite the evidence of toxic events and their potential consequences on public health, fisheries and tourism, very few countries in the region have developed management plans to address this issue. To analyze the situation, IOCARIBE, ANCA and the CCO (Colombian Ocean Commission), convened a workshop of Caribbean experts.

The objective of the meeting, held on 17th and18th August, 2016 at INVEMAR (Santa Marta, Colombia), was to propose a risk management system for the Greater Caribbean concerning HAB events, with a particular focus on Ciguatera Fish Poisoning.

Representatives from many institutions – the General Maritime Director, the National Navy of Colombia, the Ministry of Health and Social Protection, the National Institute of Health, Health Secretariats of Sucre, San Andres Island, Antioquia and Magdalena, the National Authority for Aquaculture and Fisheries, INVEMAR, National University of Colombia, and the Colombian Commission of the Ocean - attended the workshop. In addition, there were participants from Cuba (National Toxicology Center of Cuba and Fisheries Research Center) and Panama (University of Panama and the Panamanian Maritime Authority).

During the workshop, the status of ciguatera in the IOCARIBE region was discussed, with particular emphasis on medical issues. Cuba, Colombia and Panama, presented national reports, showing some similar problems and the mechanisms developed to deal with ciguatera problems in each country. The experts analyzed the main needs in the region in terms of knowledge, resources and legislation.

Resulting from the meeting was a recognition by the experts of the need for a regional risk management system for the Greater Caribbean to deal with ciguatera fish poisoning. This system should incorporate the largest number of IOC Member States impacted, coordinate actions with other regional initiatives and projects and optimize the existing infrastructure in the region.

The IOC-ANCA group, supported by IOCARIBE, should continue to serve as a coordination mechanism to strengthen this system. In this respect participants agreed upon specific commitments, such as:

- Preparation of guidelines for research and capacity building .
- Summarize the status of Ciguatera in each country.
- Assess the gaps, requirements, constraints, and bottle-necks of the system.

To contribute to the global IOC strategy on Ciguatera and reduce vulnerability through the generation of knowledge about the biology, ecology, physiology and toxicity of benthic microalgae present in marine ecosystems, the experts proposed to develop a joint macro-regional plan with WESTPAC (ANCA-WESTPAC).

Acknowledgements

We are grateful to IOCARIBE, Ministry of Health and Social Protection, CCO and INVEMAR, for financial and technical support for the meeting.

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Fig. 1. Workshop of Caribbean Experts on Ciguatera: Participants attending the IOCARIBE-ANCA-CCO Workshop on Ciguatera at INVEMAR (Santa Marta, Colombia), 17-18 August 2016

IOC/WESTPAC Scientists Shape the Future of Regional HAB Research

More than twenty experts from countries in the Western Pacific gathered in Nha Trang, Vietnam, 19-22 December 2016 to foster cooperation and define research priorities for Harmful Algal Blooms (HABs) in the region (Fig. 1).

The East and Southeast Asian countries in the Western Pacific are populated by more than 3 billion people whose activities have been inextricably linked to ocean, with large impacts on the surrounding coastal waters and marine ecosystems.

Primarily associated with escalating human activities, coastal aquaculture, nutrient inputs and water quality degradation in coastal systems are expected to increase in many areas in the region. According to recent reports, the occurrence of HABs and their social-economic impacts seem to be increasing in the WESTPAC area. Therefore, it is essential for HAB scientists in the region to provide scientific input, transfer of technology, and bridge the gap between science and management in order to better assist countries in mitigating the negative impacts of HABs.

The regional workshop entitled "The Development of a Research Strategy for Harmful Algal Blooms: what we know, and what we do not know on HABs", was initiated by the UNESCO/IOC Sub-Commission for the Western Pacific (WESTPAC), and kindly hosted by the Institute of Oceanography, Vietnam. The workshop was structured around three objectives:
1) to review current knowledge on HAB; 2) to identify scientific problems/questions/gaps, and 3) to develop a research strategy for HAB sciences in the region.

Following a series of institutional and national reports on current status and issues on HABs, scientific reviews were provided by some leading scientists to cover various aspects of HAB science in the region, including: (1) the biology and ecophysiology of HABs species (taxonomy, phylogeny and geographical distribution, bloom dynamics); (2) marine biotoxins (responsible species and their toxins, and fate of toxins in ecosystems); (3) fish kills mechanisms (causative mechanisms, including factors related to harmful substances and environment) and (4) socio-economic aspects of HAB outbreaks (economic losses, preventive measures and management) (Fig. 2).

Throughout the four-day workshop, all participants demonstrated their passion for closer cooperation among institutions and countries in the region. Lively discussions took place about the need for a regional research strategy with a view to addressing national and/or regional specific HABs and synergiz-



Fig. 1. Participants of the WESTPAC-HAB Workshop at the Institute of Oceanography, Nha Trang, Vietnam, 19-22 December 2016



Fig. 2. Talk on management and socio-economy of HABs by Dr. Kazumi Wakita.



Fig. 3. Prof. Gires Usup, IPHAB vice-Chair, and Dr. Po Teen Lim, GlobalHAB SSC member, (first and second from left).

ing the ongoing HAB research and capacity building efforts at these levels. Dr. Po Teen Lim presented the science and implementation plans of the IOC-SCOR sponsored new GlobalHAB research program (Fig. 3). It was widely recognized that national and regional efforts are complementary to global efforts, which form a strong and essential basis to achieve the global objectives of HAB science. The workshop further identified the importance of multidisciplinary work to clarify bloom forming mechanisms, fish stress and fish mortalities and shellfish toxin accumulation (including fate of toxins in shellfish and why shellfish do not depurate toxins).

After the workshop, continued efforts are ongoing to develop a draft strategy and associated outreach materials. The draft will be distributed for further inputs during the 10th WESTPAC International Scientific Conference, 17-20 April 2017, in Qingdao, China to encourage participation of HAB scientists in the region, and to strengthen networking and involvement of HABs researchers in both regional and international programs.

Acknowledgements

We are grateful to Dr. Dao Viet Ha (Vice Director, Institute of Oceanography, Nha Trang, Vietnam) for hosting the event and heartfelt hospitality provided during the workshop.

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IOC-SCOR GlobalHAB Scientific Steering Committee Meeting

The GlobalHAB programme was launched in January 2016, with financial support the the U.S. National Science Foundation (through SCOR) and from IOC. During this first year, the Scientific Steering Committee of GlobalHAB elaborated the draft of the Science and Implementation Plan of the programme. This document was based on suggestions from the international HAB community participating in the Open Science Meeting in Paris, April 2013, at the sunset of the former program GEOHAB. The draft was also enriched by many other contributions during the first year of the programme and subsequent inputs at the International Conference on Harmful Algae in Florianópolis, Brazil (October, 2016) and submitted to external revision.

The GlobalHAB SSC members will held their second meeting in Naples, Italy, from 28 to 30 March 2017. The objectives of this meeting will be to finish edition of the revised Science and Implementation Plan. This document constitutes an addendum to the GEOHAB Science Plan. An analysis of the activities that can be implemented at short (1-2 years) and longer (until 2025) term, will also be conducted. The information will be soon available at the new webpage *www.globalhab.info*.

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ICES-IOC Working Group on Harmful Algal Bloom Dynamics

The ICES-IOC WGHABD will meet on 25 - 28 April 2017, at the Finnish Environment Institute, Helsinki, Finland. Terms of reference to be addressed include production of a status report about harmful algal events in the ICES area, national reports, new findings and the ecology of *Alexandrium ostenfeldii* and cyanobacteria from the Baltic.

For more information, contact the WGHABD Chair at: *E.Bresnan@marlab.ac.uk*

13th Session of the IOC Intergovernmental Panel on Harmful Algal Blooms

The thirteenth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IOC-IPHAB) will be held from 3 to 5 of May 2017 at the IOC headquarters in Paris, France. Detailed information on the agenda, list of participants and working documents will be available at: *http://hab.ioc-unesco.org/* (Upcoming Events).

By 1st April 2017, Member States should send detail of their delegation, comments on the draft provisional agenda, name of candidates to chair discussion panels, and nominate candidates for the post of Chair and Vice-chair of IPHAB. Member States which never participated in IPHAB sessions are invited to do so and to send a letter of interest for the programme together with the detail of their delegation to the session of its intergovernmental body.

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In memory of Paul Harrison

With great sorrow, we learned of the recent unexpected passing of our colleague, mentor and friend Paul J. Harrison. Among his many contributions, Paul was a founding member of the Scientific Committee on Oceanic Research (SCOR) working Group 137 "SCOR WG 137 " Global Patterns of Phytoplankton Dynamics in Coastal Ecosystems: Comparative Analysis of Time Series Observations" and the recently-formed IOC Working Group to "Investigate Climate Change and Global Trends of Phytoplankton in the Oceans" (IOC WG TrendsPO). Few people have had a larger impact on the oceanographic community than Paul. He was exceptional through his contributions as a scientist, and his ability to motivate others. Paul had a rich life that included a deep commitment to family, and the people he worked with. He also loved to share his excitement and knowledge of the oceans and never missed an opportunity to include family when possible. He was doing both this past December, giving natural history lectures to passengers on a Caribbean cruise, accompanied by members of his family. Unexpectedly, he became very ill while on board and passed away due to complications associated with influenza.

Paul grew up on a 300-acre farm and attended a one-room schoolhouse near the town of Uxbridge, northeast of Toronto. He had a passion for plants, and after completing a Bachelor's degree at the University of Toronto, he went on to do a Master's degree in plant ecology at the University of Guelph. While attending university in Toronto, he met Victoria; they were soon married and over the next 51 years embarked on many adventures together. The first big adventure was after Paul graduated from the University of Guelph. They signed up with CUSO (Canadian University Students Overseas) and headed off to Ghana on a two-year teaching contract. They used this opportunity to travel the Nile, climbed Mount Kilimanjaro and travel throughout East and West Africa. It was also here that they charted a course for the future that included pursing a PhD in oceanography with Dick Dugdale at the University of Washington.



Paul and Victoria Harrison. Photo from Rachel Harrison

Paul entered graduate school in 1967 and was soon interrogating phytoplankton and exploring their physiological response to varying nutrient supplies. This work led to a series of seminal papers on the nutrient physiology of diatoms and was the stimulus that led Paul to begin his lifelong pursuit of understanding phytoplankton growth, their population dynamics and their central role in ocean ecosystems.

Most of Paul's career was at the University of British Columbia, where he began a faculty position in the Institute of Oceanography and the Department of Botany in 1975. He rapidly built an internationally recognized program in biological oceanography that attracted trainees from far and wide. Two of us (Curtis Suttle and Kedong Yin) were among the early students in Paul's lab, but there were many others in those beginning days, including Dave Turpin (President of the University of Alberta), Charlie Trick (University of Western Ontario), Neil Price (McGill University), Peter Thompson and John Parslow (CSIRO, Tasmania), Bill Cochlan (University of San Francisco), Greg Doucette (NOAA), Lee Jackson (University of Calgary) and Maurice Levasseur (Laval University); others that went on to productive non-academic careers ranging from medicine to environmental consulting. The students and post-docs kept coming to learn from the guru of phytoplankton, and over the years 45 graduate students, 15 postdoctoral scholars and countless undergraduates trained with him. Many of these people, too many to list here, went on to be leaders in academia, government and industry. Paul never stopped mentoring and inspiring those around him. Very appropriately and deservedly, he was honored as the inaugural recipient of UBC's graduate teaching award in 1992.

Paul's scientific contributions include over 300 refereed scientific papers, and he is one of the most cited authors in biological oceanography. His work was much broader than phytoplankton, and he co-authored "The Bible" of seaweed physiology and ecology; investigated the impact of large-scale iron fertilization experiments in the North Pacific on primary productivity, the impacts of pollution and nutrient enrichment from sewage treatment, nutrient cycling and climate change. His scholarship has attracted many honors, including being elected a Fellow of the Royal Society of Canada, and a recipient of the Timothy R. Parsons Medal. Despite all of the accolades, Paul always remained humble and well-grounded, and was always the first to put the achievements of others above his own. He was truly inspirational.

He left UBC in 2002 to direct the program in Atmospheric and Marine Science at Hong Kong University of Science and Technology, and help build the reputation and the faculty associated with marine science at HKUST. As well as instigating foundational research and inspiring researchers in Hong Kong, he cultivated the growth of biological oceanography and coastal marine science in China. His work was some of the first in the region exploring red tides, dead zones, and the role of the ocean in reducing global warming and climate change.

He returned to UBC in 2010 as Professor Emeritus, and continued to be as active as ever. He published nearly 50 papers in the last five years, mentored students and junior faculty, and promoted the research and reputations of his trainees and colleagues. He continued to organize and teach courses for advanced students and postdocs, and provide advisory and leadership roles to national and international scientific committees and government agencies as an authority on water quality and

supply, marine resources, climate change impacts and environmental protection. His voice and perspective will be sorely absent.

Paul will be greatly missed by those he touched, but especially by Victoria, his wife of 51 years, and their children Rachel, Richard and Christina.

A scholarship fund that has been set up in Paul's memory and is accepting donations can be found at: *http://memorial.supporting.ubc.ca/paul-j-harrison/.*

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Continued from page 16

During his visit, Dr. Cembella shared his knowledge on the biotransformation of toxins in clams. RedFAN also partially supported the participation of some of its members in the 17th International Conference on Harmful Algae held in Brazil in October 2016. In this event, the Mexican bid, presented by Christine Band Schmidt (CICIMAR),was the winner to become the local organizer of the 19th International Conference on Harmful Algae. RedFAN will be involved in the organization of this important event that will be held in Los Cabos, B.C.S., Mexico in October 2020.

In 2015 RedFAN gave partial financial support to some members for attending the third congress of SOMEFAN (Mexican Society for the Study of HABs) and in 2017 it will be an important support for the organization of the 4th Congress of the Society which will be held together with the 2nd Meeting of the Latin American Association for the Study of Harmful Algae (ALEAN) in October in Cancun, Quintana Roo, Mexico (*www.somefan.org*).

Further, the RedFAN published a *state of knowledge* book in Spanish, detailing the incidence of HABs, effects, ecology, taxonomy and physiology in Mexico. The book chapters are authored by members of RedFAN. In addition, a bulletin with news on HAB occurrence and RedFAN activities is published periodically. As part of the dissemination tasks, a contribution on HAB topics was published in the national newspaper *La Jornada*, and a webpage about RedFAN (Spanish and English) developed (*http://redfan.cicese.mx*; Fig. 1). RedFAN looks forward to the consolidation of an interdisciplinary working group in a short time to prepare a National Program to deal with HAB events in Mexico.

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Keynote Presenters announced for 11th ICMSS in Galway



The International Conference on Molluscan Shellfish Safety, organized by the Marine Institute in Galway, Ireland will take place from 14th May to 18th May 2017 at the National University of Ireland, Galway. The conference provide an opportunity to bring together experts from all over the world to exchange ideas, information and the most up to date findings, allowing us to review current state of the art and future prospects in molluscan shellfish safety. Keynote presentations from Dorothy Jean McCoubrey (Public Health Specialist, New Zealand), Jim Oliver (University of North Carolina, USA), Andy Turner (Cefas Weymouth, UK), Don Anderson (Woods Hole, USA), Jan Vinjé (Centers for Disease Control, Atlanta USA), Ana Gago-Martinez (EU Reference Laboratory for Biotoxins, Vigo, Spain), Philipp Hess (Ifremer, Nantes France) and Robert Atmar (Baylor College of Medicine Texas USA) will set the tone for what promises to be an exciting and timely conference.

Topics including risk assessment approaches, molecular techniques, emerging toxins and regional events will be presented in over 150 presentations, and an additional day of Science Café presentations on the Friday 19th will allow for a hands on relaxed format to present, discuss and explore advancement in HAB methods, LC-MS/MS Technology and Regulatory Matters. In addition to the HAB and Toxin issues there is a full theme of Microbiological and Virus sessions, and many of these are plenary to promote a level of cross pollination between disciplines!

No week in Galway would be complete without experiencing the hospitality that the west of Ireland is renowned for, and a full programme of social activities will be provided. The organising committee are very pleased with the standard of submitted presentations and these will be presented in plenary, parallel and poster sessions. Attendees will have the opportunity to see first-hand the latest research and developments in our field and discuss and collaborate with colleagues from all around the globe. To register for the conference please go to *www.ICMSS2017.com .*

11th International Conference on Modern and Fossil Dinoflagellates

The University of Bordeaux (France) is the organiser of the *11th International Conference on Modern and Fossil Dinoflagellates*, DINO11, which will be held from 17 to 21 July 2017 at Bordeaux, France. This series of conference was first held in 1978 in Colorado Springs, Colorado (United States) (DINO1) and the 11th DINO meeting will be held for the first time in France.

Dinoflagellates and their cysts have shown major research interest for both, biologists working with modern dinoflagellates and geologists working with fossil dinoflagellates. From a biological viewpoint, this major plankton group has major interests in their taxonomy, genetics and ecology, and particularly in the study of toxic dinoflagellates (e.g. *Alexandrium, Dinophysis*) that can endanger human health through intoxication of shellfish or other organisms higher up the food chain. From a geological viewpoint, fossils dinoflagellate cysts are routinely used in biostratigraphy and palaeoclimatological applications. The international conference DINO11 will serve as a platform for exchanges between biologists and geologists concerning all these themes.

More information can be found on: *http://www.laplf.org/dino11/calquedino11.htm*

2017 Gordon Research Conference on Mycotoxins & Phycotoxins

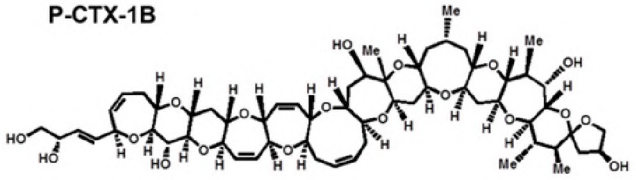
Understanding the Exposure and Global Health Risks Associated with Naturally Occurring Environmental Biotoxins in a Rapidly Changing World

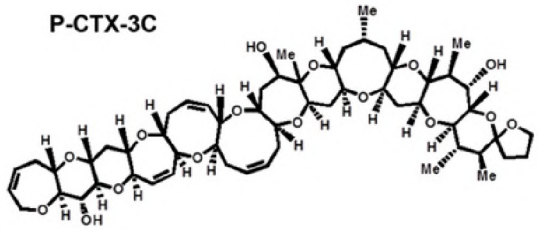
This meeting will be held from 18-23 June, 2017 at Stonehill College, Easton, MA, United States. Chairs: Kathi Lefebvre & Paul Turner.

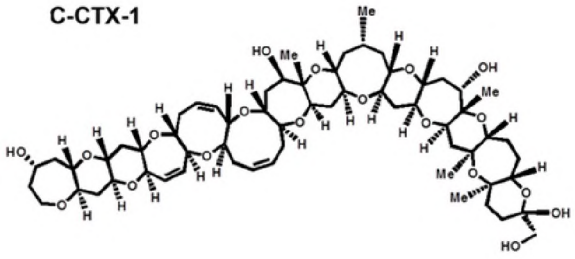
If you have any questions or would like further information, please contact Megan Totten (*mtotten@grc.org*)

Additionally, a Gordon Research Seminar will be held in conjunction with the above GRC. The Mycotoxins & Phycotoxins GRS is scheduled to take place from June 17-18, 2017 at Stonehill College, Easton, MA and will be chaired by Katherine Perri & David McMullin.

Website: *http://www.grc.org/programs.aspx?id=14794*







SAHFOS 4th International Marine Phytoplankton Taxonomy Workshop

An IOC sponsored workshop to be held from 3 to 14 July 2017 at the Sir Alister Hardy Foundation for Ocean Science (SAHFOS), Plymouth, Devon, United Kingdom.

This workshop is aimed at early career scientists, technicians, postgraduates and marine ecologists with a basic knowledge of marine phytoplankton. This interactive workshop focuses on the identification of key marine phytoplankton found in North Atlantic and European waters.

A combination of seminars and hands-on practicals led by internationally renowned experts covering sampling techniques, settling and slide preparation, cell counting, isolation and microscopy and culturing.

Please note the application deadline for the 2017 course has passed, and the workshop is fully subscribed.

More information can be found at the SAHFOS website: *https://www.sahfos.ac.uk/* (Training Courses and Workshops)

Contact: *djoh@sahfos.ac.uk*

IOC Qualifications in Identification and Enumeration of Harmful Marine Microalgae

A now traditional IOC course on identification of harmful marine microalgae, including optional workshops on enumeration and culture techniques.

The 2017 course will be held from 6 to 19 August at the IOC Science and Communication Centre on Harmful Algae in Copenhagen, Denmark.

Since 1993 the IOC has conducted training courses on harmful microalgae. The purpose has been to improve the taxonomic and identification skills of the participants for research purposes and for practical monitoring of harmful algal blooms. The course includes 100 hours of teaching and is divided into two parts.

- The first part of the course is an internet teaching programme giving general introductions to the various groups of harmful algae; this part is mainly for self-study and estimated to 40 hours of reading.
- The second part is a practical course in species identification including 2 optional workshops in enumeration and culture techniques (see tentative programme below). It may be possible to participate in both workshops, or alternatively spent the time examining mixed samples from various geographical regions and/or own samples. Part 2 includes 60 hours of teaching and a microscope will be available to each participant during the entire period.

From 2006 the IOC training in HAB identification has been offered within a new framework which gives accreditation. The present course includes now a practical exam at the end of the course with an IOC Certificate of Proficiency in Identification of Harmful Algae issued to participants who pass the exam. We know by experience that many of the more than 500 trainees we have had over the years have wished the courses to give accreditation, and in some countries, the IOC courses have become a reference for laboratories to be approved for carrying out regulatory monitoring for harmful microalgae.

Detailed information on the course can be found in "Upcoming Events" at the IOC website: *http://hab.ioc-unesco.org/*

Session on Ciguatera at the 10 IPFC

A theme session on ciguatera, *Ciguatera fish poisoning in the Indo-Pacific region: incidence, toxin dynamics, impacts on socio-ecosystems, and risk management*, will be held at the **10 Indo-Pacific Fish Conference** (10 IPFC), 2-6 October 2017, Tahiti, French Polynesia (*https://ipfc10.criobe.pf/*).

Organizers: Mireille Chinain, Susanna Piovano, Jean Turquet, Marie-Yas-mine Dechraoui-Bottein (*mchinain@ilm.pf; susanna.piovano@usp.ac.fj; Turquet.arda@orange.fr; M-Y.Bottein@iaea.org*)

The Ciguatera session (H5) is planned as part of Theme H (The Future of Fish and Human Interactions). The call for abstract is now open: *https://ipfc10.criobe.pf/program/call-for-abstracts/*

Scope: Fish products are the nutritional basis of many island populations globally. Ciguatera Fish Poisoning (CFP) results from the consumption of fish that have accumulated ciguatoxins (CTXs) produced by benthic dinoflagellates in the genera *Gambierdiscus* and *Fukuyoa*. Over the past decades, the frequency and distribution of CFP have increased significantly in the Indo-Pacific region, a likely consequence of



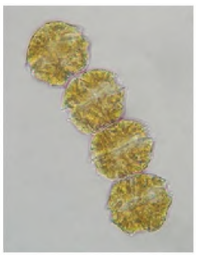
Photo from Institut Louis Malarde, French Polynesia, in Clausing et al, IOC Manual and Guides 59, 2016

numerous environmental changes in coastal and lagoon ecosystems. Despite increased knowledge about the impacts of climatic cycles and biogeography of *Gambierdiscus*, ciguatera events are still very difficult to predict. Likewise, the uptake, tissue distribution, accumulation and toxicity of CTXs in fish are still poorly understood. The existence of numerous structural variants (congeners) of the toxin, and the lack of a duly validated reference test for CTXs both constitute a major obstacle in the sustainable exploitation of fish resources. In addition to the direct effects of ciguatera on public health and the economy of nations highly dependent on fish consumption, the fear of ciguatera often leads to reduced fishing in many indigenous Pacific populations, which reminds us that the impact of ciguatera risk must also be examined from sociological and societal perspectives and not only from a public health standpoint. Recently, several initiatives to implement a more efficient management of CFP risk globally have emerged within the scientific community. The main objective of this session is to present the recent advances in these research fields and to foster networking between scientists and stakeholders concerned with seafood safety.

Expected Audience: The targeted audience includes researchers (biologists, eco-toxicologist, modelists, economists, etc.) and managers interested in marine biotoxins and seafood safety issues. A group of 20 attendees from the Pacific region, Japan, Europe, and La Réunion is expected.

Taxonomic Note

Alexandrium catenella vs. *A. fundyense*
The Nomenclature Committee for Algae has decided (Prud'homme van Reine, 2017) that the name *Gonyaulax catenella (Alexandrium catenella)* should not be rejected and that *A. fundyense* and *A. catenella* are conspecific having nomenclatural priority the name *A. catenella.* Prud'homme van Reine, 2017, Taxon 66(1):191-192. [*https://doi.org/10.12705/661.16*]

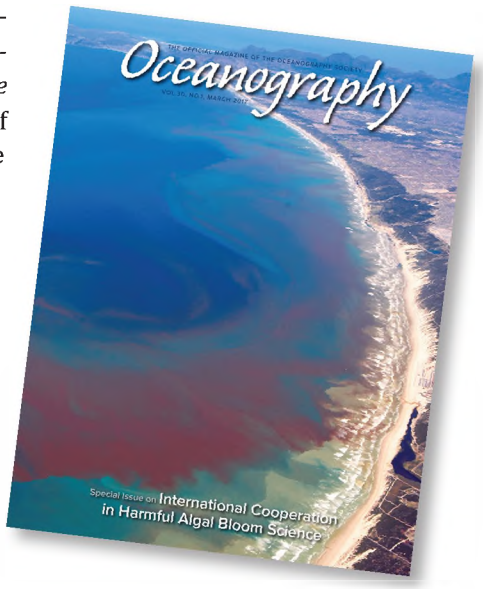


Alexandrium catenella from Huichas Islands, Aysén, Chile. Micrograph from Pablo Salgado.

Oceanography special issue

The March 2017 *Oceanography* special issue on *International Cooperation on Harmful Algal Bloom Science* will be available online by the end of March. The table of contents can be found at *https://tos.org/oceanography/issue/volume-30-issue-01*

Articles in this issue synthesize achievements and identify pending questions within different Core Research Projects from the SCOR-IOC GEOHAB Programme (2000-2013). The recently launched new programme, GlobalHAB, is introduced.



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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 57:
31 May 2017

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Harmful Algae News

AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

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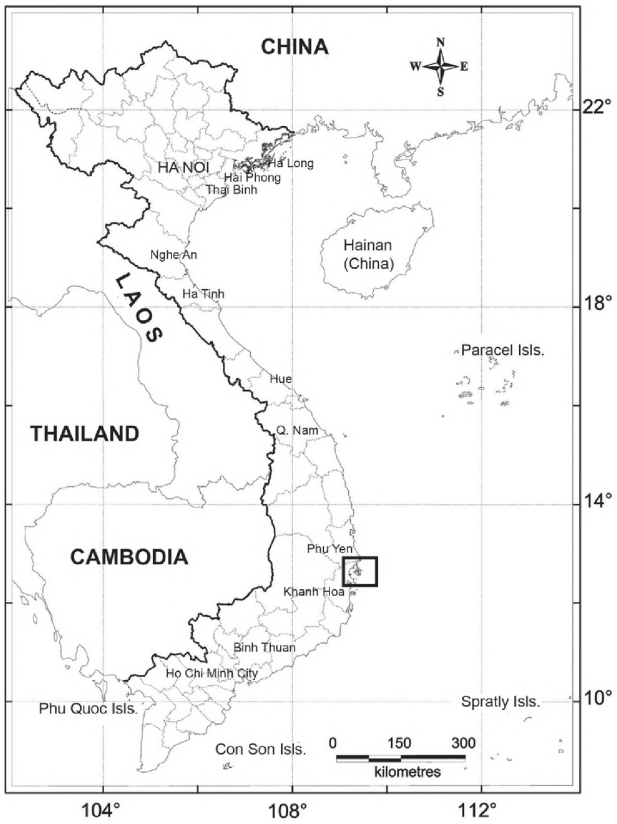
United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Unusual fish killing blooms of *Tripos furca* in Van Phong Bay, South Viet Nam



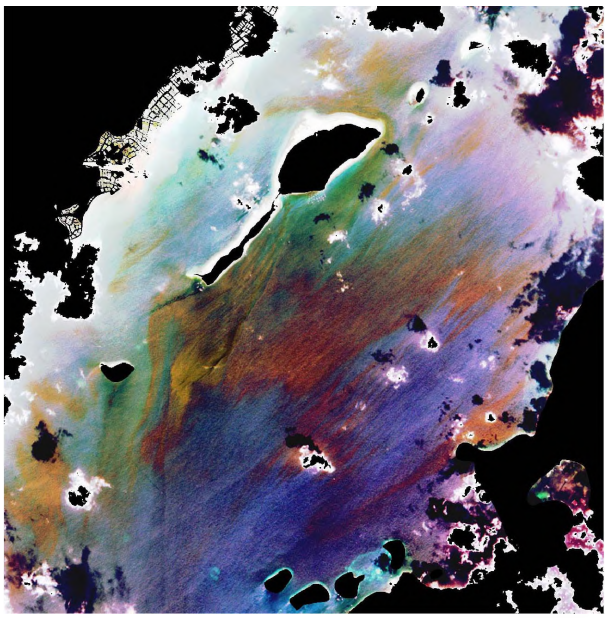


Fig. 1. a) Map of Vietnam and the study area (framed with a square). b) High resolution satellite images during this period were rare due to cloudy weather conditions. An image from 9th November shows patches of the bloom spreading over the inner part of Van Phong Bay. (Source: ESA, Sensinel-2 colour composite, processed by Tong Phuoc Hoang Son, Institute of Oceanography, Viet Nam).

In the beginning of November 2016, a phytoplankton bloom was observed by fisherman in Van Phong Bay, Viet Nam with red-brown patches spreading over the inner part of the bay. Although no fish mortality was reported at the time, the patches raised concern among fish farmers in the area, and samples were sent to the Institute of Oceanography. The bloom forming species was identified as *Tripos furca*. This bloom developed throughout November and mass mortality of wild and caged fish occurred during the last part of the month.

Bloom development

The earliest sign of a developing bloom was retrieved from satellite images on 14th October 2016 in the inner part of Van Phong Bay (Fig. 1). A sample on 30th October confirmed a high density of *T. furca*. From late October to early November heavy rainfall occurred in the Khanh Hoa Province, with a maximum of 400 mm per day. After the 6th November, rainfall was very weak or even absent in the Province (data from Provincial meteorological station, not shown). Nutrient data are not available but runoff following the heavy rainfall may have favoured the development of the bloom.

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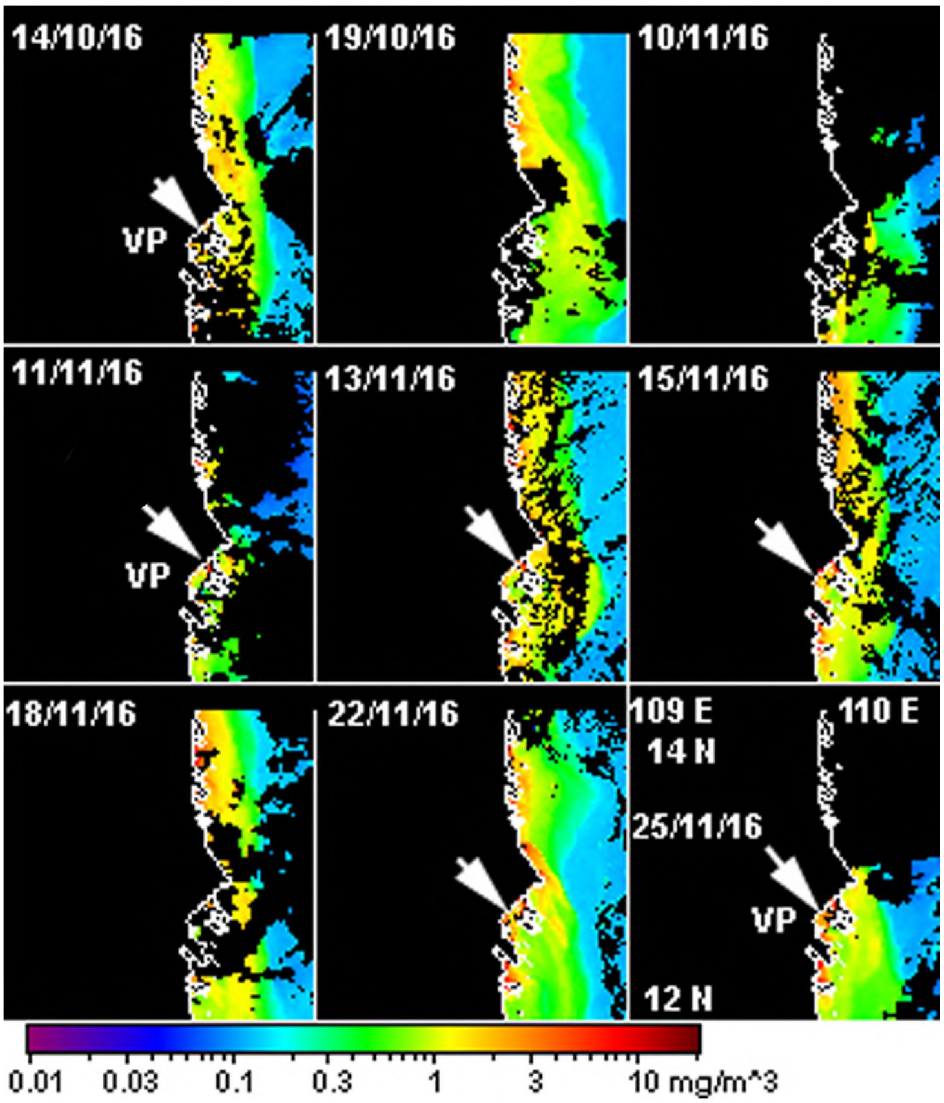

Fig. 2. Chl-a distribution in coastal waters of Khanh Hoa province during 14th October to 25th November 2016 (sources: MODIS-Aqua Level 2 images, NASA). Clear skies were observed on 14th October, and 11th, 13th, 15th, 22nd, 25th November, while the other days were very cloudy. High chl-a concentrations were seen on 11th and 13th November in the northern part of Van Phong Bay and on 22nd November, chl-a patches.

The dominant species in all plankton samples was *T. furca* with *Peridinium quinquecorne* (up to $1.3 \cdot 10^6$ cells·L^{-1} on 26th November) also blooming in a small area in the southwestern part of the bay. During the bloom of *T. furca*, the species diversity was low (2-38 species recorded in the samples). During the period from 28th November to the 1st December, cell densities reached $3.9 \cdot 10^6$ cells·L^{-1} (Fig. 4). At the same time, cell density of *T. furca* outside Van Phong Bay, near Dai Lanh Beach, was only 280 cells·L^{-1}.

Fish mortality was first observed in early November when ca. 35 t of caged fish were killed. This incident affected 260 households in Dam Mon and was attributed to the extremely heavy rainfall which may have reduced the salinity of the surface water within a very short period of time [1].

Harmful effects

During the second and third week of November the fishermen observed increasing discolouration of the water and occasional death of young and non-healthy lobsters and 3-5 kg size cobia fish (*Rachycentron canadum*) (Fig. 3b). On November 24th, mortality of marine animals mounted to an estimated 15 t and included wild fish as well as farmed fish (about 7 t of cobia, 1000 young lobsters and 500 kg of cultured snails [2] (Fig. 3a). A few days later, on 29 November in Dam Mon, an estimated 200 t of caged cobia died and the economic loss amounted to about ca. 1 million US dollars.

The dinoflagellate *T. furca* is not known to be a toxin producing species but it may form high-biomass blooms causing damage through secondary effects such as oxygen depletion. In North Viet Nam it formed a high-biomass bloom in Ha Long Bay in July 2011 killing 10 t of caged groupers and snappers [3]. There are several previous reports of blooms of *T. furca* associated with mortality of fish or other marine fauna including Hong Kong [4], Japan [5], South Africa [6], and Mexico [7]. Low oxygen concentrations may be part of the reason for the observed mortality but clogging and physical damage to fish gills leading severe mucus production almost certainly also plays a role [7].

Data on oxygen levels from the bloom area in Van Phong Bay are not available. However, there was strong indications of gill damage in dead 3-10 kg fish collected on 28th November. Dissection of fish at the Institute of Oceanography in Nha Trang showed congestion of gill arches and gill filaments and large amounts of mucus.

There is no regular government-based HAB monitoring in Van Phong Bay. During the development of the bloom information and warnings were sent directly to the fish farmers from the scientists at ION through personal contacts. According to the fish farmers, the information provided helped mitigate the harmful effects as planned new stocks for the aquacultures were postponed, feeding was reduced, and fish and lobster pens were moved to safer areas. Blooms of *Noctiluca scintillans* [8], and *T. trichoceros* [9], have been reported previously in the bay. Considering the recent expansion of aquaculture activities and the planned industrial and tourist development, there is an urgent need for regular HAB monitoring in Van Phong Bay.

Acknowlegements

Thanks to Dr. Jacob Larsen for reviewing the written English. A high resolution colour composite sentinel satellite image was provided by Mr. Tong Phuoc Hoang Son. This work is funded by the Department of Natural Resources and Environment of Khanh Hoa Province.

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Fig. 3. a) Brown colour water inside the fish cages (Photo provided by a local fish culture company) and b) Fish mortality at the pier (Photo by Hai Lang - Van Giang, Khanhhoa-Online, issue date: November 25th).

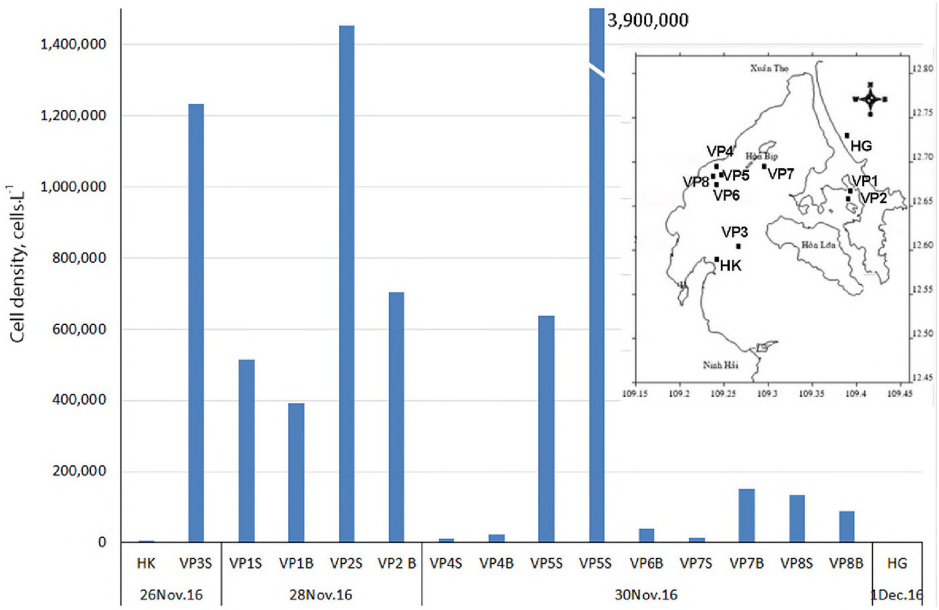

Fig. 4. Variation of cell density (cells/L) of T. furca during November 26th to December 1st 2016 at different sampling stations.

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Mortality of Chilean farmed salmon in wellboats in transit through a *Karenia* bloom

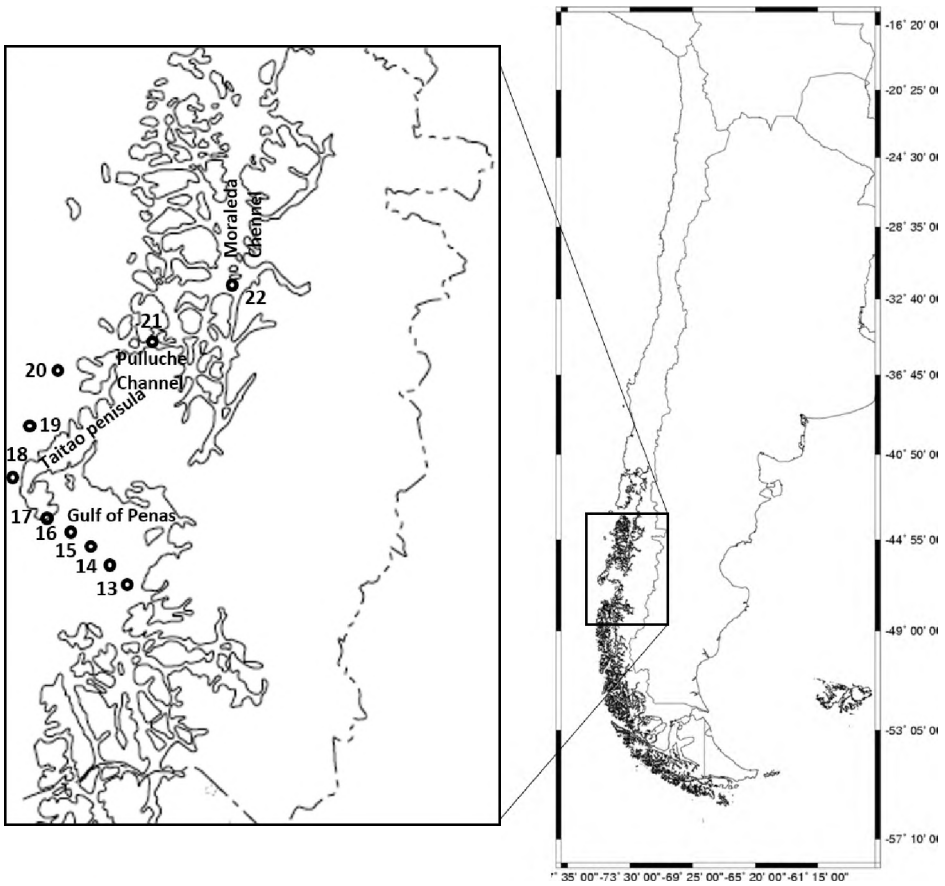

Fig. 1. Geographic distribution of the stations sampled near the Gulf of Penas, Chile, between 31st January and 1st February 2017.

A mass mortality of around 170,000 salmon, worth US$ 390,000, was recorded on wellboats in transit between the Magallanes and Los Lagos regions (Southern Chile) on January 26th, 2017. Investigations into the environmental causes were pursued after technical and operational failures (e.g. oxygen depletion) were ruled out. From the onset, harmful algal blooms (HAB) were suspected as the most probable cause as the area is known for previous large-scale blooms including non-toxic diatoms belonging to the genera *Chaetoceros* and *Rhizosolenia*, toxic phytoflagellates, such as *Heterosigma akashiwo* and *Pseudochattonella verruculosa*, and toxic dinoflagellates (*Alexandrium* and *Karenia*). Blooms of different geographical scales, including some significant events , have been recorded in the affected area [1, 2]. The occurrence of toxin producing HABs has been related to anomalous sea surface temperature (SST) and weather conditions such as those associated with El Niño [2].

Environmental variables, including SST, salinity and pH were measured in 10 sampling stations between the Gulf of Penas and Moraleda Channel (Fig. 1) between January 31st and February 2nd, 2017. Synoptic overviews of SST, chlorophyll-a concentration and oxygen saturation distributions were obtained from the analysis of MODIS/AQUA satellite images. Surface water samples were fixed with acidic Lugol's solution and analyzed according to the Utermöhl technique for identification and enumeration of phytoplankton.

Satellite images of the Gulf of Penas, Taitao Peninsula and Pulluche Channel showed the presence of sub-Antarctic water masses (ASAA) characterized by salinity values of 34-36, and temperatures between 11.5 and 13.6 ° C (Fig. 2), whereas the southern part of the Gulf of Penas (station 13) and the stations at Moraleda Channel showed modified sub-Antarctic water masses (ASAAM), which were colder and with lower (29-30) salinities due to runoff from glaciers and rivers [3]. The seawater pH varied between values close to 7 in the Gulf of Penas to around 8 in the remaining stations (Fig. 2). Satellite-derived chlorophyll-a concentrations were close to 5 μg L^{-1}, and oxygen saturation was 44.4%. The lower pH and oxygen in waters from the Gulf of Penas may have been related to coastal upwelling in the area.

Phytoplankton in the water samples was diverse, with diatoms and dinoflagellates being the most abundant taxonomic groups. A marked difference was observed between stations 13 to 20 (Gulf of Penas and Taitao Peninsula) and stations 21 to 22 (Pulluche and Moraleda Channels). The first group of stations presented a phytoplankton community typical of oceanic waters, with dominance of unarmoured dinoflagellates of the genus *Karenia* which represented 21 to 91% of the total abundance. The second group of stations (21 to 23) showed a phytoplankton community, characteristic of the fjords and chan-

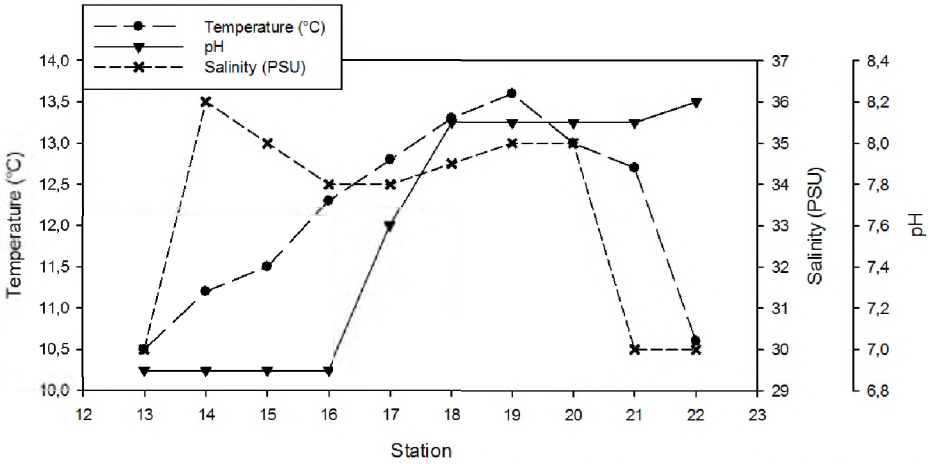

Fig. 2. Sea Surface temperature (°C), pH and salinity (PSU) across the sampled stations in the vicinity of the Gulf of Penas.

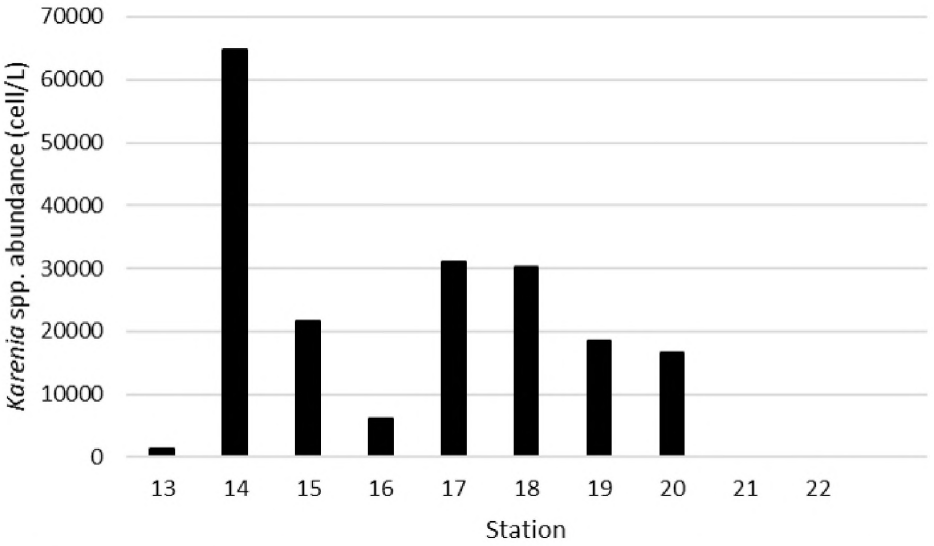

Fig. 3. Density (cells L^{-1}) of Karenia spp. in water samples of the vicinity of the Gulf of Penas.

nels, composed mainly of diatoms.

Most *Karenia* (ex *Gymnodinum*) species produce toxins that can kill fish and other marine organisms when they bloom. In addition to toxicity, some *Karenia* blooms cause animal mortalities through the generation of anoxia and at least one species, *K. brevis*, produces brevetoxins that not only kills fish, marine mammals, and other animals, but also causes neurotoxic shellfish poisoning (NSP) and respiratory distress in humans exposed to the toxic sea spray associated with the blooms [4,5].

Samples showed a high density of *Karenia* species (up to 65 x 10^3cells L^{-1}; average of 19.4 10^3cells L^{-1}) representing between 0 and 91% of the total phytoplankton community. Densities of *Karenia* spp. in the Gulf of Penas varied between 2 x 10^3cells L^{-1} (station 13) and 65 x 10^3cells L^{-1} (station 14), while stations around the Taitao Peninsula (18, 19, 20) showed densities of 31, 19 and 17 x 10^3cells L^{-1} respectively (Fig. 3). The distribution of *Karenia* spp. was restricted to areas with a high influence of oceanic waters, and was not recorded in Pulluche and Moraleda Canal (stations 21 and 22). The bloom of *Karenia* was multispecific, including a variety of forms resembling *Karenia brevis*, *K. papilionacea*, *K. mikimotoi*, *K. brevisulcata* and *K. bidigitata*. (Fig 4). The dominant *Karenia* form was *Karenia* sp 1 (Fig. 4c) and had an approximate width of 28.8 µm and length of 32 µm.

The phytoplankton communities and environmental variables varied in the study area from typically oceanic, with upwelled waters with lower-pH and oxygen levels (Gulf of Penas and Taitao Peninsula), to those characteristic from more brackish waters from fjords and channels (Moraleda Channel). The oceanic phytoplankton was dominated by ichthyotoxic dinoflagellates of the genus *Karenia*, which were identified as the most probable cause of the mortality of the salmon in wellboats during their passage through the Gulf of Penas. The high toxicity of *Karenia* spp. was evidenced by the rapid death of salmons on board.

Multispecific blooms of the genus *Karenia* have been described before [6]. Here we describe a multispecific bloom with the co-occurrence of at least 5 species of *Karenia*. The presence of multiple ichthyotoxic species of *Karenia* and the difficulty to observe features of naked dinoflagellates after fixation made identification to species level unattainable. There are studies in progress to describe the specimens after further examinations by scanning electron microscopy, and molecular studies.

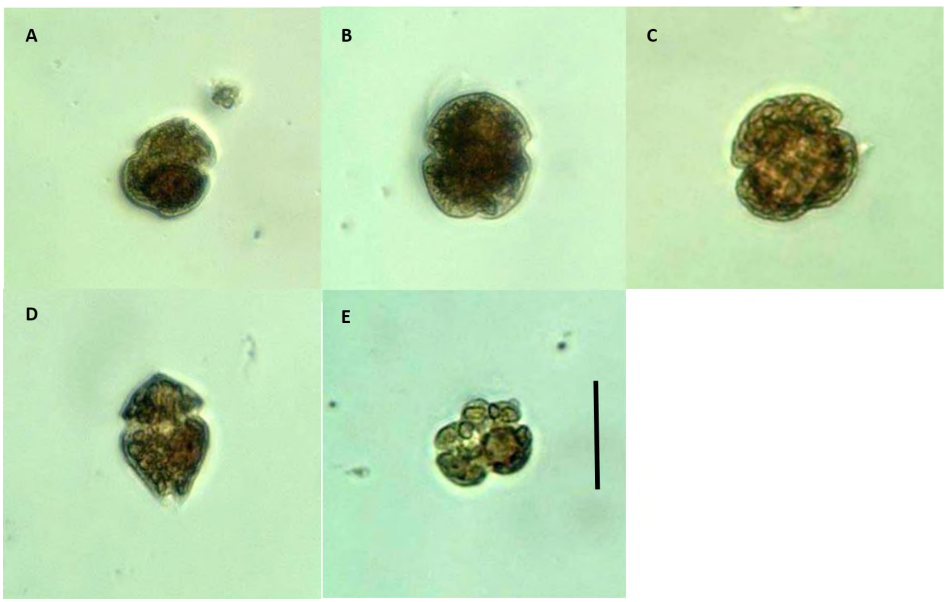

Fig. 4. Light microscope images (400X) of different species of Karenia present in the vicinity of the Gulf of Penas. A) Karenia cf mikimotoi; B) K. cf digitata; C) Karenia sp1; D) Karenia sp 2; E) K. cf papilionacea/K. cf brevis. Size bar = 30 µm.

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A bloom of *Amphidinium carterae* in Ria de Aveiro, Portugal

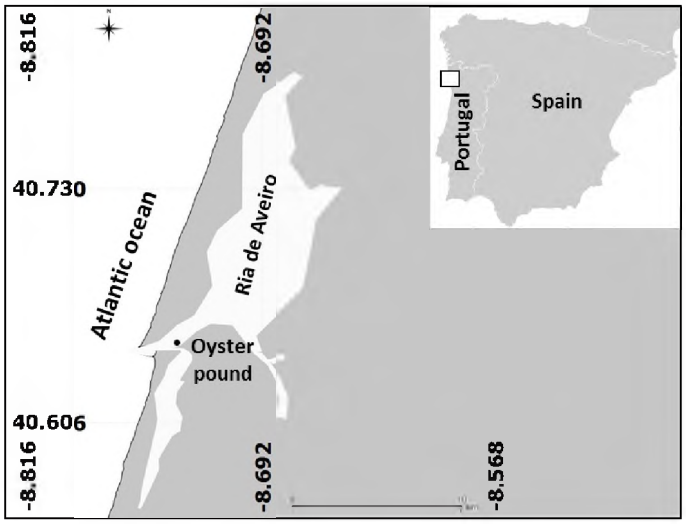

Fig. 1. Ria de Aveiro and oyster farm location.

Amphydinium carterae is a benthic unarmored cosmopolitan dinoflagellate classed as a fish killer due to its capacity to produce hemolytic compounds such as amphidinols [1] and polyhydroxyl carteraol E [2]. This species can be found in shallow and sheltered waters in intertidal or estuarine marine sandy sediments, from tropical, subtropical and temperate ecosystems [3,4].

Between March 24th to March 29th 2016, a bloom of *Amphidinium carterae* was detected in an oyster farm (area of 6 ha, water column of 30-60 cm depth with oyster production of 2kg m^{-2}) in Ria de Aveiro (Fig 1). Dinoflagellate cell densities reached 5.4 x 10^6 cells L^{-1} and oysters suffered significant weight loss.

Ria de Aveiro is the largest Portuguese tidal coastal lagoon, extending 45km N-S and about 10 km E-W, between the Aveiro city and the Atlantic Ocean. It has a single connection with the sea and has inputs from two main rivers and several smaller streams [5]. This aquatic system had ancient salt flats that were converted to fish and shellfish farms with tidally regulated water inflow. In this coastal lagoon, aquaculture has a great social and economic importance [6]. The *Amphidinium* bloom was recorded within the shellfish pond and decreased towards the main branch of the Ria. Within the pond, the water presented a brown-reddish color, was cold (12.4-12.9 ºC), brackish (20.9-28) and slightly basic (pH of 8.0-8.4). The most abundant accompanying species were *Cylindrotheca closterium* (7.6 x10^5 cells L^{-1}), *Amphyprora* sp. (5.4 x10^5 cells L^{-1}) and *Prorocentrum minimum* (9.8 x 10^3 cells L^{-1}). On the day before the sudden increase of cells in the water, the total radiation increased significantly, from 3505 to 19560 Kj m^{-2}. Values remained that high until the maximum of *Amphidinium* developed and these high light levels may have triggered the bloom [7]

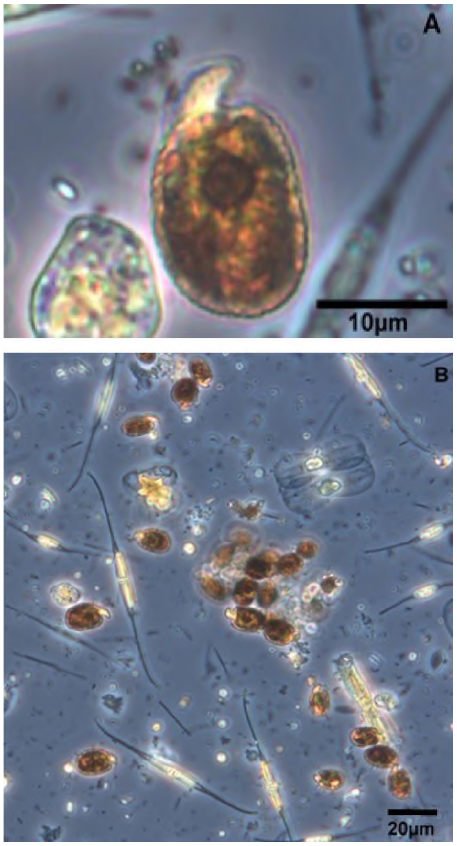

Fig. 2. (A) Amphidinium carterae cell (image taken with an LM Leica DMI 8 with 400x magnification). (B) Sample from Amphidinium carterae bloom (image taken with an LM Leica DMI 8 with 200x magnification).

Amphidinim carterae was morphologically identified by inverted microscopy (Leica DMI 8) at 400/200x magnification, in natural and in Lugol's preserved samples (Fig. 2A, B). Cell dimensions (n=21) averaged 17µm in length(15.0-20.0 µm) and 10.4 µm in width (10.0-12.5 µm), within the range defined for the species [8]. *Amphidinim* was isolated and cultured for molecular identification based on the Large Ribosomal Subunit (28S) with the primers pair D1R/D3Ca [9]. In a Blast search, our sequences showed 99% similarity with 100% cover and E-value of zero compared to the Genbank sequence JQ394805.1.

Although the presence of the species is expected to be among those listed in the National HAB monitoring plan, the last reported occurrence of *A. carterae* in Portuguese estuarine waters dates back to 1985 [10]. This event reinforces the need to monitor the species occurrence and the associated environmental conditions in estuarine systems to forecast blooms and minimize social-economic impacts.

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Harmful dinoflagellates in the Gulf of Guinea, Nigeria, West Africa

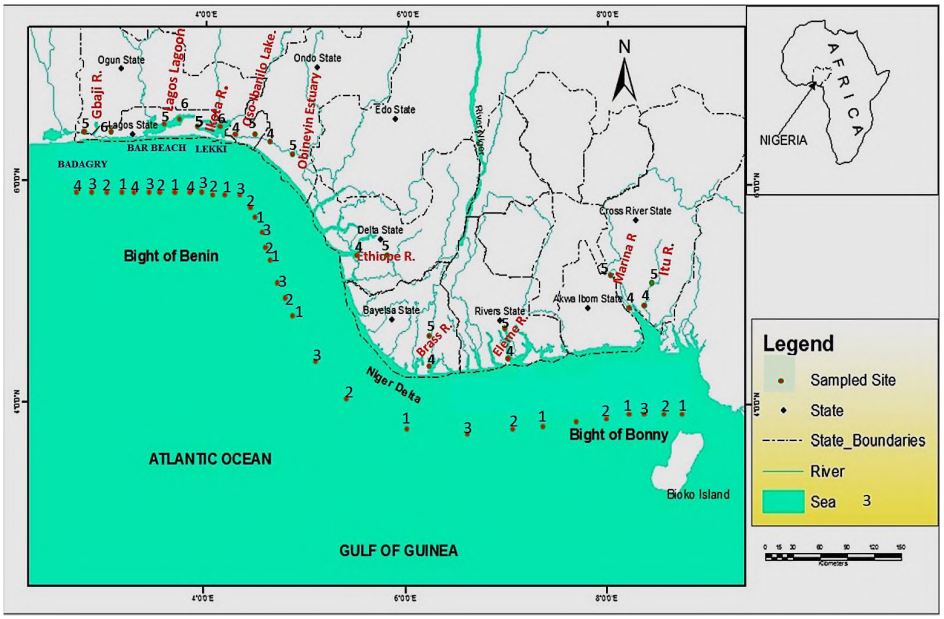

Fig. 1. Coastal map of Nigeria showing the study area.

Harmful algal blooms (HABs) are worldwide phenomena [1]. The increasing spread, frequency and severity of harmful algal incidents have been well documented and have led to the establishment of harmful algal monitoring programs in coastal states in developed countries around the world. This is not the case in several West African countries, including Nigeria, for a variety of reasons. These include a lack of awareness of HABs and the threats they pose, funding difficulties and others. Several coastal communities in Nigeria depend heavily on seafood for their nutritional and economic subsistence and as such will be directly affected by the health, economic and ecosystem disruption impacts caused by HABs.

This report is based on quarterly HAB surveys of the Nigerian coast situated in the Guinea Current Large Marine Ecosystem, in the Gulf of Guinea which span eight coastal states in the south (Akwa Ibom, Bayelsa, Cross Rivers, Delta and Rivers states) and south west (Badagry, Bar Beach, Lekki, Ogun and Ondo states) of Nigeria (Fig. 1). The country coastline, between latitude 4°10′- 6°20′ N and longitude 2°45′- 8°35′ E, is approximately 853 km long. It is composed of four distinct geomorphological units, namely: the Barrier-Lagoon complex; the Mud coast; the Arcuate Niger Delta; and the Strand coast [2]. This study marks the first extensive study on HABs in the entire coast of Nigeria. Prior studies [3,4] were based on small spatio-temporal scale data collection.

Sampling was carried out from March 2014 to January 2015; samples were collected with a 10-µm mesh size net and a known volume of water sedimented for qualitative and quantitative analyses respectively. Harmful dinoflagellates were quantified following the drop count method described by Lackey [5]. Net samples were analysed using an OLYMPUS optical binocular microscope (CX40RF200) fitted with an AMSCOPE digital imager (Model no: MU1403).

Two hundred and twelve phytoplankton samples from 54 locations collected during each quarterly sampling, were analysed for quantitative and qualitative assessment. From these, a total of 74 dinoflagellates taxa were documented. Cell concentrations were found to be generally higher in the southwest locations (Fig. 2a), with total cell concentrations ranging between 13 and 811 x 10^3 L^{-1} compared to the south locations (Fig. 2b) where total cell concentrations ranged between 13 and 351 x 10^3 L^{-1}. We found that the major contributors to the total cell counts in the south were high densities of species of the genera *Alexandrium* (352 x 10^3 L^{-1}), *Prorocentrum* (195 x10^3 L^{-1}), *Protoperidinium* (159 x 10^3 L^{-1}) and *Tripos* (=*Ceratium)* (117 x 10^3 L^{-1}) in July. In the southwest locations, the major contributors varied depending on the sampling season. For instance *Prorocentrum* spp. (754 x $10^3$$L^{-1}$), *Scrippsiella* sp. (702 x 10^3 L^{-1}), *Tripos* spp. (455 x $10^3$$L^{-1}$) and *Protoperidinium* spp. (312 x 10^3 L^{-1}) were most abundant in March; *Alexandrium* spp. (546 x 10^3 L^{-1}) and

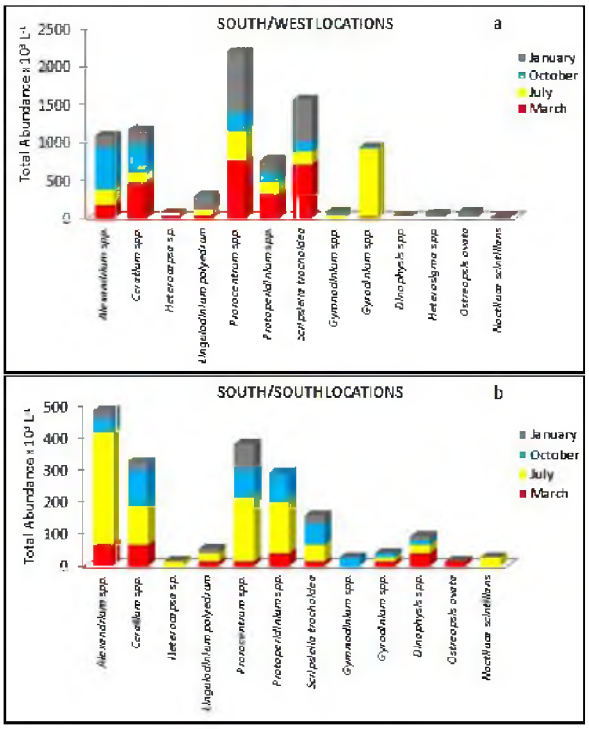

Fig. 2a-b. Total density of harmful dinoflagellates in the south-west and southern coastal locations of Nigeria.

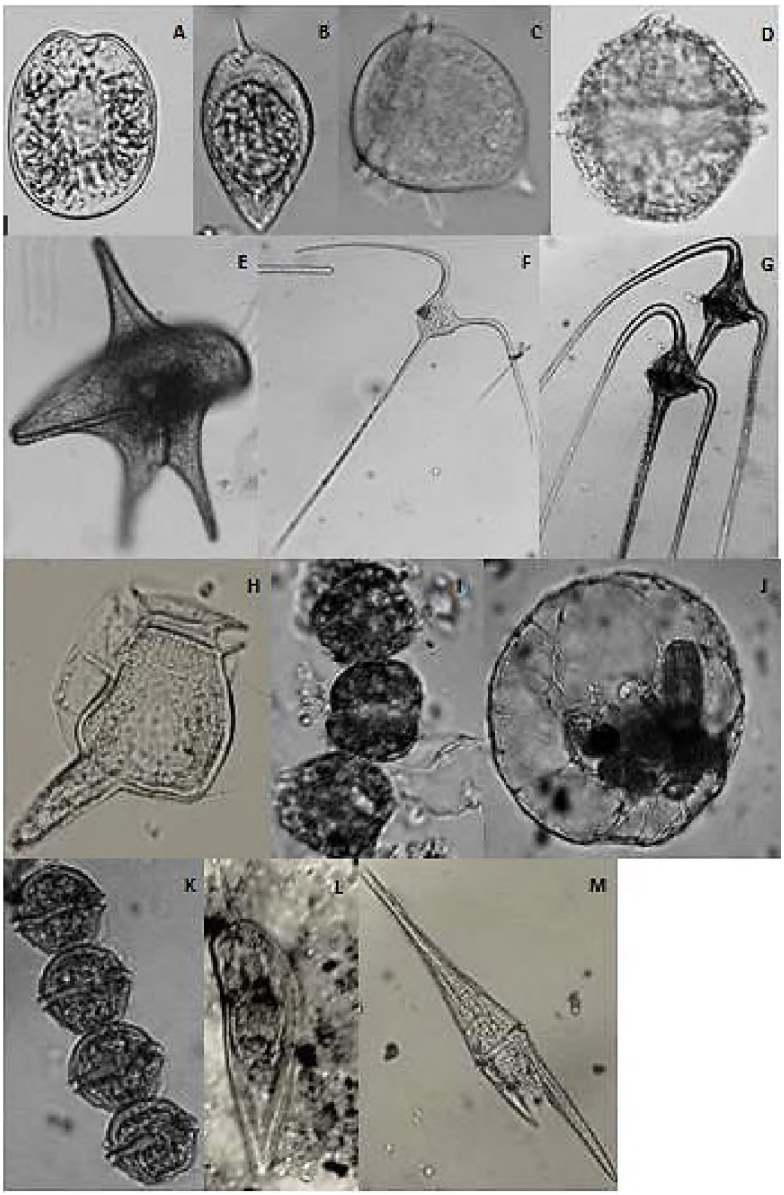

Plate 1. Light micrographs of some dinoflagellates from Nigerian coastal waters. A. Prorocentrum sp.; B. Prorocentrum micans; C. Phalacroma hastatum; D. Lingulodinium polyedrum; E. Protoperidinium elegans; F. Tripos (=Ceratium) macroceras; G. Tripos cf vulture; H. Dinophysis caudata; I. Gymnodinium catenatum; J. Noctiluca scintillans; K. Alexandrium sp; L. Prorocentrum gracile; M. Tripos furca.

Tripos (354 x 10^3 L^{-1}) in October while *Prorocentrum* spp. and *Scrippsiella* spp. showed cell maxima of 871 x 10^3 L^{-1} and 549 x 10^3 L^{-1} respectively in January.

Qualitative data showed *Alexandrium* spp., *Tripos furca, Tripos fusus, Prorocentrum micans, P. gracile, Lingulodinium polyedrum, Heterocapsa niei* and *Scrippsiella trochoidea* as the most widely distributed taxa in the study area (Plate 1).

Increased occurrence of harmful algae in recent years have been ascribed to coastal eutrophication, global climate change as well as increased awareness [6]. Higher cell numbers of harmful dinoflagellates in the south-west locations relative to the other locations may be due to increased nutrient inputs via effluent discharge from various industries found especially in Lagos and Ogun States and several coastal development projects particularly in Lagos. On the other hand, low cell densities of harmful dinoflagellates observed in the south are attributable to lower salinities owing to the diluting effect of the Niger deltaic inflow into the Gulf of Guinea [7]

A number of harmful effects [8] have been ascribed to the dinoflagellates found in our studies, including species that can form high biomass blooms and cause indiscriminate kills of fish and invertebrates through oxygen depletion, clogging and excess mucus stimulation of fish gills. Included among these are *Tripos furca*, *T. fusus, T. tripos, Prorocentrum micans, Noctiluca scintillans* and *Scrippsiella trochoidea.* Also notable are toxic harmful dinoflagellates that produce a suite of toxins, including *Alexandrium catenella* and *Gymnodinium catenatum* (saxitoxins), *Dinophysis caudata* (okadaic acid congeners), and benthic HAB (*Ostreopsis* cf *ovata,* palytoxins, and *Prorocentrum lima,* okadaic acid). These toxins can directly impact humans when consumed via contaminated seafood.

This study has shown that harmful dinoflagellates are present in Nigerian waters in high cell densities characteristic of bloom levels. There is the need to undertake a continuous surveillance programme for harmful algal bloom detection. Implementation of routine harmful algal monitoring programmes for Nigeria will provide valuable information for the management of coastal resources and help provide early warning signals of harmful algal bloom events in the region.

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Satellite detection of *Trichodesmium* blooms in the Southwestern Pacific

In the oceans, a large portion of the subtropical and tropical pelagic areas are dominated by oligotrophic conditions. The cyanobacterial diazotroph community, composed of unicellular, filamentous, and diatoms-symbiotic (DDAs, diatom-diazotroph associations) forms is dominant. Major species are *Trichodesmium erythraeum* Ehrenberg and *T. thiebautii* Gomont, two species of *Katagnymene* (*K. pelagica* and *K. spiralis* Lemmerman), and *Richelia intracellularis* as components of DDAs [1]. Weak winds (< 4 m s-1) and calm conditions are independent factors which allow accumulations of *Trichodesmium* on the surface of the sea, in particular *T. erythraeum,* which has a stronger positive buoyancy than *T. thiebautii* and *T. contortum* [1]. Observations of *Trichodesmium* accumulations (termed 'mats') around New Caledonia by merchant ships, French Navy, R/V *Alis* and voluntary observers are the result of positive cell buoyancy which brings together trichomes and colonies at the sea surface every summer [2]. *Trichodesmium* is mainly observed around New Caledonia and Vanuatu and a *Katagnymene* form was observed between Niue Island and Tonga in December 2002 [2].

In the southwest tropical Pacific, satellite and aerial observations showed the presence of 3000 km long surface blooms of *Trichodesmium*, mainly around islands of the Melanesian archipelago, i.e. Vanuatu, New Caledonia, and as far east as the Fijian archipelago and the Tonga trench [2]. This area coincides with high nitrogen fixation rates [3]. There are numerous correlations between the observations of surface mats in the ocean and high reflectance on the MODIS satellite, as for example at the East of New Caledonia in 2002 (Fig. 1a) and 2004 (Fig. 1b, Fig. 1c ,Fig 1 d) and in January 2017 (Fig. 1e). In summer 2003, at the peak of a moderate 2001-2003 El Niño, a *Trichodesmium* spp maximum of 4,500 trichomes L^{-1} was observed in the Loyalty Channel, a concentration much lower than in New Caledonia lagoon surface accumulations which can reach 20,000 trichomes L^{-1} [4]. *Trichodesmium* blooms may spread to cover enormous sea surface areas. In December 2014, a huge surface bloom detected by MODIS coincided with observations of *Trichodesmium* mats (Fig. 2a,b) during a SPOT cruise on board R/V *Alis* [5] and was followed until March [6]. Mat observations are

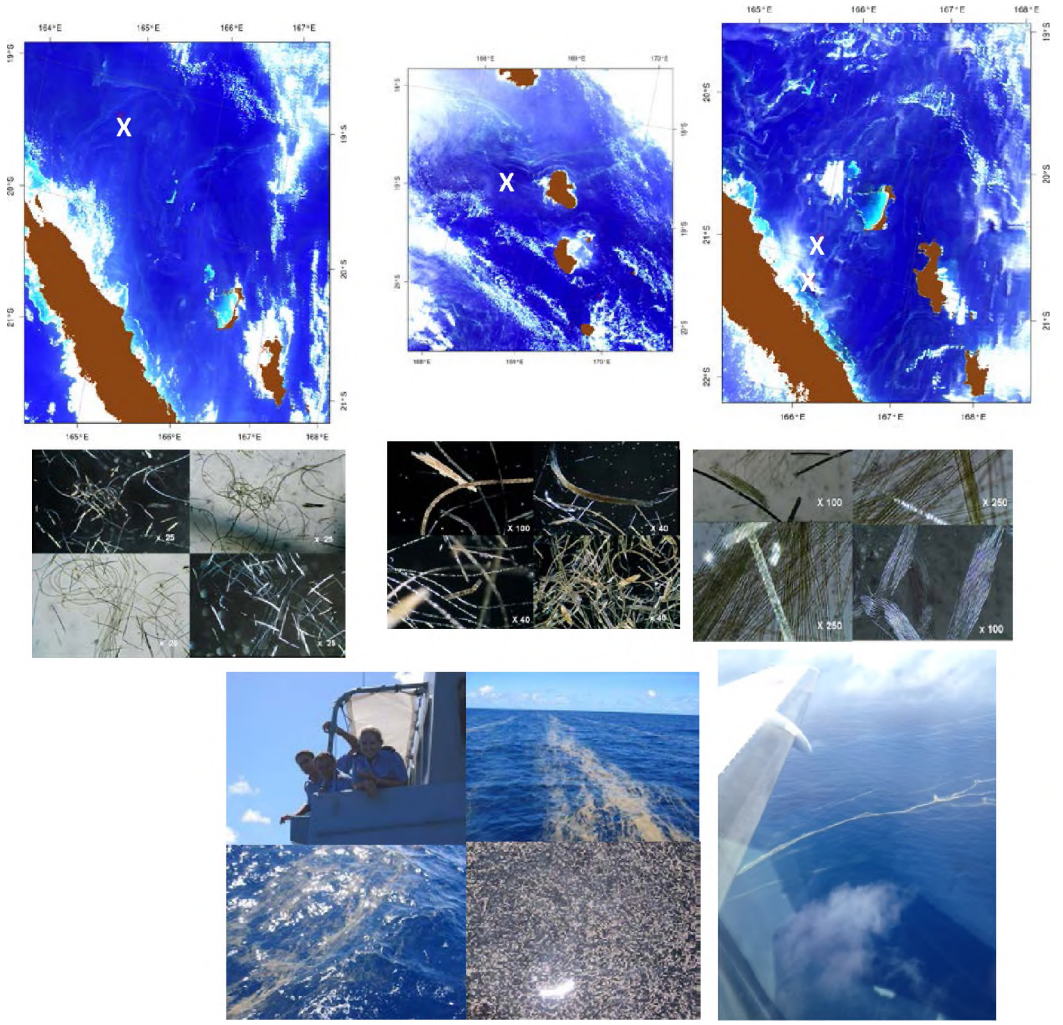

Fig. 1a-c) "True color" MODIS images off New Caledonia showing mats as high reflectance in white around the islands. White crosses: Visual observations corresponding to microscope micrographs of Trichodesmium in formalin fixed samples collected by the French Navy in: November 2002 (a); January 2004 (b); February 2004 (c). d-f): A mixture of different forms of Trichodesmium observed: T. erythraeum, T. thiebautii, and Katagnymene spiralis found in observations on satellite images indicated by a cross. g) Surface Trichodesmium mats (from the French Navy ship La Glorieuse in January 2004). h) Picture of a mat captured during a New Caledonia Gardian airflight in January 2017 (courtesy Jérôme Aucan).

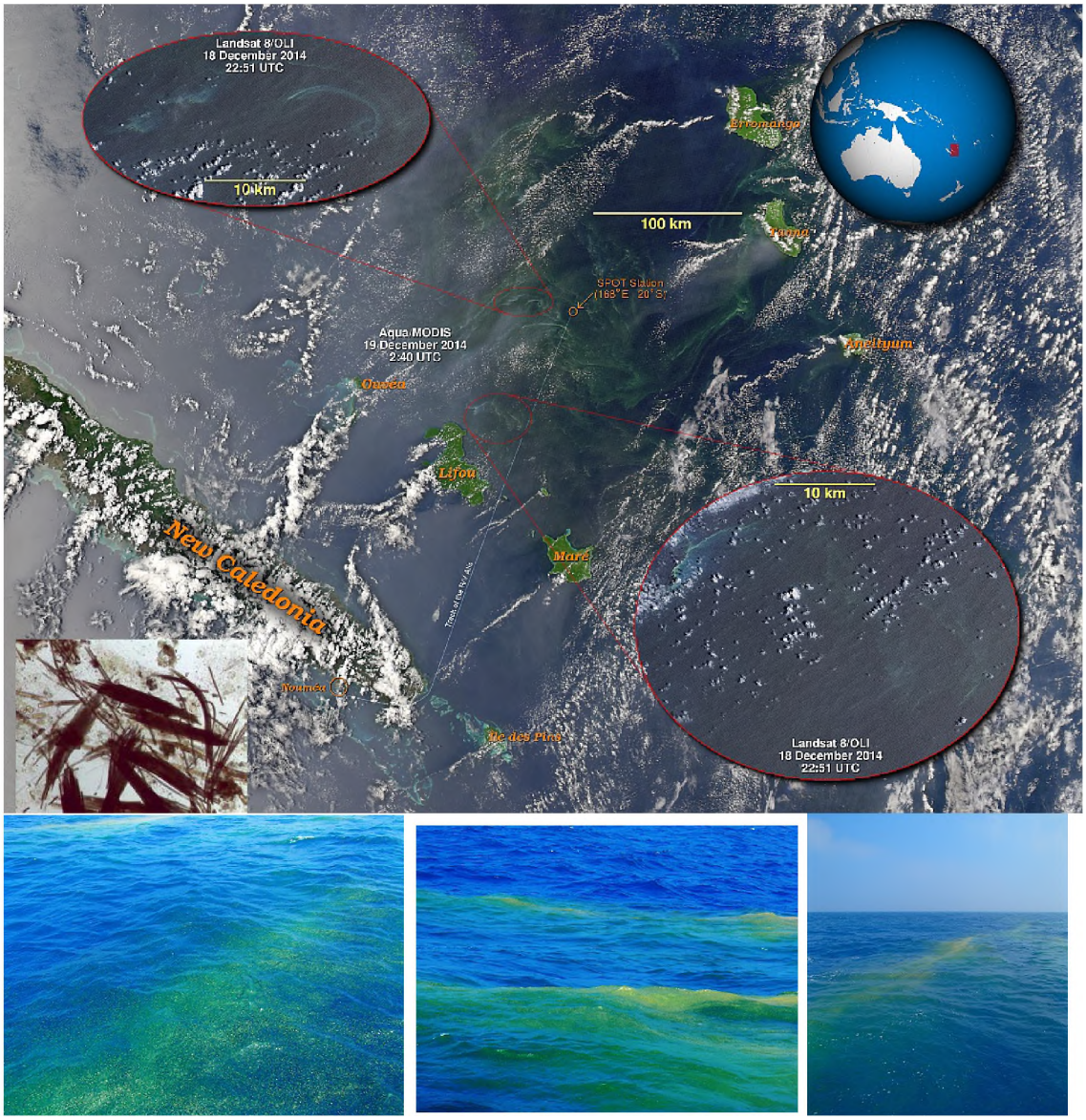

Fig. 2a) MODIS image of the Trichodesmium bloom with white and green mats between Vanuatu Islands and New Caledonia in December 2014; with Landsat 8 zooms on mats superimposed and the microscopic observation of Trichodesmium colonies in surface samples at the SPOT station visited with R/V Alis; b) Pictures of Trichodesmium mats as photographed from R/V Alis. http://earthobservatory.nasa.gov/IOTD/view.php?id=85073

essential for calibrating ocean colour algorithms aimed at detecting filamentous diazotrophs from their particular optical properties. Some of those are pigments (mainly phycoerythrins) absorption and particles backscattering [7] or their ability to float at the surface and present a characteristic optical signature [8]. Application of an algorithm for the satellite detection of *Trichodesmium* mats in oceanic waters of the South Western Tropical Pacific is in progress at the Centre IRD of Noumea [9]. Other phytoplankton blooms around islands in the Tropical Pacific can be detected by MODIS and recent satellite Sentinel sensors. Microscopic identifications of the species involved should be more systematic and observation data centralized.

Acknowledgements

We are grateful to the French Navy and the Aeronaval Base of New Caledonia for their active participation in the observation programme as well as to the captains and crews of the IRD R/V *Alis*. We thank Norman Kuring for the NASA MODIS and Landsat 8 images at the NASA web site. We thank Isabelle Biegala for the SPOT (South Pacific Time Series) programme.

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Plankton Planet in New Zealand

Plankton Planet is the first citizen science programme designed for biological oceanography. It is a co-operation between scientists and volunteers from the sailing community based on mass sequencing of DNA barcodes from the extracts of plankton communities collected worldwide. The results of the analysis of samples collected during the 2015 pilot project demonstrated that the quality of the samples was equivalent to that of those obtained during the *Tara* Oceans scientific expeditions. More than 200 million DNA barcodes from 300 samples were generated in the pilot phase. The programme's ability to reach out to sailing communities and recruit citizen "Planktonauts," equip vessels, and coordinate open-ocean sampling of plankton communities across the world oceans has proven it can provide information about plankton communities over spatio-temporal scales never before possible.

The programme is now preparing its next steps for an expansion in geography and duration. To these ends, a week-long scientific workshop is being held in Auckland, New Zealand, in July thanks to a Seeding Fund grant from the New Zealand Ministry of Business, Innovation & Employment awarded and administered by the Royal Society of New Zealand. It brings together scientists from New Zealand, Europe, and the United States to share recent developments and discuss innovations in collaborative plankton research. Of particular importance is the incorporation of plankton biodiversity (DNA meta-barcoding) data into global oceans models. The scientists will also use this opportunity for outreach to school groups and the general public.

Thanks to the generous support of the New Zealand Maritime Museum and the Sir Peter Blake Trust, an evening programme will be hosted by the Maritime Museum for the general public on 6 July, 2017, at 6:30 p.m. "An Epic Voyage through Our Changing Seas" will bring together New Zealand experts in Māori waka and sailing tradition with international scientists including Prof. Mick Follows, Dr. David Gruber, Dr. Emmanuel Boss, and Dr. Manu Prakash for a series of presentations and discussion with the audience.

From 6 to 30 July, 2017, "Plankton Arts" a display of never-before-seen images of plankton will be on exhibit at the Maritime Museum. Giant prints of plankton, 3-D models, lenticular prints, and videos aim to bring the microscopic world closer to the public and increase their appreciation of the role plankton plays as basis of the ocean's entire food chain and a regulator of the planet's climate.

For more information see *www.planktonplanet.org.*

To register for "An Epic Voyage through Our Changing Seas" at the Maritime Museum Auckland, 6 July, 2017, at 6:30 p.m. go to www.EVENTBRITE

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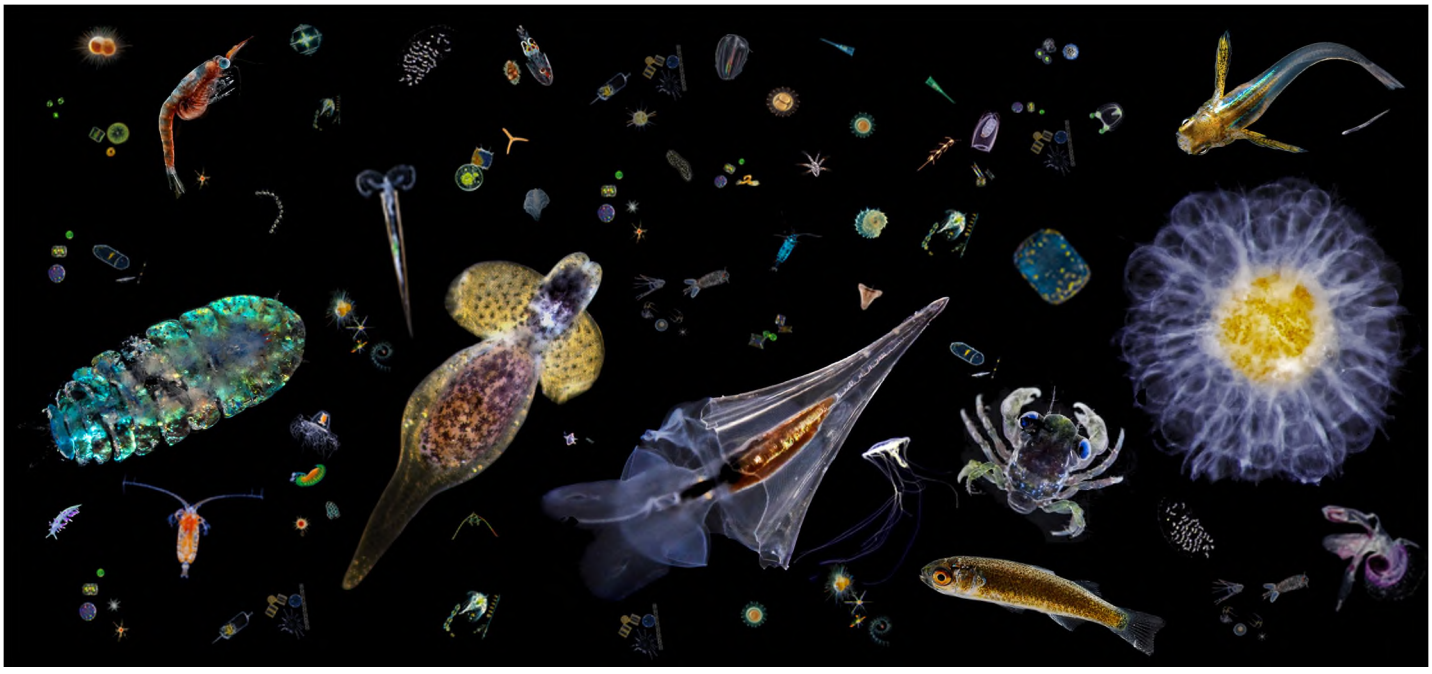


Fig. 1. Marine Plankton. Courtesy of Christian Sardet

Molluscan Shellfish Safety Conference in Ireland

Over 230 international delegates from 27 countries participated in the 11th International Conference on Molluscan Shellfish Safety (ICMSS) from Sunday 14 to Thursday 18 May 2017 at the National University of Ireland, Galway. Convened by Ireland's Marine Institute, the ICMSS is the only international conference that focuses specifically on molluscan shellfish safety, and has previously been hosted by countries including New Zealand, Canada, France, Spain, Australia, USA, and Chile. The 2017 conference programme addressed the themes of protecting consumers, assuring supply and growing confidence. Participants including sea-food safety professionals, scientists, regulators and industry experts discussed these topics and focused on success and innovation in seafood safety and the latest research that will lead us into the future.

Conference Convenor Joe Silke of the Marine Institute said, "The conference was a huge success and was very timely with recent developments in areas of HABs including the occurrence of Tetrodotoxin in European bivalves, blooms of *Alexandrium pseudogonyaulax* and its goniodomines in the Limfjorden (Kattegat) and innovative risk management and science based models from around the world." The conference hosted sessions on emerging toxin methods and new technology where advances in novel instrumentation and testing methods were presented. Mr Silke said "Ireland has a distinguished track record in the area of molluscan shellfish safety, in developing state- of-the-art monitoring protocols, forecasting tools and a variety of cutting-edge research activities. The ICMSS will help to further increase our knowledge and showcase our commitment to shellfish safety."

The keynote speaker for ICMSS was public health specialist Dorothy-Jean McCoubrey, who has more than 30 years of experience in regulatory roles in New Zealand. Ms McCoubrey said the conference gathers together the world's best experts to achieve two important goals; "The first goal is to dream big with our visionary food safety research. The second is to translate this imaginative foresight into practical and useful material that has the potential to benefit all of society".

Over 70 oral and 46 poster presentations were delivered in 16 themed sessions. The conference keynote presentations were streamed live and over 800 viewers tuned in to these presentations from all over the world. These keynotes were presented by Jim Oliver Bonnie Cone, Distinguished Professor and Professor of Microbiology at the University of North Carolina Charlotte (USA), and Andrew Turner, Principle Chemist at Cefas Weymouth Food Safety Group (UK). There were also keynote presentations from Donald Anderson from Woods Hole Oceanographic Institute (USA), Jan Vinjé from the Centers for Disease Control and Prevention (USA), Ana Gago-Martinez from the EU Reference Laboratory for Marine Biotoxins (Spain) and Philipp Hess from IFREMER, Nantes (France)

In addition, the ICMSS included a series of five 2 hour workshops on the last day to explore new technologies and regulatory matters in food safety. For more specific information on the content and abstracts at the 2017 conference see *www.icmss2017.com*

The international conference was hosted by the Marine Institute in association with Bord Iascaigh Mhara, The Food Safety Authority of Ireland, The Sea Fisheries Protection Authority, The Irish Shellfish Association and NUI Galway. The next ICMSS will be hosted in Mexico in May 2019 and further information on this will be published in due course.



Members of the local organising committee attending the official opening of the 11th ICMSS Conference

13th Session of the Intergovernmemntal Panel on Harmful Algal Blooms (IPHAB)



Participants in the 13th IPHAB session. IOC, Paris, 3-5 May 2017

This year marks the 25th anniversary of the Intergovernmental Panel on Harmful Algal Blooms (IPHAB) established under the auspices of IOC of UNESCO. Over that period IPHAB has provided the international framework for regional and global coordination and prioritization of capacity development, research and strategic initiatives on HABs. The IPHAB met for its 13th session at UNESCO Headquarters, Paris, from 3-5 May 2017. The Panel reviewed the many activities and results completed during 2015-2016 and noted the continued broad engagement of the science and management community in activities. The major achievements presented at IPHAB included:

(i) the formal establishment of GlobalHAB, appointment of science steering committee members, and finalizing of the Science and Implementation Plan;
(ii) development of an IOC-IAEA-FAO-WHO inter-agency strategy for research and monitoring on Ciguatera;
(iii) implementation of sixteen training courses and training-through-research projects;
(iv) continued development of the IPHAB-IODE Harmful Algae Information System including HAEDAT;
(v) continued publication of the IOC Harmful Algae News;
(vi) progress on the preparation of the first Global HAB Status Report;
(vii) progress on the preparation of a Guidance Manual on HABs and Desalination;
(viii) development of regional activities within ANCA, FANSA, HANA, WESTPAC-HAB and WESTPAC-TMO;
(ix) the work of the ICES-IOC WGHABD and ICES-IOC-IMO WGBOSV; and
(x) the continued vital partnerships with SCOR, ICES, PICES, IAEA and ISSHA. The Panel was also briefed on the IOC Capacity Development Strategy; the UN Sustainable Development Goals 14; and the OBIS database by IOC staff.

The IOC-coordinated HAB Programme is the only global intergovernmental effort to understand, manage and mitigate the harmful effects of algal blooms, and has therefore maintained a critical leadership role via IPHAB. Particularly noteworthy is the progess on inter-sessional IPHAB activities, including efforts by Task Teams to maintain an updated taxonomic list of HAB taxa and revisions to the nomenclature, identification and structures of novel phycotoxins, and associated toxicological effects. Combined with IPHAB-sponsored initiatives on mapping biogeographical information on HAB events, these data-bases are essential to sustain both research and monitoring strategies.

The Panel has shown flexibility and adaptive capacity since its inception, modifying its functions and tasks to address emerging global priorities for ocean research and monitoring as defined by IOC. The IPHAB has aligned its priorities to address the four IOC overarching themes:

i. Healthy Ocean Ecosystems;
ii. Early Warning for Ocean Hazards;
iii. Climate Change Adaptation and Mitigation and
iv. Enhanced Knowledge of Emerging Science.

Exciting new elements of HAB research have benefited immensely from the recognition by IPHAB of the importance of linkages to ocean observational systems and the effects of global change.

The Panel made decisions for priorities 2018-2019 for international HAB activities to be implemented by the IOC UNESCO and IPHAB partners. The Decisions concern the following:

(i) IOC/HAB Training and Capacity Building Programme;
(ii) Regional HAB Programme Development, continuation with revised Terms of Reference of the IPHAB;
(iii) Task Team on the development of a Global HAB Status Report;
(iv) Task Team on a Global Strategy on Ciguatera for Improved Research and Management; (v) Task Team on Harmful Algae and Desalination of Seawater;
(vi) Task Team on Biotoxin Monitoring, Management and Regulations;
(vii) Task Team on Algal Taxonomy, and
(viii) Task Team on Harmful Algae and Fish Kills.

Dr. Gires Usup (Malaysia) was reelected as Chair and Dr. Allan Cembella (Germany) was reelected as Vice-Chair.

Contact: hab.ioc@unesco.org. More information and updates on activities at *http://hab.ioc-unesco.org*

Forthcoming events

11th International Conference on Modern and Fossil Dinoflagellates

The University of Bordeaux (France) is the organiser of the *11th International Conference on Modern and Fossil Dinoflagellates*, DINO11, which will be held from 17-21 July 2017 at Bordeaux, France. This series of conference was first held in 1978 in Colorado Springs, Colorado (United States) (DINO1) and the 11th DINO meeting will be held for the first time in France.

Dinoflagellates and their cysts have shown major research interest for both, biologists working with modern dinoflagellates and geologists working with fossil dinoflagellates. From a biological viewpoint, this major plankton group has major interests in their taxonomy, genetics and ecology, and particularly in the study of toxic dinoflagellates (e.g. *Alexandrium, Dinophysis*) that can endanger human health through intoxication of shellfish or other organisms higher up the food chain. From a geological viewpoint, fossils dinoflagellate cysts are routinely used in biostratigraphy and palaeoclimatological applications. The international conference DINO11 will serve as a platform for exchanges between biologists and geologists concerning all these themes.

More information can be found on: *http://www.laplf.org/dino11/calque-dino11.htm*

Session on Ciguatera at the 10 IPFC (Grant applications still open!)

A theme session on ciguatera, *Ciguatera fish poisoning in the Indo-Pacific region: incidence, toxin dynamics, impacts on socio-ecosystems, and risk management,* will be held at the **10 Indo-Pacific Fish Conference** (10 IPFC), 2-6 October 2017, Tahiti, French Polynesia (*https://ipfc10.criobe.pf/*).

Organizers: Mireille Chinain, Susanna Piovano, Jean Turquet, Marie-Yasmine Dechraoui-Bottein (*mchinain@ilm.pf; susanna.piovano@usp.ac.fj; Turquet.arda@orange.fr; M-Y.Bottein@iaea.org*)

The Ciguatera session (H5) is planned as part of Theme H (The Future of Fish and Human Interactions). The call for abstract is open: *https://ipfc10.criobe.pf/program/call-for-abstracts/*

Scope: Fish products are the nutritional basis of many island populations globally. Ciguatera Fish Poisoning (CFP) results from the consumption of fish that have accumulated ciguatoxins (CTXs) produced by benthic dinoflagellates in the genera *Gambierdiscus* and *Fukuyoa*. Over the past decades, the frequency and distribution of CFP have increased significantly in the Indo-Pacific region, a likely consequence of numerous environmental changes in coastal and lagoon ecosystems. Despite increased knowledge about the impacts of climatic cycles and biogeography of *Gambierdiscus*, ciguatera events are still very difficult to predict. Likewise, the uptake, tissue distribution, accumulation and toxicity of CTXs in fish are still poorly understood. The existence of numerous structural variants (congeners) of the toxin, and the lack of a duly validated reference test for CTXs both constitute a major obstacle in the sustainable exploitation of fish resources. In addition to the direct effects of ciguatera on public health and the economy of nations highly dependent on fish consumption, the fear of ciguatera often leads to reduced fishing in many indigenous Pacific populations, which reminds us that the impact of ciguatera risk must also be examined from sociological and societal perspectives and not only from a public health standpoint. Recently, several initiatives to implement a more efficient management of CFP risk globally have emerged within the scientific community. The main objective of this session is to present the recent advances in these research fields and to foster networking between scientists and stakeholders concerned with seafood safety.



Photo from Institut Louis Malarde,French Polynesia, in Clausing et al, IOC Manual and Guides 59, 2016

Expected Audience: The targeted audience includes researchers (biologists, eco-toxicologist, modelists, economists, etc.) and managers interested in marine biotoxins and seafood safety issues. A group of 20 attendees from the Pacific region, Japan, Europe, and La Réunion is expected.

Student Grants Opportunities

Two grants could be of interest to HAN readers:

Grant 3: Ciguatera fish poisoning in the Indo-Pacific region: incidence, toxin dynamics, impacts on socio-ecosystems, and risk management

Grant 5: Women in Marine Sciences in the Indo-Pacific

Depending on the number and the quality of applicants, each grant could include one of the following three options: 1/ flight, accommodation and registration fees; 2/ accommodation and registration fees; or 3/ only registration fees.

Applications must be submitted before the 30th June 2017.

6th International Symposium

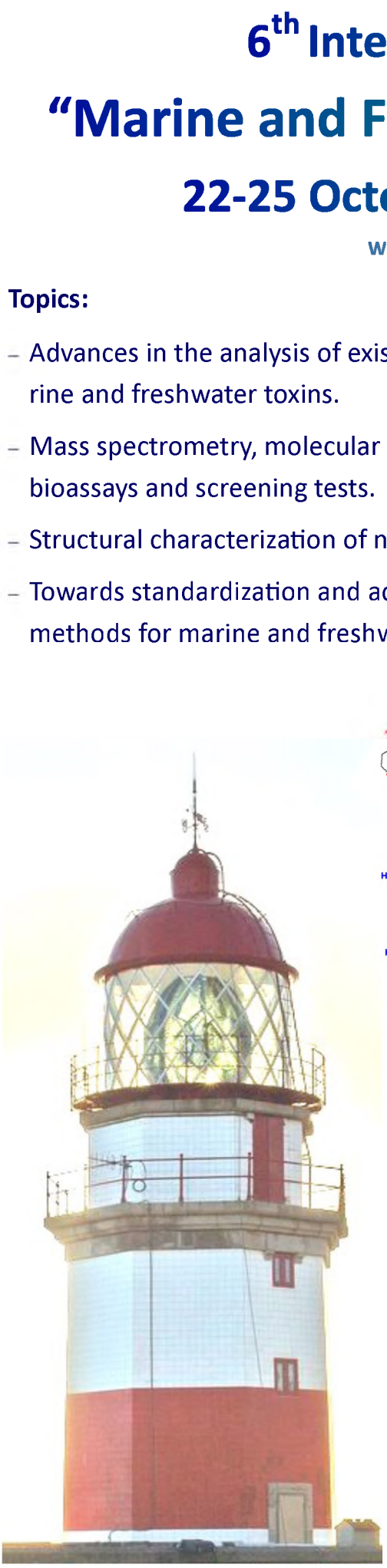
"Marine and Freshwater Toxins Analysis"

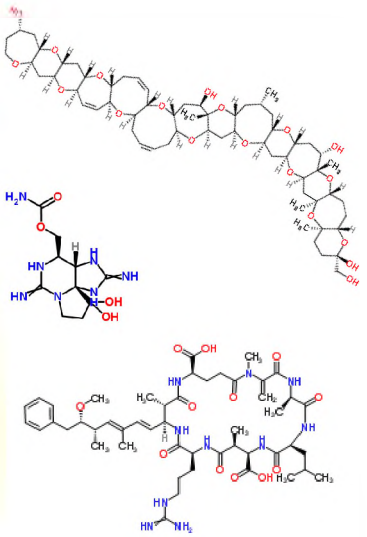
22-25 October 2017, Baiona, Spain

Website: http://biotoxinsmeeting.org/

Topics:

- Advances in the analysis of existing and emerging marine and freshwater toxins.
- Mass spectrometry, molecular methods, toxicity assays, bioassays and screening tests.
- Structural characterization of new potential toxins.
- Towards standardization and accreditation of analytical methods for marine and freshwater toxins.





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Ana Gago-Martinez, UVIGO, ES

Triantafyllos Kaloudis, EYDAP, EL

Important dates:

30 July 2017: Abstract submission
30 July 2017: Early bird registration
30 Aug 2017: Acceptance of abstracts

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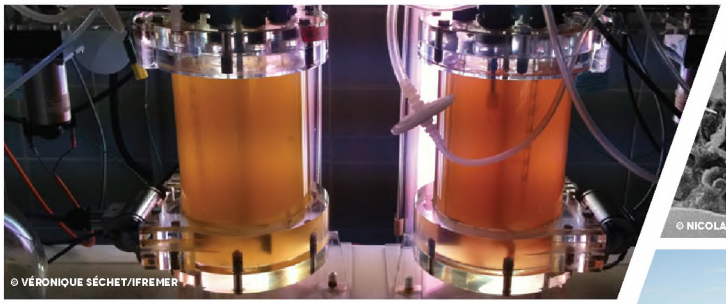
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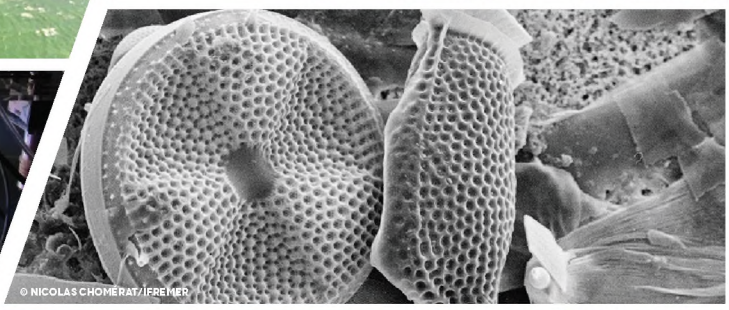
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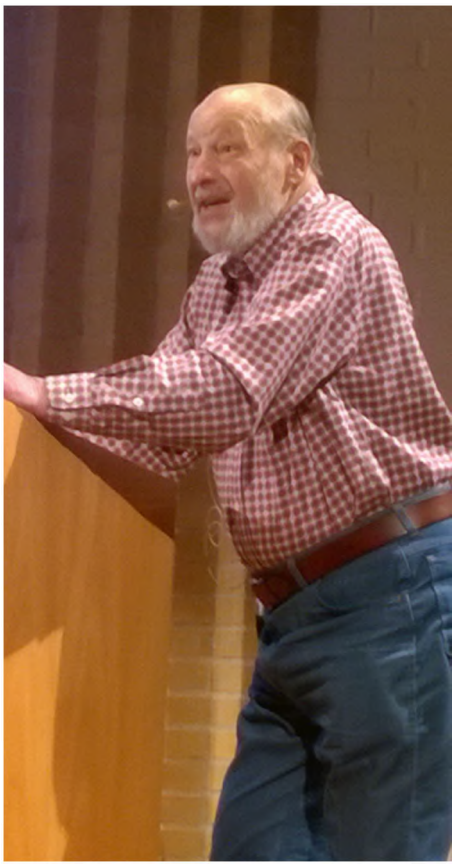
Ted Smayda in Memoriam
10 April 2017

This year the HAB and research community lost another giant intellect. Professor Theodore J. Smayda, (Ted as he preferred to be called) passed away after a period of illness. A graduate of the Braarud School of phytoplankton ecology of Oslo, Norway, Ted held unique skills and insights into phytoplankton dynamics including those of HAB species. Author of over 150 peer reviewed publications and numerous book chapters, he remained a prolific writer and active researcher until the last days. His command of the literature in all languages was legendary and his ability to synthesize information from disparate sources was truly a skill few mastered with his proficiency. His inquisitive mind probed continually for major principles governing the dynamics of phytoplankton which were constantly used to stimulate his many students and colleagues alike. Always willing to listen, Ted developed ideas, questions and testable hypotheses with novice students to well established colleagues alike. Generous with his thoughts and ideas, he freely shared these through his many presentations at meetings, symposia and invited seminars. Among his many scientific accomplishments, Ted took pride in the excellent library holdings at the GSO Pell Library in which he personally scoured sources for pertinent literature. This was in keeping with an overriding philosophy of providing an environment where the mind was the limiting factor and all else was provided. Another scientific accomplishment he was particularly proud of was the long term Narragansett Bay Time Series, a data set beginning in 1959 and continuing weekly into the late 1990s. These data, obtained for various stations in Narragansett Bay but specifically for Station 2 that was sampled weekly over the entire period, remains one of the most complete phytoplankton data sets to date. Unlike other long-term data sets, this was one conducted on whole water samples, detailed species observations to a significant level and included physical chemical data. The legacy of this data set will be available to all in the near future. The data set was also a cohesive element for his students who all contributed to its completion and often generated the hypotheses realized in their thesis research and subsequent publications. Analysis of these data resulted in Ted's understanding of the open niches provided for HAB species development, phytoplankton patterns occurring over unexpected periods spanning several years and trends significant to understanding effects of climate change. Ted's unique view of looking at HAB events as "Rosetta Stones", giving us insights into functioning of complex marine environments where we have few tools to dissect their elements arose from investigating this dataset. He continuously looked through a microscope, knew the species and was able to document their changes in time and in various environments. This occurred at a time when skills at the taxonomic level were disappearing for more convenient but less informative and labor intensive methods. The foundation of these insights will provide stimulus for ideas for many years to come. In a personal vain, Ted was a happy family man, enormously proud of his children and their accomplishments and dearly loved nature including his beloved gardens and pond environment. His love for languages continued and was reveled in his private writing of poetry. A mentor, teacher, perpetual student and lover of knowledge, Ted will be sorely missed but deeply remembered through his enormous legacy of literature, ideas, and continued stimulus to know more. His contributions will continue for yet many years.



Ted Smayda at the Scientific Symposium on Harmful Algal Blooms and Climate Change in Gothenburg, Sweden May 19–22, 2015 (Photo Joe Silke)

Carmelo R. Tomas
University of North Carolina Wilmington, USA

Some of Ted key publications

Smayda TJ 2008. Complexity in the eutrophication-harmful algal bloom relationship, with comment on the importance of grazing. Harmful Algae 8, 140-151

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 58:
8th September 2017

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Intergovernmental Oceanographic Commission

Call to Contribute to Global Harmful Algal Bloom Status Reporting

From 25 to 28 September 2017 sixteen HAB experts from 13 countries gathered at the headquarters of the IOC IODE (International Oceanographic Data Exchange) Programme Office in Oostende, Belgium (see group picture in the back page), to receive training in data entry into the Ocean Biogeographic Information System (OBIS; *http://www.iobis.org*) and the Harmful Algae Event Database (HAEDAT; *http://haedat.iode.org*). Editors within defined HAB regions were requested to collect publications associated with the **occurrences of toxic algae,** even with no recorded impact, and enter these records in OBIS. The aim is to trace reliable geographic ranges for the genera/species that are included in the IOC Taxonomic Reference List of toxic and ichthyotoxic algae (*http://www.marinespecies.org/hab*). This activity will complement the compilation of **records of harmful events with impacts** (including cases of intoxicated seafood, discolorations, mucilages, etc.) collected in HAEDAT, with new event data being entered from regions for which this information is currently missing. The effort of compiling and increasing data sets is being intensified in order to provide a substantial part of the basis for a first Global HAB Status Report.

This report series will provide the scientific community as well as decision makers with a reference on HAB occurrence and impacts on ecosystem services. IOC-UNESCO project partners include the International Atomic Energy Agency (IAEA), the International Council for Exploration of the Sea (ICES), the North Pacific Marine Science Organization (PICES) and the International Society for the Study of Harmful Algae (ISSHA). We hope that the establishment of these data bases will allow us to convincingly answer key questions on the probability of change in HAB frequencies, intensities, and geographic range resulting from environmental changes at local and global scales. While the available data sets are still incomplete, we here provide examples of analyses from HAEDAT (Figs. 1, 2, 4) and OBIS (Fig. 3) data. While the results are preliminary and conclusions are likely to be modified as more data become available, we present them as an encouragement for HAB workers to contribute to this important initiative.

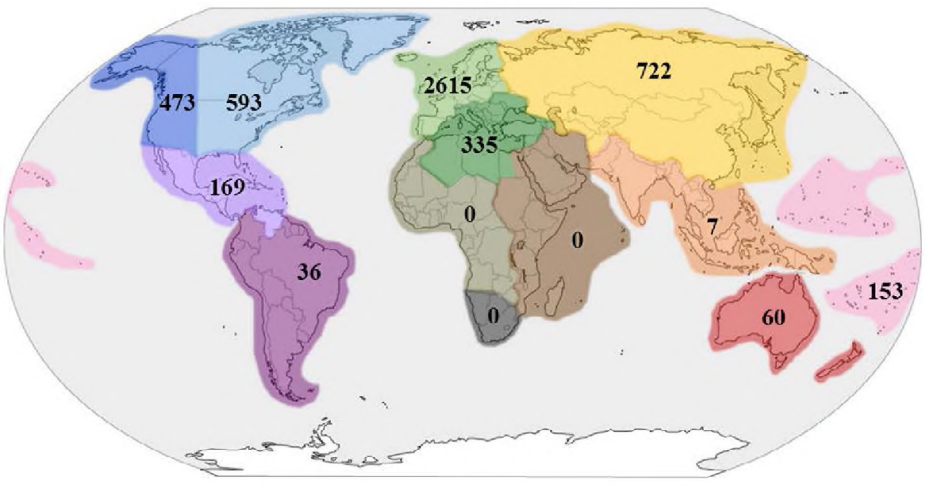

Fig. 1. Total number of HAEDAT records in the different OBIS regions of East Coast America, West Coast America, Caribbean Central America, Northern Europe, Southern Europe, Mediterranean, Australia/New Zealand, North Asia and Pacific. The regions of South America, Africa and South East Asia represent key missing data sets. Data as of 1/3/2017. Compiled by Laura Schweibold who worked for 6 months on a GHSR Masters project supervised by G.Hallegraeff.

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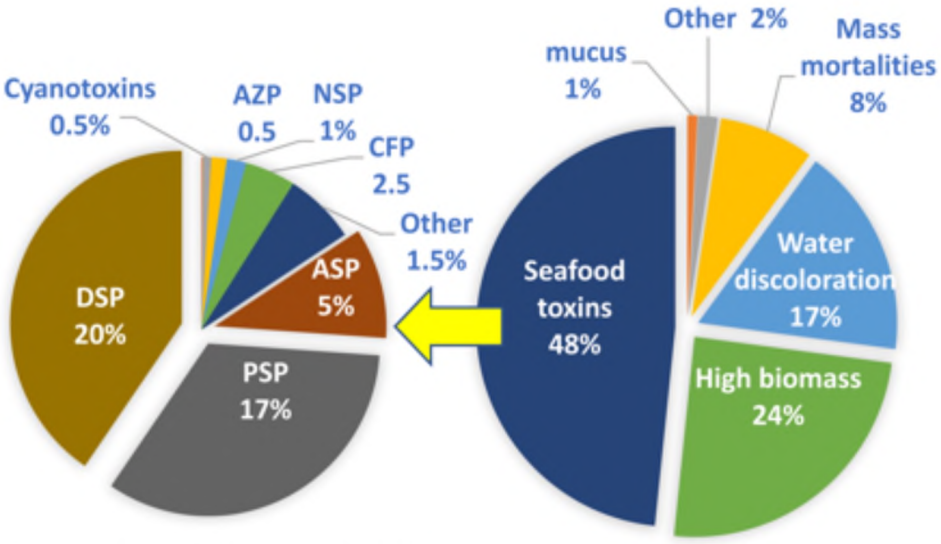

Fig. 2. Partitioning of 5136 global HAEDAT events into seafood toxins, high biomass water discolorations, fauna mass mortalities, and the further breakdown of seafood toxins into DSP, PSP, ASP, NSP, CFP, AZP and cyanotoxins. Data as of 1/3/2017. Compiled by Laura Schweibold.

Different regions and countries suffer from different types of HABs, and this is reflected in the way countries/regions enter their data. North America and Europe operate highly sophisticated shellfish toxin monitoring programs. PSP is the dominant seafood toxin syndrome in North America, NSP in the Gulf of Mexico and DSP in Europe. The effectiveness of these monitoring programmes is well reflected in the fact that only an estimated 1 to 10% of toxin-producing blooms lead to human poisonings. In contrast in less well monitored areas such as Australia/New Zealand and Central America up to 50 to 60% of toxin producing algal bloom events can lead to human victims. In the extreme, Pacific HAEDAT data are based exclusively on human ciguatera poisonings diagnosed by medical practitioners.

OBIS HAB species occurrence data are even more incomplete, and heavily biased by European records. Time series data for location records of the key target genera *Dinophysis* and *Pseudo-nitzschia* (Fig. 3) exhibit an increase in frequency over the past 30 years, undoubtedly reflective of increased awareness and monitoring effort.

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) have used HAEDAT to map the distribution of harmful algal events during the period from 2014 to 2016 in the North Atlantic. Examples for PSP, DSP and AZP are presented in Fig 4. These maps highlight the regional aspect to harmful algal events in the North Atlantic area with PSP events recorded across the USA, Canada and Europe during the three year period, a higher incidence of DSP events recorded in Europe, and AZP events restricted to the SW coast of Ireland with a low number also recorded in the UK and Norway.

Planned outputs of the Global HAB Status Report include: (1) a dedicated session during ICHA18 in Nantes in October 2018; (2) a special issue of Harmful Algae in 2019; (3) regular 2-3 year reports on the status of global HABs, the first of which will be launched in Nantes.

Follow the development of the Global HAB Status Report at *http://haedat.iode.org/* and see who is involved and how you may engage.

Acknowledgements

We thank Ward Appeltans and Pieter Provoost at the IOC/IODE Project Office for hospitality and technical support. The meeting was funded by the Flanders Government through the Ocean Teacher Global Academy and DIPS-4-ocean assessments projects.

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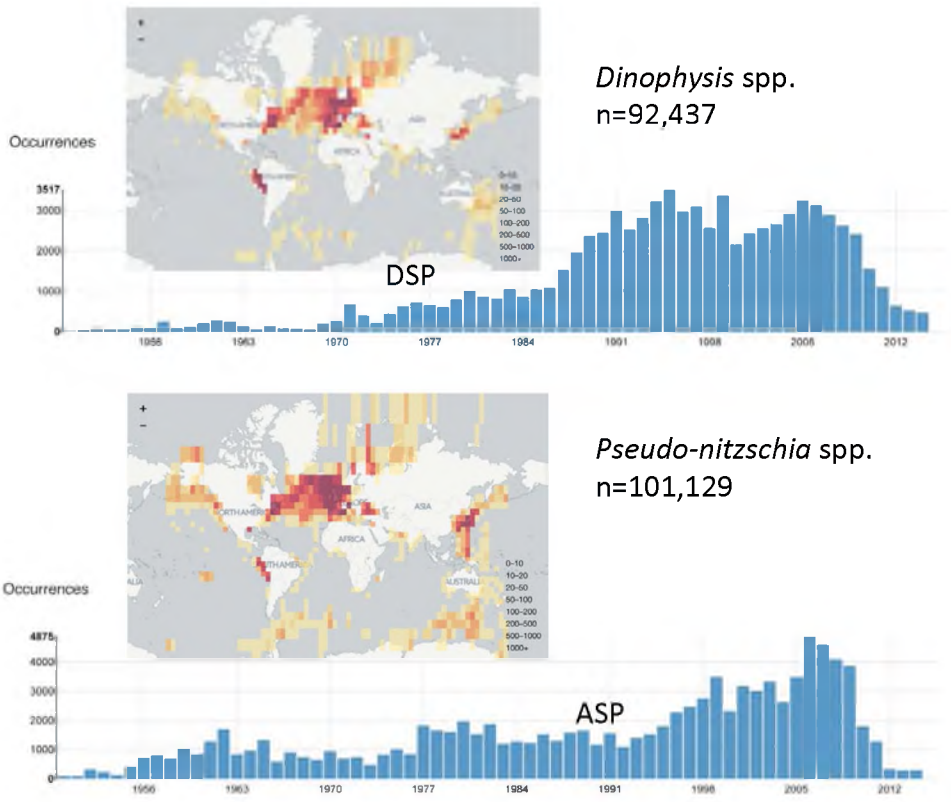

Fig. 3. Time series of OBIS location records of the HAB genera Dinophysis and Pseudo-nitzschia between 1950 and 2014, with the time of description of their associated syndromes DSP and ASP indicated. Records are heavily biased towards Europe.

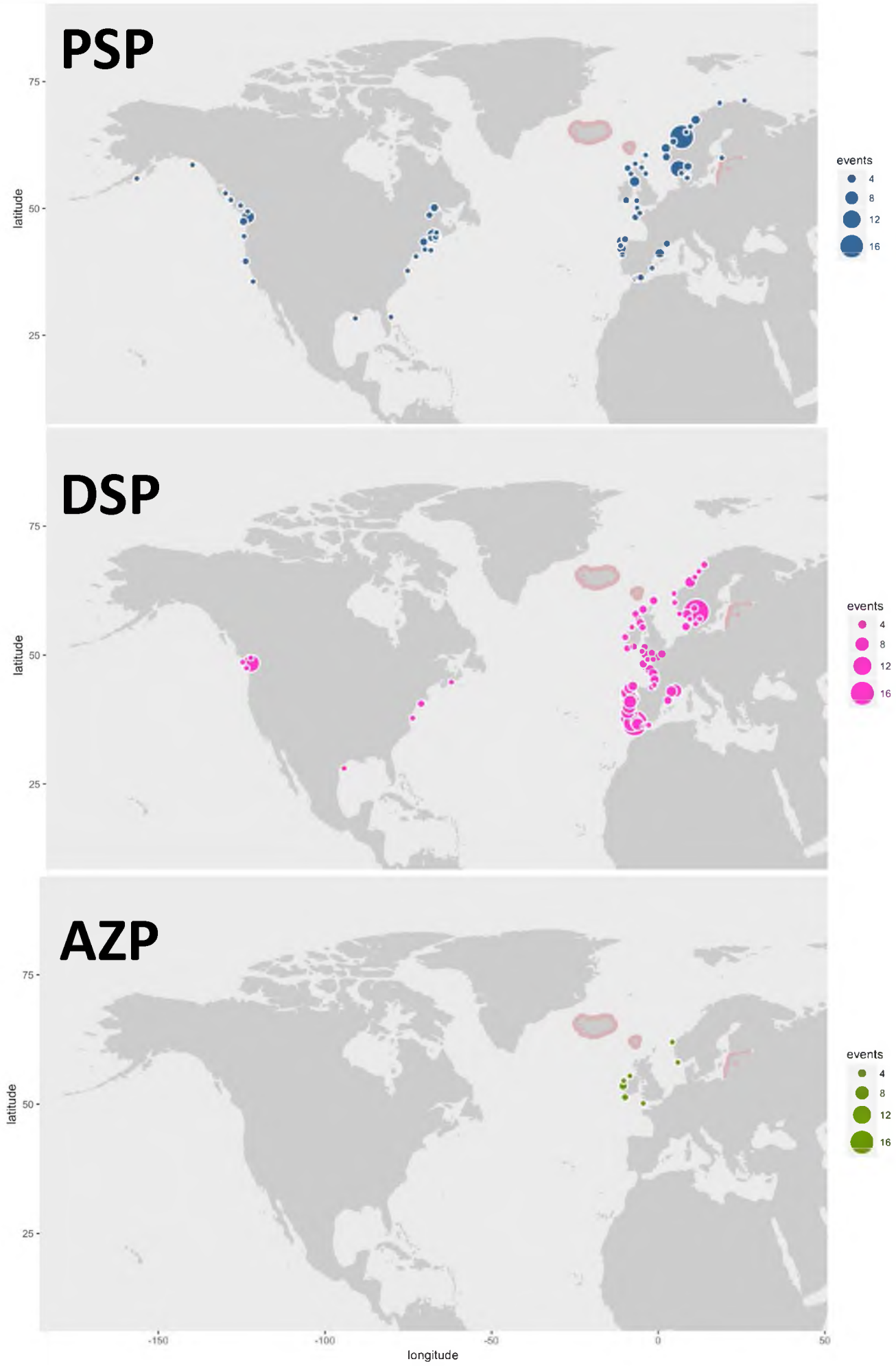

Fig. 4. Maps showing the incidence of PSP, DSP and AZP during the period 2014 to 2016 in the North Atlantic as reported by the ICES-IOC WG HABD. Areas such as Northern Canada and Greenland are not routinely sampled and countries with pink borders have still to submit data. Data from the Pacific shorelines of Canada and the USA are also included.

Pelagic *Sargassum* reaching Serranilla Bank, Caribbean Colombia, may pose a risk to baby turtles



Fig. 1. Location of Serranilla Bank in the Caribbean Sea (Photo Wikipedia)

Floating *Sargassum* has been known from centuries to occur in the Atlantic Ocean, in a region named the Sargasso Sea. Floating *Sargassum* mats can form a pelagic ecosystem comprised by two species, *S. fluitans* and *S. natans*, which support a high variety of species associated with them [1]. It has been estimated that the Sargasso Sea harbors about 10 million tons of wet biomass [2]. Although deposits of *Sargassum* occur naturally and regularly on beaches, since 2011 enormous quantities of these seaweeds have been washed ashore in the Caribbean Sea, Gulf of Mexico, and, in 2011, even reached the West coast of Africa [3-5]. Multiple hypotheses have been proposed to explain such events, amongst which stand the excess of nutrient loads, a change in trade currents, and global climate change [6].

To some extent, *Sargassum* biomass may be beneficial to the environment, because it provides food and shelter to a number of organisms, it may help fight beach erosion and provides nutrients to beach habitats [6]. However, when biomass is very high, it may have negative effects: the accumulation of algae on the sea surface precludes light penetration and affect corals, sea grasses and benthic macroalgae [7]. Drift algae on the beach may become a barrier to nesting turtles and/or to baby turtles finding their way to the ocean [7-9]. Cleaning up the excessive biomass along the beaches may enhance beach erosion [6] and decomposition on the shore may change water chemistry and induce hypoxia, with consequent fish die-off [10].

Serranilla Bank is an ancient atoll in the Caribbean Sea, at 15º 50' N and 79 º 50' W (Fig. 1). It has several small keys emerging from the water to form some permanent islands. These small islands, far from other emerged territories, have been recognized recently as important nesting areas for sea turtles (Barrientos-Muñoz and Ramirez-Gallego, pers. comm.).

In September 2017, the Colombian Commission for the Ocean (CCO), with the participation of other Colombian agencies and institutions (Colciencias, Dimar) and the logistic support of the Colombian Navy (Armada de Colombia), organized a scientific expedition to Serranilla Bank. During this expedition, which is the first and largest effort to date to study the biodiversity of this remote area of Colombia, we observed a large amount of floating *Sargassum* reaching the beaches of Beacon Key, the largest island in Serranilla Bank (Fig. 2). The algae accumulated on the beaches, and formed a thick 40 cm high mat (Fig. 3). On the beach, there were a large number of turtle nests (Barrientos-Muñoz and Ramirez-Gallego, pers. comm.) which, by the time of the *Sargassum* wave, were ready to disclose and the baby turtles go to sea. Some baby turtles were observed having troubles passing the barrier posed by the *Sargassum* mat (Fig. 4), and were vulnerable to predation by ghost crabs, rats and other predators.

Considering that all the sea turtles species in the Caribbean are at risk of extinction, large amounts of *Sargassum* in Serranilla Bank may pose an additional threat to the survival of these organisms.

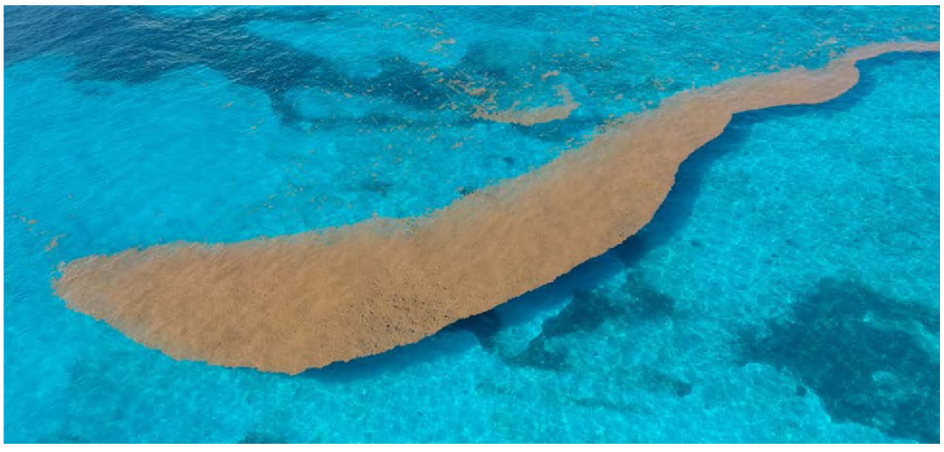

Fig. 2. Aerial photo of drifting Sargassum reaching Beacon Key. (Photo credits: Santiago Estrada-Robledo)

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The authors are greatly indebted to the Comision Colombiana del Océano (CCO), to the Armada Nacional de Colombia, to Colciencias, and to Dimar for organizing the Scientific Expedition *Seaflower* 2017, Serranilla Bank. We thank Karla Georgina Barrientos-Muñoz and Cristian Ramirez-Gallego, from the Fundación Tortugas del Mar, for sharing their knowledge on sea turtles and Santiago Estrada-Robledo, from the Reef Shepherd Scuba diving school, for the aerial photo of *Sargassum*. The present study was financed by Universidad Nacional de Colombia, sede Caribe.



Fig. 3. Sargassum accumulation as a thick mat on the nesting beach



Fig. 4. A baby turtle struggling to find its way through the Sargassum mat

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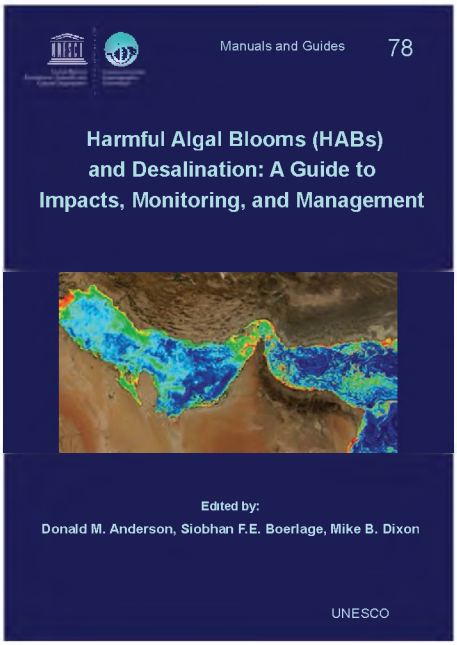
NEW!! Harmful Algal Blooms (HABs) and Desalination: A Guide to Impacts, Monitoring, and Management



UNESCO's Intergovernmental Oceanographic Commission has launched the first ever guidebook on the growing problem HABs pose to seawater desalination plants. The launch took place last October at the International Desalination Association World Congress in São Paulo Brazil.

This guidebook is published to help the desalination industry tackle an issue that represents a potential threat both to human health and to the distribution of desalinated water on which an increasing number of arid countries rely for their fresh water needs.

The 517-page manual is a groundbreaking achievement built on the cooperation of 63 HAB and desalination industry specialists from multiple disciplines, some of which had rarely interacted in the past.

The publication was sponsored by the Middle East Desalination Research Center (MEDRC), the United States Agency for International Development (USAID) and the IOC of UNESCO. It was edited by Don Anderson, Siobhan Boerlage and Mike Dixon.

Copies of the manual can be ordered online at *http://www.ioc-unesco.org/HAB-desalination*

A red tide event associated with the dinoflagellate *Karenia mikimotoi* in the Firth of Clyde, Scotland

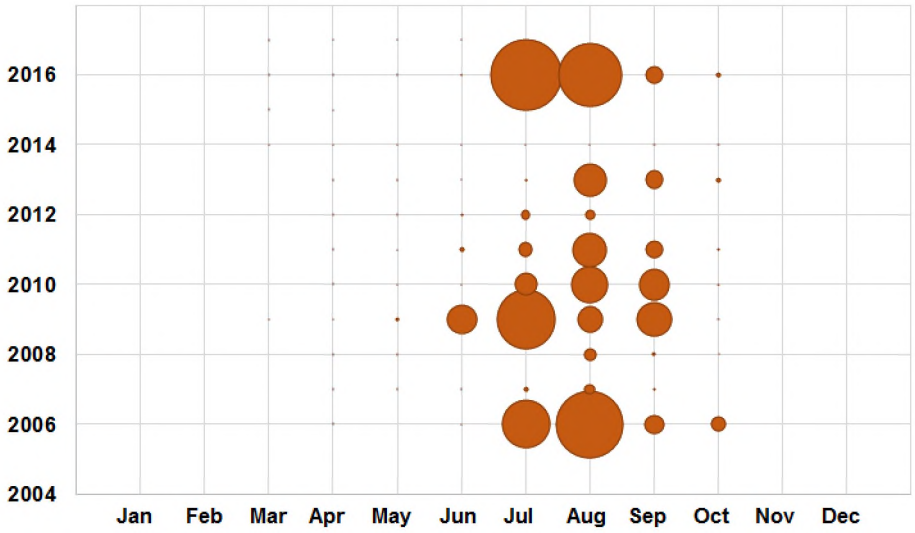


Fig. 1. Maximum abundance of K. mikimotoi by month and year for monitoring sites in Scottish coastal waters. The densest bloom observed exceeded 4 million cells per litre in July 2016.

The potentially harmful dinoflagellate species *Karenia mikimotoi* has been an occasional red tide forming species in Scottish waters since the 1980s. In 2006 a bloom detected off the west coast of Scotland covered an exceptionally large area, progressing around the Scottish coast and leading to mass mortalities of benthic invertebrates and wild fish [1]. Since then regular monitoring has been undertaken by the Scottish Association for Marine Science (SAMS), revealing recurrent blooms in late summer (Fig. 1). In addition to this, occurrences have been recorded at monitoring stations administered by Marine Science Scotland (MSS) and the Scottish Environment Protection Agency (SEPA).

In July 2016 SAMS alerted SEPA and MSS to increasing observations of *K. mikimotoi* in seawater samples collected from coastal sites around southwest Scotland. Soon after, members of the public made contact with SEPA regarding water discolouration and large numbers of dead marine life washed ashore along the coast at a number of locations in the Firth of Clyde. Samples were sent for identification by SEPA and MSS analysts, who made contact with SAMS, confirming there was an ongoing bloom event covering the Firth of Clyde area (Fig. 2).

Combined SAMS and SEPA data illustrate the coverage and development of this bloom through summer 2016 (Fig. 3). The bloom began in late July along the Ayrshire coast, and persisted at high abundance into September in Loch Ryan (Fig. 4). Cell density was sufficient to bring about hypoxic conditions three times [2, 3], and mass mortalities of a variety of marine organisms were reported during these periods. One SEPA sample in Loch Ryan contained 3.5×10^7 cells per L^{-1}, which would have the potential to bring about total anoxia in the immediate vicinity upon termination of the bloom [2] (Fig. 5). This is reflected in the greater variety of organisms reported washed ashore dead in the Loch Ryan area.

Considerable effort has been made to describe and predict the conditions that initiate these blooms [4, 1], as the species has the potential to cause economic damage to the important finfish aquaculture sector on the west coast of Scotland. Blooms can occur as a result of advection from offshore [1, 5], or sparsely-distributed overwintering vegetative cells may act as a seed population when environmental conditions become favourable [5]. *Karenia mikimotoi* has also been observed to have a resting stage in the form of non-motile spherical cells [6] that could have the potential to initiate a bloom.

For part of the bloom period, the electronic bathing water advice signs administered by SEPA were automatically warning against swimming, due to heavy rainfall in the area which overloads wastewater treatment capac-

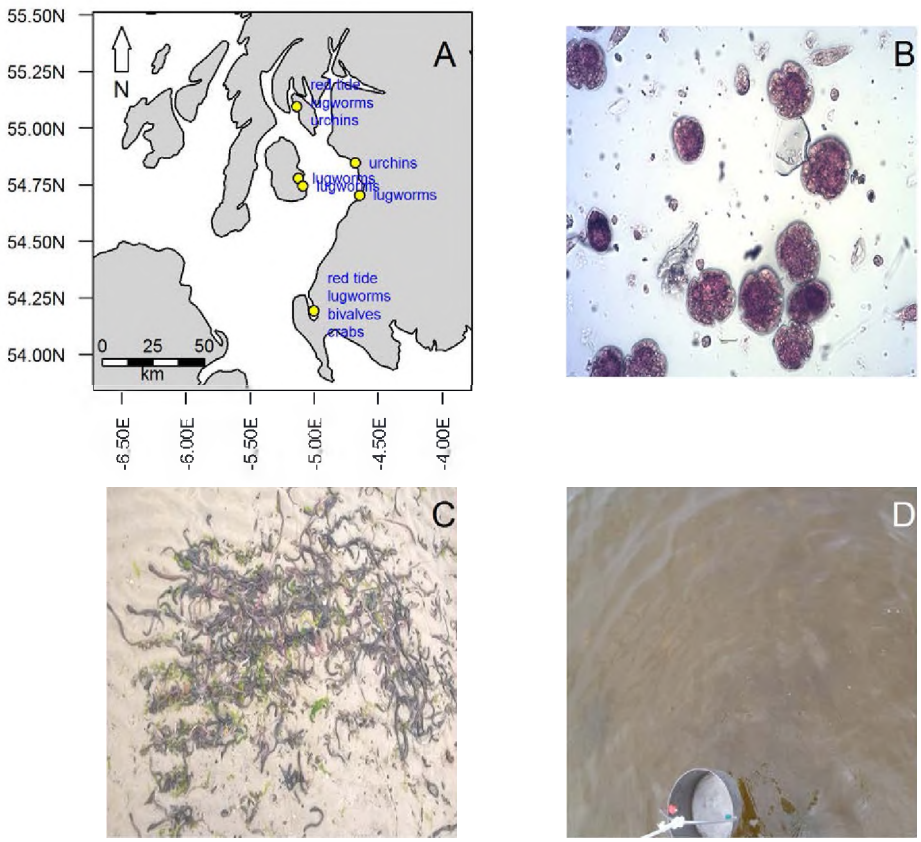


Fig. 2. The 2016 K. mikimotoi bloom. A: locations of reported mass mortalities; B: K. mikimotoi cells sampled at Ettrick Bay, isle of Bute; C: dead lugworms washed up on the shoreline at Ettrick Bay; D: discoloured water in Loch Ryan.

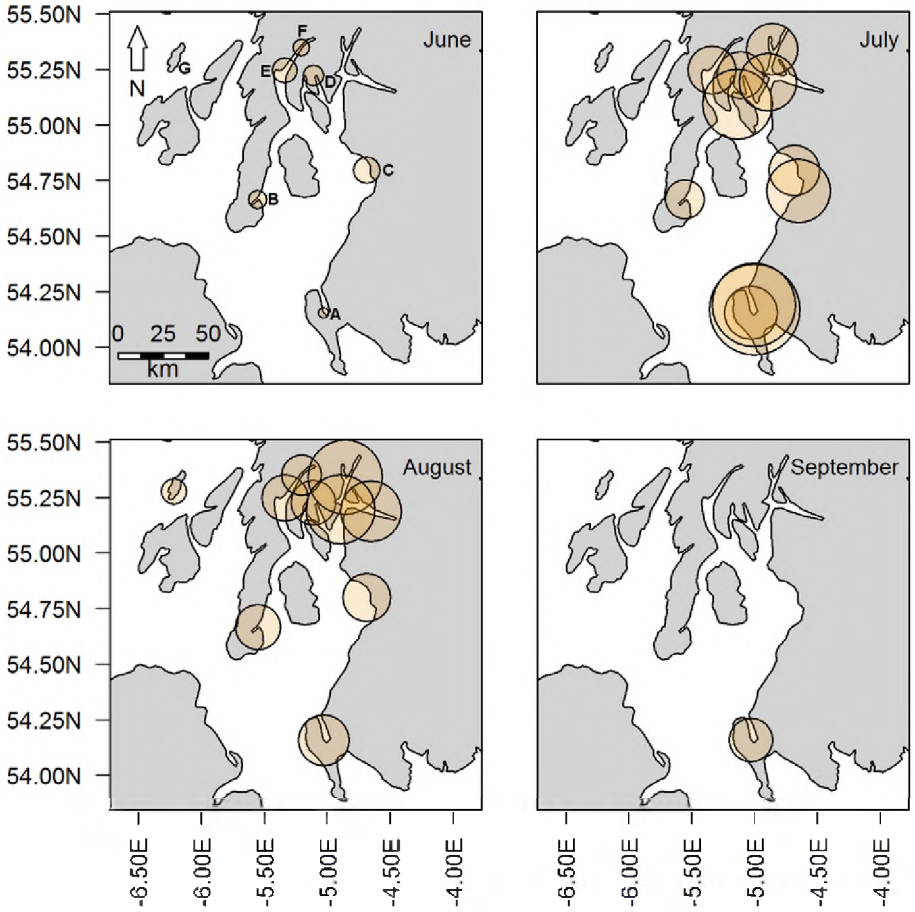


Fig. 3. The progression of the bloom in the Firth of Clyde area. Letters indicate locations as described in Fig. 4.

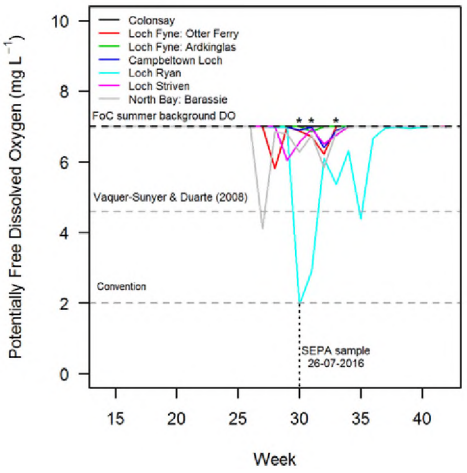


Fig. 5. Theoretical effect of the K. mikimotoi bloom on background dissolved oxygen concentration (black horizontal dashed line: Firth of Clyde summer averaged SEPA data 2005-2015). Cell density was sufficient to cause hypoxia on 3 occasions at 4.59mg L^{-1}[8] or once at 2mg L^{-1} (grey dashed lines). An exceptionally dense bloom recorded by SEPA in Loch Ryan had potential to cause local anoxia (black vertical dotted line). Mass mortalities around the Firth of Clyde were reported on three dates (black asterisks).

ity resulting in discharge of untreated wastewater into the marine environment. This may contain additional nutrients of use to *K. mikimotoi* but these were not measured at the time of phytoplankton sampling. Rainfall is a factor in the initiation of *Karenia* blooms in the English Channel [7, 8] and more generally can establish conditions that lead to red tides set out by Smayda [9].

Acknowledgements

We thank Sheila Fraser at MSS for providing a second opinion on the identity of the cells. We also thank the phytoplankton monitoring team at SAMS and Lucy Kennedy, Lynne McDonald, and Michelle Tibbles at SEPA for assistance in sampling soon after reports of mass mortalities.

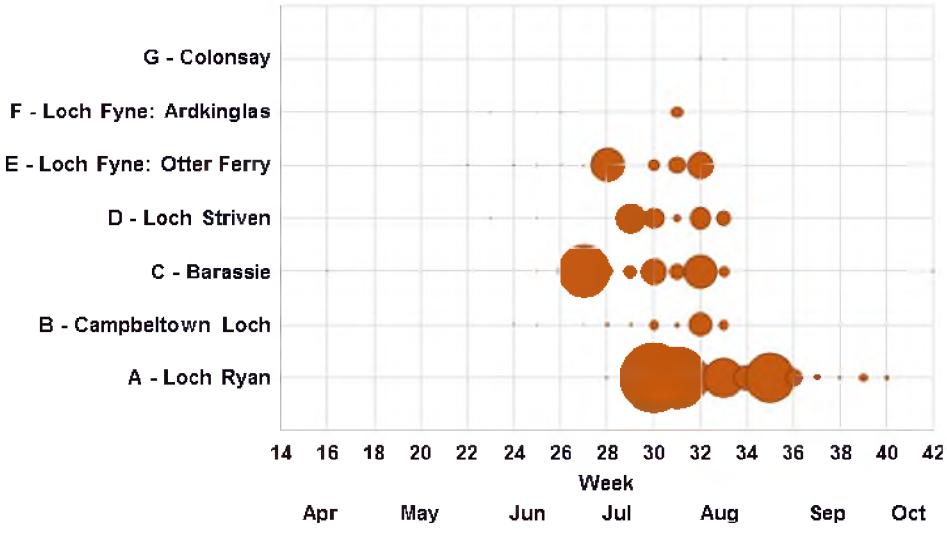


Fig. 4. The progression of the K. mikimotoi bloom by week in 2016, recorded at coastal monitoring sites around the Firth of Clyde.

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First evidence of high saxitoxin concentration in *Crassostrea iridiscens* associated with *Gymnodinium catenatum* blooms at Banderas Bay, Jalisco México

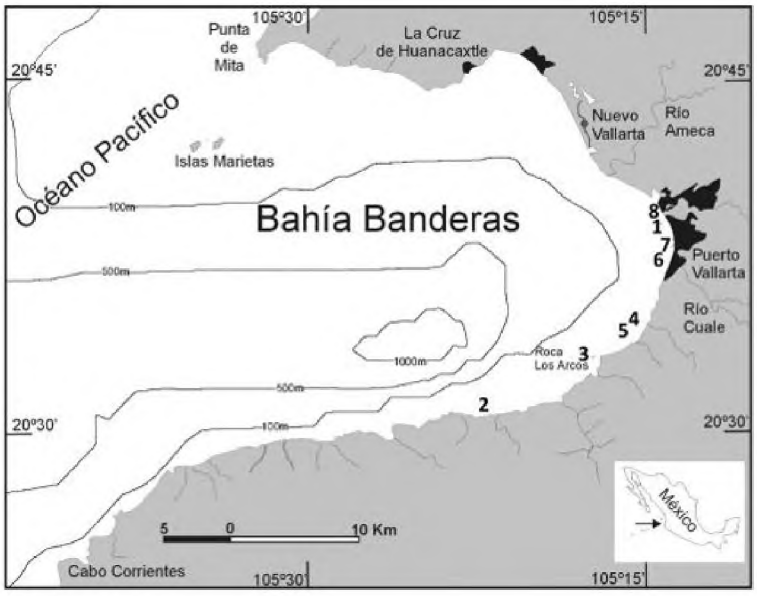

Fig. 1. Location of sampling stations in Banderas Bay Jalisco México during Gymnodinium catenatum bloom from March to June of 2017.

Banderas Bay has a long-standing history of toxic HABs. The presence of saxitoxins (STXs) had been associated with dinoflagellates belonging to the genus *Alexandrium* and with *Gymnodinium catenatum* [1, 2]. Blooms of both of these microalgae can pose a high risk for marine food resources to become contaminated with STXs, some of the most potent neurotoxins (PSP syndrome) which can prove fatally toxic to humans [3]. During 2017 the highest density of toxic *G. catenatum* and the first evidence of high STX concentrations in rock oysters (*Crassostrea iridiscens*), one of the most iconic species for the shellfish industry in the region, were recorded. A review of this event was carried out by the CIC-CUCOSTA-U de G in collaboration with the local health authorities of the Epidemiology Section (Department of Health Jalisco) for health protection and shellfish toxins regulatory purposes.

Banderas Bay is located on the western Mexican coast (20° 15´ to 20° 47´ N, 105° 15´to 105° 4´ W) [4]. It has a total area of ~ 975 km^2, and is limited by Punta de Mita (north) and Cabo Corrientes (south), with a range of ~40 km between the two points (Fig. 1). The mean sea surface temperature is 26.4ºC with a seasonal range from 23.3 ºC during winter-spring, to 30.0 ºC during summer-autumn. The area has a complex hydrodynamic regime due to its location in a transitional area seasonally influenced by three important current systems: (1) the California Current that flows southward, bringing cold and low salinity water to the region, (2) the Costa Rican Costal Current that flows northward, transporting warm waters of intermediate salinity, and (3) the warm and dense water of the Gulf of California, which is transported southward into the Banderas Bay region [4].

Samples of native *C. iridiscens* were obtained from three commercial oyster harvesting areas and the shellfish extracts prepared for toxin analysis at the CCAYAC-COFEPRIS (Comisión del Control Analítico y Ampliación de Cobertura) laboratories, according to the Mexican Official NOM-242-SSA1-2009 standards and procedures [5]. Samples from *G. catenatum* bloom areas were collected from March to June at eight stations (E1-E8), stored in 500-ml plastic bottle and preserved with Lugol-acetate solution for identification and cell counts at a Leica® light microscope. Phytoplankton quantitative analyses were carried out with 20x and 40x magnification, using a 1 ml Sedgewick-Rafter settling chamber. Temperature and salinity were measured *in situ* using a YSI 85 multiparameter probe, and micrographs of *G. catenatum* obtained with a digital camera.

Based on mouse bioassay analysis (MBA), the maximum toxin concentration in *C. iridiscens* samples, 389.82 µg STX eq 100 g^{-1} shellfish flesh , was observed at Station E-7 (Sheraton), the same location where the highest density of *G. catenatum* (2.42 x 10^6 cells L^{-1}) was found (Fig. 2). Regulations from the health authorities stipulate that bivalve molluscs for the market must not contain more than 80 µg STX eq 100 g^{-1} tissue [5]. Given the magnitude of this PSP event, a temporary ban was immediately implemented by COFEPRIS authority, and a suspension of the extraction, commercialization and consumption of rock oysters was enforced through notification advice SST-CRS/599/2017, until STXs and *G. catenatum* densities in sea water dropped to safe levels. There were differences of three orders of magnitude (range, 3 x 103 - 3 x 106) in *G. catenatum* cell densities between the 8 sample sites (Fig. 3a). These densities were the the highest recorded since 2001 to date [1, 2]. During the toxic event, the mean surface temperature

Table 1. Toxin content (µg STX eq 100 g^{-1}) in soft tissue of Crassostrea iridiscens (rock oyster) from Banderas Bay, Jalisco, México at stations. E-8 (Velas), E-7 (Sheraton) and E-6 (Malecón).

Date	µg STX eq 100g^{-1}	Station	Mean Temperature ° C	Mean Salinity ups	Mean pH
March 14th	32.52	E-8	22.85	33.48	8.28
March 24th	389.82	E-7	24.52	33.43	8.34
April 6th	48.86	E-7	22.5	33.26	8.18
April 7th	not detected	E-7	22.5	33.26	8.18
May 8th	38.61	E-7	-	-	-
June 6th	not detected	E-7	25.1	33.16	8.14
June 23th	59.52	E-6	23.12	33.25	16.23

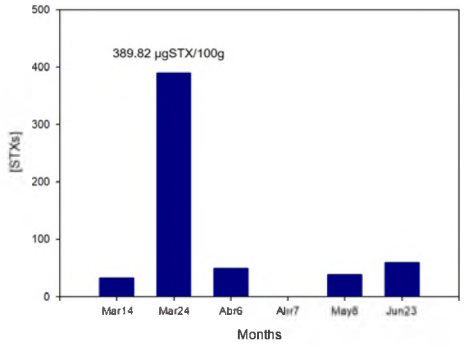

Fig. 2. High saxitoxin concentration in Crassostrea iridiscens associated with Gymnodinium catenatum blooms in Banderas Bay, Jalisco México.

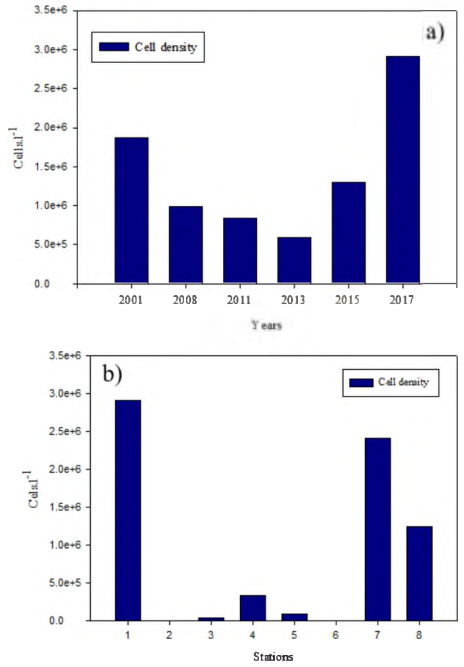

Fig. 3. Temporal (a) and spatial (b) variability of G. catenatum bloom in Banderas Bay, Jalisco México.

ranged from 22.19 to 25. 26 ºC, salinity remained almost constant (33.67-33.01) and pH values from 8.81 to7.92. All these data coincide with "normal" seasonal patterns for this coastal area [4]. Previous data from the area have shown that increased in *G. catenatum* cell blooms exhibits a high spatial and temporal variability and are frequent in Banderas Bay (Fig. 3b). The highest densities are observed during winter-spring every year coinciding with the upwelling season.

Our findings during March and June 2017 suggest the accumulation of toxic cells of *G. cateantum* (Fig. 4) could explain the highest STXs concentrations determined in shellfish from the Bay.

The standard mouse bioassay was used for regulatory purposes by the health authorities and there were no cases of public health illness during this event. This emphasizes the relevance of established monitoring programmes as an essential tool for timely detection and surveillance of the presence of HAB species and their toxins to protect public health in the coastal waters of Banderas Bay.



Fig. 4. Micrographs of live cells of Gymnodinium catenatum from Banderas Bay at 400x (a) and 200'x (b); Seawater discoloration due to a G. catenatum patch during the bloom period in March-June 2017 (c) (Photos: M.C. Cortés-Lara)

Acknowledgements

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ICES-IOC Working Group on Harmful Algal Blooms Dynamics

The report of the International Council for the Exploration of the Sea (ICES) and Intergovernmental Oceanographic Commission of UNESCO (IOC) Working Group on Harmful Algal Bloom Dynamics (ICES-IOC WGHABD) is now available on the ICES website (*http://www.ices.dk/community/groups/Pages/WGHABD.aspx*). ICES-IOC WGHABD met on three occasions to address 11 terms of reference (ToRs) during the three year reporting period; April 2015 (hosted by Teresa Moita and Alexandra Silva, IPMA, Lisbon, Portugal), April 2016 (hosted by Raffaele Siano, IFREMER, Brest, France), and April 2017 (hosted by Anke Kremp, SYKE, Helsinki, Finland (Fig. 1)).

Three terms of reference contributed to the work of the IOC Intergovernmental Panel on Harmful Algal Blooms (IOC IPHAB). A manuscript on fish killing algae produced by ICES-IOC WGHABD will feed into the IPHAB task team on fish killing algae. ICES-IOC WGHABD have updated and quality controlled data in the IOC-ICES-PICES Harmful Algal Event Database (HAEDAT). Maps showing the regional distribution of harmful algal events presented at ICES-IOC WGHABD using data from HAEDAT from 2014 – 2016 are included in the report. ICES-IOC WGHABD is producing a Harmful Algal Event status report for the ICES area during 2018. This status report will be the ICES contribution to the Global HAB Status Report currently in production (see article by Hallegraeff et al. in this issue).

A dynamic range of new findings were presented each year with summaries included in ICES-IOC WGHABD annual reports from 2015 - 2017. These presentations encompassed studies performed with the latest technologies or approaches and include the use of technologies such as the Imaging Flow Cytobot to examine HABs in the USA and Sweden, citizen science in France, the use of sediment cores to reconstruct the history of toxic *Nodularia spumigena* blooms in the Baltic Sea and Oslofjorden, development of early warning systems in the UK and social unrest in Chile as a result of HABs.

ICES-IOC WGHABD also reviewed the OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition and participated in the subsequent advisory drafting group ADGJAMP during 2015. The WG also contributed to the organization of the ICES-PICES-IOC scientific symposium on climate change and harmful algal blooms held 19 – 22nd May 2015, Gothenburg, Sweden. More than 60 scientists from across the globe participated. A major output of this symposium is the peer reviewed publication, Wells, M.L. et al., (2015). Harmful algal blooms and climate change: Learning from the past and present to forecast the future. Harmful Algae, 49:68–93.

The WG also reviewed the use of molecular probes, qPCR methods targeting toxin producing genes and metabarcoding of HABs, as well as the state of knowledge of BMAA, the amino compound ß-methylamino alanine. The dynamics of *Gymnodinium catenatum* in the Iberian Peninsula, *Alexandrium minutum* in the Bay of Brest and *A. ostenfeldii* and cyanobacteria in the Baltic were also reviewed with summaries of presentations included in ICES-IOC WGHABD annual reports.

The next meeting of ICES-IOC WGHABD will take place from 23rd – 26th April 2018, hosted by Dr Margarita Fernandez Tejedor from IRTA, Sant Carles de la Ràpita, Tarragona, Spain.

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Fig. 1. Participants at the ICES-IOC WGHABD hosted by Dr. Anke Kremp at SYKE, Helsinki, Finland, April 2017.

SEAFDEC-MFRD Regional Training Course in Malaysia

Harmful algal blooms (HAB) and their socio-economic impacts are recognized internationally due to the negative impacts from HABs on the the coastal ecosystem, safety and security of food and drinking water, and human health hazards. Some incidents and occurrences are cross national borders and cover wide sea areas.

HAB events especially affect the rapidly growing aquaculture industries in Southeast Asian countries, HAB monitoring programmes have been established and implemented. These programmes have been hampered by the lack of trained manpower in HAB monitoring. The Southeast Asian Fisheries Development Center (SEFDEC) through the Marine Fisheries Research Development (MFRD) program has implemented a series of training workshops to enhance the expertise of personnel from its member countries. A regional training course with the theme "Specimen Preservation and its Application in HAB Monitoring and Studies" was successfully conducted at Bachok Marine Research Station, Institute of Ocean and Earth Science, University of Malaya.

The 4-day training course, hosted by Dr. Po Teen Lim, was attended by 21 participants from nine Southeast Asian countries (Brunei, Cambodia, Indonesia, Laos, Malaysia, Philippines, Thailand, Singapore, and Vietnam) (Fig. 1). The Chief of MFRD, Mr. Soon Eong Yeap in his official remarks, stressed the continuous commitment to support HABs and biotoxin programmes initiated through the Japanese Trust Fund on HABs and biotoxins. In the welcoming remarks, Dr. Lim highlighted the need for capacity building and strengthening of HAB research collaboration in the region (Fig. 2).

The project leader of the UNESCO IOC WESTPAC HAB program, Dr. Mitsunori Iwataki, provided support by contributing reference and teaching materials. All lectures were followed by practical sessions on HAB monitoring and studies. An introductory lecture given by Dr. Lim highlighted HAB occurrences, types, causative species and impacts. Dr. Chui Pin Leaw provided lectures on the current detection technologies applied in HAB research and monitoring. The practical sessions included sampling devices and sampling processing, type of preservatives and stains, single-cell polymerase chain reaction (PCR), and real-time quantitative PCR for species detection and enumeration (Fig. 3). During the practical session, a contest on the best sketch of a HAB species was held and three participants from Thailand, Cambodia, and Philippines were awarded prizes. During the discussion session, participants expressed their interest on certain topics for the future training course. The 4-day training course ended with a certificate presentation ceremony.

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Fig. 2. Observing phytoplankton specimens by microscopes.



Fig. 3. Demonstrating qPCR assay for species detection.



Fig. 1. Participants of the SEAFDEC Regional Training Course at Bachok Marine Research Station, University of Malaya.

Forthcoming events

Workshop on morpho-molecular methods for the study of dinoflagellate cysts

Monday 12th and Tuesday 13th February 2018

Location: Cawthron Institute, 98 Halifax Street East, Nelson 7010, New Zealand

Organisers: Kirsty Smith, Lesley Rhodes (Cawthron Institute), Kenneth Neil Mertens (Ifremer, France)

We invite you to participate in this workshop which will discuss traditional morphological methods and novel molecular methods (e.g. qPCR, metabarcoding) and how to combine these techniques to achieve the best results when identifying dinoflagellates and their cysts. Other protists can also be included if there is interest from participants. During the workshop the pros and cons of traditional microscopic techniques versus novel molecular techniques will be discussed and demonstrated; incubation techniques will also be demonstrated.

Participants are invited to present their research during the workshop, and bring samples/slides to study. If coming from overseas, please check with Kirsty Smith for quarantine requirements.

The workshop, which includes complimentary lunches on Monday and Tuesday, is free and will require registration only (no charge). Participants have to pay their trip and accommodation. Please contact the organisers for suggestions on travel to Nelson and accommodation.

Please let us know as soon as possible if you wish to attend as the number of participants is limited to 20.

Hope to see you there!

Kenneth Mertens, Kirsty Smith & Lesley Rhodes, Cawthron Institute, New Zealand

Email: *kirsty.smith@cawthron.org.nz*

ICES-IOC-IMO Working Group on Ballast and Other Ship Vectors

5-7 March 2018

The next meeting of the Working Group on Ballast and Other Ship Vectors (WGBOSV) will be held in Caniçal, Madeira, Portugal from 5 to 7 March 2017.

If you, or any expert from your country, wish to attend, please contact the chair of the WGBOSV, Dr. Baily: *Sarah.Bailey@dfo-mpo.gc.ca*

SETAC Europe Conference, Rome

13-17 May 2017

In the framework of the next SETAC Europe conference (Rome, 13-17 May 2017), within Track 4 "Ecological risk assessment and human health risk assessment of chemicals mixtures and stressors and risk mitigation strategies", the following session has been proposed.

4.6 Do human activities and global change influence the occurrence, toxicity and pathogenicity of marine harmful microorganisms affecting seafood safety?

Co-chairs: Jorge Diogène, Angelo Maggiore

Variations of environmental factors associated with human activities and global change may have direct and indirect impacts on the occurrence, dominance, persistence, adaptation, toxicity and pathogenicity of marine microorganisms. For example, changes in climate may allow originally tropical phytoplankton species to expand their spatial and temporal distribution to previously temperate waters. Shellfish, finfish and other marine organisms can accumulate in their organs pathogenic microorganisms or the toxins they can produce, which are transferred within food webs reaching humans through consumption of seafood. Changes in the biology and ecology of pathogenic and toxin-producing microorganims could therefore determine an increase in the number and intensity of seafood-related diseases. Microorganisms and toxins not previously reported in European seas are now appearing, including areas that are intensive producers of seafood. This is a cause of concern as some of these emerging hazards are not included in current legislation and monitoring programs.

This session has different objectives. A major objective will be to gather information and data on variations in the occurrence, intensity, species composition, toxicity and pathogenicity of harmful marine microorganisms. Particular attention will be focused on biologically relevant areas (hatching, nourishing areas), sensitive areas (estuaries and coastal zones), harvesting, fishing areas or areas occupied by aquaculture facilities. These data should include information on environmental variables linked to climate change (pH, temperature, salinity, oxygen, nutrients etc).

This information is crucial for the development of models that may be able to predict the spatio-temporal distribution of harmful microorganisms, their toxicity/pathogenicity and the bioaccumulation of marine toxins in marine organisms. These models may provide management tools for the design of sampling programs and the implementation of mitigation strategies, taking into consideration the possible co-occurrence of different harmful organisms. This session will also identify research needs and knowledge gaps in understanding how environmental changes will affect the microbial community, in particular the mechanisms underlying their sensitivity to changes (acclimation, physiological plasticity, strain diversity etc). It will also intend to favour a critical analysis of how human activities and global change are impacting the ocean microbial community and discuss future trends in research to better assess this issue, with particular focus on the impact on seafood safety and public health.

The deadline for abstracts submission is November 29th, 2017.

More info at: *http://bit.ly/2yj1Ogv*

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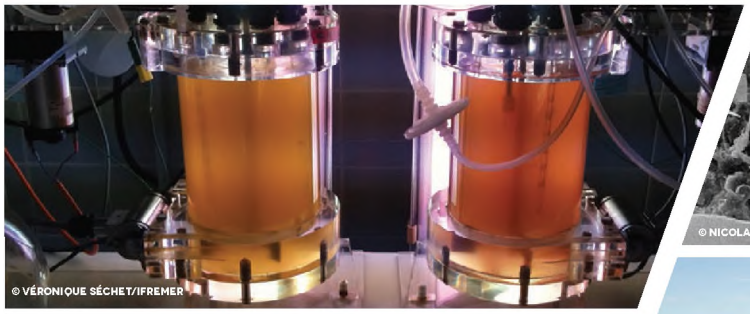
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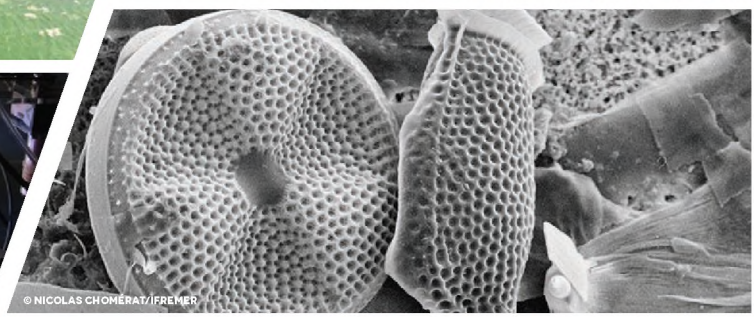
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Rex Munday in Memoriam

Dr Rex Munday, an internationally renowned toxicologist, sadly passed away on the 20th July this year. His wife Christine, daughter Sarah (Finch) and son John were with him. Rex published with all his family at different times, an example being the paper Munday, Munday and Munday, 2005, J. Agric. Food Chem. 53, pp 9695–9700!

Rex, an organic chemist (graduated Sheffield University, UK), arrived in 1979 for a two year stint at AgResearch, Hamilton, New Zealand, as a facial eczema research fellow and ended up staying for nearly four decades. He was instrumental in developing the zinc bolus technology which provides a practical solution to protect animals from facial eczema. In 2009 his research was recognised with a Kudos Lifetime Achievement Award (Kudos Science Trust, Waikato, New Zealand). It is worth checking out his own explanation of his research at *https://www.youtube.com/watch?v=bimDOJPbi_o*.

Rex also researched isothiocyanates, which are present in Brassica vegetables, and demonstrated that they afford protection against bladder cancer in experimental animals, raising the possibility that these natural, non-toxic substances could be valuable for cancer prevention in humans.

As "Principal scientist, toxicology" at AgResearch, he rapidly became internationally recognised for his work for the government funded Safe New Zealand Seafood programme, collaborating with the Cawthron Institute to determine the implications of micro-algal biotoxins for human safety. The work saw him involved with the FAO/WHO/IOC expert panel on shellfish toxins and expert panels of EFSA on setting regulatory limits for biotoxins.

He was on the organising committee for the 16th International conference on Harmful Algae, Wellington, New Zealand, 2014, and presented a plenary lecture on "Risk assessment of seafood toxins". Many readers of HANews will remember him for his succinct, informative and often entertaining presentations over many years. He has chapters on biotoxin toxicity in many books, for example:



- Munday R 2013. Toxicity of Cyclic Imines. In 'Toxins and Biologically Active Compounds from Marine Microalgae', G.-P. Rossini (ed.),

- Munday R 2013. Toxicology of Seafood Toxins: A Critical Review. In 'Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection', Third Edition. L. M. Botana (ed.) and, about to be published,

- Munday R 2017 Toxicology of Seafood Toxins: Animal Studies and Mechanisms of Action. In Comprehensive Analytical Chemistry: Recent Advances on the Analysis of Marine Toxins. J Diogene and M Campas (eds.).

Rex was very sure that oral toxicity was the way to go for the setting of biotoxin regulations. He wrote a comment about Paralytic shellfish toxins (PSTs), which was to be submitted to HANews, and which stated with his usual clarity that: *"New analytical techniques allow the concentration of the various PSTs in a seafood sample to be determined with accuracy and precision. But the toxicity of the different compounds in the mixture of toxins varies, so in order to determine the risk of the sample to human health, we need to know the relative toxicities of the different compounds in the mixture. This permits the calculation of 'Toxicity Equivalence Factors (TEFs)'. A major compound in the mixture (in the case of the PSTs this is saxitoxin) is given a TEF of 1.0. If the toxicity of a compound (Compound X) in the mixture is half that of saxitoxin, its TEF is 0.5. If the toxicity of another compound (Compound Y) is twice as toxic as saxitoxin, its TEF is 2.0. So, if the analytical data shows concentrations of saxitoxin, Compound X and Compound Y in the sample of 0.5 µg/g, 0.2 µg/g and 1 µg/g respectively, the overall toxicity can be calculated as 1 x 0.5 + 0.5 x 0.2 + 1 x 2 = 2.6 µg/g.*

In the past, TEFs for PSTs have been based on toxicity by intraperitoneal injection in mice. This is inappropriate, since human exposure to such toxins is via the oral route, and there is no correlation between toxicity by intraperitoneal injection and that by oral administration. The acute oral toxicity of a number of PSTs has now been determined in mice, and these data have been used to calculate TEFs that are relevant to the human situation. The new data on oral toxicity will lead to a more precise evaluation of the levels of PSTs in seafood that are safe for human consumption."

Rex was highly regarded by his colleagues and was a mentor to many younger scientists. We will all miss his sage advice and his delightfully dry sense of humour. His contribution to HAB science has been immense.

A small sample of Rex's >150 key HAB related publications

Munday R, Briggs L, Truman P, Gooneratne R, Edwards P, Pascal S M 2012. Novel toxins produced by the dinoflagellate *Karenia brevisulcata*. Harmful Algae, 13, 47-57.

Munday R, Quilliam M A, LeBlanc P, Lewis N, Gallant P, Sperker S A, Ewart H S, MacKinnon S L 2012. Investigations into the toxicology of spirolides, a group of marine phycotoxins. Toxins 4, 1-14.

Munday R., Selwood A I, Rhodes L 2012. Acute toxicity of pinnatoxins E, F and G to mice. Toxicon, 60, 995-999.

Munday R. 2011. Palytoxin toxicology: Animal studies. Toxicon, 57, 470-477.

Lesley L. Rhodes, Tim Harwood, Andy Selwood, Kirsty Smith, Lincoln Mackenzie
Cawthron Institute, Nelson, New Zealand

Paul McNabb
IECU Manager, Thermo Fisher Scientific, Nelson, New Zealand

Rex Munday's HAB research highlights

Discovery of Tetrodotoxin in grey side-gilled sea slugs: "Rex had a sharp wit, best illustrated with an anecdote from 2013. We had collected hundreds of samples to explain why dogs were dying on Auckland beaches and sent twelve to Rex for toxicity screening using live mice. The next day we didn't dare phone to ask for an update but waited for an email, knowing Rex would be hard at work. Soon that email arrived and briefly, but accurately, described the materials and method used. This was followed by a list of each sample in numerical order with a short description of symptoms observed, most being 'no symptoms'. But next to one sample were the words 'death in seconds'. We immediately phoned Rex and asked what he thought of this result. His reply was 'Well, yes, that is an interesting sample. I have diluted it 10,000 fold and it's still killing a mouse in under one minute'. I'll never know if he was having us on or if he was just that truly objective and professional, he was often both."

Paul McNabb

The toxicity of anatoxin congeners from cyanobacteria: "Working in Hamilton on toxins from cyanobacteria I had heard the tales of the legendary Rex Munday and had always longed for the chance to work with him. A year ago, the opportunity was realised as we set out to investigate the acute toxicity of the anatoxin congeners, which are produced by the *Phormidium* which plagues many rivers around New Zealand. For a young scientist Rex's level-headed guidance was a god-send and allowed us to navigate a safe course through the minefield which research can be. Anyone who has worked near me whilst I'm extracting *Phormidium* samples knows that the aroma is slightly off-putting. Rex had the following succinctly simple statement on the difficulties of trying to feed these musty extracts to mice; 'For experiments on toxicity by feeding, mice are trained to eat small quantities of cream cheese. When they eat the offered cream cheese within a matter of seconds, the appropriate weight of freeze dried test material is mixed with the cheese and given to the mice. However, mice trained to eat cream cheese refused to eat the mixture with *Phormidium* extract and a different approach was required.' Rex's solution ended up being quite simple and effective; a positive displacement pipette was used to place the food on to the mouse's tongue and the food was instantly/instinctively gobbled it up. Rex's innovation and experience will be sorely missed by the toxic cyanobacteria research community of New Zealand."

Jonathan Puddick

Oral toxicity of paralytic shellfish toxins: "Rex was a key cog in the Safe NZ Seafood research programme, well-liked and respected by his colleagues both in NZ and abroad. He had an easy-going nature and great sense of humour. His work on the toxicity of various marine toxins over a long period of time made him an expert in this field. Rex published his findings in numerous journal articles, book chapters and featured on a number of expert working groups. He was firm in his belief that the toxicity assessment of marine toxins should be based on oral exposure rather than intraperitoneal administration, as this more accurately represents their route of exposure in humans. In recent years, Rex undertook a large piece of work on the acute toxicity of paralytic shellfish toxins, which represent a class of structurally related marine toxins that are problematic in NZ and other parts of the world. His findings are internationally important and will result in changes to the way this toxin class is regulated and will undoubtedly improve human health protection from exposure to these potent marine toxins.

On a personal level Rex was both a mentor and a friend. He always took the time to ask how I was going and how my family was tracking. He was terrific to bounce ideas off and was able to provide guidance on how to deal with work matters that were not going so well. I really appreciated this gesture. Thanks Rex, you will be missed."

Tim Harwood

The identification of pinnatoxins in pacific oysters: "I worked with Rex on some very interesting projects over the past 15 years. He was a fantastic person to collaborate with as he was an extremely good scientist, an inspirational mentor and had a great sense of humour to boot. My job as a natural products chemist was to provide Rex with compounds for toxicological evaluation. Rex always enjoyed giving us chemists a hard time, and because of his background in chemistry he knew how to do this really well. He would take great joy in seeing the reaction on our faces when he explained how much toxin would be required for a toxicological study, knowing that this would be a painstaking and near impossible task.

One of the highlights of my early career was working with Rex on a research project to identify unknown toxins in shellfish from South Australia. Several oyster growing areas in South Australia had been closed down due to the presence of an unidentified fast acting toxin. The project was extremely successful; we discovered three novel toxins, pinnatoxins E, F and G and determined their acute toxicity. This resulted in the re-opening of oyster growing areas in South Australia and in New Zealand. To be honest, I was nervous about taking on such a complex project due to my lack of experience. Rex's support and mentorship gave me the confidence to be able to complete that challenging task."

Andy Selwood



Oostende OBIS/HAEDAT training workshop participants.

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 59:
10 December 2017

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25 YEARS

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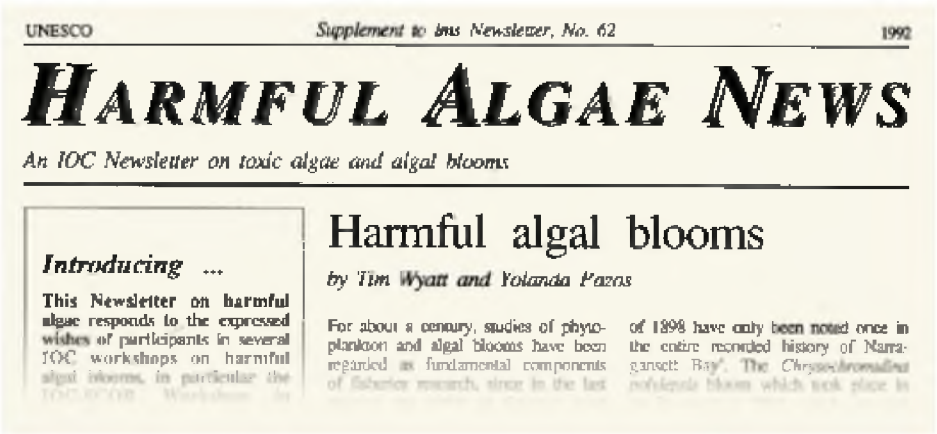
Harmful Algae News was first published in early 1992 in response to requests from the participants at a number of IOC meetings and workshops on harmful algae, in particular the IOC-SCOR *ad hoc* Meeting in Rhode Island (USA) 2-3 November 1991, hosted by Ted Smayda where the final draft of the Harmful Algal Bloom Programme was prepared. At this time, e-mail was in its infancy, web sites still very simple but there was demand for a channel to disseminate information about harmful algal events and research, as well as to disseminate information on management/research programmes, conferences, meetings etc. The field of HAB science was new, the community multidisciplinary and at a national level, often scattered between many different institutions. The need for a communication channel to strengthen networks and co-operation was obvious and *Harmful Algae News* became a core element in the IOC Harmful Algal Bloom Programme.

Harmful Algae News started as an annex to the IOC UNESCO newsletter '*International Marine Science*' (IMS), with the enthusiastic support of IMS editor Gary Wright and assistant Michelle Turner. However, *Harmful Algae News* became independent and eventually survived the IMS which ceased some years later. *Harmful Algae News* has been in publication for longer than any other IOC newsletter and its viability reflects the sustained focus on harmful algae in the IOC programme, as well as the continued interest by governments, institutions, scientists and those im-



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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

Introducing ...

This Newsletter on harmful algae responds to the expressed wishes of participants in several IOC workshops on harmful algal blooms, in particular the

Harmful algal blooms

by Tim Wyatt and Yolanda Pazos

For about a century, studies of phytoplankton and algal blooms have been regarded as fundamental components

of 1898 have only been noted once in the entire recorded history of Narragansett Bay". The *Chrysochromulina*

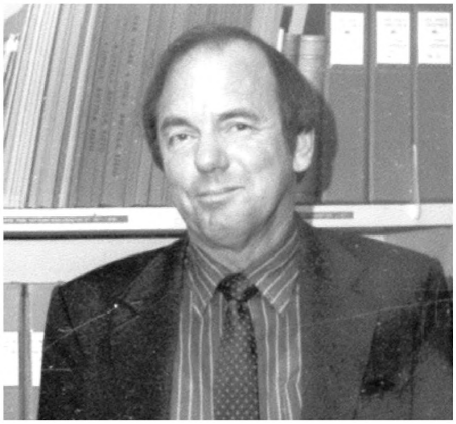


Vladimir Ryabinin, Exec. Secretary, IOC

"The Decade of Ocean Science will be a unique ten-year, global, large scale cooperative programme to seek urgently-needed scientific solution to support effective ocean management, stewardship and sustainable development" *(continue next page)*







pacted by harmful algal events. Since *Harmful Algae News* turned 20 years old in 2012, it has been a web based e-newsletter which meant longer issues were possible and back issues easily accessible. We are currently working on a searchable index for all *Harmful Algae News* issues.

The start of *Harmful Algae News* would not have been possible without a dedicated professional editor, Tim Wyatt. Tim was already an experienced journal editor and author, and a main contributor to *Harmful Algae News* himself. In the early years he was assisted by Yolanda Pazos, also at the Institute for Marine Investigations (IIM) in Vigo, Spain. Later, staff at the IOC Science and Communication Centre at the Spanish Institute of Oceanography (IEO) in Vigo (Jorge Diogéne, Ángeles Aguilera, Mónica Lion and Cristina Sexto) provided invaluable support in the compilation and layout. Since 2012 Leif Bolding at the Department of Biology at the University of Copenhagen has been producing the layout and providing the appropriate server facilities. Tim Wyatt edited 48 issues of *Harmful Algae News* plus some special issues over his 22 years as Editor. When Tim decided to retire as Editor we were lucky to have Beatriz Reguera (IEO Vigo) and Eileen Bresnan (Marine Scotland) willing to take over and continue the same high standard. No doubt these standards have stimulated submissions and made *Harmful Algae News* what it is today. We hope the international HAB science and management community will continue to submit their news items and announcements and that *Harmful Algae News* can continue to serve all its readers including the International Society for the Study of Harmful Algae.

To subscribe to HAN, send an e-mail to "han@sympa.iode.org" with empty subject line and in the text body: subscribe han *name* e.g.: for Peter Black —> "subscribe han Peter Black" or just "subscribe han"

Henrik Enevoldsen, Intergovernmental Oceanographic Commission of UNESCO, Head, IOC Science and Communication Centre on Harmful Algae



Key people in the early days of the HAB programme. From top to bottom: Ted Smayda (USA), host of the IOC-SCOR Workshop, Newport, October 1991, where the HAB programme was outlined (photo DM Anderson); Tom Osborne, IOC Technical Secretary when the programme was launched; Bernd Dybern (Sweden), 1st IPHAB Chair, 1992, and last, Adriana Zingone (Italy), 2nd IPHAB Chair during the III IPHAB meeting in June 1997, Vigo, in the middle with Henrik Enevoldsen (IOC) on the left and Vice-Chair Rhodora Azanza (Philippines) on the right.

Dear Readers

The fact that this year we are celebrating the 25th anniversary of the IOC newsletter *Harmful Algae News*, makes me, as Executive Secretary of the Intergovernmental Oceanographic Commission of UNESCO, proud of the strategic foresight of the Commission. Continuous publication of this newsletter shows the interest in the subject by the coastal zone management community; is evidence of successful delivery of new knowledge by the interdisciplinary research community; and is the result of effective prioritization of IOC work by its Member States. The harmful algal research domain is an example of ocean science which is of primary importance for sustainable development. Indeed, people's health, nutrition, availability of fresh water, and many economic interests are directly affected by the emergence of harmful algae. Successful research on and predictions of harmful algae demonstrate that oceanography is becoming more and more capable of responding to people's needs. To make decisive and major improvements in making ocean observations, research, sustaining services and responding to growing societal needs is the main objective of the United Nations Decade of Ocean Science for Sustainable Development (2021-2030). The Decade is going to shape much of the activity of the IOC and its partners in the years to come. Let me invite you all to engage and contribute.

Vladimir Ryabinin, Executive Secretary, Intergovernmental Oceanographic Commission, UNESCO, Paris

Intergovernmental Panel on Harmful Algal Blooms also turned 25!

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission









IPHAB Technical Secretary Henrik Enevoldsen IOC UNESCO Secretariat

New IPHAB Chair Gires Usup (Malaysia)

Outgoing IPHAB Chair Rob Magnien (USA)

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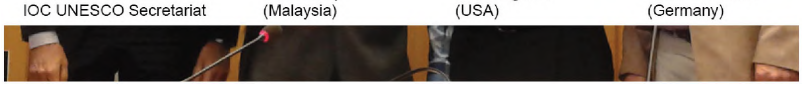

During 1992, the same Year as the IOC published the first issue of *Harmful Algal News*, it also established an *Intergovernmental Panel on Harmful Algal Blooms* (IPHAB) which has met every second year since it was formed. The Panel is composed by IOC Member State representatives and representatives of organizations working on different aspects of HABs. The Panel identifies and decides on priorities for international cooperation on HABs and also works to identify resources to facilitate the diverse work schedule to solve some of the real problems caused by harmful algae.

Over the last 25 years the IOC Intergovernmental Panel on HAB has initiated a large number of initiatives and activities leading to training opportunities, manuals, guides, projects and cooperative research. A special issue of the journal *'Oceanography'* includes a paper on the history and role of the IOC HAB Programme in the development of international HAB science. The paper can be downloaded at *http://dx.doi.org/10.5670/oceanog.2010.25.*

We here wish to pay a tribute to those who have chaired the Panel, and their Vice-chairs, the past 25 years and their contribution to developing the HAB research and management community at a global scale.

From top to bottom: Beatriz Reguera (Spain) and Phil Busby (New Zealand), elected as IPHAB Chair and Vice-Chair in 2002; Flowers to the outgoing Chair (Adriana Zingone, Italy) (left) and Vice-Chair (Rhodora Azanza, Philippines) (right); Leonardo Guzmán (Chile) (middle), Chair from 2007 to -2011 welcomes the newly elected Chair, Rob Magnien (USA) (left) and Vice-Chair, Gyres Usup (Malaysia) (right) for the next term; Bottom: Results from last elections, at the 12th IPHAB Session, Paris, April 2015

A retrospective look at the early days of HAB cyst research, and a look to the future

On this occasion of the 25th anniversary of the publication of *Harmful Algae News*, several of us were asked to look backwards in time to some of the earlier days of HAB science. One area of study that has been a major part of my own research programme and that of many others in our community is the role of cysts and resting stages in the bloom dynamics of HAB species. Here, I offer a personal perspective on the early stages of development in that field and a brief look towards the future as well. My apologies at the outset if there are omissions of people or findings that should have been included – this is not meant to be a scholarly synthesis, but rather a personal retrospective.

To set the stage, let's go back nearly fifty years to a time when the term *harmful algal bloom* did not exist and when researchers worked individually or in very small teams in scattered locations without any major research programmes or international initiatives. In 1972 when I was just starting graduate school, there was a massive New England red tide caused by the dinoflagellate we now call *Alexandrium catenella*, but which then had multiple names, including *Gonyaulax tamarensis* and *G. excavata*. This outbreak dealt a devastating and unexpected blow to the New England region of the United States, causing shellfish closures along the coasts of multiple states. Considerable attention by the press and public covered this new and worrisome threat to public health and fisheries resources and ultimately motivated the *First International Conference on Toxic Dinoflagellate Blooms* in Boston, Massachusetts in 1974.

At the time of that conference, I was a long-haired graduate student in the Civil and Environmental Engineering Department at MIT (Fig. 1), searching for a thesis topic. People often ask me how someone in an engineering department could end up a biologist working on HABs, and the answer to that lies in part with the 1972 New England red tide, but also with the 1974 conference and a series of events and discoveries that came shortly before and after.

The conference was very small compared to the current ISSHA meetings, with approximately 100 participants from four countries versus 500 or more participants representing 50 or 60 countries at our current meetings. The focus of that meeting was almost entirely on blooms of *Alexandrium catenella* and *Karenia brevis* (the species was then called *Gymnodinium breve*), as the discoveries of diarrhetic shellfish poisoning (and its linkage to *Dinophysis* spp.) and amnesic shellfish poisoning (and *Pseudo-nitzschia*) were 10-15 years in the future. The ciguatera fish poisoning syndrome was known, but the identification of *Gambierdiscus toxicus* as the causative organism was also several years away. Among the contributions at that meeting, several are noteworthy in the context of the theme of this narrative. The first was by David Wall, a geologist at the Woods Hole Oceanographic Institution (Fig. 2). In the late 1960's and early 70's, Wall (working with Barrie Dale at the time) discovered that the fossilized cell walls of organisms thought to be extinct (called hystrichospheres) were in fact dinoflagellate cysts, and that many of the forms that they were using in paleontological reconstructions were still living in the ocean and producing cysts that could be germinated to establish taxonomic affinities. (This same line of work was concurrently being pursued by Bill Evitt and colleagues at Stanford University). In order to isolate and germinate living cysts from modern sediments, Wall and Dale developed a technique that is used to this day involving sonication and sieving – the former used to disaggregate sediments and the latter to separate size fractions containing cysts. In his paper in the 1974 conference proceedings and in other papers published near that time [1-3], Wall highlighted the important roles that cysts likely played in dinoflagellate bloom dynamics, including determining where and when blooms might originate, allowing survival through environmental extremes, and facilitating genetic recombination through sexuality. He told us *"Despite these seemingly important considerations, the encystment-excystment cycle in dinoflagellates has not been expressly studied in relation to any specific red tides or related instances of paralytic shellfish poisoning. Details of the cyst cycle have been accumulated independently of red tide research....."* [1]. This contribution and other papers at the time show considerable foresight that was fully validated through a sharp increase in the cyst-based HAB research of the subsequent decades.

Another talk at that conference was equally insightful, this one by Karen Steidinger (Fig. 3) ("Basic factors influencing red tides [4]"). In her talk and paper, Karen outlined the fundamental stages of blooms, focusing on initiation, transport, and development. Like Dave Wall, she also highlighted the importance of cysts: *"The recent investigations of Dr. David Wall .....lend credence to the speculation that pelagic, toxic dinoflagellate blooms might originate from*

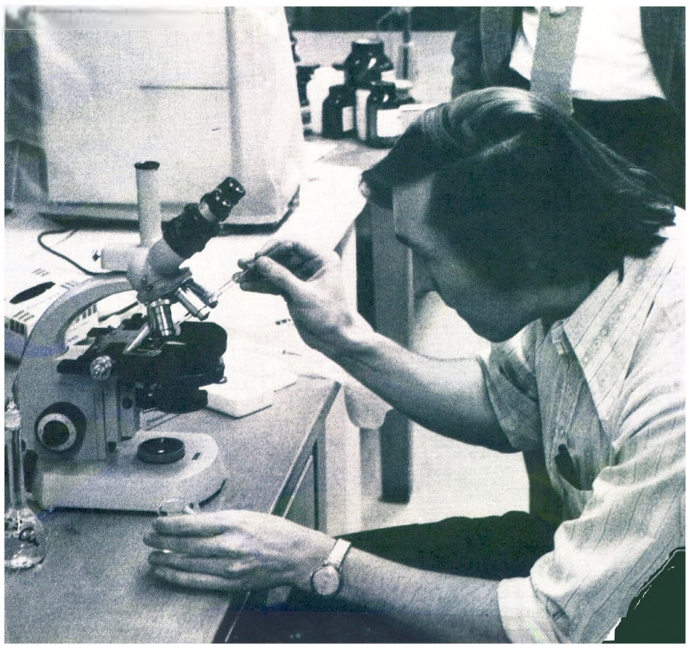

Fig. 1. Don Anderson as a graduate student in the Civil and Environmental Engineering Department at the Massachusetts Institute of Technology (MIT), 1975

dormant stages and that these stages might be associated with certain bottom sediments. This then brings up the question, if benthic resting stages of certain dinoflagellates actually "seed" coastal red tides, are there localized areas of accumulation, or what we could call "seedbeds"?.....The possibility of benthic seed populations and seedbeds for at least some Gonyaulax (=Alexandrium) and Pyrodinium has a much higher probability than for Gymnodinium breve..... It would seem that this avenue of research should have a high priority among phytoplankton systematists and ecologists."

As a graduate student sitting in the audience, I was motivated by these talks and papers and immediately started to look at my own research from a new perspective. At the time, I was in a water chemistry laboratory doing trace metal (copper) sensitivity experiments with my *Alexandrium* cultures, reasoning that there was some chemical process that could explain the link between *Alexandrium* blooms, river runoff, and low salinity coastal waters (I still believe that this is the case, but I dropped this line of inquiry and it has not been pursued to any significant extent since). Many of my cultures contained rounded, non-motile cells, which I thought could be cysts, but no cyst stage had yet been described for *A. catenella*. Fortunately for me, Woods Hole was a short drive away for me, so I was able to bring my cultures to Dave Wall. After a quick examination, he gave me the disappointing news that these were not what he would term true cysts, but rather a temporary stage with much thinner walls (he and I eventually named these "*pellicle cysts*" after the resistant layer that surrounds them [5]). Next, one of those pivotal moments in a career occurred in which luck or good fortune played a role. Instead of just sending me back to my lab with this negative news, Dave suggested that we sample a nearby salt pond where PSP toxicity had been recurrent in the years after the big 1972 bloom. We collected surface sediments with an old plankton net that was dragged across the bottom, and Dave demonstrated the sonication and sieving method. In short order, we were looking at a number of unknown cysts types that I set about trying to isolate and germinate. One produced an elongate cell (Fig. 4) that after germinating, ultimately divided to produce cells of *Alexandrium catenella*. I was already aware of the importance of this discovery after the talks and papers of Wall and Steidinger, and now I had proof that at least some of their speculations were valid. The door was suddenly open to some very exciting and new work with living cysts, and this new perspective on *Alexandrium* bloom dynamics totally changed the directions of my thesis research. Furthermore, I had found perfect study locations for my work – the Cape Cod salt ponds where these blooms occurred regularly every year, and in which they were localized due to restricted tidal exchange with nearby coastal waters. We continue to work in these systems today – they are essentially natural mesocosms. Several publications ensued [5,6], accompanied by a paper by Barrie Dale [7] who had left Woods Hole and was working independently on this same species using sediment from Norway.

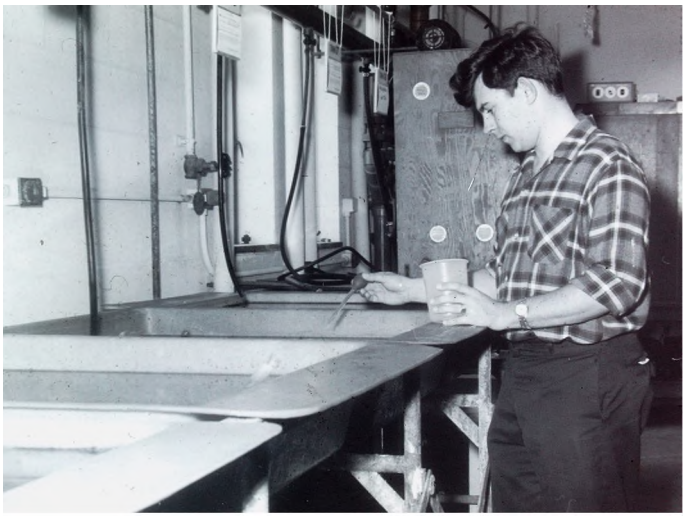

Fig. 2. David Wall, an early pioneer in dinoflagellate cysts, collecting cysts from settling trays in Woods Hole in 1974

In one of these papers [6], I was able to document the extreme seasonality of *Alexandrium* blooms in the salt pond, raising fundamental questions about the mechanisms underlying the patterns of excystment and encystment. I characterized the mandatory dormancy interval, which begins at cyst formation and can last several weeks to months until maturation is completed. Cysts were unable to germinate during this interval, thus explaining the absence of *Alexandrium* cells in the water column in the months following each spring bloom. What remained a mystery was why the bulk of the *Alexandrium* cysts in bottom sediment did not germinate later in the summer or fall or even early winter after they had matured, but instead remained in a resting state until the next spring. One part of the answer was found some years later when we showed that high temperatures can inhibit cyst germination [8]. Yet, the absence of a major bloom in the late summer and fall when temperatures had decreased enough to be favorable for germination remained a mystery. Only in recent years have we begun to understand that mature *Alexandrium* cysts can re-enter dormancy – one cause may be unfavorably warm summer temperatures. But we have also learned that exit from that dormant state into quiescence (a state in which cysts will germinate if conditions are favorable) occurs in response to a quantifiable amount of chilling. By quantifying the duration and severity of cold, *A. catenella* tracks the passage of winter, delaying germination until spring when the outlook is more favorable for bloom success. This groundbreaking work by one of my students, Alexis Fischer, demonstrates that dinoflagellate cysts possess physiological behavior that is similar to that observed in terrestrial plants where a period of chilling is needed for optimal seed germination, bud flowering, or bulb emergence [9]. We now know that *Alexandrium* cysts can cycle between quiescence and dormancy multiple times as seasons and years progress, and equally importantly, that the frequency and timing of the cycling may well be determined by the history of the chilling those cysts experience. Recent work by Cary López on *Pyrodinium bahamense* is revealing a similar environmentally-induced cycling behavior in tropical dinoflagellate cysts.

Those early days as a graduate student and then as a postdoc were times of rapid discoveries, as nothing was known about the distribution of *Alexandrium* cysts in the region, or of the role of temperature, nutrients, light, oxygen, and other environmental pa-



Fig. 3. Karen Steidinger, Penrose Conference on Fossil and Modern Dinoflagellates, 1978

rameters on the formation, deposition, and germination of cysts. One of several challenges at the time was that we were unable to produce true hypnozygotic resting cysts in our cultures. There was good reason to believe that sexuality was involved, so compatible mating types needed to be combined. Further, nutrient limitation seemed to be a trigger to initiate the process. We quickly learned that dinoflagellate species varied considerably in the ease with which they could be induced to form cysts in cultures. Species like *Scrippsiella trochoidea* produced prolific numbers of cysts without special precautions, whereas our *Alexandrium* cultures were far more recalcitrant. Eventually, with deliberate modifications to the content of the culture medium as well as the manner in which it was prepared (i.e., minimizing contaminants and precipitates) we were finally able to produce cysts in *Alexandrium* cultures [10], opening yet another door to new studies and discoveries.

It is clear that nutrient limitation can induce sexuality and cyst formation in cultures of *A. catenella* and other dinoflagellate species, but to this day, we still do not know if there are other factors at work – perhaps a density-dependent or quorum-sensing type of response, or even a response to the presence of grazers or parasites. Exploration of this response has long been limited by the constraints associated with laboratory cultures, but now we are fortunate to be entering a new era of in situ investigations thanks to biosensors like the Imaging FlowCytobot (IFCB), that allow us to observe the encystment process underwater in the field with extraordinary resolution. Among many other recent findings, IFCB observations by Mike Brosnahan [11,12] have documented mass gametogenesis (75 – 90% of all cells) at the end of *A. catenella* blooms under conditions that do not appear to be nutrient-limited. I believe that the tools are now in hand to resolve the longstanding mystery of what induces cyst formation in the field and suspect that the final story will be much more complicated than simple nutrient limitation.

Not long after our early successes with sexual induction and cultures, my attention shifted to studies of cyst distribution and dynamics in the field. We were documenting the abundance and dynamics of vegetative cells in the plankton during blooms, so I wanted to quantitatively link those observations to cyst abundance and distribution in the sediments before and after those blooms. At the time, however, studies of dinoflagellate cysts were still heavily influenced by geologists working with sediment cores. Typically, those investigations enumerate cysts at different depths in cores, expressing results in terms of cysts per gram of dry sediment. Although this approach is appropriate when one is studying vertical profiles of dead, empty, or fossilized cysts in sediments dating back hundreds or thousands of years, this type of parameterization is not useful for biological studies in which one wants to map out the distribution and abundance of cysts and estimate the number of germinated cells inoculating the overlying water column. To address the latter topic, we needed to know how many cysts were present per square meter of sediment, so we started doing experiments to see how reproducibly we could quantify cysts per cubic centimeter or ml of sediment. Our results were consistent and scientifically appealing so we published the method [13] and began to use it in the salt ponds and other nearshore locations. This approach was not well received, however, as I was repeatedly criticized at conferences when these results were presented. Geologists argued that the water content and lithology of surface sediments differs sufficiently from site to site, or from layer to layer, and that normalization of cyst abundance to a volume of sediment introduces substantial errors compared to the dry weight approach. I remember being told at an international conference that my results and the population dynamics models based on them were essentially meaningless! This disagreement went even further, as one publication [14] recommended that "*future studies use standardized methods based on measurements of cyst concentrations per gram of dry sediment*". This recommendation was repeated in training manuals for those working with living dinoflagellate cysts [15]. Despite this strong and vocal opposition, I was still firmly convinced that cysts could be quantified per unit volume of sediment, and so I initiated a study over multiple years in which we quantified cysts both ways (i.e., cysts per gram dry weight and cysts per ml). We did this in the Gulf of Maine across a wide range of sediment types and cyst concentrations, and found a strong and statistically significant relationship between the two parameters, for the top cm of sediment (Fig. 5). The relationship for the top three cm of sediment is even stronger. This of course does not mean that this relationship will apply in every location, but it does show that the hypothetical concerns raised about this

approach did not stand up to data at my study locations.

I raise this issue in this narrative because I want to correct what I feel are unjustified recommendations that may prevent those working on cysts from obtaining the type of biological data that can advance our understanding of certain types of HABs. With the proper precautions, we can map out cyst distributions in a way that is complementary to the manner in which we study the vegetative stages in the water column. There's no question that both populations are patchy and difficult to adequately represent with limited sampling, but it can be done. Knowing the cyst abundance per ml of surface sediments allows the distribution to be mapped in a way that has biological meaning, as has now been seen in many studies. If one assumes that the cyst germination flux comes from a thin layer at the sediment surface of a defined thickness, it is possible to calculate germling emergence per square meter, and thereby quantify the inoculum to the overlying water column. This is simply not possible if the cysts are expressed per gram of sediment, unless additional measurements are made that determine the density (grams per ml of the sample). Once the cyst distribution is expressed in terms of area, it is then possible to use laboratory- or field-derived germination rates to calculate the germination input to a body of water, an approach that is fundamental to bloom dynamics modeling efforts for cyst-forming species.

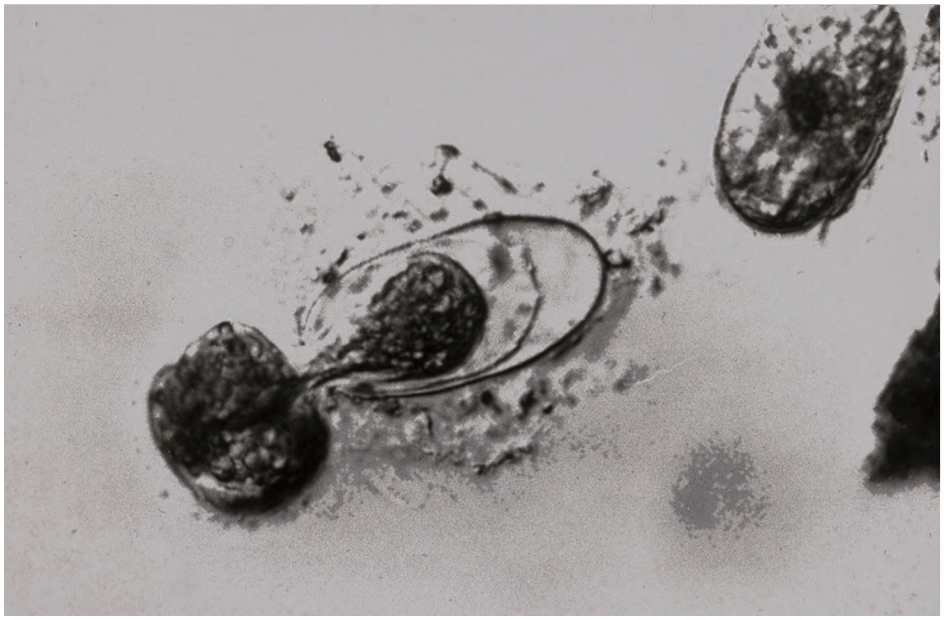

Fig. 4. Germinating Alexandrium catenella cyst. (Photo: Don Anderson)

My guidance to those working on cysts in natural sediments is to demonstrate that you can sample surface sediment reproducibly, and that the distributional data obtained makes sense in the context of bathymetry, currents, and sediment type. I hope that those working on dinoflagellate cysts recognize the validity and value of the two different approaches for cyst enumeration – cyst number per gram dry weight for paleontological reconstructions of past populations and sediment transport modeling, and cysts per unit area or unit volume for studies of the dynamics of living cysts. Another option would be to measure cysts per gram dry weight and determine the density of each sediment layer, as this would allow a simple calculation that would also provide cysts per unit volume data.

Armed with the knowledge that was possible to map out cysts over large areas, my team set about to do this in salt ponds, bays, and open coastal waters. These efforts confirmed Dave Wall and Karen Steidinger's original speculation – that areas of accumulation (seedbeds) existed and were a critical element in bloom dynamics. Some seedbeds were small, and others huge – like the ones we documented in the Gulf of Maine (see figure 5) that covered an area as large as 22,000 km^2, varying 3–4-fold in area and 10-fold in cyst abundance among years [16]. Those studies also demonstrated that the Gulf of Maine system has only two seedbeds with the bathymetry, sediment characteristics, currents, biology, and environmental conditions necessary to persist for decades or longer. The value of these mapping studies was even more evident when strong positive correlations were confirmed between the abundance of cysts in surface sediments and the size of the blooms and resulting toxicity in the subsequent year [16,17].

Our next challenge was to measure or estimate the flux of emerging cells that inoculates blooms. Efforts by my lab but in particular those of Ishikawa, Naksuike, and others [18,19] have yielded some promising devices and approaches that consistently indicate that germination is occurring in only a thin veneer a millimeter or so thick at the sediment surface, leaving the large

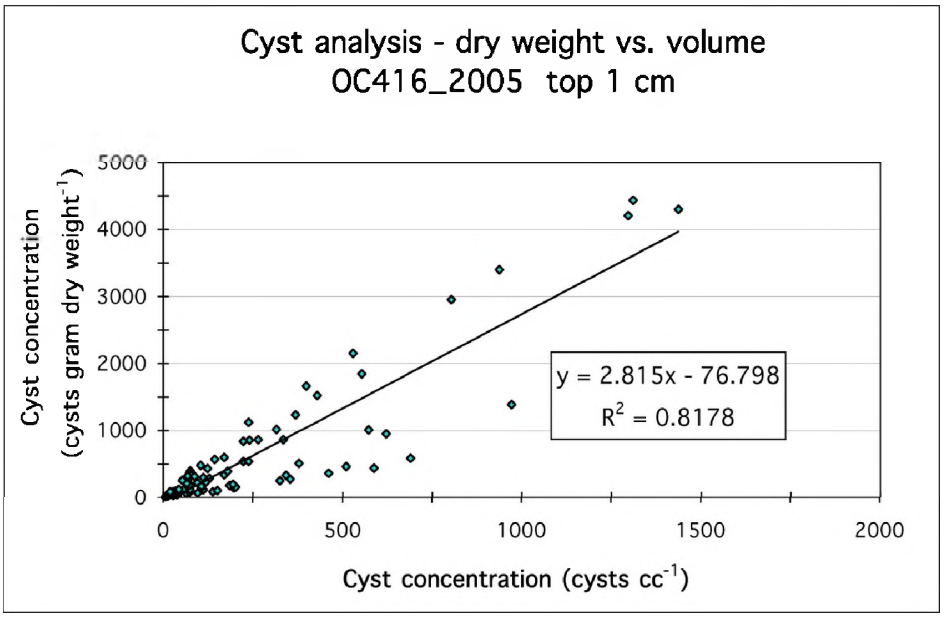

Fig. 5. Significant relationship ($p < 0.0001$) between cysts per cm^3 and cysts per gram dry weight in the top cm of sediment at 85 stations throughout the Gulf of Maine. From [16]

number of cysts in subsurface layers unable to germinate or emerge, presumably due to lack of oxygen or to the tortuous pathway posed by sediment grains and detritus. Many might think that major storms and waves can erode significant layers of sediment and transport cysts long distances, but here again, our studies in the Gulf of Maine have shown that other than in very shallow waters, even major storms only erode a millimeter or less of sediment [20] and that the cysts and other eroded material don't travel very far before settling again [21]. Specifically, the depth of sediment eroded ranged from about 0.05 mm at a sandy 70 m deep station, to about 1.2 mm in clayey-silt sediment at 250 m [20], with the majority of the resuspended material remaining within 20 km of the source locations [21].

I could go on, but lack of time and space argues that I bring this narrative to a close here. As I look back, I am fortunate and grateful to have been part of an era of discovery that has developed the methods and approaches to study cyst dynamics in a manner analogous to the way we study bloom dynamics in surface waters. It was not easy, as there was strong opposition to some of the methods that were used, but the scientific process and good data have overcome the speculation. There are certainly challenges and uncertainties ahead in the study of cysts and life history dynamics, but significant progress has been made and much knowledge gained. The future looks bright for future discoveries, facilitated by our growing recognition that marine dinoflagellate cysts have much in common with terrestrial plants and that their distribution and abundance can be quantitatively mapped – this valuable information will enable us to estimate deposition and germination fluxes. We clearly need continued study to help elucidate the mechanisms that trigger sexuality and cyst formation, and that will likely involve observations using in situ sensors like the IFCB. After more than 60 years, the study of living dinoflagellate cysts remains a vibrant and important element in HAB research, and I am grateful for the foresight and guidance of David Wall, Karen Steidinger and others, and to have been part of that evolution myself.

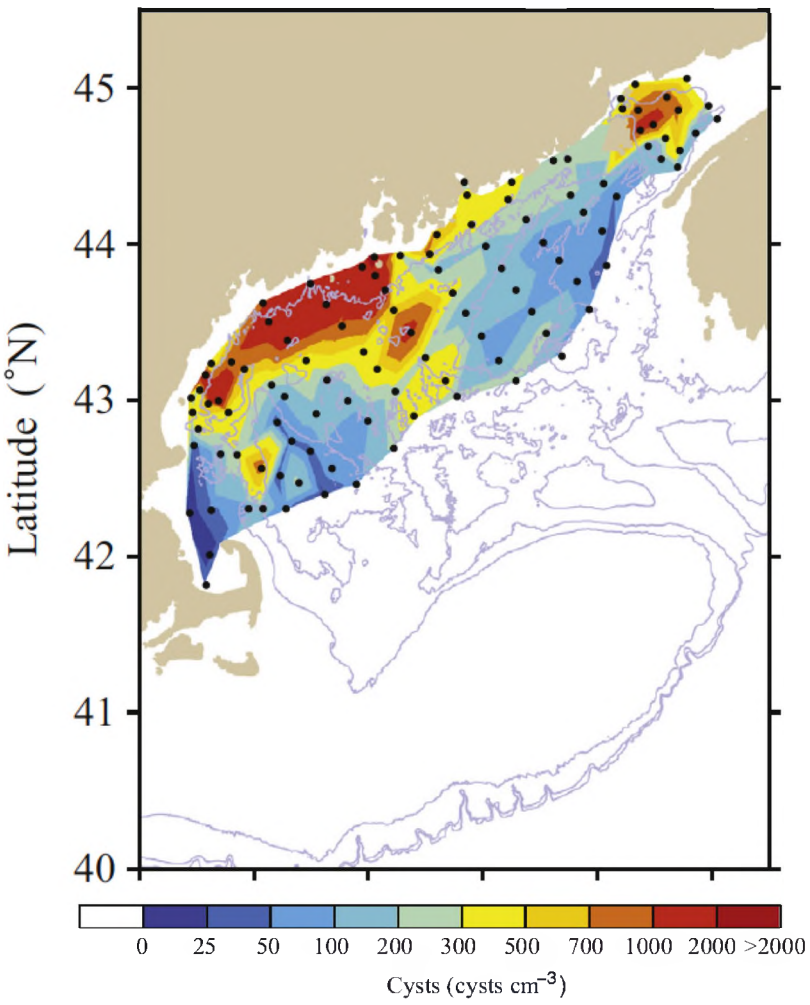

Fig. 6. Map of Alexandrium catenella cyst abundance in the Gulf of Maine. This image depicts a multi-year (2004–2011) arithmetic mean (cysts cm3) of the surface (0–1 cm) sediment-layer. Two seedbeds are clearly visible -one at the mouth of the Bay of Fundy in the north, and the other in mid-coast Maine to the south. From [16]

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How do algal blooms kill finfish and how can we mitigate their impacts?

Algal blooms, water discolorations and their association with fish kills have been recorded since historic times, such as the description in the Bible (1000 years BC) *"all the waters that were in the river were turned to blood. And the fish that was in the river died; and the river stank, and the Egyptians could not drink of the water of the river"* (Exodus 7: 20-21). In this case, a non-toxic bloom-forming alga became so densely concentrated that it generated anoxic conditions resulting in indiscriminate kills of both fish and invertebrates. Similarly, water discolorations and massive fish kills in Florida coastal waters have been reported by Spanish explorers since the 15th century (now known to be caused by the dinoflagellate *Karenia brevis*).

Finfish held captive in intensive aquaculture systems are extremely sensitive to HABs, and the impact of fish-killing algal blooms on human society thus has been exacerbated by the increase of finfish aquaculture. Shilo 1967 [1] first investigated problems for the Israel *Tilapia* pond aquaculture industry caused by the haptophyte *Prymnesium parvum*, but research efforts to understand fish killing mechanisms intensified in 1973 when *Chattonella marina* raphidophyte blooms (Fig. 1) devastated yellowtail aquaculture in the Seto Inland Sea [2] and in the 1980s when the dinoflagellate *Karenia mikimotoi* started to impact on the fledgling salmon farming industry in Norway [3].

Fish-killing algal species are responsible for much greater economic impacts (summarized in Table 1) than HAB events leading to seafood biotoxin contamination. The 2016 fish kills in Chile by *Pseudochattonella* and *Alexandrium catenella* which led to losses of US$800M triggered social unrest. It is therefore surprising that progress in our understanding of how HABs kill fish has been so slow. Financial support for and research interest in HABs by the fish farm industry has often been short-lived, and ranked lower than investments in fish husbandry, nutrition, and disease control. With the exception of *Karenia brevis*, so-called fish-killing toxins or "ichthyotoxins" are not known to have human health impacts and therefore also have not been a priority for seafood regulators. Scientific progress has been hindered by the use of widely different bioassay systems and lack of chemical analytical methods to quantify and characterize ichthyotoxins from seawater medium. Assay systems have included *Artemia* or *Daphnia* assays, the use of fish or mammalian erythrocytes, and a wide range of juvenile or adult fish (damselfish, sheepshead minnow, mountain minnow, zebrafish, salmon, sea bass) tested under different exposure regimes. The novel application of a standardised and highly sensitive and reproducible rainbow trout RTgill-W1 cell line assay in Australian, Chilean, Danish, Mexican and US laboratories has allowed significant progress to be made in the past 10 years [4,5]. This assay has been automated in a plate reader measuring cell viability dyes, and been successfully applied also to screen freshly collected natural seawater samples from fishkill events in Australian and Korean waters.

What is the precise mechanism causing fish gill damage?

While all high biomass algal blooms (>100 x 10^3 cells L^{-1}) can cause mechanical stress to the sensitive gill tissues of fish and trigger excess mucus production, of much greater concern for the aquaculture industry are the highly potent, taxonomically unrelated flagellate groups *Cochlodinium, Karenia, Chattonella, Pseudochattonella, Heterosigma* and *Prymnesium*. One feature that these algal groups have in common is that they are all fragile cells, which can lyse even upon impact on the gills of fish, especially during the end of blooms, or conditions of osmotic stress or during upwelling. For less fragile fish-killing algae such as *Karlodinium* or the armoured *Alexandrium* the conditions that cause cells to exude ichthyotoxins and/or cause cell lysis are critical, and ichthyoxicity by these genera tends to be more variable.

The first point of attack by all above algal groups are the fish gills (Fig. 2), resulting a generalized necrotizing degeneration of the epithelium of the secondary lamellae with associated sloughing, often accompanied by swelling of the primary lamellar epithelium and congestion of branchial vessels [3]. Fish gills tend to respond in a singular way and gill pathology is remarkably similar for different ichthyotoxic algae and in different fish species [3, 6-9]. Once the fish gills are compromised, algal neurotoxins if present can penetrate the blood stream, cause fish behavioural changes, and loss of the blood haemoglobin's oxygen binding affinity [2].

Several competing theories have been proposed as to the precise mecha-





Fig. 1. Mass mortality of (above) yellowtail fish in the Seto Inland Sea in Japan (photo courtesy Prof T.Okaichi) and (below) blue-fin tuna in South Australia (photo courtesy Barry Munday), both caused by Chattonella marina blooms. Higher potency of Australian blooms (kills occurred at 66,000 cells/L) compared to Japan (500,000/L) is attributed to higher sensitivities of tuna but also higher ichthyotoxicity by Australian high-light adapted algal strains [5].

Table. 1. Economic losses from algal blooms for finfish aquaculture in different parts of the world

HAB species	Country	Financial Losses
Chattonella *Heterosigma*	Japan	USD500M, 1973 USD135M,1980/90
Cochlodinium *polykrikoides*	Korea, China Canada	USD95M, 1995 USD2M, 1999
Heterosigma *Chaetoceros*	British Columbia	USD35M, 1980-90
Heterosigma	New Zealand	NZD12M
Karenia digitata	Hong Kong	USD32M, 1998
Karenia mikimotoi	Norway	USD6M, 1988
Alexandrium catenella *Pseudochattonella/* *A. catenella*	Chile	USD60M, 2002/ USD800M, Jan-Mar 2016
Chattonella *Karenia*	South Australia Tasmania	AUD45M, 1996 AUD3M, 2003

nism of how algae kill fish. These include: free fatty acids (FFA), reactive oxygen species (ROS), and phycotoxins such as brevetoxins, karlotoxins, gymnocins, or varying combinations of all previous compounds.

Polyunsaturated fatty acids (PUFA)

Shilo working with fish-killing *Prymnesium* pointed out how "lipid micels" released by the algae can impact on the fish gill lamellae and interfere with osmoregulation [1]. Okaichi, investigating *Chattonella,* focused on free fatty acids damaging fish gills [2]. This was pursued by French researchers including Patrick Gentien [10] working with *Karenia mikimotoi* (as *Gyrodinium aureolum*), who identified free fatty acids such as OPA (octadecapentaenoic acid) and EPA (eicopentaenoic acid) as having the highest ichthyotoxic potency. Mardones [11] also confirmed the ichthyotoxicity by DHA (docasahexaenoic acid) from *Alexandrium catenella.* Paradoxically these compounds are well-known to have a beneficial effect for human health, notably heart disease, when consumed in moderation and used to replace saturated fat. These PUFA compounds are prone to oxidative degradation, in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. In whole fish experiments it has been convincingly demonstrated that exposure to OPA and EPA did cause fish gill damage at concentrations in seawater of approximately 3ppm [7,12]. This is short however of what dense algal blooms would generate.

Reactive Oxygen Species (ROS).

The role of Reactive Oxygen Species in ichthyotoxicity has long been suggested from whole fish experiments with *Heterosigma* and *Cochlodinium*, where application of ROS mopping enzymes such as catalase and peroxidase significantly improved fish survival [13, 14]. Japanese researchers led by Oda [15] focused on the fish-killing raphidophyte *Chattonella* which is a potent producers of ROS notably when algal cells are ruptured. Several other fish-killing algae such as *Karenia* and *Alexandrium catenella* are also strong ROS producers, but for *Heterosigma, Karlodinium* and *Prymnesium* ROS on its own cannot explain ichthyotoxicity. Using a xanthine-xanthine oxidase chemical reaction to generate superoxide at concentrations equivalent to fish-killing *Chattonella*, Marshall [7] demonstrated that superoxide on its own does not kill fish. Similarly, Trick and co-workers [16] showed that hydrogen peroxide produced by *Heterosigma* did not explain ichthyotoxicity. ROS also exhibited negligible impact in the RT fish gill assay [5].

Synergism between ROS and free fatty acids

Pursuing the role of ichthyotoxicity by EPA, Marshall [7] demonstrated that when damselfish were challenged with EPA in the presence of ROS, this increased the potency of EPA by up to 15 fold (Fig.3). Similarly, DHA in synergism with ROS became 9 times more ichthyotoxic [11]. The precise nature of the lipid peroxidation products generated remains poorly known, and new analytical methods for the detection of such compounds in seawater during fish kills (e.g. using SPATT collectors) are much needed.

Role for true phycotoxins?

The dinoflagellate *Karlodinium* is known to produce chemically well-defined linear polyketide *karlotoxins*, which at ecologically realistic concentrations can account in full for their fish-killing potency [17]. While prymnesins from the haptophyte *Prymnesium* have attracted considerable interest [18] it remains to be shown whether these compounds can account for their ichthyotoxic potency. Similarly, cytotoxic chemical compounds such as *gymnocin* from *Karenia mikimotoi* remain to be shown to play a quantitative role in ichthyotoxicity [19]. Polyether ladder *brevetoxins* from the Florida *Karenia brevis* are well known as the cause of Neurotoxic Shellfish Poisoning in humans but purified brevetoxin PbTx2,3 exhibited limited ichthyotoxicity against RTgill cells [5], suggesting that peroxidation pathways may need be invoked in fish kills. The production of brevetoxins by raphidophytes is disputed [20]. Neuroactive compounds (but not brevetoxin) have recently been claimed also for the raphidophyte *Heterosigma* [21]. Mardones [11] found no evidence for a role of *saxitoxins* in fish gill damage, which instead could be explained by DHA and ROS synergism. Using the fish-gill ichthyotoxicity assay, karlotoxin and DHA could account for fish kills in their own right, but not EPA, STX, PbTX, OPA nor OTA. Synergisms between DHA x ROS and EPA x ROS were able to explain fish kills, and so could fatty acid aldehydes.

Implications for mitigation of fish-kill events

Several different strategies are currently practised in the fish farm industry to mitigate the ichthyotoxic effects of HABs. These include: cessation of fish feeding, towing away of cages from affected areas, perimeter skirts to protect against algal surface slicks, aeration or

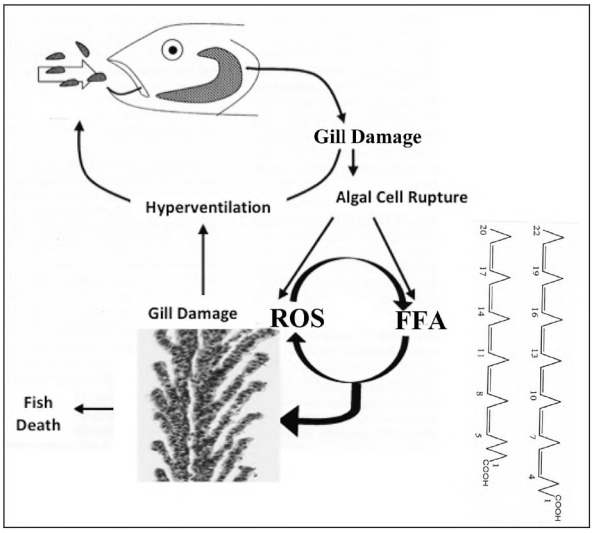

Fig. 2. Diagrammatic representation of algal bloom mediated fish kills, involving rupture of fragile algal cells to release a cocktail of Reactive Oxygen Species (ROS), Free Fatty Acids (FFA) but which rarely involves true Phycotoxins [modified after 7]. The chemical structures of two dominant microalgal polyunsaturated fatty acids EPA and DHA are indicated

airlift upwelling to dilute harmful algal concentrations, or clay flocculation to reduce numbers of harmful algal cells [22]. Mass fish mortalities lead to tonnes of dead fish being dumped in land fills (Port Lincoln, Australia, tuna mortality 1996) or dumped offshore (Chilean red tide 2016), but this material is rarely used for fish meal. As argued here, with perhaps a single exception (Florida *Karenia brevis*), none of these "ichthyotoxins" are of human health significance, meaning that recently killed fish are still fit for human consumption. Triggered by autonomous HAB monitoring systems, once fish start to die a decision thus could be made to instigate emergency harvest operations. To prevent the build-up of histamines, fish should be kept alive as long as possible during harvesting. This can be achieved by diluting algal concentrations via airlift upwelling, or by targeted in-pen emergency application of clays [23] that mop up ichthyotoxins at clay loadings significantly lower than those considered harmful to benthic marine invertebrates.

Laboratory experiments with fish gill cell lines or even whole fish tank experiments can only serve as proxies for real-life fish farm situations. HAB researchers need to forge better collaborative links with the fish farm industry to conduct research on fish kill events that involve the *in situ* measurement of ichthyotoxins in seawater using new analytical methods. Progress in understanding fish killing mechanisms by HABs will pave the way towards more effective mitigating strategies which are much needed to feed an ever growing world population with high quality seafood.

Acknowledgements

This overview is partially based on a keynote lecture presented at the ICHA17 Brasil Conference, a more complete write up of which can be found in the proceedings of that meeting. Numerous PhD students worked with me on fish-killing algae over 20 years, including Juan Dorantes-Aranda, Graeme Lush, Judi Marshall, Jorge Mardones, Ben Mooney, and Andreas Seger. I benefited from valuable collaboration with the late Barry Munday (fish pathology), Peter Nichols (lipids), Prof David Waite (ROS) and Prof Al Place (karlotoxins).

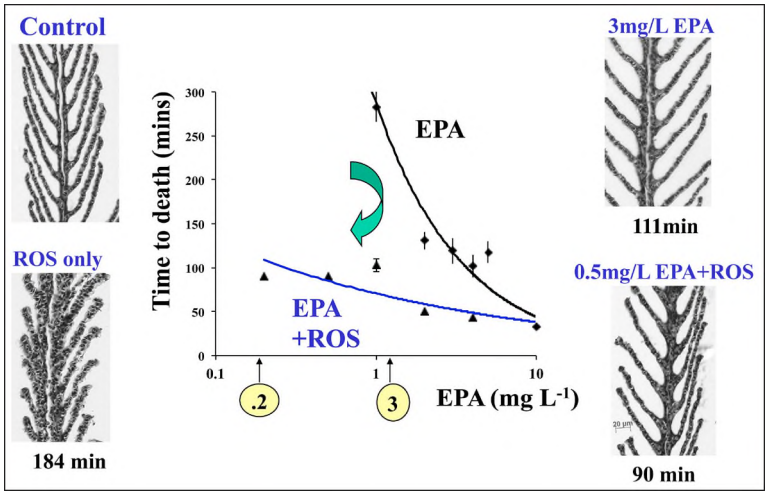

Fig. 3. Toxicity of the free fatty acid form of eicosapentaenoic acid (EPA) to damselfish: (top line) EPA on its own, requiring 3 mg L^{-1} to kill fish in 300 min; (bottom line) EPA in the presence of xanthine generated superoxide, where 0.2 mg L^{-1} EPA could kill fish in 100 min. Histopathology of damselfish gill tissues under selected treatment regimes is illustrated. Modified after [7]

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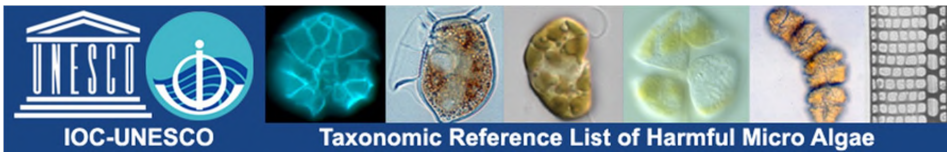

As part of the 25th anniversary issue of *Harmful Algal News* I am providing an overview of the *IOC-UNESCO Taxonomic Reference List of Harmful Algae* (*www.marinespecies.org/hab/*) and will highlight some of the problems which have faced or are facing the Intergovernmental Panel on Harmful Algal Blooms (IPHAB) *Task Team on Taxonomy* that is responsible for updating the list.

Historical timeline

1993

A task team on the taxonomy of harmful algae was established at the Second Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms in Paris in October 1993, in line with the HAB Programme Plan. The aim of the task team was to make taxonomic recommendations of harmful algal species, and membership comprised Y. Fukuyo, Japan, M. Elbrächter, Germany, and Ø. Moestrup, Denmark, the latter serving as chairman. During the 1993 session it was "encouraged" to create a computerized taxonomic data base of harmful species, an activity which at the time was planned between the *Expert Centre on Taxonomic Identification* (ETI) in Amsterdam and the *Botanical Institute*, University of Copenhagen.

1997

A major step forward was taken at the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms, which was held in Vigo, Spain 1997. There it was decided to establish a broader Task Team on Algal Taxonomy, with the following terms of reference:

I. to provide an agreed reference list of harmful algal species, including correct author citation, date of valid publication of the species, and a list of synonyms for each species
II. to provide guidelines to reduce nomenclature instability of harmful algal species
III. to organize round table discussions on nomenclature and taxonomy of harmful algal species
IV. to organize inter-calibration exercises to standardize the identification of harmful algal species

2000

The contents of the list were discussed in detail at the HAB Conference in Hobart in 2000, and it was decided to aim at including additional information in the list such as the basionym of each species (the first name applied to the species), reference to the article in which the species was described, reference to the article in which the species was given its current name, information on the type locality, information on the main harmful effects of each species, including up to three references with information on toxicity, toxins or toxic effects.

2002

The list was completed in 2002 and made available online on the homepage of the Department of Biology, University of Copenhagen.

Post 2002

During the following years the list was regularly updated, based on information from committee members and others. The list was also discussed at the meetings of the Intergovernmental Panel on Harmful Algal Blooms in Paris and suggestions for improvements were made.

2008

A major change took place in 2008 when the list became part of WoRMS, the World Register of Marine Species. This change was decided following a visit by Gert Hansen, Henrik Enevoldsen and me to the headquarters of WoRMS at VLIZ in Oostende, Belgium. The merits of becoming part of WoRMS were discussed and the technicalities of how to include and update species in WoRMS were explained and demonstrated. WoRMS is a very large database which includes almost half a million species of marine organisms. It was decided that the HAB list should both be included in WoRMS and be accessible as a separate entity of WoRMS. The WoRMS list is harvesting algal taxonomic data from ALGAE-BASE in Ireland. It was subsequently decided that the IPHAB Task Team should coordinate the List of Harmful Microalgae with ALGAE-BASE by using the same names of the relevant algal species. The names from ALGAE-Base are therefore automatically used in the List of Harmful Microalgae. When name changes become necessary, these are discussed and agreed with ALGAE-BASE and inserted in both lists.

Which species should be included in the list?

The list was born as a result of the growing interest in harmful algae. The many name changes which regularly took place for many harmful algae, following publication of new information, was/is a source of confusion to ecologists, toxicologists, people involved in routine monitoring and others, thus construction of an agreed and updated list was considered important. A decision had to be made on which species to include in the list. Early on the distinguished member of the Task Team, Prof Y. Halim from Egypt, of *Alexandrium minutum* fame, suggested that all harmful species should be included in the list, in other words the list should include also species which at some stage had formed blooms, causing low oxygen levels in the water at night, and leading to mortality of fish and/or or bottom invertebrates such as certain species of diatoms and silicoflagellates. While these were and still are very real issues, to include all such species in the list would have made the list too long and difficult to handle. It would have entailed including numerous non-toxic and usually harmless species. The Task Team eventually agreed on including only species known to form a toxin or suspected to do so. The lack of knowledge about toxins was particularly relevant for algae causing fish kills, as many of the compounds responsible for the fish kills were/are still not well known. The next problem was to decide on the definition of the term toxic, did it mean toxic to humans, fish, *Artemia*? It was agreed to concentrate on species toxic to humans or to fish. When in doubt, a comment was included under the relevant species.

Nomenclature problems

One reason for the development of the list was that incorrect author citations were recorded in the literature. In fact, the poor general knowledge of and interest in nomenclature is still a problem.

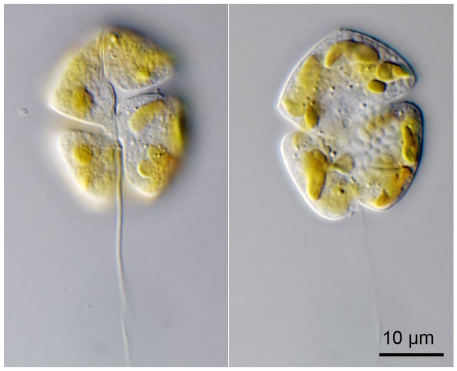

Fig. 1 Karenia mikimotoi (strain K-0260), a historic strain isolated in 1977, as Gyrodinium aureolum, from Oslofjord, Norway, by Karl Tangen (micrographs from Gert Hansen)

Thus mix-up of zoological and botanical nomenclature sometimes appears, even in major phycological journals. It may result in the creation of invalid species names, or it causes uncertainty about the validity of names. An example is the description of the genus *Stoeckeria*, which first appeared in 2005 in Journal of Eukaryotic Microbiology. The description lacked the Latin diagnosis required in botany at the time. The authors apparently intended this heterotrophic dinoflagellate to be described using zoological nomenclature. That is acceptable, a species which is validly described according to one Nomenclature, automatically becomes valid in the other. However, in the title of the article in which *Stoeckeria* was first described, the authors used the class name Dinophyceae, which is botanical, not zoological. Since the authors did not state specifically in the article which nomenclature they used, it caused some readers to conclude that because of the word



Fig. 2. Dinophysis acuminata from Mariager Fjord, Danish coastal waters (micrographs from Øjvind Moestrup)

Dinophyceae the authors intended the new genus to be described according to the botanical nomenclature. The lack of a Latin diagnosis therefore made it invalid. The confusion has actually still not been resolved, and the problem needs to be discussed and decided upon by the International Nomenclature Committee, before it can be fully resolved.

Name changes

One of the problems facing the Task Team on Algal Taxonomy that maintains the List of Harmful Microalgae, is name changes (e.g. *Karenia mikimotoi*) (Fig1). This phenomenon shows no sign of disappearing, and it has recently become an even more serious problem, following the increased use of molecular sequencing.

Invention and application of new techniques that allow species to be examined in more detail have commonly resulted in old species being split into two or more species. This happened when transmission electron microscopy (TEM) became a routine tool in the 1950s, used in detailed studies of small flagellates, morphological details of which are difficult or impossible to see using light microscopy (LM) but readily visible in the TEM. It resulted in the description of numerous new flagellate species, some harmful, others not. This became a problem for ecologists and others who did not use TEM in their investigations and identification to species level was no longer possible. A similar situation took place some time later, notably for diatoms, when scanning electron microscopy (SEM) became a general tool. In recent years, the problem has reached major proportions, following introduction of molecular methods. At the moment there is a tendency to split species into ever smaller units, discernible only by molecular methods. In other cases, some species have been merged, commonly based on studies of one or a few genes, or on observations of polymorphic life cycles (e.g. small cells of *Dinophysis* species) (Fig. 2). Controversy arises when certain genes of morphologically different species (morphospecies) are found to be very similar. Do such species in fact belong to a single species? This has become a major problem for the Task Team on Taxonomy, as it results in discussions on how to define species, genera, families, and in fact all taxonomic levels. Such decisions have always been a problem, but molecular techniques have enabled discrimination of very small parts of the genome, inducing some researchers to define only the genetically most similar strains as belonging to the same species. Categories, subspecies or varieties are presently rarely used by phycologists. If the genes examined are found to be slightly more different, the material is considered to represent different species, and if even more different, to belong to different genera, families, and so on.

Is splitting or merging more correct? There are no rules for this problem in the Code of Botanical Nomenclature; it is up to the researcher her/himself to decide on how to define what constitutes a species, a genus, a family, etc.

How to define a species has been discussed for longer that I care to remember, but there is still little agreement on this question. The same applies to the higher taxonomic levels. It is amazing that there is so much confusion, following so many years of careful study. Boenigk et al [1] in their discussion at the VI European Congress of Protistology in Berlin 2011, expressed it clearly: "*Scientists have been trying in vain for hundreds of years to find the 'correct' definition of species, but the simplest conclusion is that one does not exist*" (p 99 in [1]). The species concept is a practical means that allows us to communicate about our organisms. However, there are currently more than twenty definitions of what defines a species, and "*The advent of molecular data ... compels us to reconsider how species may be most effectively and informatively delineated*" (p 99 in [1]). The so-called biological species concept is often mentioned, also in phycology: if two populations can cross and produce fertile offspring, they belong to the same species. This concept has strong limitations, however, as shown in both mammals and vascular plants. The two known species of buffalo, the American and the European, readily form fertile offspring. If they were to be placed together, they would most likely soon merge into a single species. Yet, we prefer to consider them two different species. The same applies to many other geographically separated organisms. The situation is further complicated by some populations of

geographically widespread species being able to form fertile offspring, while other populations of the same species are not.

Molecular techniques have contributed very significantly to solving many taxonomic problems, but they have not resulted in the emergence of a finite species concept. We have to accept that all levels of classification are subjective rather than objective, and it has always been that way. To cite Charles Darwin: "*I mean by species, those collections of individuals, which have commonly been so designated by naturalists*" (cited by M. Ereshefsky in [1]).

At the generic level, splitting into many smaller genera is occurring rapidly at the moment. The genera containing a large number of species are presently being split into many smaller genera, and if this trend continues, these diverse genera will become a thing of the past. In some cases, some of the new genera are monophyletic rather than polyphyletic, and splitting becomes a matter of personal preference. In other cases separation into smaller genera can result into a better understanding of relationships of the species in this particular part of the old genus. All these problems obviously leave the work of the *Task Team on Taxonomy* difficult. One problem facing taxonomists is that results of taxonomic studies are used not only by academics themselves but also by non university people. With this in mind, the taxonomic systems suggested should not be overly academic; a pragmatic approach to taxonomy will help taxonomy to retain its usefulness in the general public.

A pragmatic approach is currently not held in high esteem, as exemplified by the case of *Cochlodinium*. *Cochlodinium polykrikoides* (Fig. 3) is a very serious fish killer in South-East Asia, but *C. polykrikoides* is not the type species of *Cochlodinium*, and evidence has existed for some years that the type species, whose morphology had not been studied in detail, is so different genetically that the two species should not be included in the same genus. If the genus were to be split, the type species, *Cochlodinium strangulatum*, would then retain the name *Cochlodinium*, while unrelated species presently included in *Cochlodinium*, including *C. polykrikoides*, would need to be given one or more new generic names. Such a change is bound to cause considerable confusion in countries like South Korea, where the name *Cochlodinium polykrikoides* is well known by the general public. There are, however, ways to solve such problems; it is possible to change type species of a genus if this is deemed to be critically important. It is a solution which has to be discussed and accepted by the internal *Nomenclature Committee*, but it has happened before (e.g. the type species of *Acacia*). In the case of *Cochlodinium polykrikoides* it would have been pragmatic to suggest that *C. polykrikoides* should become the new type species for *Cochlodinium*, as this would reduce confusion. There is no guarantee that the suggestion would have been accepted by the *Nomenclature Committee*, however, but in the meantime a new generic name, *Margalefidinium*, has been suggested for *Cochlodinium polykrikoides* [2].

A common problem facing the Task Team has been that in some cases sequencing of single or a few ribosomal genes have proved insufficient to decide on the species level to be used. This was particularly clear in the case of the dinoflagellate *Peridinium aciculiferum* complex, which comprises *P. aciculiferum*, *P. malmogiense* (as *Scrippsiella hangoei*), *P. baicalense*, and *P. euryceps*. The ribosomal genes of the four species are very similar [3], in fact Logares et al. [4] found no difference in ITS1, ITS2, 5.8S, SSU and partial LSU between *P. aciculiferum* and *P. malmogiense*. Yet these species are morphologically and ecologically different and it would be meaningless to merge them into a single species.

Studies on the ribosomal genes therefore do not always provide the final answer. Will these taxonomic problems be easier to solve when more genes or whole genomes become available for use by the taxonomist? Some changes are bound to occur, and an example of such a change is the case of the protist group Heliozoa, suggested by Ernst Haeckel in the late 1800s. The group was found to be polyphyletic based on sequencing of the SSU gene [5], and Haeckel's old name Radiolaria was therefore in for modification. However, subsequent studies of transcribed genes (single cell transcriptomics) resulted in the opposite result, Haeckel was now right once more; the group was again monophyletic [6].

Taxonomy is going through a difficult time at the moment. The Danish phycologist Tyge Christensen, whose name is associated with the *Tyge Christensen Prize* awarded annually by the *International Phycological Society*, wanted genera to be identifiable with the use of a hand lens or at least in a light microscope. If the present trend continues, most unicellular species and genera will eventually become identifiable only by using molecular sequencing. It will be a challenge to handle this situation in a pragmatic way.

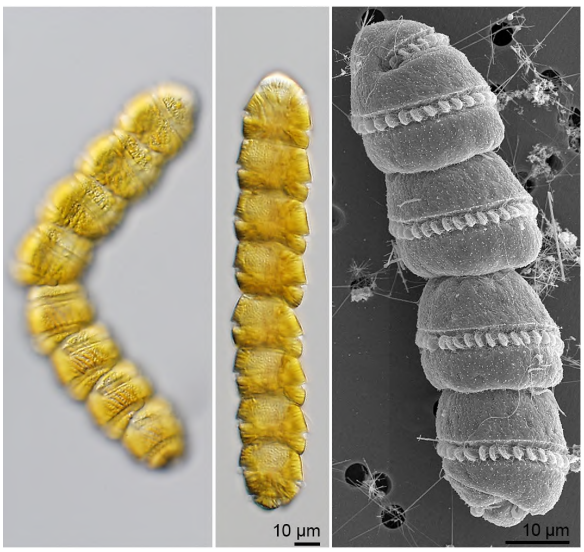

Fig. 3. Margalefidinium polykrikoides (strain K-1292) from Tsushima Island, Nagasaki, isolated by Mitsunori Iwataki in 2006 (micrographs from Gert Hansen)

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Algal toxin discovery, management and regulation over the last 25 years

Algal toxins in the dark ages (pre-1992)

From a historic perspective, knowledge about algal toxins can be divided into truly prehistoric occurrences such as known from paleontological studies [1-2] and more recent historic records. In these historic records, there are descriptions of poisoning incidents that clearly point towards the occurrence of the algal toxins centuries ago, such as the description of Captain George Vancouver, whose crew suffered from paralytic shellfish poisoning during the exploration of the Pacific Northwest in 1793 [3]. Other examples include ciguatera [4] and paralytic poisoning [5-6]. During the 19th century, modern taxonomy emerged as a science with developments in microscopy; by 1900, rather systematic studies of phytoplankton communities are common [7-10] and provide the basis for the biogeography of many toxic genera. In the early to mid-20th century the links between algae and toxins, or at least the toxic effects of algae, are being made [11-15].

By the beginning of the 1990s, many major algal toxins that cause acute poisoning had already been discovered, including brevetoxins [16-19], ciguatoxins [20-27], domoic acid [28-36], okadaic acid and analogues [37-40], prorocentrolide [41], and saxitoxins (STXs) [42-46]. Quite a few analogues of the main toxins had already been discovered [47-50], as well as several groups of compounds produced by dinoflagellates that provoke death in mice used for the mouse bioassay (MBA) but are not necessarily related to human poisoning events, such as pectenotoxins [50] and yessotoxins [51].

Discovery of toxins over the last 25 years

While discovery was mostly driven by human poisoning prior to the 1990s, afterwards the discovery of further compound groups that are produced by dinoflagellates and their metabolites in shellfish was facilitated by the introduction of the MBA for lipophilic toxins in routine shellfish safety testing in European legislation [52-53], as well as by several technological advances. One of the main technological drivers in discovery was certainly the onset of the use of liquid chromatography coupled to mass spectrometry (LC-MS) [54-56], which became quite widespread by the beginning of the 2000s [57-61].

The number of toxin groups that were discovered over the period from 1966 to 1990, (fifteen), was not much less than those discovered over the last 25 years, (nineteen) (Fig. 1). However, the number of analogues in each group has rapidly increased. A good example of this is the STX-group where a review in 1990 counted nineteen observed analogues with a further five predicted from plausible metabolization or chemical transformation pathways [62]. In 2010 a review reported over 50 observed analogues [63]. A simi-

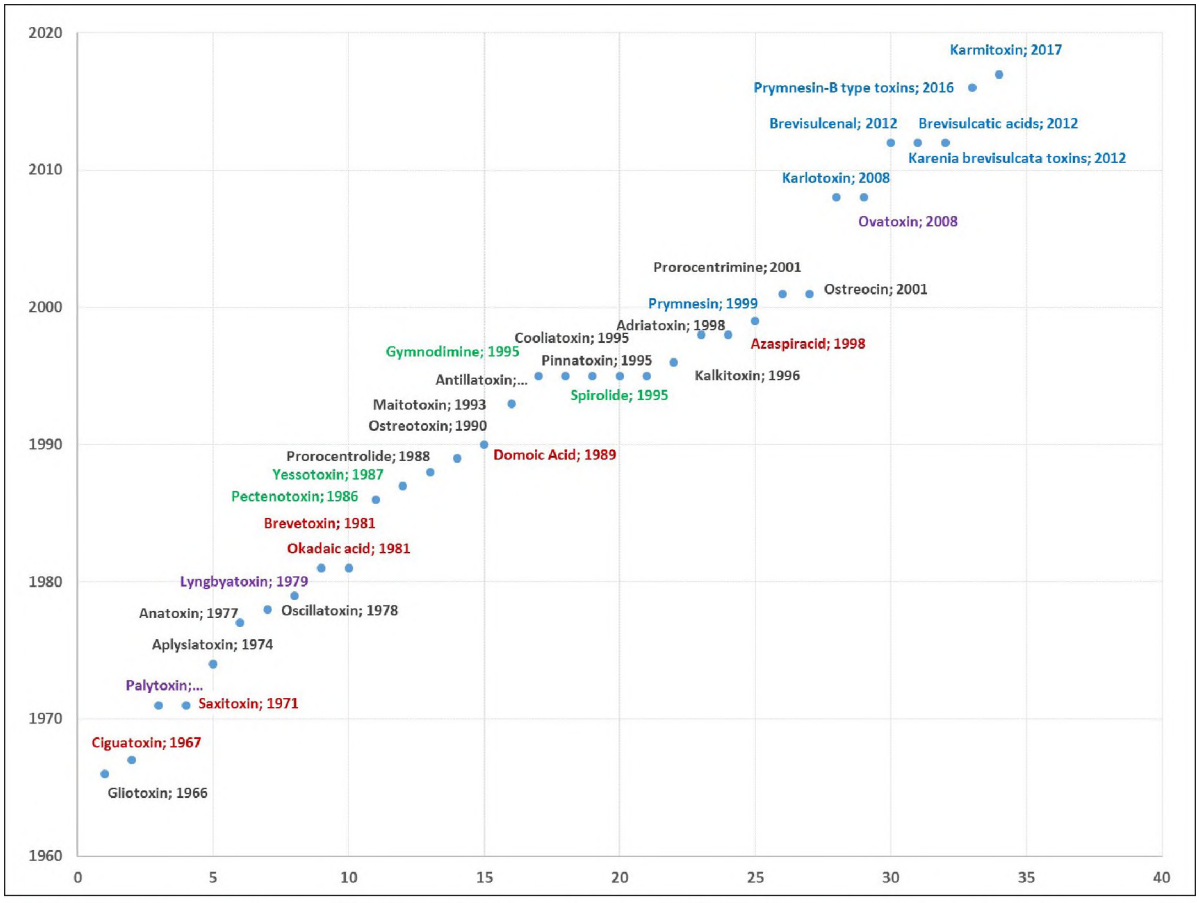

Fig. 1. Discovery or description of the structure of the first analogue of 34 toxin groups (1966 – 2017). Colour code: blue: toxins involved in fish kills; red: toxins involved in human poisoning, violet: toxins causing skin irritation or respiratory problems (BTXs should be red, blue and violet), green: toxins known for 20-30 years and not proven to have negative effects on humans or aquatic organisms, black: toxic compounds yet to be related to effects in humans or aquatic compounds. Nota bene: not many toxin groups relevant to human poisoning are being discovered while more and more toxins related to fish kills are (toxins of Karenia brevisulcata may be related to the Wellington Harbour syndrome)[79-108]

lar rapid increase in known analogues has been observed for the azaspiracid (AZA) group, with the first analogue described in 1998 [64] and a review in 2014 reporting 30 analogues [65]. Only three years later, over 50 analogues are known for this group, including novel phosphate derivatives [66-71]. This discovery rate could have been even more rapid if the causative organisms of AZAs had been elucidated earlier. However, the delay from the first poisoning report in 1995 [72] to the discovery of the culprit organism [73] in this case was likely due to: i) the initial misidentification of the heterotrophic dinoflagellate *Protoperidinium crassipes* (a vector of AZAs upon its feeding on *Azadinium*) as the causative agent, and ii) the difficulties in identification of such a small organism (<15 µm) by optical microscopy in water samples fixed with acidic Lugol's solution, the most common way to preserve samples in routine plankton monitoring.

Another phenomenon that has appeared repeatedly over the past 25 years is the discovery of slightly modified base skeletons for toxin groups. The ciguatoxin (CTX) or CTX1B (= P-CTX-1B) had been reported relatively early on and had been isolated from the moray eel [74]. The algal precursor CTX4A was only described in 1997 [75], yet a slightly modified base skeleton had been reported a few years earlier from *Gambierdiscus*, i.e. CTX3C [76]. Prymnesins are another example of such skeleton variation which is indeed very labour-intensive in natural product discovery as basically the full discovery pipeline has to be completed: bioguided fractionation and isolation of the compound, purification and structural characterisation including mass spectrometry, nuclear magnetic resonance (NMR), UV, infrared and potentially many other studies [77-78].

Finally, it should be noted that only a few compound groups discovered since 1992 have been clearly related to human health issues. These include AZAs (diarrhoea), ovatoxins and to a lesser extent the toxins of *Karenia brevisulcata* (aerosol and direct contact exposure).

There is a significant increase of compounds that appear related to fish kills, e.g. karlotoxins, karmitoxins, prymnesins (A, B and C-Type) and *K. brevisulcata* toxins. The need to clarify the agents involved in fish kills has also been highlighted by a recent systematic review of toxic and harmful algae [109], as well as by the Intergovernmental Oceanographic Commission of UNESCO (IOC) Intergovernmental Panel on Harmful Algal Blooms (IOC-IPHAB), that included the topic in its list of Task Teams.

The systematic inventory of toxins has also been updated [78-108] by the IOC-IPHAB Task Team on Biotoxins, Management and Regulation over the past few years and international databases, e.g. the Harmful Algal Event Database (HAEDAT) updated accordingly. This same panel also contributes to other IPHAB activities whenever chemical expertise is required (e.g. fish kills, HABs and desalination etc.).

Drivers of change in management and regulation

There have been many drivers of change in management practises (e.g. detection methodology) and regulation. These include (i) increased awareness by governments of poisoning events and fish and shellfish mortalities through IPHAB communication with member states (ii) increased pressure from shellfish industry against the MBA for lipophilic toxins due to its qualitative character, false positive results and delays in reporting (iii) technological advances. The conflicts caused by the disadvantages of the animal assays (mouse and rat) for lipophilic toxins has been subject to much debate [110] and decade-long efforts to produce the necessary standards and reference materials for the validation of alternative methods, which have been aided by researchers in Canada (e.g. Michael Quilliam), Ireland, Japan (e.g. Takeshi Yasumoto), New Zealand and many other countries [111-122]. Again the IPHAB panel played a pivotal role in pushing this issue at European and international levels for several years with the help of Phil Busby† (New Zealand Food Safety Authority), a long battle for which the international community will remember him.

Monitoring systems, management practises and legislative changes have been recently reviewed for different trade blocks [123-125]. A major step has been made with the switch from the mouse bioassay to chemical testing by LC-MS/MS for lipophilic toxins, first in New Zealand, then Europe [126] and most recently Japan.

Outlook

Several points can be raised looking foreward from the historic perspective. Climate change is one of the most striking challenges that has been raised with regards to prediction of harmful algal blooms (HABs), and while certain trends appear to manifest themselves [127], much more research is needed to fully anticipate the impacts of climate change on our ecosystems, HABs and their impacts on society [128]. As mentioned above, the need to improve our understanding of the impacts of micro-algae on other aquatic organisms, in particular those that serve as major food resources, i.e. fish and shellfish, has been recognised and requires major international efforts. The multiplicity of compounds in the marine environment only emerges with the recent onset of the omics and while recent studies have shown the feasibility to explore this chemical diversity in the marine environment with techniques such as metabolomics [129-131], more systematic studies will be required to effectively monitor our coastal waters to protect our resources and consumers. Finally, it should be noted that one of the longest-known groups of toxin, i.e. the ciguatoxins, still continue to cause the highest number of seafood poisoning globally [132] and thus deserves the attention of the scientific community over the next few decades.

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Butterflies in Brazil

Abstracts are not always reliable guides to authors' intentions. They are not expected to reveal a great deal about the evidence to be deployed in support of the science, evidence that may not even exist before deadlines for writing abstracts! Nevertheless, as examples of a minor art form, we are entitled to subject them to criticism. These comments were provoked by reading the abstracts of the Florianópolis conference. Here the focus is on two topics, i) climate change, and ii) suggestions that we follow Ben Schneiderman [1] into the convoluted procedures of "Science 2.0". A word count of the Florianópolis conference abstracts reveals that *climate* occurs 73 times, 33 times in the phrase *climate change*, 4 times in presentation titles [2].

Here are some incantations from the abstracts: "*Emerging*[1] *evidence suggests that climate change is impacting marine and freshwater phytoplankton communities"; "Climate change and anthropogenic activities in the coastal zone are increasing the risk of Harmful Algae Blooms"; "The worldwide distribution, frequency and duration of cyanobacterial blooms are driven by eutrophication and climate change"; "El Niño ... is increasing in frequency and magnitude due to climate change"; "As a result of climate change and non-climatic factors, algal bloom frequency, composition and spatio-temporal distributions are changing."* These quotations are mostly assertive; such formulae are not unique to the Florianópolis conference, but were much more prominent there than in earlier conferences in the series.

The idea that harmful blooms are a growing problem was expressed in the first HA conference by Anand Prakash who wrote; "*... there is evidence that paralytic shellfish poisoning outbreaks are increasing in intensity and spreading to new areas*" [3]. He invoked eutrophication to account for increased *intensity* of blooms, and cited Tokyo Bay and the inner Oslofjord as examples. His only example of *spreading* was that of PSP to the western Gulf of Maine in 1972, which was the primary raison d'être for convening the conference. In subsequent discussions of this theme, we often find some combination of the words *frequency, intensity* and *spreading*. Ted Smayda used the phrase *global epidemic* [4].

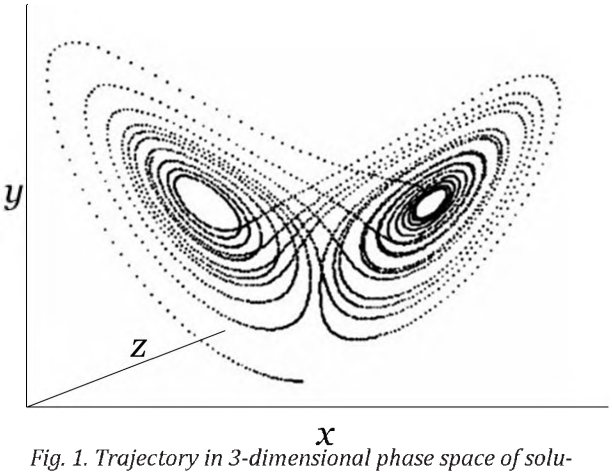

Fig. 1. Trajectory in 3-dimensional phase space of solutions of a simple model of atmospheric convection. Each point represents an instantaneous state of the system. The region to which the points are confined is called a strange attractor. This is Lorenz's butterfly.

In a widely cited statement of concern with climate change and harmful algae Gustaaf Hallegraeff wrote that "*public health and economic impacts of such events appear to have increased in frequency, intensity and geographic distribution*" [5]. Note here, it is not red tides that have increased, and the public health and economic problems *appear to have* (not *have*) increased. This distinction is often missed. Hallegraeff also discussed the possibility that there is a real epidemic (in Smayda's sense). Although he concluded that this too is apparent rather than real, he listed various putative causes, amongst them eutrophication and climate variations.

Revisiting the theme nearly two decades later, Hallegraeff [6] provided a succinct guide to what might happen under a conventional IPCC climate projection; he discussed the potential impact of warming seas, changes in mixed layer depth, wind regimes, runoff, upwelling rates and other variables – we can call them underwater weather – as well as various feedbacks, and overfishing. These variables are standard fare for marine ecology, appealed to routinely to explain the changing population dynamics of HABs as well as other marine species.

The key ideas of climate change are not always examined critically, nor with the natural suspicion that their frequently doctrinal character should excite. So what do we mean by *climate change*? On decadal to secular time scales, we can distinguish *mean* climates, *modal* climates, and *model* climates. Climates result from the interactions of many different elements within the atmosphere, hydrosphere, cryosphere, and biosphere, including phytoplankton. The future states of each of these systems are determined by many interactions within and between them, multitudes of signals with differing amplitudes and phase relations, positive and negative feedback mechanisms, in short, complex dynamics. Complexity really is complex! The mean climate of a region is the average of all the processes we call weather, conventionally calculated over a thirty year time span (World Meteorological Organization) from surface records of temperature, humidity, precipitation, winds, etc. But the mean deceives; it hides variations due to internal *modes*, the best known of which are the El Niño Southern Oscillation (ENSO), and the North Atlantic and Pacific Decadal Oscillations (NAO, PDO); so on decadal to secular time scales, regional climate fluctuations are largely due to modes. Model climates are those we build in our imaginations and in our computers.

Climate *changes* are persistent trends identifiable in meteorological records, usually considered as responses to changes in external forcing rather than modes; recent examples are the cooling from 1940 to 1970 or the warming from 1970 to 2000. "Anthropogenic global warming" (AGW) considers recent warming a result of industrial growth, and many AGW models focus on forcing by atmospheric carbon dioxide concentrations [CO_2]. As time scales grow from secular to millennial, we meet palaeoclimates with well known phenomena like Pleistocene Ice Ages and the Holocene climatic optimum. But the perceived HA epidemic is

1) *Emerging* is a fashionable prefix for evidence, as it is for markets, equities, roles, ... A sciencedirect.com search finds the phrase *emerging evidence* in nearly 30, 000 titles; time to give it a rest?

on a decadal time scale, and identified palaeoclimatic oscillations are not necessarily a useful guide to its interpretation.

An obvious obstacle to detecting climate signals in HAB data is posed by anthropogenic eutrophication. Another obstacle is the fact that phytoplankton respond directly to the increased [CO_2] blamed for warming in many models. Phytoplankton, macroalgae and sea grasses respond to raised [CO_2] by increased nitrate uptake and growth. Thus higher [CO_2] can lower the impact of eutrophication. Increased [CO_2] consumption by phytoplankton also raises pH, hence counters any tendency for acidification.

In the original examples of increased intensity of blooms (Tokyo Bay and inner Oslofjord), eutrophication seems a highly likely cause since both are semi-enclosed water bodies with increasingly urbanized watersheds; but eutrophication cannot have driven the sudden spread of PSP into the western Gulf of Maine, open waters with low human population density along the coast. The message from these examples is that we must analyse the frequency, intensity, and spreading of HABs as separate phenomena, on a species by species basis, and not lump them all together as manifestations of a global epidemic. The same message emerges from more recent examples, for example those summarized by Hallegraeff [6] from his antipodean perspective.

Edward Lorenz [7] asked, "*Does the flap of a butterfly's wings in Brazil set off a tornado in Texas? Is the behavior of the atmosphere unstable with respect to perturbations of small amplitude?*" There is a *nonlinear* relationship between the two variables, butterflies and tornados, meaning that an equation relating them is not of the first degree; nonlinear relationships are the hallmark of *dynamical* systems (Fig. 1). Lorenz wrote: "*Since we do not know exactly how many butterflies there are, nor where they are located, let alone which ones are flapping their wings at any instant, we cannot, if the answer to our question is affirmative, ..., accurately predict the occurrence of tornados at a sufficiently distant future time.*" A practical corollary of this sensitivity to initial conditions, called *the butterfly effect*, is that forecasts of the future are nearly impossible. Climate

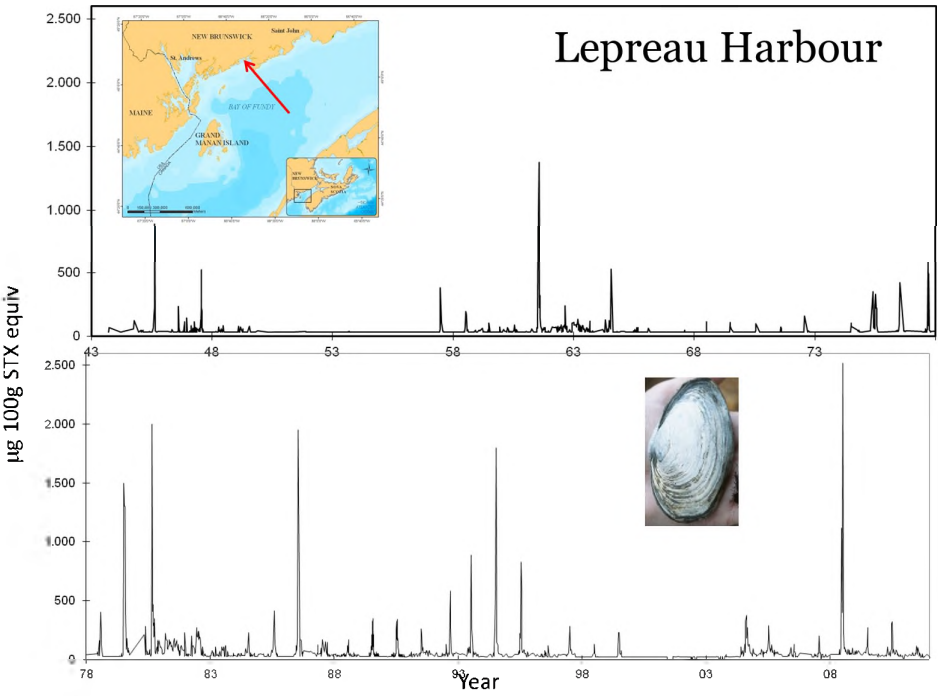

Fig. 2. PSP toxicity maxima identified by White [11] are centered on years 1945, 1961 and 1980; these lag minima of lunar declination in 1940, 1959, and 1978 by a few years. Had the trend continued, further peaks might have been expected around 2000 and this year. The Lepreau Harbour toxicity time series shown here has the three earlier peaks, and more recent ones in 1995 and 2009 which precede the lunar declination minima in 1997 and 2015. Clearly the nodal signal is not the whole story! (Figure courtesy of Jennifer Martin, St Andrews Biological Station, NB, Canada).

then is the prototype of chaotic systems.

We all know that algal growth and the likelihood of blooms are linked to wind regimes, water column stability, runoff, mixing rates, and so on, that is to the local weather, all modulated by regional climate variations. One might therefore anticipate that time series of harmful algal events contain signals provoked by multiannual trends in these oceanographic parameters, hence act as proxies for climate trends. But it seems to be very hard to extract unambiguous signals from records of HABs and algal toxins. In an issue of the *Journal of Sea Research* [8], 14 papers examine phytoplankton time series 1 to 4 decades in length, with special attention to climate variability and eutrophication. "*The initial hope that these data sets do contain significant signals, and that they can be identified and described, is not realized*", at least on these time scales; and, "*despite indisputable and often major changes in coastal waters caused by human activities, no clear signals are detectable in phytoplankton composition and dynamics, nor any which are distinguishable from changes in areas relatively unaffected by such impacts.*" [9]. Claims to the contrary make much use of the subjunctive tense. But Kedong Yin & Jianzhang HE may have distinguished eutrophication and climate variation in a time series (1983 to 2014) of Hong Kong red tides [10].

In the case of toxins, PSP records from the Bay of Fundy (1944-1983) have yielded evidence of a multi-annual lunar signal, the nodal tide, with a period of 18.6 years (Fig. 2) [11]. This finding merits more attention than it has so far received given that the same signal has emerged from analyses of the PDO, the NAO, and many oceanographic and meteorological time series [12, 13]. Chaotic systems are conceptually remote from the clockwork universe of Isaac Newton and Pierre-Simon Laplace. But the nodal tide and other astronomical forcings are deterministic, they do run like clockwork, and can in principle provide forecasts of future climate variation.

The first conference in this series took place in Boston in 1974. From then until the Lund conference (1989), the word *climate* was not indexed in the proceedings. At the Newport conference (1991), El Niño was mentioned twice, and there were suggestions that as a result of hypothetical climatic

trends, *Karenia brevis* might appear more often in the South Atlantic Bight of the US and *Gymnodinium catenatum* bloom more often in northwestern Iberian waters. There was also a warning by Barrie Dale germane to such speculations, that large scale climate models cannot predict local changes.

Little more was heard of supposed links between HABs and climate until two decades later in Hersonissos (2010). The preface to the Hersonissos proceedings claims that "*...this conference included ... a solid body of 50 papers and posters on climate change*"; if true, few of them appeared in the proceedings, where the phrase *climate change* appears in a single contribution about *Prymnesium parvum* in some Texan lakes, and the phrase *global warming* only twice. There were speculations that the appearance of *Chattonella globosa* and *Dinophysis tripos* in Norwegian coastal waters might be related to global warming, similarly for *Ostreopsis ovata* in the upper Adriatic.

Changing patterns of abundance on decadal time scales were reported at the Changwon conference (2012) for species of *Karenia*, *Cochlodinium*, *Chattonella*, and *Heterosigma* in Korean and Japanese waters, *Pyrodinium* in the Philippines, *Gymnodinium* in Portugal, *Prorocentrum* and *Heterosigma* in Narragansett Bay. As pointed out at the time [14], some of these patterns may be linked to regional effects of phenomena like the PDO or ENSO; and some almost certainly, as Patricia Glibert argues, to eutrophication. As already mentioned, disentangling the separate impacts of climate and nutrient loading is a major problem. AGW was not invoked, indeed it is clear that we do not yet have an adequate data base to do so [15]. Also at Changwon, Barrie Dale warned that "available time series plankton data are inadequate for establishing species' responses to natural climate variations and therefore offer no sound basis for predicting effects of climate". The Wellington conference (2014) abstracts contain the word *climate* only six times; in the two contributions in which climate change was addressed directly there, its impact on HABs was again enmeshed with eutrophication.

So what did participants at Florianópolis mean when they wrote of climate change? Was warming intended? From the abstracts alone, it is hard to know. A few clearly mean *weather* in the sense used above. Are others jumping on a bandwagon? If so, they might recall Gustaaf Hallegraeff's admonition: "*Crying wolf does not serve our discipline and we need to refrain from making unsubstantiated climate predictions*!" [16].

Science 2.0 was represented at Florianópolis [17, 18] by Jianguo Liu's "*coupled human and natural systems*" or CHANS [19] and Tim Lang's "*ecological public health*", EPH [20]. Both combine ecosystem models with other complex systems. CHANS are built by linking models of economic, social, hydrological, atmospheric, and biological subsystems and interactions among them. EPH combines "*more than just evidence, and includes the open pursuit of social values, highlighting the role of interest groups, and debate across society not just within restricted scientific circles*." Climate change is central to both schemes. The novelty then is to weld ecosystem dynamics with climate dynamics, add some flavours from outside science, run some models, and see what might happen.

Shneiderman's Science 2.0 combines applied science, engineering, and design and attempts to tackle problems like disaster response systems or environmental sustainability [1]. These are not purely scientific problems, and their complexity makes it necessary to break them into smaller pieces for analysis. One of Shneiderman's five strategies for doing this is classical reductionist science [21]. It is therefore impossible to accept the viewpoint of one participant in the conference, that taxonomy, physiology, biogeography, and population dynamics are no longer useful research topics, that "they will not adequately inform on purported climate change: HAB linkages" [22].

Even though complex models of the climate or an ecosystem can exhibit chaotic dynamics, with all the technical challenges entailed, Science 2.0, EPH, and CHANS propose to combine several such systems in search of solutions to economic and political problems. These aspirations read like recipes for "cybernetic gigantism" [23]. We can agree that solutions to complex problems demand multidisciplinary approaches, and that collaborative projects which combine expertise from diverse disciplines are potentially fruitful. But if science is to be part of these activities, it must be real science, not a debased imitation.

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Red tides in Kamchatka coastal waters (Bering Sea, Russia) are a barrier for the salmon fishery and Pacific salmon

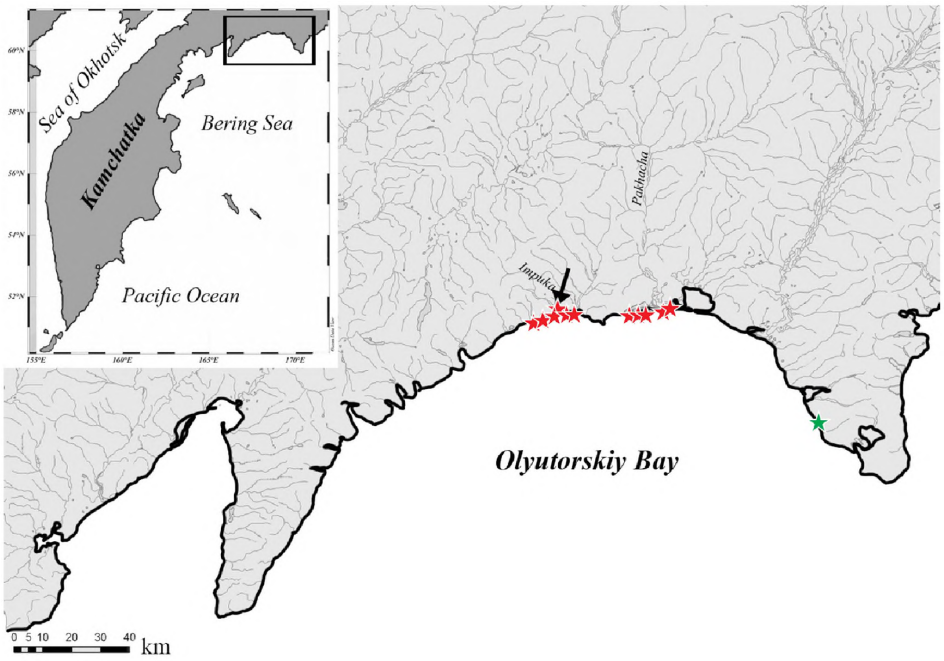

Fig. 1. Map of Olyutorskiy Bay (Kamchatka, Bering Sea) where a red tide, reported by fishermen, occurred in July 2017. The stars denote fishery sites: red, affected by the bloom; green, not affected; the arrow indicates the sampling area

In mid-July 2017 a red tide occurred in Olyutorskiy Bay, Bering Sea (Fig. 1). Fishermen of the company *Delfin* witnessed this phenomenon at their fishery sites (Fig. 2). The first sign of the bloom was observed on July 11th, following a flood during July 8th-9th caused by a strong cyclone. By July 15th the bloom extended along the central coastal zone of the bay, in a layer approximately 5-6 m in depth. The fishermen's attention was attracted by the unusual behavior of pink salmon (*Oncorhynchus gorbusha*) that were approaching the rivers to spawn and entered the bloom area: "*Fish were slack, looked tired and died soon in the trap net*". Additionally, natives of the Pakhachi settlement reported that nearshore waters were reddish-brown, had an unpleasant smell, and dead salmon were washed ashore.

To investigate this phenomenon, a surface water sample was taken on 15th July 2017 from the reddish-brown discoloured plume one mile from the mouth of the Impuka River (Fig. 1). Bright-field and epifluorescence microscopy [1] were used to identify the phytoplankton present. Saxitoxin content in seawater samples was determined by an immune-enzyme assay (IEA) with the help of the test-system RIDASCREEN® FAST PSP SC, R-Biopharm AG, Germany. The test-system detection limit is 5 µg kg^{-1} with specificity to different PSP toxins of: PSP, 100%; decarbomoyl PSP, 20%; gonyautoxin II and III, 70%; neosaxitoxin, 12%.

Alexandrium tamarense (Lebour) Balech was the dominant species (up to 1.32 x 10^5 cells L^{-1}) (Fig. 3). Other dinoflagellates, such as *Tripos fusus* (Ehrenb.) Dujard., *Protoperidinium* sp. and *Alexandrium* cf. *leei*, co-occurred. The diameter of *A. tamarense* vegetative cells ranged from 31 to 40 µm, and that of *A.* cf. *leei* was around 36 µm. *A. tamarense* was found both as vegetative cells (Fig. 3A-C) and in different stages of cyst formation (Fig. 3F, G).

Saxitoxin concentration of the seawater sampled was 0.33 µg L^{-1}. The neurotoxic impact of the bloom on fish in Olyutorskiy Bay was observed by the "slack" behavior of pink salmon passing through the bloom patches, and mortality of some individuals. There are no standards for saxitoxin concentration in sea and fresh water in Russia, but the established regulatory level is 800 µg kg^{-1} for the edible tissues of mollusks and crabs [2, 3].

Visual observation from the air of the Pacific salmon in the spawning

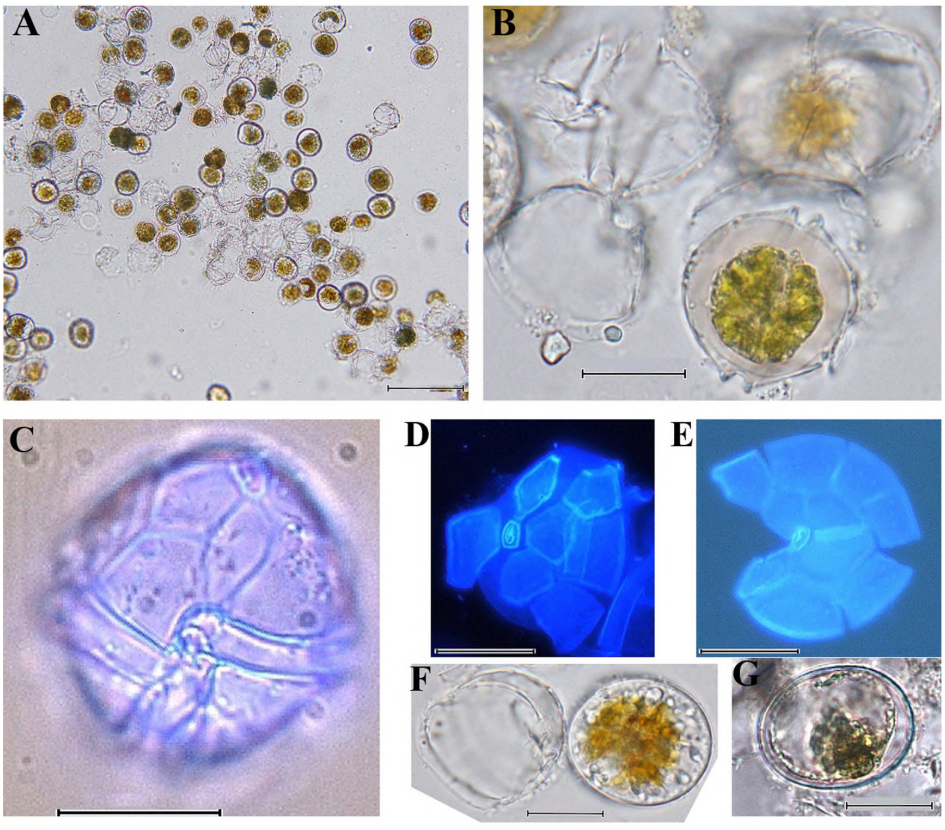

Fig. 3. Alexandrium tamarense in a water sample from Olyutoskiy Bay during the bloom event: A. General view of cells in the sample; B. Cells with content and empty thecae; C: Ventral view of the theca; D-E. Calcofluor-stained epithecal plates; F. Shedding of the theca (cyst formation); G. Cyst. Scale bar = 100 µm (A), 20 µm (B-G).



Fig. 2. Red tide in Olyutorskiy Bay on 15th July 2017

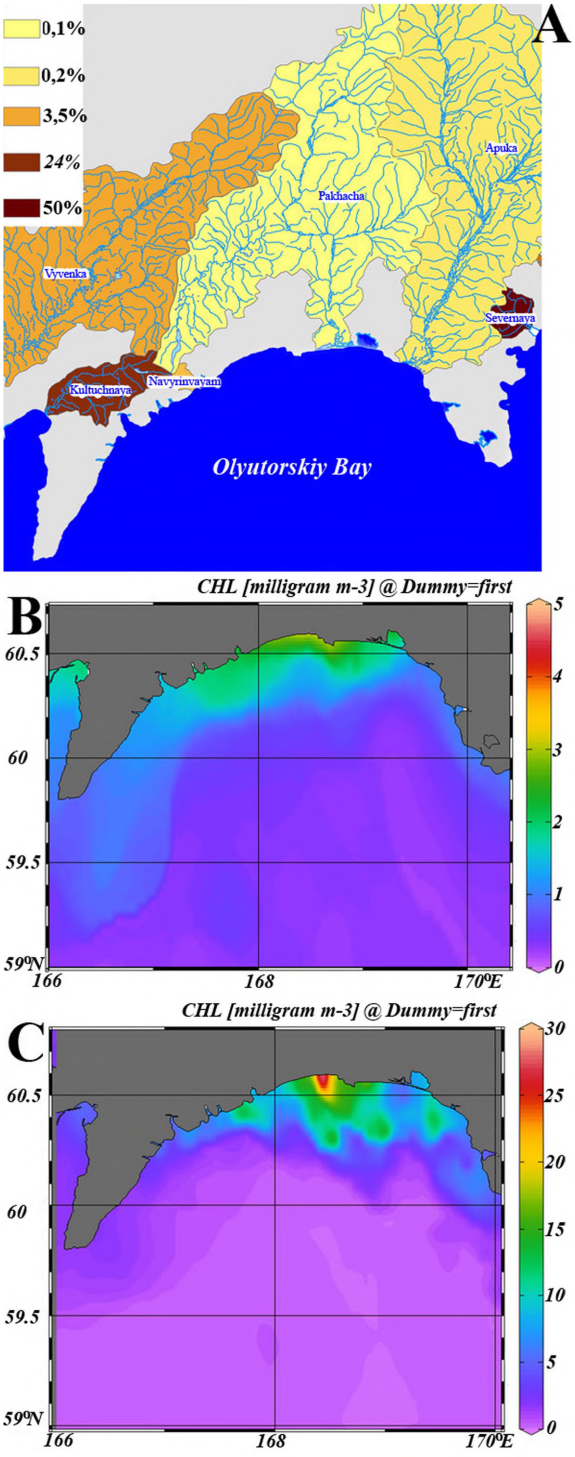

Fig. 4. Distribution of Pacific salmon in river basins (A) and bloom patches as detected from the chlorophyll-a concentration (B:15th July 2017; C: 21th July 2017; http://marine.copernicus.eu).

grounds of the Olyutorskiy Bay river basin performed during the second half of August showed an atypical distribution in the rivers. Maximal escapes were recorded in river basins located in the western and eastern parts of the Olyutorskiy Bay area, whereas in its central part, the abundance of the target species (*Oncorhynchus gorbusha*, *O. keta* and *O. nerka* (all called Pacific salmon) was extremely low (Fig. 4).

A map of salmon distribution over the spawning grounds (Fig. 4A) was compared with that of satellite images of chlorophyll-*a* (Fig. 4B, C) which suggested that the toxic bloom caused a re-distribution of the migratory paths for salmon, with most of them moving to the rivers adjacent to Olutorsky Bay. Changes in anadromous migratory paths of Pacific salmon caused some problems for the fishery in the bloom zone and additional unscheduled expenses.

This is the first documented event for Kamchatka, where a coastal bloom of the toxic microalgae appeared to be a barrier both for fisheries and Pacific salmon spawning migration into freshwater.

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First report of *Gambierdiscus* in the Western Mediterranean Sea (Balearic Islands)

Gambierdiscus (Dinophyceae) species are benthic dinoflagellates living in marine littoral zones of circumtropical areas and have recently been described in temperate waters [1]. Some species are producers of potent neurotoxins: ciguatoxins (CTXs) and maitotoxins (MTXs). Ciguatoxins are linked to Ciguatera Fish Poisoning (CFP). Ciguatera used to be restricted to tropical and subtropical areas, but since the last decade, it appears to be expanding to more temperate latitudes. For example, outbreaks of ciguatera have been reported in the Canary Islands and Madeira (eastern Atlantic Ocean), where several species of the genus *Gambierdiscus* have been identified [2].

In the Mediterranean Sea, no thorough evidence of cases of ciguatera exist. The only reports of CTX-like toxins in fish, which are not confirmed, were based on the Cigua-Check Fish Poison Test kit (Oceanit, Hawaii), a method that has proved to be unreliable [3]. Nonetheless, *Gambierdiscus* species have been reported in the last decade in Crete and Cyprus (eastern Mediterranen Sea) [4-6]. One species of *Fukuyoa (F. paulensis)*, a genus that includes species previously included in the genus *Gambierdiscus*, was reported in the Balearic Islands in 2015 [7]. Little is known about diversity, distribution and toxicity of *Gambierdiscus* spp. in the Mediterranean Sea. The present study confirms the presence of *G. australes* in the two Balearic Islands of Majorca and Minorca, and this constitutes the first report of *Gambierdiscus* genus in the western Mediterranean Sea.

In this study, microalgal samples were collected from macroalgae and rocky substrates in 19 stations in Majorca and Minorca in September 2017. Water temperatures ranged from 24 to 27 °C and salinity from 36.2 to 38.0. In the laboratory, samples were observed under the microscope and individual cells were isolated with micropipettes to establish cultures for morphological and molecular analysis. Calcofluor white stain was used for morphological identification. Cells were observed with a compound microscope equipped with epifluorescence at 630X (Leica DMLB). The *Gambierdiscus* cells observed were anterior-posteriorly compressed. Morphology of the epitheca and the hypotheca is shown in Fig. 1 in which the plate terminology employed follows Fraga and collaborators [8]. The epitheca has a rectangular-shaped 2' apical plate and the P_0 plate is ventrally oriented; the hypotheca has a narrow 2'''' plate equivalent to 1p plate in Chinain [9]. The cell surface is smooth. The cell length and width of 62 individuals were measured. Length ranged from 60.9 to 92.3 µm (mean of 75.6 µm) and width ranged from 64.1 to 90.8 µm (mean of 78.6 µm). The original description [9] described a length range of 76-93 µm and a cell width of 65-84 µm. Further morphological analysis will be performed using electron microscopy.

To facilitate molecular identification to species level, DNA was extracted from individual or a few clonal cells using the Arcturus™ PicoPure™ DNA Extraction Kit (Applied Biosystems, CA, USA). Afterwards, the domain D8-D10 of the LSU rRNA gene was amplified by a Polymerase Chain Reaction (PCR) using the pair of primers FD8 and RB [9], and products were sequenced. The D8-D10 sequences obtained in this study were deposited in GenBank under accession numbers: MG708117- MG708130. DNA sequence analysis of amplified rDNA fragments confirmed that all *Gambierdiscus* spp. corresponded to *G. australes*, which was in accordance with the morphological identification. *G. australes* was present in 10 out of the 19 sampling stations in Majorca and Minorca (Fig. 2), indicating that this species is well established at different locations around the coasts of both islands. It will be important to evaluate the temporal distribution of this species.

The first report of *G. australes* was in the Australes archipelago (French Polynesia). This species is also widely distributed in areas such as New Zealand and the Canary Islands, but it had not been reported yet in the Mediterranean Sea. Some studies mentioned that the spatial expansion of *Gambierdiscus* and CFP may be related to the increase of temperatures caused by climate change

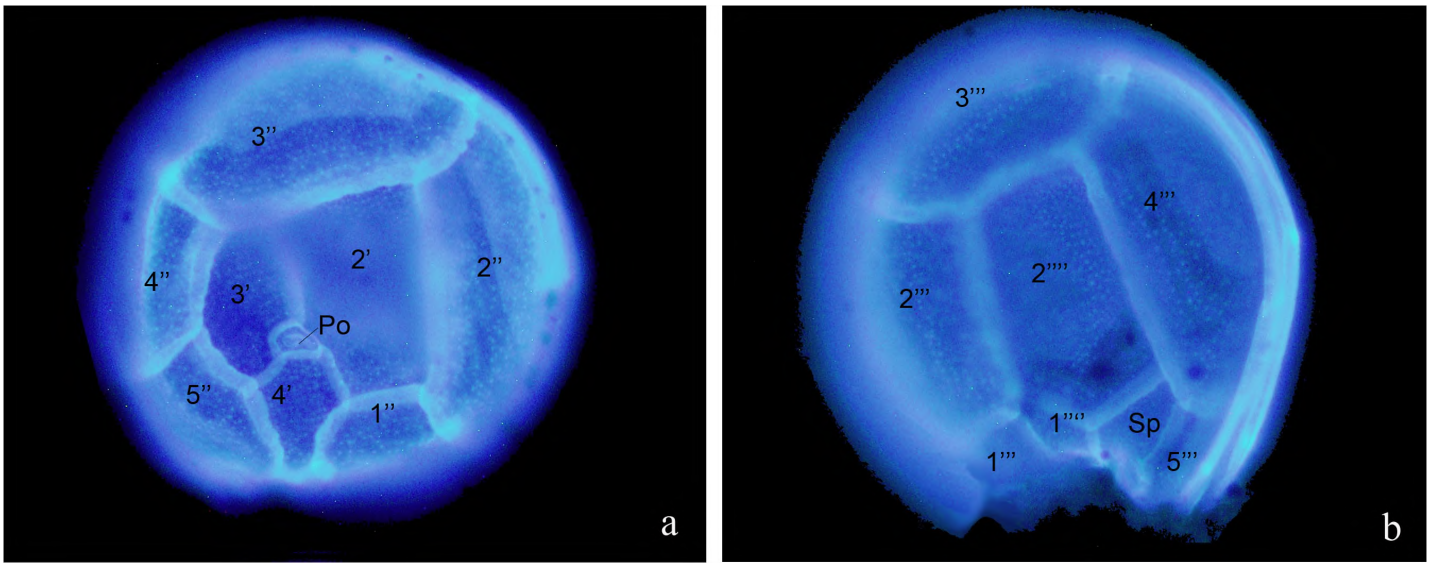

Fig. 1. Epitheca (a) and hypotheca (b) of Gambierdiscus australes cells stained with Calcofluor White.

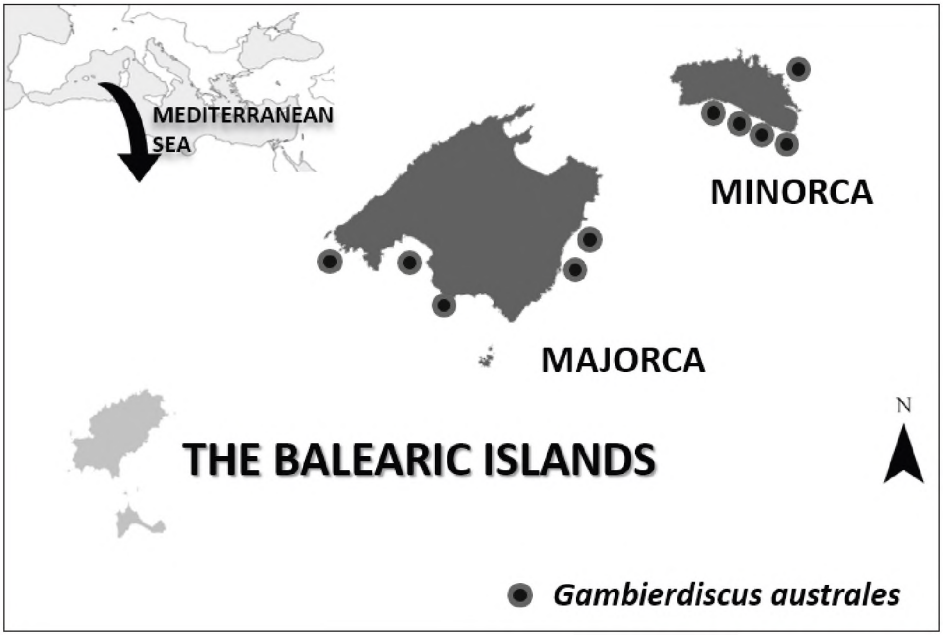

Fig. 2. Locations where Gambierdiscus australes was recorded in the Balearic Islands (39° 30'N, 3° 00' E), Spain.

[10]. The Mediterranean Sea, which is a semi-enclosed sea, seems to be one of the regions strongly affected by the rising of temperatures, and this makes this region more suitable for tropical species [11]. A recent study describes a high diversity of *Gambierdiscus* species in the Canary Islands which would suggest that this genus is not a recently introduced taxon in that area, although climate change may contribute to increase the populations density [2].

It will be important to understand the origin of *Gambierdiscus* in the Mediterranean and the effect that climate change may have on *Gambierdiscus* populations. Moreover, it will be necessary to study whether the Balearic Islands could be a new spot of ciguatera.

Improving our knowledge about diversity and toxicity of these benthic dinoflagellates will provide a better characterization of health risks taking into consideration climate change trends.

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Forthcoming Events

ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

The next ICES-IOC WGHABD, hosted by Margarita Fernandez-Tejedor at IRTA, Sant Carles de la Rapita, Tarragona, Spain, will meet from the 23rd – 26th April (inclusive) to work on the following terms of reference:

A. Deliver National Reports on harmful algal events and bloom dynamics for the year 2017.
B. Review progress and summary of fish killing algae activities underway during the reporting period 2017–2020.
C. Updating of the ICES-PICES-IOC Harmful Algal Event Database (HAE-DAT.
D. Global HAB Status Report for the North Atlantic area: report using data and products generated from HAE-DAT and supplementary time series data as appropriate.
E. New findings in harmful algal bloom dynamics.
F. HABs and the EU Marine Strategy Framework Directive (MSFD). Approaches in Europe to including HABs in the assessment of the Good Environmental Status for the EU Marine Strategy Framework Directive.
G. New results about how physical, chemical and biological interactions control the dynamics of selected harmful micro-algae.
H. Ciguatera Fish Poisoning (CFP) in the ICES area: review new developments in methodology to research the issue, modelling efforts, risk assessments to protect human health, initiatives in other bodies (IPHAB, PICES etc.).

Comparison by light microscopy and qPCR of potentially ichthyotoxic microalgae in Danish on-shore lagoons producing European flounder (*Platichthys flesus*): Pros and cons of microscopical and molecular methods



Fig. 1. Lagoon used for production of European flounder at Fishlab, Denmark.

Evaluation of phytoplankton communities is frequently used to determine the ecological status of water bodies. Hence, species diversity of phytoplankton has become an integral component of national assessment programs. The most commonly used technique for counting phytoplankton is the Utermöhl method [1] and the precision of the enumeration is evaluated with standard statistical analyses techniques [2]. Despite long-term reliance on the Utermöhl method, new techniques for algal identification and enumeration are continuously being explored. Particularly, the recent explosion in molecular tools has resulted in an influx of alternative methods (e.g. real-time qPCR, microarrays and FISH-FC). While many of these methods are promising, their results often differ from those of the conventional method that they were intended to supplement (or replace). Given the importance of algal community assessments, significant efforts have been put into the quality assurance of phytoplankton counts [3]. This has led to the development of standardised procedures.

This study aimed to compare cell counts of seawater samples by light microscopy (LM) and qPCR from on-shore production lagoons of European Flounder (Fig. 1). A total of six lagoons had been filled with untreated seawater from the nearby Limfjorden. Samples for phytoplankton analyses were taken twice weekly from 7 March to 18 May, 2017. A total of 55 samples were examined by LM and qPCR. Potentially ichthyotoxic species (Table 1) were identified either quantitatively or qualitatively by qPCR using species-specific primer sets and hydrolysis (Taqman) probes. Results from qPCR were compared to microscopic cell counts performed by Fishlab. Examples of two representative lagoons are provided in Fig. 2A-D.

Data gathered from all six lagoons revealed ca. 20 groups or species by LM (not shown) and the qPCR assays available detected 8 out of the 11 potentially fish killing species (Fig. 2A, C). There were very few cross overs in terms of species identification between the two methods. LM failed to detect seven of the potentially ichthyotoxic species and *Dictyocha* was the only microalga detected by both methods. Despite this, the cell densities differed markedly. The qPCR assay only detected *Dictyocha speculum* cells in lagoon six at a density of 2,600 cell L^{-1}, whereas LM detected much higher numbers of 56,560 and 36,210 *Dictyocha* 'sp.' in lagoons 1 and 6, respectively. *Pseudochattonella farcimen* and *P. verruculosa* were detected in all lagoons by qPCR and a succession pattern for *P. farcimen* to *P. verruculosa* was evident. When temperatures were low (8-9 °C) *P. farcimen* was dominant but as temperatures increased above 9 °C a switch occurred and a decline in *P. farcimen* concentration coincided with an increase in *P. verruculosa* cell numbers (Fig. 2A, C). During the production period, lagoons were replenished with an addition of ca. 10% of newly collected seawater. The additional water appeared to re-inoculate each lagoon with additional *Pseudochattonella* cells. Fish survival rates in the lagoons were between 0.5 and 13 %.

In terms of monitoring, the discrep-

Species	Lagoon number 1	2	3	4	5	6
Alexandrium tamarense						
Alexandrium ostenfeldii	+			+	+	+
Karenia mikimotoi						
Prymnesium parvum				+	+	
Pseudochattonella farcimen	+	+	+	+	+	+
Pseudochattonella verrculosa	+	+	+	+	+	+
Karlodinium veneficum	+	+	+	+	+	+
Pfiesteria shumwayae	+		+	+		+
Pfiesteria piscicida	+	+	+		+	+
Luciella masanensis						
Dictyocha speculum						+

Table 1. List of species for which qPCR assays are available for quantitative (cell abundance) and qualitative (presence/absence) of potentially ichthytoxic microalgae in this study. '+' = presence of a species in the lagoons used for production of European flounder.

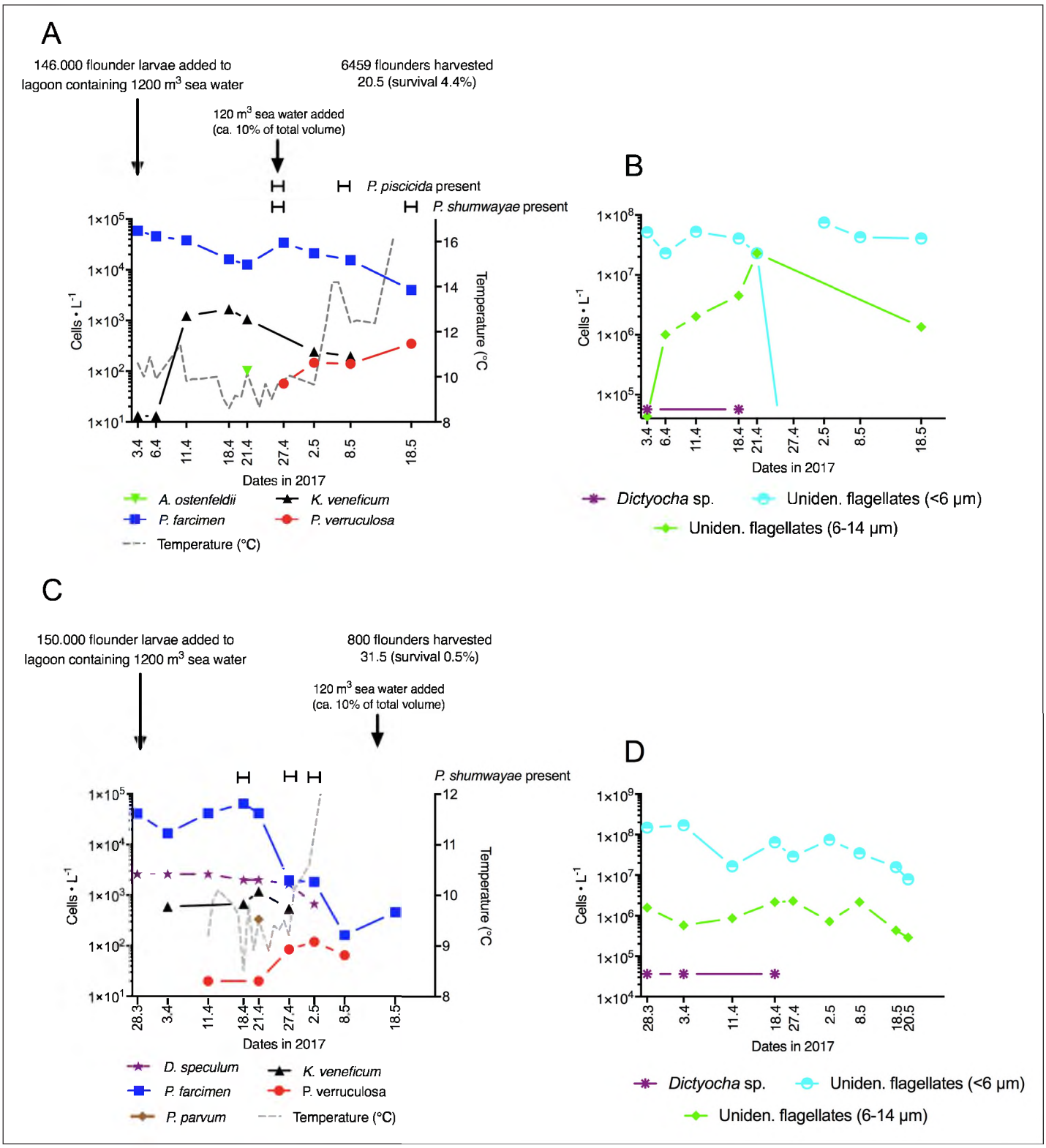

Fig. 2. Comparison of phytoplankton species identification and cell densities (cells L^{-1}) by qPCR (A and C) and light microscopy (target species and groups which potentially could comprise ichthyotoxic organisms) (B and D) in lagoon 1 (A and B) and lagoon 6 (C and D), respectively. The right Y-axis shows the water temperature during the growth period. Presence of Pfiesteria piscicida and P. shumwayae at specific dates are indicated by H. Information on the number of flounder larvae added and the survival rates in percentage at the harvest dates are provided at the top of A and C.

ancy between the qPCR and LM results is obviously quite worrying. There are clearly problems with one or even both methods and it raises many questions over their accuracy. When evaluating phytoplankton data, each inaccuracy associated with sampling, sub-sampling and sample preparation should be taken into account. As each individual step from sampling to counting comes with its own forms of variation, we will critically analyse each method and discuss potential problems and sources of error.

Microscopic analysis

Traditionally light microscopy has been the gold standard for phytoplankton identification due to its relatively low costs and equipment requirements (basically an inverted microscope and a settling chamber). One of the main advantages of LM over qPCR is its ability to identify at least theoretically all organisms present in the sample in contrast to qPCR, which will only target species of interest, e.g. toxic or nuisance organisms. If there were any new species

present in a sample then qPCR would miss those probably due to lack of a developed assay. However, LM does require high levels of taxonomic skills and the precision in identification is only as accurate as the taxonomist allows. Different taxonomists trained in different ways using different identification literature can cause large person-person differences. The ease of identification is also species dependent. For example highly plastic species, or those with a variable life cycle are harder to identify and can often be easily misidentified. The naked form of *Dictyocha speculum* can easily be confused with the rounded cells of *Pseudochattonella*. Some species of the genus *Alexandrium* cannot be identified to species level due their very subtle morphological differences in their thecal plates. When dealing with toxic species, false positives are less problematic but can cause substantial financial losses if they result in the closure of a fishery, but when toxic or problematic organisms are missed completely this could have dire consequences. To reduce confusion, each taxonomist should be provided with a checklist of common species with up to date taxonomic names.

Undertaking frequent inter-comparison exercises, e.g. the ring test or the International Phytoplankton Intercomparison (IPI) exercise, provide feedback on how individual laboratories and taxonomists perform. This forum also provides an opportunity to convene a discussion on nomenclatural changes and new technological advances in monitoring techniques.

As error can be introduced in various different forms, in order to get the most accurate and reproducible results each individual step from: collection, storage, subsampling, homogenisation, filling the chamber, settling and counting strategies all require their own standardised protocols.

All aspects of the protocol need to be considered from the storage containers to the type of fixative used. Many cells e.g. *Pseudochatonella* spp. are sticky and can adhere to plastic walls and as plastic bottles is often preferred over glass especially when transporting samples this can become problematic for accurate cell enumeration. The choice of preservative is important and often the optimal preservation methods are taxa specific. It is not always easy to obtain reliable estimates from fixed material; preservatives can alter the sample in various ways creating a biased measurement. Lugol's iodine [4] has long been the fixative of choice due to its relatively low toxicity and high stability. However, it is known to introduce artefacts such as changes to cell size, a reduction in cell number and in some instances it may fail to preserve certain taxa all together [5-6]. Each alternative fixative comes with its own issues [7].

Settling chambers themselves can be another source of variation. For reliable cell counts, specimens must be completely randomly distributed within the chamber. If cells do not follow a poisson distribution then it will bias the enumeration and any statistical analysis will be affected.

To prevent uneven settling, the samples must be at a constant temperature during the settling period. For a higher chance of getting a well-mixed distribution then samples must first be homogenised. The best way to homogenise a sample is the 'Paul-Schatz' figure of eight rotation method where samples are mixed 100 times in a rhythmic pulsating motion. Even when all precautions are taken, it is still almost impossible for cells to be randomly distributed due to issues such as cell clumping caused by polysaccharide fibrils or inconsistent settling conditions. Due to radial abundance gradients cell abundances at the periphery can be up to 50% lower than at the center, causing a settling bias. Uneven settling will affect the counting strategy. For any counting strategy a predetermined number of units must be observed. The number of units differs depending on the organism and the research objectives. Typically to reach an accuracy of 10% at least 400 cells must be counted [8]. Whole chamber cell counts should be carried out where possible but other counting strategies are often used such as transects and random fields.

qPCR

When carefully designed, with optimisation and validation, qPCR assays are highly accurate and sensitive, but without due care and optimisation, qPCR can be plagued by reproducibility and reliability problems.

The quality of the starting material is one of the key determinants for obtaining reliable and reproducible data. As with microscopy fixatives and storage techniques play a large part in quality of the samples. Fortunately for short term storage Lugol's iodine is the most ideal fixative and the same sample can be used for both microscopic and qPCR analysis [7]. Before amplification DNA must be extracted from the cellular material and commonly commercial extraction kits are applied. To get purified genomic DNA the sample must undergo a number of steps to lyse the cells, remove contaminants and purify the resulting DNA. In cases where the purification step is inefficient the resulting DNA may not be representative of the sample and/or contain compound(s) that will cause assay interference. In these cases the performance of the reaction will be sub-optimal, causing a reduction in the sensitivity and/or amplification efficiency. Inhibition of amplification can occur in different ways. Firstly, when high molecular weight compounds, e.g. humic acids or complex carbohydrates, combine with metal ions to sequester the nucleic acids away from the polymerases and prevent amplification. While some molecules block or inhibit the polymerase or alter the specificity of the primers, inhibitors which block or delay polymerase activity are highly problematic and they can lead to an underestimation of material in the sample or false negatives [9]. Typical approaches to combat inhibition include alternative DNA extraction kits, dilution, specialised polymerases, addition of adjuvants and internal controls.

When designing a qPCR assay it is important to select an appropriate target and to design specific primers with no cross reactivity with other organisms. This study used previously validated species-specific hydrolysis probes in combination with primers to add an extra level of specificity. To ensure accuracy, time is required to optimise the efficiency of the assay and validate it multiple times. This sometimes means altering the constituents of the master-mix used, e.g. nucleotides, magnesium chloride or polymerase concentrations. This is extremely relevant when tackling issues arising from multiplexing assays [9-10].

Once optimised users can still face a number of precision related challenges.

As qPCR measures genetic material rather than viable cells an over estimation of cell numbers can occur due to the inclusion of dead or dying cells. Problems may also occur when targeting multiple copy genes where the organism carries different numbers of the target depending on nutritional status, stress or replication stage. This can lead to an over or under estimation of total cell numbers. Common problems associated with cell number enumeration and copy number do not occur until late exponential-stationary phase meaning that cell numbers can be accurately quantified until this point [11]. One way of potentially overcoming this issue it to use standards created using cells from all parts of the growth curve to produce an 'average' copy number. However, this will decease the overall accuracy of the assay.

As with microscopic analysis qPCR requires standardisation/normalisation for both the laboratory protocols and statistical analysis strategies. To aid this, in 2009 the MIQE guidelines, *Minimum information for publication of Quantitative Real-time PCR Experiments*, were published. These guidelines are designed to 'encourage better experimental practice'. The guidelines establishes a framework for conducting qPCR experiments in the laboratory and are designed to improve experimental workflow and should be followed when designing any qPCR assay [12].

Conclusions

Clearly despite efforts to standardise procedures for both techniques there are still many problems affecting the accuracy and the quality of the results. The comparison of enumeration techniques that was carried out in this study has highlighted the difficulties in obtaining comparative data especially of small-sized, ichthyotoxic microalgae. Enumeration by LM missed many important species, which emphasizes how difficult it is to identify phytoplankton from Lugol's fixed material. We are now moving into the era of 'bio-monitoring 2.0' and with the reduction in costs for meta-barcoding based techniques it is still to be seen if these molecular techniques will eventually replace LM. Yet improvements need to be made across the board for all techniques. The low survival rate of European flounder observed in the 2017 production may be explained by the diverse assemblage of potentially ichthyotoxic microalgae in the lagoons. In previous years the survival rate has been 40-50%.

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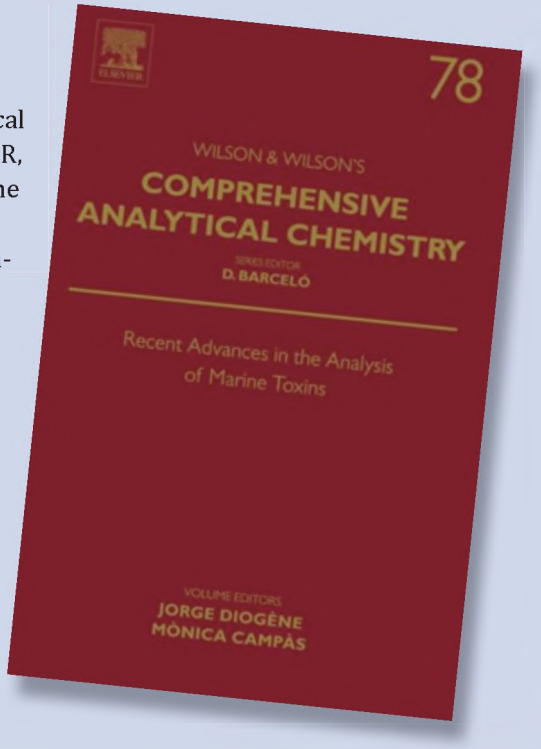
Recent Advances in the Analysis of Marine Toxins

Recent Advances in the Analysis of Marine Toxins, Volume 78, edited by J. Diogéne and Monica Compás, the newest release in the Comprehensive Analytical Chemistry series, provides chapters from well known authors in the field. Updated sections include topics such as:

- The importance of toxin detection and quantification: environmental issues, public health, food safety, animal health, bioterrorism, bioactive compounds, medical approach, an LC-MS/MS analysis of marine toxins;
- Animal bioassays: identification of toxins and mechanism of action;
- Receptor binding assays for the analysis of marine toxins;
- Immunoassays and optical biosensors (visual, SPR, fluorescence) for marine toxins, and
- Electrochemical biosensors for marine toxins.

Details on the table of content and others can be found at: *https://www.elsevier.com/books/recent-advances-in-the-analysis-of-marine-toxins/diogene/978-0-444-63941-7*



The Cawthron Institute Culture Collection of Micro-algae (CICCM)

The CICCM is designated as a "nationally significant database" by the New Zealand government and so receives partial funding for its continued existence. Isolates from 13 classes of micro-algae are maintained either as live cultures or cryopreserved. The approximate 500 isolates include benthic, epiphytic and planktonic marine harmful algal bloom species as well as a collection of freshwater cyanobacteria. Most of the isolates are toxin producers and there are also species that are unique to New Zealand waters. Every isolate in the collection has an associated body of information. The collection underpins research programmes, for example the Safe New Zealand Seafood programme, and is the focus of many student projects both nationally and internationally.

Over the summer of 2017/18 the *Cawthron Foundation* (see box) provided a *Kathleen Curtis* scholarship for an undergraduate student to assist the curators in populating a new CICCM database with the relevant information for each isolate, including site of isolation, culturing tips, molecular and toxin information and associated publications. The scholarship was awarded to Kendall Morman, a biomedical science student at the Auckland University of Technology, New Zealand (Figure 1). To quote Kendall, "It has been a rewarding experience helping this team to construct the CICCM database and I have been fascinated to learn how research involving the collection has increased understanding of marine and freshwater biotoxins in New Zealand."

The database, built using Microsoft's 'Share Point Online' platform, will meet the strategic aim for all collections and databases in Australasia to be easily interrogated. Initially the database will only be accessible 'in-house', but will enable the curators to provide full information with ordered isolates. Online ordering continues via the website: *www.cultures.cawthron.*

The CICCM is distinct from larger collections in the Northern Hemisphere and this has attracted commercial interest in potential micro-algal products. A key user of the collection is the Cawthron Natural Compounds group which aims to make a wider range of toxins available to the global market through a partnership with Sigma-Aldrich to supply algal toxins via their renowned catalogue (see HANews 51 August 2015).

The CICCM will continue to generate economic benefits to New Zealand by underpinning the development of improved and cheaper toxin and molecular tests for the New Zealand shellfish industry and by supporting capability development in all aspects of HABs through provision of cultures to post-graduate students and post-doctoral researchers.

CAWTHRON FOUNDATION is a charitable trust which raises donations, bequests and endowments for public-good science. The Foundation also funds scholarships to support talented emerging scientists and delivers community education programmes: *http://www.cawthn.org.nz/foundation/scholarships/*

Acknowledgements

The 2017/18 Kathleen Curtis scholarship was made possible with generous financial support from the Emery Family.

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Fig. 1. Kendall Morman being welcomed at a powhiri (a Maori welcoming ceremony) led by Harvey Ruru, Cawthron Institute's kaumatua (senior elder member of the group)

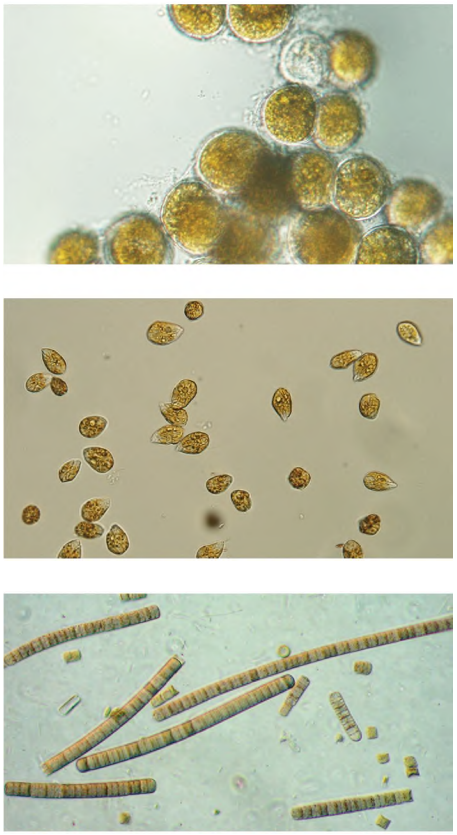

Fig. 2. A selection of cultures held in the CICCM. From top, Vulcanodinium rugosum (non-motile cells), Ostreopsis siamensis, and Phormidium sp

ISSHA's CORNER

The XVIII International Conference on Harmful Algae is approaching! It is time for nominations for achievement awards (Yasumoto Life Time and Patrick Gentien Young Scientist), registration to the conference as a student if you wish to participate in the Maureen Keller Award competition and fund-raising for the auction.

Nominations for the 2018 Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award

ISSHA members are invited to submit nominations for the Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award. Further information on the appropriate profile of the nominees can be found at *www.issha.org* (click on "Awards").

Any ISSHA member in good standing may submit nominations for either achievement award, which should include a description of the nominee's contribution (not more than one page). Please, make sure that you and your nominee have renewed your memberships for the period 2017-2018.

Nominations should be sent by e-mail to Dr. Marta Estrada (*marta@icm.csic.es*) Chair of the Committee on Achievement Awards. Deadline: May 15, 2018.

Please write "ISSHA Achievement Awards 2018" in the message subject. Nominations will be considered by the ISSHA Council, and the awards will be presented at the 18th ICHA Conference Nantes, France, 21-26 October 2018.

The Maureen Keller Student Award for best student presentations at ICHA

The Maureen Keller Award is given to the outstanding graduate student oral and poster presentation at the ICHA2018. Conference participants are chosen in advance to review students' presentations. The evaluations are processed by the ISSHA Awards Committee and awardees are announced during the ICHA closing ceremony.

The presentations are evaluated based on the following criteria:

- Clearly stated hypothesis or objective
- Scientific merit and originality
- Coherence (consistency between introduction, data, conclusions)
- Intelligibility (selection of important points, brevity, accuracy, and clarity of expression)
- Presentation (appearance, layout, clarity)

Candidates can ask to be considered for these awards during the abstract submission process (*https://www.icha2018.com/abstracts/call-for-abstracts/50*)

ISSHA AUCTION

Donations for the ISSHA Auction 2018

ISSHA auctions have been a tremendous success during past International Conferences on Harmful Algae and are becoming established as a tradition. It is an opportunity for conference participants to bid on a broad range of donated items such as books, signed reprints, photos, jewelry, T-shirts, paintings, algae-related items, liquor, and scientific equipment. The auction items are donated by ISSHA members from around the world. Funds collected during the auction are an important income for ISSHA. ISSHA was able to offer partial travel support to 25 students to attend the Conference in Florianópolis, Brazil (9-15 October 2016).

Please, contact Dr. Wayne Litaker (*wayne.litaker@noaa.gov*) who is responsible for the auction about your donations (including "ISSHA Auction 2018" in the message subject). You may wish to include a picture of your item. A list of items will be presented on the ISSHA website before the conference. Dr. Wayne Litaker (*wayne.litaker@noaa.gov*) can provide a letter template to use to request donations from companies. The address where items may be sent in advance of the meeting will be provided on the conference website.



ISSHA conference

Preparation of the ISSHA conference 21-26/10/2018, Nantes, France (*https://www.icha2018.com*) is progressing well.

Five **plenary speakers** have already confirmed their attendance:

- Prof. Elena Litchman (Univ. Minnesota, Michigan State University, USA)
- Prof. Bill Gerwick (Univ. California San Diego - Skaggs & Scripps, USA)
- Prof. Erik Jeppesen (Aarhus University, NL)
- Dr. Mireille Chinain (Institut Louis Malardé, French Polynesia)
- Prof. Thomas Hartung (John Hopkins University, USA)

Check out the **session topics** here: *https://www.icha2018.com/scientific-program/session-topics/35*

And note the **abstract submission deadline** (15/4/2018)

Please submit abstracts here: *https://www.icha2018.com/abstracts/call-for-abstracts/50*

Forthcoming Events

First announcement of the 11th International Conference on Toxic Cyanobacteria (ICTC)



We are pleased to disseminate the first announcement of the *11th International Conference on Toxic Cyanobacteria* (ICTC) that will be held in Krakow, Poland from May 5 – 10, 2019. The ICTC is a periodic scientific meeting that includes members of the international community and focuses on the science and study of cyanotoxins and toxic cyanobacteria. The theme of the ICTC 11 is: "*Learning from the past to predict the future*". Please mark your calendars and make plans to join us in Krakow during May, 2019! Conference website: *http://ictc11.org/*



Harmful Algal Bloom (HAB) sessions at the upcoming 2018 ASLO summer meeting in Victoria, British Columbia

Ted Talks: The career, contributions, and impact of Theodore J. Smayda

Session SS04 convened by:
Tracy A. Villareal, The University of Texax at Austin (*tracyv@austin.utexas.edu*)
James A. Yoder, WHOI (*jyoder@whoi.edu*)
Edward G. Durbin, Univ. of Rhode Island (*edurbin@uri.edu*)

Ted Smayda's career in marine science spanned 60 years on topics ranging from phytoplankton suspension, ecology, succession, community structure, growth rates, biogeography, and the problems of harmful algae. Through his students and colleagues, his reach extended into phytoplankton-zooplankton interactions and the structuring of planktonic ecosystems. With his passing in the spring of 2017, we invite the community to join us in a celebration of his career with contributions on any aspect of phytoplankton or oceanography that were inspired or affected by his work.

Keywords: Biogeography, Ecosystem, Ecology, Marine, Phytoplankton

There will be another three Harmful Algal Bloom (HAB) related sessions:

- Crossing disciplinary boundaries across the freshwater-marine continuum to advance the understanding of HABs (SS71).
- Cyanobacterial and algal metabolites: occurrence, ecology, prediction, and management (SS07), and
- Cyanobacterial ecology as a basis for their mitigation and control under global change (SS51).

The first session will focus on broad HAB research and management solutions, whereas the second session will focus specifically on cyanobacterial and algal metabolites. The third session will focus on aspects of cyanobacterial ecology in relation to environmental change and bloom management. Session abstracts are located at *https://aslo.org/victoria2018/special-sessions*

If you are a HAB researcher planning on attending the 2018 ASLO summer meeting, please consider submitting an abstract to either session. Abstracts are due 16-Feb-2018; registration can be completed at *https://aslo.org/victoria2018/main*

International Coordination of Research on Harmful Algal Blooms
From GEOHAB to GlobalHAB

International cooperation is fundamental to advance understanding of HAB dynamics and to improve our ability to predict them. Fostering this international cooperation was the mission of GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms), the first worldwide research programme focusing exclusively on harmful marine microalgae. From 1998 to 2013, GEOHAB, under the sponsorship of the Intergovernmental Oceanographic Commission (IOC) of UNESCO and the Scientific Committee on Oceanic Research (SCOR), focused on the physiological, behavioral, and genetic characteristics of harmful microalgal species, and the interactions between physical and other environmental conditions that promote the success of one group of species over another. GEOHAB implementation was based on a multidisciplinary, multi-scale and comparative approach, and stimulated the development of new experimental, observational and modelling tools.

During the 15 years of GEOHAB' activity, the international community has contributed to understanding mechanisms underlying HAB population dynamics within an ecological and oceanographic context. At the end of GEOHAB, advances and existing challenges were summarized in six papers in *Oceanography* (The Official Magazine of the Oceanographic Society) in March 2017, (*http://tos.org/oceanography/issue/volume-30-issue-01*). A GEOHAB synthesis, *Global Ecology and Oceanography of Harmful Algal Blooms*, edited by Patricia M. Glibert and co-editors will be published by Springer (*Ecological Studies, Analysis and Synthesis*, Volume 232) in spring 2018.

As GEOHAB ended, the community recognized the benefits ofinternational cooperation and encouraged continuation of a new program built on legacy provided by GEOHAB, but now including freshwater systems, and addressing the effects of HABs on human society now and in a rapidly changing world. Thanks to the invaluable support of IOC and SCOR, the new programme, GlobalHAB, was launched in January 2016. Since then, the GlobalHAB Scientific Steering Committee has been elaborating the Science and Implementation Plan that provides a scientific framework for the integration and coordination of research and expertise of many individual scientists in the study of HABs in different aquatic ecosystems. As in GEOHAB, the direct implication of the international community in the programme is fundamental for GlobalHAB success. Information on the GlobalHAB programme and how to participate can be found at *http://www.globalhab.info*.

Elisa Berdalet, Chair, and the GlobalHAB Scientific Steering Committee

GlobalHAB GOAL AND MISSION

The overall Goal of GlobalHAB is to improve understanding and prediction of HABs in aquatic ecosystems, and management and mitigation of their impacts.

The Mission of GlobalHAB includes the following elements:

- Foster international coordination and cooperative research to address the scientific and societal challenges of HABs, including the environmental, human health and economic impacts, in a rapidly changing world.
- Consolidate linkages with broader scientific fields and other regional and international initiatives relevant to HABs.
- Foster the development and adoption of advanced and cost-effective technologies.
- Promote training, capacity building and communication of HAB research to society.
- Serve as a liaison between the scientific community, stakeholders and policy makers, informing science-based decision-making.



The Themes integrated in GlobalHAB range from small-scale (e.g., cellular) subjects (e.g., biodiversity, adaptive strategies) to studies at ecosystem scale and climate change-related processes.



THE 18TH INTERNATIONAL CONFERENCE
ON HARMFUL ALGAE
FROM ECOSYSTEMS TO SOCIO-ECOSYSTEMS

ICHA 2018
21 – 26 OCTOBER
NANTES, FRANCE

18th International Conference on Harmful Algae
www.icha2018.com

IMPORTANT DEADLINES

Abstract submission deadline: 15 April 2018
Early bird registration: 15 July 2018
Get the 17 ICHA Proceedings at www.issha.org



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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 60:
15 May 2018

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Harmful Algae News

AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

No. 60 - July 2018 · www.ioc-unesco.org/hab

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Marine eukaryote and HAB monitoring in Japan with next generation technology

Sequencing technologies such as Illumina MiSeq have made it possible to obtain billions of sequence reads in a single experiment. Massively parallel sequencing (MPS) is revolutionizing surveys of eukaryotic diversity, including harmful algal bloom (HAB) species. This technology enables us to detect several hundreds of operational taxonomic units (OTUs) of eukaryotes from seawater samples and facilitates the detection of low-abundance populations in complex eukaryote communities. The eastern part of Hokkaido facing the Okhotsk Sea is where the coldest water (−2°C) flows in from Sakhalin in the winter. The marine ecosystem in this area has been considered sensitive to perturbations in environmental conditions. Mombetsu city is thus recognized as the most important monitoring base for observing plankton biodiversity in the coldest waters of Japan. Plankton biodiversity has been performed using MPS-based technology once a week, from April 2012 until present day at Mombetsu city. The Mombetsu city office also has carried out daily environmental monitoring such as water temperature and salinity since 1996, and morphology-based monitoring of zooplankton sampled using nets more than once a week since 1997. In this study, a monitoring survey of eukaryote biodiversity using the MPS-based technique was performed. Surprisingly, many HAB species were detected in northern Hokkaido.

Water temperature, salinity, and chlorophyll-fluorescence were measured weekly with a CTD from April 10 2012 to June 2, 2014 (n = 112) at the Okhotsk tower in Okhotsk Sea (Mombetsu city, Japan) (44°20.22'N, 143°22.85'E) and

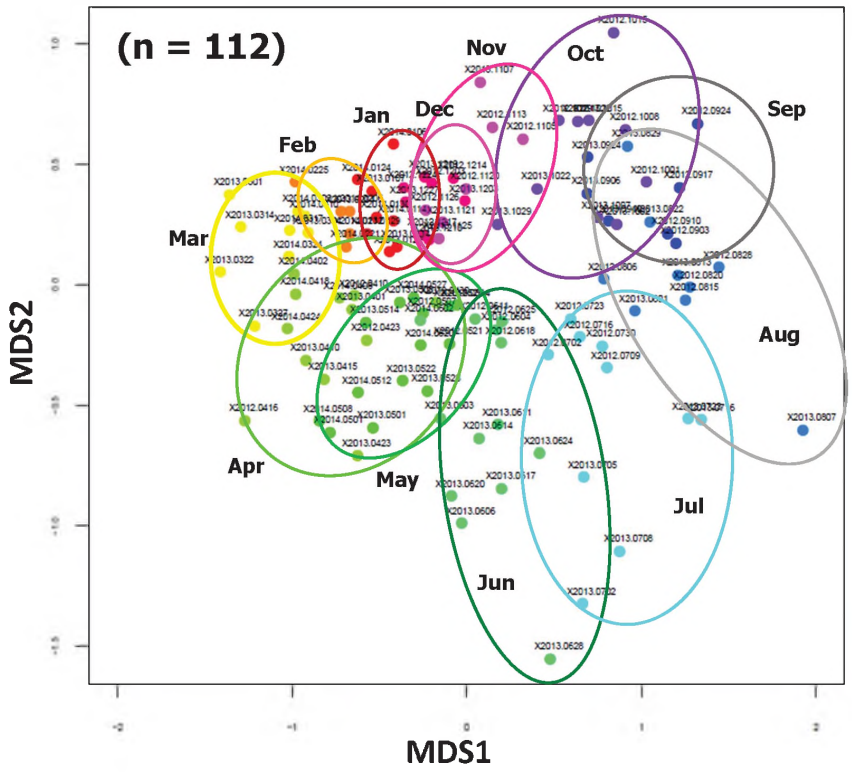

Fig. 1. A plot of non-metric multidimensional scaling (NMDS) analysis for the eukaryotic biodiversity survey using the MPS-based technique in the seawater samples of Okhotsk Sea. The similarity index was calculated using the Jaccard index.

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A follow-up special session on dinoflagellate cysts is scheduled during the ICHA 2018 in Nantes, and will ensure the transfer of expertise and uptake of methods internationally (see p. 17).

Anyone interested in this session can contact: *Kenneth.mertens@ifremer.fr* or *Kirsty.Smith@cawthron.org.nz*

the surface data was used in this study. Surface seawater (0.5 L) was collected weekly with a plastic bucket during the same period. For MPS-based monitoring based on amplicon-seq of 18S-rRNA gene (V7–9 region), PCR amplification and 454 pyrosequencing were performed according to Nagai *et al* [1, 2].

Water temperature, salinity, and chlorophyll *a* measurement ranges were -1.6-22.4°C, 18.3-33.7 PSU, and <0.0-9.8 μg L^{-1} during the survey period. Even during mid-summer, the surface water temperature did not reach 23°C, and it decreased to below -1.5°C in mid-winter. Relatively high chlorophyll *a* concentrations produced by microalgal blooms were observed in samples taken during the warm water seasons during the survey. This is not a common phenomenon as dense microalgal blooms, i.e. red tide formation, have often been confirmed below sea-ice in mid-winter.

The number of OTUs (Operational Taxonomic Units = species richness) ranged from 101 to 454 (266 ± 70 = average ± SD). To minimize the influence of this bias on further analyses, random re-sampling of the MPS was performed on samples with the minimum number of MPS (2,908), and the OTUs were rarefied. The re-calculated OTUs were 89-267 (179 ± 44). These numbers were similar to those at the Seto Inland Sea and Yatsushiro Sea in Japan, which are considered to be eutrophic waters (Nagai *et al* unpublished data), suggesting nutrient rich environments in the Okhotsk Sea [3]. The observed richness (= OTUs) per eukaryotic environmental sample estimated using the MPS-based method was 383-2,133 [4], 1,229-1,823 [5], and 942-1,756 [6]. Estimations of the number of OTUs vary depending on the platform used for MPS data treatment, and are also affected by universal primers. Therefore, the plankton richness or diversity in different ecosystems estimated using different platforms could not be compared directly.

The variability of plankton biodiversity among samples was evaluated by applying similarity indices (the Jaccard index), and non-metric multidimensional scaling (NMDS) analysis was performed (Fig. 1). Samples taken in the same month but from different years were more closely plotted and also the samples of closest months were located in the closest positions, showing the consecutive transition of species composition and the clear seasonal pattern in Okhotsk Sea. Interestingly, the samples were plotted more closely in winter than those at summer seasons, indicating that limited species can appear in severe winter conditions.

For comparison among the super group levels, frequencies of the OTUs and the MPSs, which were identical to those in International Nucleotide Sequence Databases (INSDs) showing > 0.990 blast top hit similarity, were calculated in each super group (Fig. 2). In the Apusozoa, Amoebozoa, and unclassified groups, the detected OTUs were less than 10 (data not shown). Their frequencies within the OTUs and the MPSs were 20.6-79.4% and 55.7-96.0%, and these data varied depending on the super groups (Fig. 2). The frequencies of OTUs and the MPSs, which were identified as a single species, were 11.9-41.2% and 30.9-70.4%, respectively. The taxonomic identification power was relatively high in Archaeplastida, Opisthokonta, and Viridiplantae, but low in Alveolata and Rhizaria, especially in Rhizaria (less than the half of Alveolata). The Rhizaria data probably results from the difficulty of the classification by morphology-based observation due to the delicacy of the cells (good fixation methods are not available) and the difficulty to maintain them as culture strains (heterotrophs require appropriate prey sources). The frequencies of OTUs and the MPSs, which were identical to those at more than two species including sp., were 5.5-38.2% and 0.6-58.6%, respectively. The frequencies of OTUs and the MPSs, which were identical to a single top hit but identified as sp., were 0.0-10.6% and 0.0-33.5%, respectively. Three important factors that influence the power of taxonomic identification are 1) the variability of the target gene sequences among species, 2) the quality and the length of next generation sequences, 3) the depth of INSDs [7, 8].

Surprisingly, the genera *Alexandrium*, *Azadinium*, *Chattonella*, *Cochlodinium*, *Karenia*, *Karlodinium*, *Prorocentrum*, *Pseudochattonella*, and *Takayama* were detected, including toxic and fish-killing species in Okhotsk Sea. *Cochlodinium polykrikoides* was detected mainly during summer survey periods. To ascertain the origin of the Sea of Japan population of *C. polykrikoides* (East Asian clade [9]), 13 samples, isolated from 11 different localities in Japanese and Korean coastal waters, were analyzed using 10 polymorphic microsatellites [10]. Population genetic analyses identified three clusters – the Sea of Japan populations, Yatsushiro Sea (Kumamoto Pref.) populations, and other populations – indicating genetic

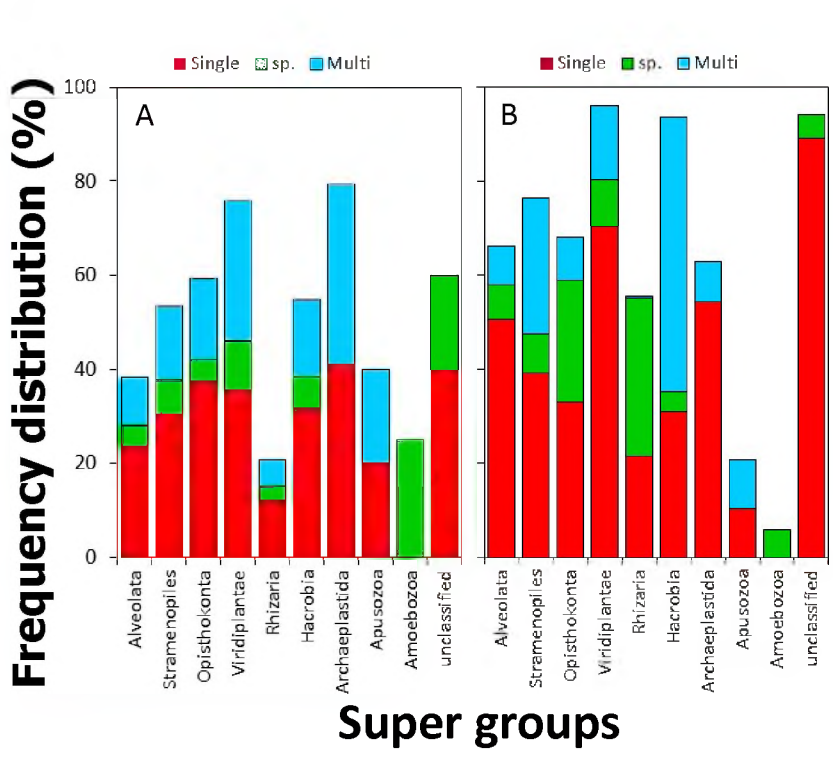

Fig. 2. Frequencies of the number of OTUs showing >0.990 BLAST top hit similarity in the INSDs calculated for each supergroup (A) and their MPS frequencies (B).

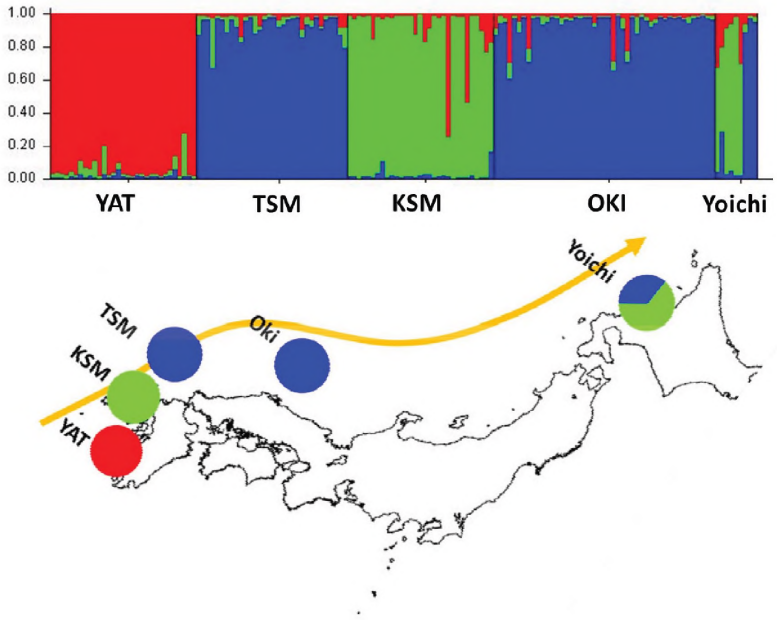

Fig. 3. Results of the assignment test (bar plot analysis) by Structure [11]

structuring of the 13 samples. In the summer of 2014, 47 and 9 clonal strains were isolated from Oki islands, Shimane Pref. (36°19.61'N, 133°33.93'E) and Yoichi, Hokkaido (43°12.11'N, 140°46.33'E), respectively. To verify the origin of the Yoichi sample, population genetic analysis was performed using 10 microsatellites. To identify the origin of the newly isolated strains in 2014, a Tsushima sample (TSM) as the Sea of Japan populations (n = 32), a Yatsushiro Sea sample (YAT) as the Yatsushiro Sea populations (n = 31), and a Nagasaki sample (KSM) as the other populations (n = 31) were used as the reference source populations [10]. As the result of the assignment test (bar plot analysis) by Structure [11], Oki islands sample was identified as the Sea of Japan population, and Yoichi sample was suggested as the admixture of the Sea of Japan and other populations. However, a large genetic break between these two populations was seen [10]. Unfortunately, a good hydro dynamic model to simulate the current system around the Sea of Japan and Hokkaido is not currently available. We speculate that these two populations, which were forming dense blooms in Korean coastal waters and in Kujukushima (Nagasaki Pref.), were separately transferred from west to east and north by the Tsushima warm current, but mixed before reaching Yoichi.

In this study, our data strongly suggested that these HAB species were transported from the Korean and Kyusyu coastal waters by the Tsushima warm current in the summer season. In Funka Bay and Okhotsk Sea in Hokkaido, scallop aquaculture is a very popular and important industry. Therefore, we need to continue the time-series monitoring using the MPS-based technology, not only for understanding the biodiversity but also for HAB monitoring to protect the shellfish industry.

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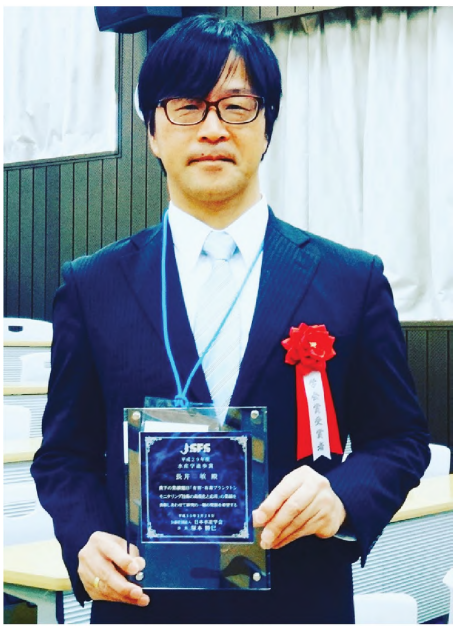

Satoshi Nagai receiving the Research Progress Award from the Japanese Society of Fisheries Science during the Society meeting in 2018.

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ON HARMFUL ALGAE

International Society for the Study of Harmful Algae
ISSHA

Quantifying dinoflagellate cysts in bottom sediments: a response to Anderson 2018

Problems associated with quantifying dinoflagellate cysts in bottom sediments featured prominently in Don Anderson´s personal retrospective view of his contribution to the early days of HAB cyst research published recently in this newsletter [1]. He correctly recognizes that cysts/g dry weight is the appropriate method for quantifying cysts in core samples as well as in surface sediment samples to be compared with cores for geologically based studies. However, the impression given that his work has been criticized for not using this method for biological studies is in my experience incorrect, and I wish to address this here.

The reference cited by Anderson to my criticism of the "wet method" utilizing cysts/volume wet sediment is incorrect – the correct reference is: *Dale B, 2001. Marine dinoflagellate cysts as indicators of eutrophication and industrial pollution: a discussion* [2]. Readers will see that this criticizes the wet method as inappropriate for a study of cores for eutrophication signals, and my recommendation to standardize methods based on the dry method for such work (on recommendation by one of the referees for this 2001 paper) has been adopted by others in the field ever since. This was not a criticism of biological studies by Anderson or others, nor do I know of such criticism from elsewhere.

However, criticism of some of the biological work investigating cysts in sediments has been expressed at meetings when these studies ignored basic elements of the science of sedimentology, and I believe sedimentation processes should be the main focus of this discussion on quantitative methods. Cysts in sediments have the potential to act as seedbeds for some HAB species, as first pointed out by Karen Steidinger, but the cysts also have to be recognized as just one (often very minor) component of finer-grained particles in a dynamic process of transport and deposition in the broader sedimentary regime. Modelling the role of cysts as seedbeds not



Professor Barrie Dale

only requires an understanding of phytoplankton ecology from the biological sciences (e.g. Dale & Murphy, 2014, [3]), but it also necessitates an understanding of the basic elements of sedimentology developed as an important field in geology.

This is not the place to summarize "Sedimentology 101", but it should suffice to point out that geologists have studied modern sediments extensively, using sophisticated methods to investigate the full range of processes of transport and sedimentation down to the micro-fabric of bottom sediments. The extent to which knowledge of sedimentology is critical for studies of cysts in sediments depends very much on the objectives of the study. For example, our research provides quantitative data of cysts/volume of sediment (i.e. the wet method) to help the Environment Agency, Abu Dhabi, with environmental impact assessments for sediment dredging projects. The objective, in this case, is simply to estimate the numbers of HAB cysts potentially released from sediment dredged by the project, a factor which is not dependent on sediment characteristics. In contrast, attempts to quantitatively model cysts in sediment after the occurrence of a HAB to forecast blooms for the following year (e.g. Anderson and co-workers in the Gulf of Maine) necessitate much more consideration of sedimentology.

The main aspect of sedimentology that has to be considered in attempts to model a HAB from cysts in sediments is that bottom sediments are a dynamic feature of, rather than a benign end product of a complex sedimentary system.

Dynamics of the sedimentary regime that affect cyst concentrations in bottom sediments

There are areas of quiescent sedimentation where bottom sediments accumulate layer upon layer without disturbance for long periods of time (varved sediments), almost always in deeper marine basins with extremely low oxygen levels that prevents disturbance by benthic organisms (bioturbation). Since low oxygen levels are known to inhibit excystment, these low-oxygen sediments are not likely to be of interest for modelling seed beds. For better oxygenated sediments that may be of interest, two main sedimentological parameters affect cyst concentrations: transport and disturbance, often constantly working together.

Bodies of water do not usually remain still for long periods of time; turbulence from wind, waves and currents affect the water column, and sediment traps suspended at different levels confirm that fine-grained particles including cysts may be transported for long distances from where they enter the system, before reaching bottom sediment. Once on the bottom, fine particles are often subjected to further turbulence and bioturbation that tends to transport and concentrate finer-grained sediment including cysts into deeper parts of the system (seen on sea charts where mud often characterizes the bottom in deeper water while more sandy sediments are found on "higher ground"). When particles reach their point of accumulation they are subject to bioturbation by benthic organisms which, particularly in productive waters, are constantly mixing the sediments – burying new particles and re-suspending older material from deeper sediment. In addition to these natural processes coastal waters are increasingly affected by intensive bottom trawling, and dramatic pictures are emerging from robot cameras that show large areas of seabed crisscrossed with scour-marks from fishing gear that caused massive resuspension and re-sedimentation of bottom sediment. Ob-

viously, sample sites should be chosen to avoid such areas if possible.

Clearly, there is room for questions or criticism when this dynamic system is modelled from the assumption that bottom sediments, for example in Anderson´s work in The Gulf of Maine, are in a static stable condition whereby a sample taken in the fall one year is considered representative of the seed bed the following spring. The fall sample may well contain many freshly formed cysts from that summer´s bloom, but by next spring these would be expected to be at least partially disseminated into deeper sediment and possibly replaced by some older cysts of more questionable viability by the processes mentioned above. The crucial question for modelers of how many viable cysts germinate from the proposed seed bed to initiate the next bloom will always be difficult/practically impossible to demonstrate. How can one know which cysts from the variety of seed beds in shallower to deeper water, potentially excysting at different times, produce which motiles in the water column in coastal regions with a complex interconnected hydrographic system offering different ecological opportunities? Modelers promising long-term forecasting of HAB from cysts in sediment should be expected to take account of the basic factors affecting the sediments, and they are obliged to check simple assumptions made about sediments where possible. For example, if modelers of HAB in the Gulf of Maine had calculated cyst concentrations in the postulated "seed bed" closer to the postulated time of excystment in the spring, and checked cyst concentrations again soon after "excystment", they could see if cyst concentrations were reduced by the amounts required to support the excystment rates assumed in the models. One of the often stated justifications for modeling is that it can expose aspects of science needing further study. I hope the modelers will accept the explanations presented here as an example of this: that inadequate consideration of the dynamics in the sedimentary system contributes to the high level of uncertainty in the models.

Behind Anderson`s complaint about unjust criticism from geologists lies an important principle that is fundamental to how science progresses: scientists have a responsibility to question/criticize each other's work. This is particularly valuable where different disciplines overlap (in this case biology and geology). Geologists know that water content varies between sediment samples, and biologists were made aware of this only when they were not accounting for it in their work. There are simple, standard methods for checking water content, and these were eventually employed by biologists to account for this factor – surely a positive response to valuable criticism.

From working with both geological and biological aspects of dinoflagellate cysts, I have benefitted greatly from being exposed to and learning from expertise in both fields. I know that HAB science will only benefit from a better understanding of the dynamics of the sedimentary system.

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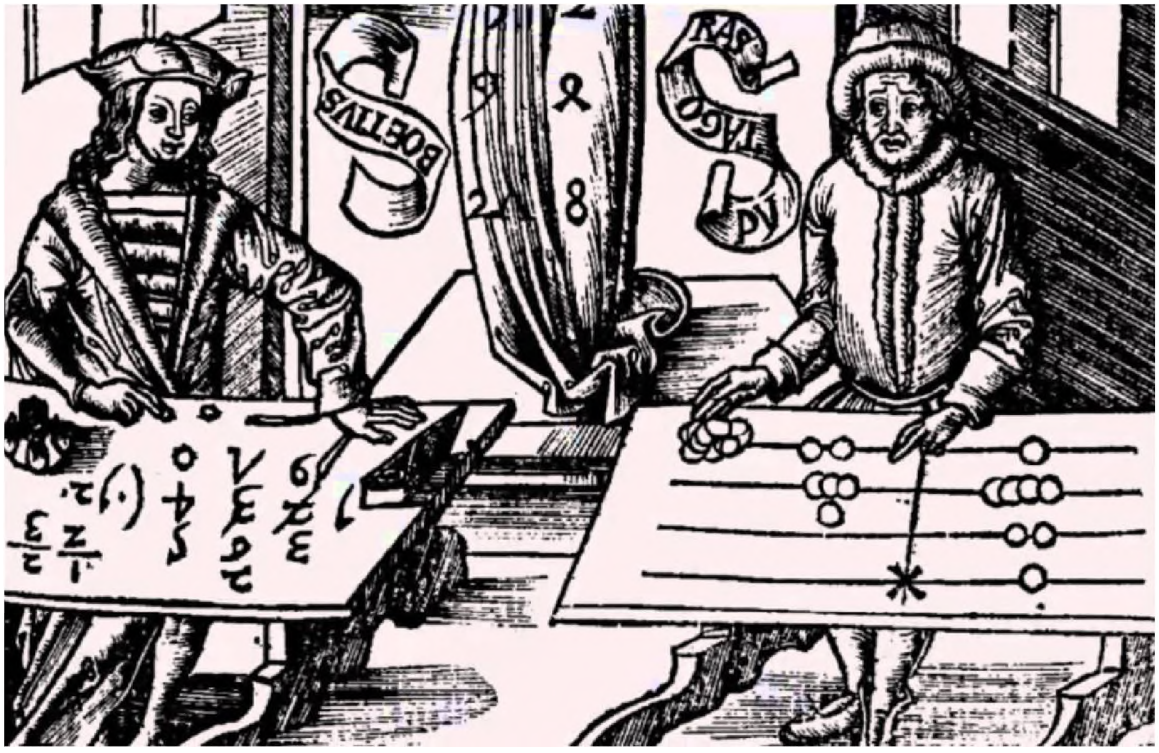

HAN contributors follow an honorable tradition of disputation; differences instead of differentials. Figure shows Isaac Newton and Leibniz, protagonists of the most famous intellectual dispute in history.

Contribution of marine invertebrates to Ciguatera poisoning : the case study of French Polynesia

Reported as early as the 15th century by explorers, ciguatera fish poisoning (CFP) is the most prominent non-bacterial seafood poisoning worldwide. Widespread in tropical and subtropical regions, CFP results from the consumption of coral reef fish contaminated with ciguatoxins (CTXs) produced by *Gambierdiscus* and *Fukuyoa* species. These potent neurotoxins can pose a severe health hazard causing gastrointestinal, neurological and cardiovascular disorders, with cold allodynia being a typical symptom of ciguatera.

Yet, other seafood products such as several species of marine invertebrates highly prized by many island communities in the Pacific region, have also been episodically involved in ciguatera-like poisoning incidents. Indeed, the first mass poisoning outbreak ever documented in the literature following the ingestion of the giant clam *Tridacna maxima* occurred in 1964 in Bora Bora Island (French Polynesia). This poisoning event which affected a total of 33 patients who displayed mainly gastrointestinal and neurological disorders, eventually led to the death of 3 people [1]. Years later, from 2001 onwards, similar incidents were again reported from French Polynesia, but also New Caledonia and the Cook Islands. Of note, besides the digestive and neurological manifestations typically evocative of CFP, patients displayed additional symptoms, such as the rapid onset of the disease, an unusual severity of symptoms including paralysis in some patients, and a rapid burning of their mouth and tongue [2, 3]. Additional poisoning cases following the ingestion of sea urchins, i.e. *Tripneustes esculentus* and *Tripneustes gratilla*, have also been described in the West Indies [4] and French Polynesia [5, 6], respectively, while other marine invertebrates such as the big blue octopus *Octopus scyanea*, the nimble spray crab, *Percnon* spp., and the large worm shell *Dendropoma maxima* have been involved in sporadic poisoning events in the Cook Islands [2]. In addition, the lobsters *Panulirus penicillatus* and octopus from the Republic of Kiribati [7] as well as two starfish from Madeira waters, *Ophidiaster ophidianus* and *Marthasterias glacialis* [8], have been recently found to also bioaccumulate CTXs.

Since many of these edible marine invertebrate species represent a valuable source of protein and revenue in Pacific island countries and territories (PICTs), it is therefore important to document these atypical forms of ciguatera further. The following data describe 2 recent mass-poisoning events which occurred in Anaho Bay (Nuku Hiva Island, Marquesas archipelago, Fig. 1) following the consumption of toxic specimens of *Tectus niloticus,* (gastropods) and *Tripneustes gratilla* (echinoids) in 2014 and 2015, respectively [9-11]. Regardless of the toxic seafood, all patients (9 tourists and 3 tourists, respectively) experienced gastrointestinal and neurological disorders. Cardiovascular symptoms were recorded only in patients that had ingested *T. niloticus,* a likely explanation for this being that patients who consumed toxic sea urchins delayed presenting at hospital, [10, 11]. In the case of the *T. niloticus* poisoning event, the unusual severity of both gastrointestinal and neurological symptoms necessitated the hospitalization of 6 out of the 9 patients.

Unfortunately, in both cases, no food remnants were available for confirmation analysis. Instead, field investigations were undertaken periodically in the toxic area in order to survey the toxicity level in these 2 groups of marine invertebrates and assess the identity



Fig. 1. Study site of Anaho Bay in Nuku Hiva Island, Marquesas Archipelago, French Polynesia

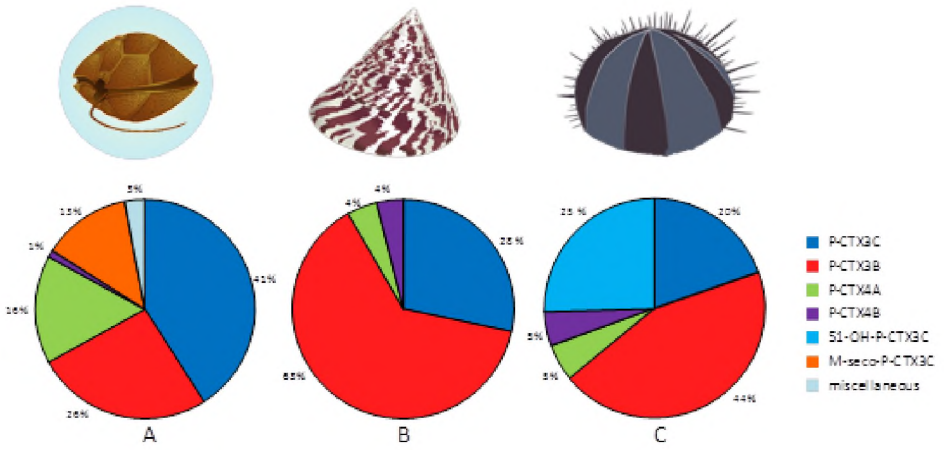

Figure 2. Comparison of Pacific ciguatoxins (P-CTXs) profiles in A) in vitro cultures of Gambierdiscus polynesiensis *(TB-92 [14]), B)* Tectus niloticus *(Nuku Hiva, July 2014 [9]) and C)* Tripneustes gratilla *(Nuku Hiva, July 2015 [11])*

of the toxins involved [9, 11]. Toxicity analysis using the neuroblastoma cell based assay (CBA-N2a) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) revealed the presence of several congeners of P-CTXs in *T. niloticus* and *T. gratilla* toxic samples, at levels consistently above the safety limit recommended for human consumption (0.01 ng P-CTX1B/g of tissue [12]), *i.e.* 11.47 and 20.19 ng P-CTX3C equiv/g of tissue, respectively. These results suggest that *T. niloticus* and *T. gratilla* are able to naturally bioaccumulate P-CTXs in their tissues [9, 11].

Interestingly, these findings were consistent with *Gambierdiscus* abundance data from Anaho Bay obtained using both the natural (i.e. macroalgae) and the artificial (i.e. window-screens) substrate methods [13]. Enumeration of *Gambierdiscus* cells achieved both microscopically and by way of quantitative polymerase chain reaction (qPCR) assays allowed to confirm that *Gambierdiscus* populations were present in this area, while qPCR estimation of the relative distribution of *Gambierdiscus* species in Anaho Bay revealed that *G. polynesiensis* was the dominant species in this toxic area [9, 11]. This species is known to produce P-CTX3C, 3B, 4A, 4B and M-seco-CTX3C as the major CTXs congeners in cultures [14]. The LC-MS/MS analyses performed on *T. niloticus* and *T. gratilla* samples showed that P-CTX3B was the major congener present, followed by P-CTX3C and to a lesser extent, P-CTX4A and P-CTX4B (Fig. 1) [9, 11]. Of note, P-CTX3B is also the major congener found in *Tridacna maxima* experimentally fed *G. polynesiensis* cells [15]. All these observations suggest that *Gambierdiscus* is the likely source of the P-CTXs congeners detected in these marine invertebrates. Sea urchins also contained a significant amount of 51-OH-P-CTX3C (Fig. 2), an analog considered to be favored through metabolization in trophic food webs [11].

Additionally, the field-surveys conducted in Anaho Bay at different time periods emphasized the slow depuration rate for CTXs in both *T. niloticus* and *T. gratilla* toxic samples. The mechanisms controlling the uptake, metabolization and depuration of CTXs in CFP-prone marine invertebrate species are still poorly addressed, thus stressing the need for further investigations. Such knowledge will undoubtedly benefit both ciguatera risk management programs and predictive models of CTX accumulation in these organisms.

In conclusion, these recent results provide evidence that a variety of marine invertebrates actually represent a potential bioaccumulation pathway for ciguatera toxins in marine food webs. Considering that most CFP monitoring programs currently rely on the survey of *Gambierdiscus* cell densities and species composition and/or the monitoring of CTXs in fish, these novel findings highlight the importance of also taking into account the toxicity of marine invertebrates whose consumption may pose a prominent health risk for populations in CFP prone areas.

Acknowledgments

The present work was supported by funds from the countries of France and French Polynesia (Contrat de Projet "Caractérisation et gestion des risques environnementaux et sanitaires liés au développement du phytoplancton ciguatérigène en Polynésie française: CARISTO-Pf" n°7937/MSR/REC of 4th of December 2015 and Arrêté n°HC/491/DIE/BPT of 30th March 2016).

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Solid Phase Adsorption Toxin Tracking (SPATT) technology for field monitoring of *Gambierdiscus* toxins with passive samplers

Ciguatera poisoning is a seafood intoxication classically associated with the consumption of tropical coral reef fish contaminated with ciguatoxins (CTXs), although some marine invertebrates such as bivalves, gastropods or echinoids are also potential vectors of ciguatera [1-3]. CTXs are polyether neurotoxins produced by dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa*, and are responsible for severe acute digestive, neurological, and cardiovascular symptoms in humans [4]. Ciguatera has major health and economic impacts on vulnerable island communities whose subsistence strongly relies on the sustainable exploitation of marine resources, such as in the Pacific island countries and territories (PICTs) where high incidence rates have been consistently reported in the past two decades [5].

Most surveillance programmes currently rely on the survey of cell densities and species composition of *Gambierdiscus* populations, as well as on the evaluation of CTXs concentrations in marine products. However, such methods are time consuming and expensive, thus emphasizing the need for supplementary tools, based on the spatio-temporally integrated sampling of dissolved algal toxins directly in marine environments. The SPATT (solid phase adsorption toxin tracking) technology, first introduced in 2004 [6], uses porous synthetic resins capable of adsorbing dissolved toxins directly from the water column (Fig. 1). Numerous laboratory and field studies, testing different adsorbent substrates of which Diaion® HP20 resin appears to be the most versatile substrate, have been carried out worldwide to assess the applicability of these passive monitoring devices to the detection of lipophilic and hydrophilic toxins produced by a variety of marine and freshwater microorganisms [7]. Regarding the monitoring of toxins associated with ciguatera, one laboratory study has demonstrated the efficacy of HP20 resin for the detection of dissolved CTXs and maitotoxins (MTXs) in *Gambierdiscus* cultures [8].

The efficacy of SPATT technology to detect *Gambierdiscus* toxins in the field in ciguateric biotopes was recently confirmed by the deployment of SPATT devices filled with 10 g of HP20 resin for 48 h in two French Polynesian sites: main village of Kaukura Island (Tuamotu archipelago) and Anaho Bay in Nuku Hiva Island (Marquesas archipelago), characterized by a moderate *vs.* high risk of ciguatera [9]. The presence of CTXs in SPATT devices was assessed using the neuroblastoma cell-based assay (CBA-N2a) and results showed that SPATT devices deployed in Anaho Bay were able to retain 1.30 ± 0.41 ng P-CTX3C equiv. g^{-1} HP20 resin. Liquid chromatography - tandem mass spectrometry (LC-MS/MS) analyses confirmed that P-CTX3B and P-CTX3C were primarily retained on SPATT devices. These results are coherent with environmental observations that confirmed the presence of significant amounts of *Gambierdiscus* cells in Anaho Bay, most notably in the species *G. polynesiensis* known for its very high toxic potential, and with toxicological analyses that demonstrated the presence of CTXs in all marine products tested [2,3]. In contrast, no CTXs were detected in SPATT devices deployed in Kaukura Island. However, as with SPATT devices deployed in Anaho Bay, LC-MS/MS analyses revealed the adsorption of a putative MTX analogue, known as MTX3, on SPATT devices deployed in Kaukura Island. Recent studies indicate that putative MTX3 is ubiquitous within *Gambierdiscus* genus [10], suggesting its potential use as a biomarker of the occurrence of *Gambierdiscus* cells in the natural environment. Since a very low density of *Gambierdiscus* cells was observed in Kaukura Island, the detection of putative MTX3 but not of CTXs on SPATT devices suggest either the presence of non-toxic *Gambierdiscus*

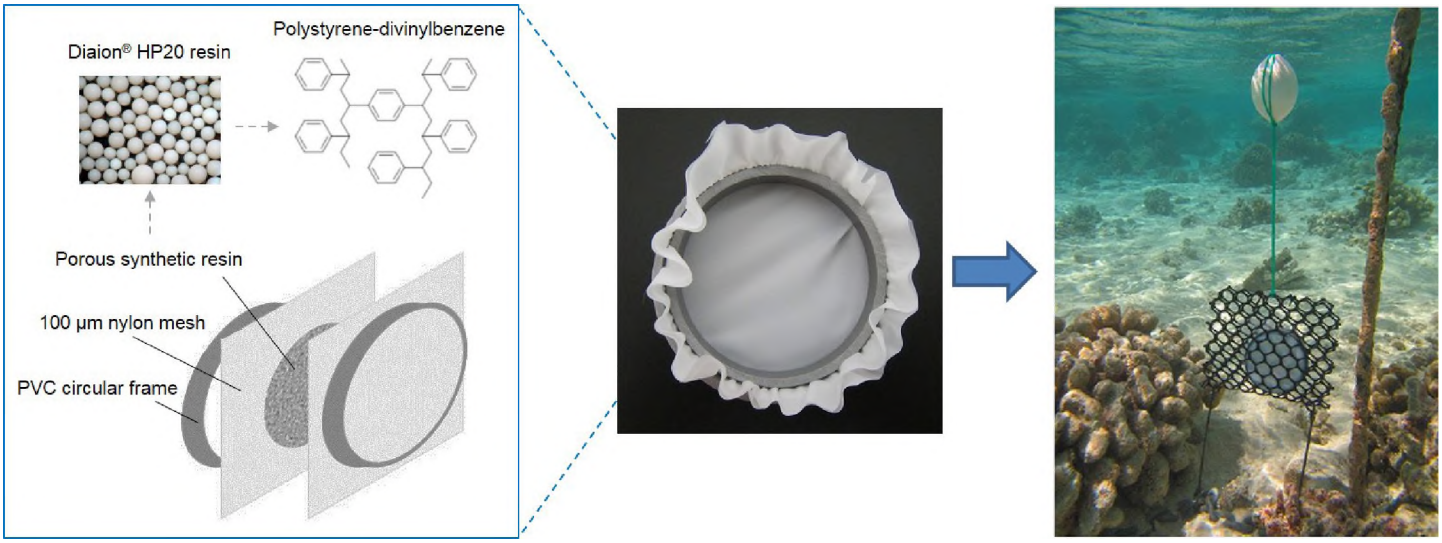

Fig. 1. Example of a design of SPATT device assembly and field deployment. Left and middle: the SPATT device is made of two nylon mesh layers filled with a porous synthetic resin (Diaion® HP20, polystyrene-divinylbenzene matrix, is the most used resin), and fixed between two PVC circular frames. Right: in the field, the SPATT device is inserted in plastic grids to prevent its damage and grazing by fish, and maintained in a vertical position in the water column using weights and floats. © M. Roué (modified from [7] and [9])

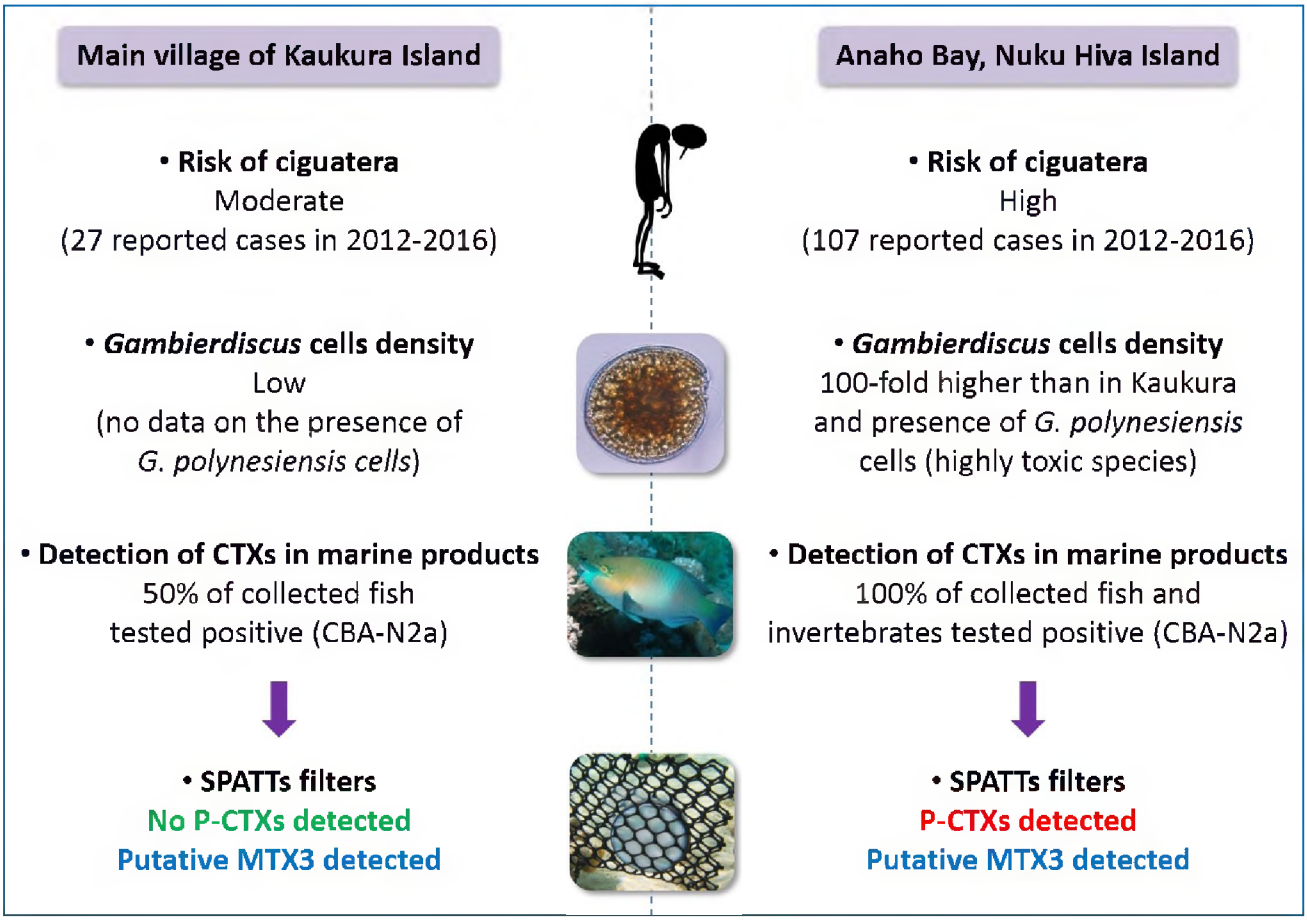

Fig. 2. Summary of the results demonstrating that SPATT *passive sampling could advantageously contribute to the reinforcement of ciguateric risk assessment and management programmes as a supplementary tool.*

or of very low densities of toxic *Gambierdiscus* cells. However, CTXs were detected in numerous fish collected in Kaukura Island (CBA-N2a), suggesting that blooms of toxic *Gambierdiscus* had previously occurred in this area, leading to the bioaccumulation of CTXs in marine products, but had disappeared at the time of the SPATT devices deployment. Another explanation could be that SPATT devices allow for the detection of CTXs in the field only when high levels of CTXs are dissolved in the seawater and are thus relevant only in highly ciguateric locations.

In all cases, these results (summarized in Fig. 2) confirmed the ability of SPATT technology to detect CTXs in the field and thus emphasize the relevance of this toxin-based technique as a useful supplementary tool when assessing the risks associated with *Gambierdiscus* proliferation in locations highly prone to ciguatera. It should be highlighted that SPATT devices are only able to give indications of the presence of *Gambierdiscus* cells (detection of MTX3) and their toxicity (detection of CTXs) in an area at a given time, but they are not effective to guarantee the edibility of marine products that could have bio-accumulated toxins in their tissues. The simplicity, low-cost and logistical advantages (e.g. storage and transport) offered by these easy-to-use devices make SPATT technology a valuable tool well adapted to the survey of CFP risk, most notably in widely dispersed and remote sampling locations in PICTs. Moreover, given the risk of simultaneous accumulation of multiple biotoxins in seafood products in tropical environments, the use of SPATT technology, when combined with downstream analyses such as LC–MS/MS multi-toxin screenings, also offers promising prospects in the framework of emerging toxins surveillance.

Acknowledgments

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New limits of *Ostreopsis* distribution in the Bay of Biscay: a first report of *Ostreopsis* in Santander Bay, Cantabria (Northern Spain)

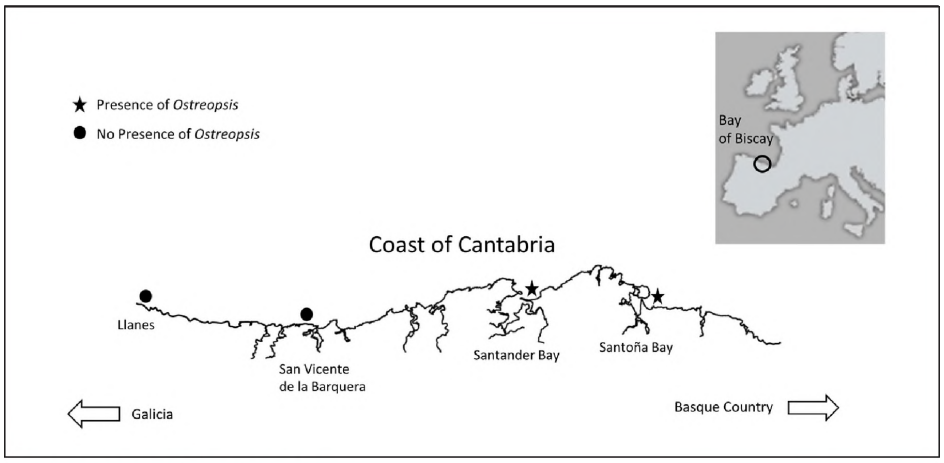

Fig. 1. Location of sampling sites

The genus *Ostreopsis* encompasses benthic dinoflagellates that represent one of the main microalgal threats for beach tourism in warm-temperate waters. Blooms of species within this genus can produce toxins, mostly belonging to the palytoxin group [1], which may cause problems affecting the respiratory system and/or skin irritation, due to their association with toxic marine aerosols. Not all the *Ostreopsis* species have been confirmed as toxin producers with only 4 out of the currently 11 species described considered toxic [2].

Ostreopsis proliferates forming a thin pellicle on the rocks or on macrophytes, but it is possible to also find it in the water column detached from the substrate forming aggregates, usually after very hydrodynamic episodes. The distribution of this genus has been typically restricted to tropical and temperate waters. In the last years, reports of *Ostreopsis* blooms in temperate waters have increased, some of them occurring in places where they had not been observed before [3]. Such is the case of the Bay of Biscay, where *Ostreopsis* spp. were recurrently observed in the southeastern part of the Bay in studies conducted during 2010 and 2011 [4]. In those surveys, the western limit of *Ostreopsis* distribution was established at the locality of Santoña [4].

Santander Bay is the largest estuary in Northern Spain, with important commercial, fishing and tourist activities. On September 7th, 2017, *Ostreopsis* was recorded for the first time west of Santoña from a survey performed in Loredo Beach, at the mouth of the Bay, (Fig. 1). Two weeks later, on September 21st, samples from two sites to the west of Santander Bay, Llanes and San Vicente de la Barquera, were collected to establish the new limits of *Ostreopsis.* No cells of this genus were detected. Additional samples close to Santoña confirmed the presence of *Ostreopsis* in this location. Up to date, Santander Bay represents the new western limit of *Ostreopsis* distribution in the Bay of Biscay. Considering the socio-economic importance of Santander Bay, the presence of *Ostreopsis* in the area should be regularly monitored.

Cultures of *Ostreopsis* isolated from epiphytic cells on macroalgae at the sampling stations in Santander Bay and Santoña were analyzed genetically to identify the species. The amplification of the ITS1 rDNA region performed with specific primers [5] confirmed that the strains belong to *Ostreopsis siamensis*, corroborating our first suspicion based on the cells' morphology (Fig. 2). These strains are currently kept at the Microalgae Culture Collection of the University of the Basque Country.

Temperatures close to the sampling site, in the mouth of Santander Bay, were above 19 °C from mid June until the end of September 2017. Our observations agree with the hypothesis posed by David *et al* [4], that temperatures above 19.5 °C during at least three consecutive months are necessary for the populations of *Ostreopsis* to develop in the Atlantic coast of the Iberian Peninsula.

Additional surveys should be planned, both to the north and west of Santander Bay, to definitively assess the distribution of *Ostreopsis siamensis* in the Bay of Biscay.

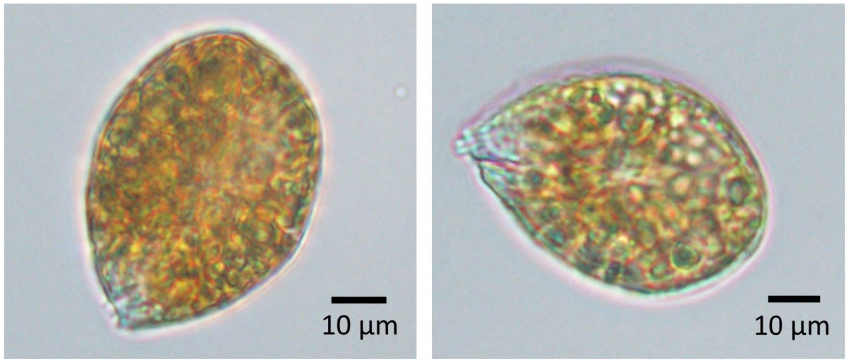

Fig. 2. Ostreopsis siamensis *from Santander Bay*

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Bloom of *Ostreopsis* cf. *siamensis* in Lisbon Bay

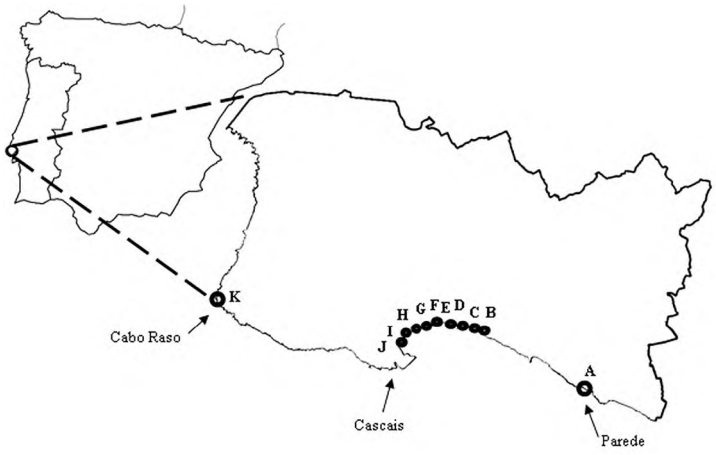

Fig. 1. Sampling sites on Lisbon Bay

Reports of benthic HAB events have increased during the last decade in temperate regions. These have been associated with proliferations of benthic toxic dinoflagellates, in particular species of *Ostreopsis.* The Mediterranean coast of the Iberian Peninsula has been regularly affected by blooms of the highly toxic species *O.* cf. *ovata* [1]. This species produces potent toxins of the palytoxin group and has been responsible for several outbreaks leading to mortality of marine organisms, human health problems and economic losses due to beach closures in tourist areas [2]. A bloom of *O.* cf. *ovata* was recorded in 2011 at Dona Ana Beach, Lagos, one of the most picturesque tourist beaches on the Algarve coast in Portugal, with no significant harmful effects [3]. So far, this is the only area on the Atlantic coast of Iberia where *O.* cf. *ovata* has been reported [3, 4]. A second *Ostreopsis* species, *O.* cf *siamensis*, is also present in the Mediterranean and on the Atlantic Iberian coast. *O.* cf *siamensis* has a more restricted distribution than *O.* cf. *ovata* in the Mediterranean but the opposite is observed on the Atlantic coast: whilst *O.* cf. *ovata* is only known to be present in the south of Portugal, *O.* cf. *siamensis* has a wide, albeit fragmented, distribution from southern Portugal to the Bay of Biscay [5].

Sampling in Lisbon Bay during summer-early autumn has been carried out every year since 2001, and cells of *O.* cf. *siamensis* regularly found, both in the benthos and in the water column, although in very low densities.

In September 2017, a survey was carried out to study the benthic dinoflagellate community in Lisbon Bay. The area is characterized by a succession of sandy and rocky beaches and sampling was carried out in intertidal pools. During the sampling of the first site (Fig. 1, G), the presence of mucilaginous threads in several of the tide pools was immediately observed (Fig. 2). A thin yellow/brownish pellicle covered the substrate, dominated by small, mostly finely branched and foliose thalli, forming a diverse cespitose assemblage; specimens of *Dictyota dichotoma*, *Codium* sp., and *Halopteris scoparia*, among others, were also present (Table 1). Samples of representative macroalgae from the tide pools with surrounding water were collected by hand with plastic bags, vigorously shaken for 1 minute to detach the epiphytic community, and the resulting water samples stored in bottles after sieving through a 100-µm net. The macroalgae were kept in the plastic bags for fresh (FW) and dry weight (DW) determination. To estimate cell densities in the water column, water samples were also collected in plastic containers and fixed with 2% neutral Lugol's solution. Fresh sample was used for observation, single- cell isolation and establishment of monoclonal cultures. The epiphytic community was immediately checked under the microscope, and the occurrence of an *Ostreopsis* sp. bloom was confirmed (Fig. 2).

Following these observations, samples were collected the next day at 11 sites along an east-west transect (Fig. 1) to study the geographical extension of the proliferation. Samples were collected and processed as above. Living cells



Fig. 2. Top left: mucilaginous brown filaments in a water pool. Top right: Ostreopsis *cells under the optical microscope; Bottom: Station G in the Bay of Lisbon.*

Table 1 - List of the macroalgae community species

Asparagopsis armata	*Corallina* sp.
Caulacanthus ustulatus	*Cystoseira* sp.
Ceramiacea	*Derbesia* sp
Ceramium spp.	*Dictyota dichotoma*
Champia sp.	*Gelidium pusillum*
Champia parvula	*Gracilaria foliifera*
Cladostephus spongiosus	*Halopteris scoparia*
Codium tomentosum	Rhodomelaceae
Colpomenia sp.	*Ulva* spp.

were isolated under the microscope, using a glass micropipette and placed into wells of a 24-multiwell plate with 1 mL of filtered seawater enriched with f/20-Si medium, kept under a 12:12 L/D cycle.

Ostreopsis spp. density (cells L^{-1}) in the water column was estimated using the Utermöhl method and that of epiphytic cells with a Sedgwick-Rafter chamber, and results expressed as cells g^{-1} FW and cells g^{-1} DW of macroalgae. For the macroalgae DW estimates, samples were dried for at least 24 hours at 60º C. Five strains were established in culture and are kept in the Algal Culture Collection of the University of Lisbon (ALISU). As *Ostreopsis* spp. are known to be cryptic species, molecular analyses were performed for species identification. About 30 mL of each culture were centrifuged, and the rDNA from the pellet was extracted, amplified and sequenced. Amplification of the ITS1-5.8S-ITS2 rDNA was performed with ITSA and ITSB primers [6]. Phylogenetic analysis (not shown) revealed that all the studied sequences grouped in the *Ostreopsis* cf. *siamensis* clade. The highest cell density, 1.04 x 10^5 cells g^{-1} DW (3.4 x 10^4 cells g^{-1} FW) was found at station G. The geographical distribution of the samples identifies this site as a hotspot, with densities of *O.* cf. *siamensis* decreasing to the east and west of station G (Fig. 3). The cell density in the water column at this site was 2.1 x 10^3 cells L^{-1}.

The densities reported here are low compared with those reported for *O.* cf. *ovata* in the Mediterranean Sea (5.3 x 10^4 cells L^{-1} [7]; 6.8 x 10^4 cells L^{-1} [8]; 8.54 x 10^6 cells g^{-1} FW [8]; 1.3 x 10^6 cells g^{-1}FW and 1.6 x 10^7 cells g^{-1}DW [9]). However, reports of *Ostreopsis* cf. *siamensis* blooms in the Atlantic and Mediterranean are rare. To our knowledge, only two reports have been published before: one from the Atlantic coast of Morocco (3.7 x 10^3 - 10^{-5} cells L^{-1}) [10] and the other from Tunisia (3.75 x 10^4 cells L^{-1}) in the Mediterranean [11]. Work on the toxicity of *O.* cf. *siamensis* has shown nearly undetectable levels of palytoxin per cell [12], thus the species represents a low risk for the environment and human health. These findings suggest that blooms may go unnoticed due to the lack of apparent harmful effects. *O.* cf. *siamensis* was first detected on the West coast of Iberia in 2008 [13] and has since been regularly detected at very low concentrations. In the present work, we report for the first time, bloom densities of an *Ostreopsis* species on the West coast of Iberia. This raises questions regarding the environmental conditions that may trigger these blooms and how these may be related to the present trend of ocean warming. Very little is known on the ecology of *O.* cf. *siamensis* and more work is needed to better understand the distribution and bloom dynamics of this species in temperate waters.

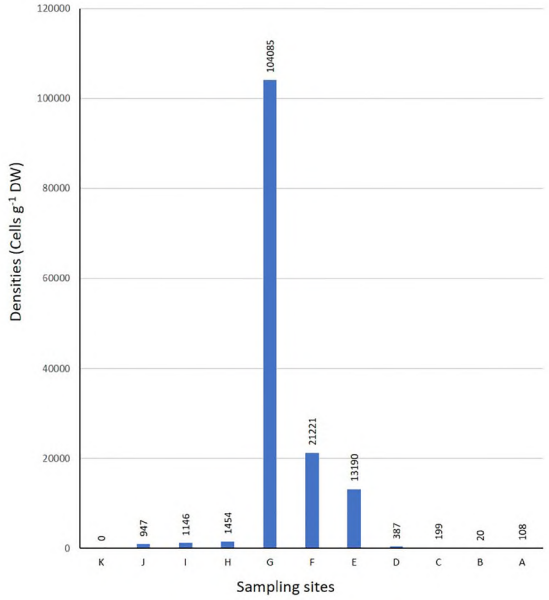

Fig. 3. Densities of epiphytic Ostreopsis *cf.* siamensis *cells in grams per dry weight.*

Acknowledgements

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A possible link between the breakdown of a fertilizer tank and a toxic *Pseudo-nitzschia* bloom

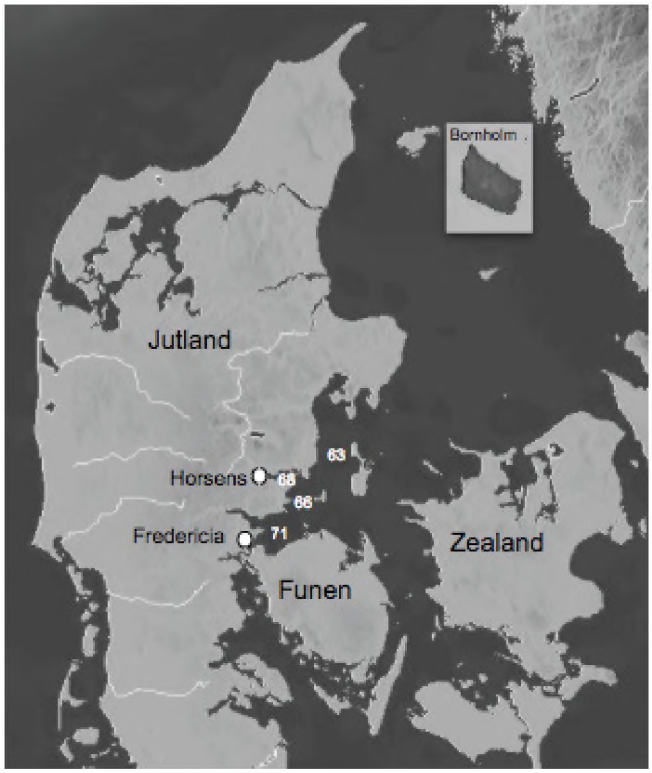

Fig. 1. Map of Denmark with approximate locations of the respective mussel harvesting areas (63, 66, 68, 71), Fredericia (the origin of the fertilizer outlet) and Horsens. Credit: openstreetmap.org

In March and April 2016, a major bloom of toxic *Pseudo-nitzschia* (PN) caused an extensive closure of mussel harvesting areas in Denmark due to raised levels of domoic acid (DA). Areas close to the east coast of Jutland (Denmark) were heavily affected by the bloom and several productive shellfish areas where closed for up to five weeks. Cell concentrations and the PN proportion of the total planktonic community were relatively low at the time. What caused the high toxicity?

DA is a potent neurotoxin that bioaccumulates as shellfish and copepods vector the toxin up the food web. Hereby DA can potentially cause problems with intoxication of consumers such as fish, birds, and mammals. The toxin is known to cause Amnesic Shellfish Poisoning (ASP) in humans. Intoxication of humans is usually avoided due to the existence of monitoring and testing programs of commercially harvested bivalves worldwide. No human intoxication was reported during the spring bloom in Denmark in 2016. Harvest of blue mussel (*Mytilus edulis*) by the industry in the affected areas was closed as soon as toxin was detected in mussel meat and the mussels already harvested were discarded. The areas concerned are usually very productive and the closure resulted in a weekly lost revenue of >100.000 DKK to local fishermen. The fishery was reopened after 4-5 weeks, under intensified surveillance.

The bloom probably originated northeast of Horsens Fjord (Fig. 1). The first measurements of DA in mussel samples were detected on March 7, 2016. During the following weeks, DA was detected in adjacent areas along the east coast of Jutland. Toxic PN blooms are rare in Denmark but these specific areas have previously been affected [1]. Other areas of Danish coastal waters might likewise be subject to DA accumulation but toxin levels are only measured in areas subject to mussel fishery, leaving toxic blooms undetected in other areas.

By the time of the bloom, the diatom community primarily consisted of *P. seriata* (Fig. 3). Identification of *P. seriata* as the potential culprit diatom for the toxicity was confirmed by TEM (Fig. 2). *P. seriata* as the responsible diatom was not surprising, as it has previously formed highly toxic blooms in the area [1]. The cell concentration peaked at the beginning of the bloom at 110,800 cells L^{-1} (Fig. 1), while the toxin content in mussel meat peaked two weeks later with 47 mg DA kg^{-1} of mussel meat. This is a relatively high amount of toxin, considering the low cell concentration.

The reason for the two-weeks temporal difference between DA peaks and peaks in cell numbers might be due to the sampling procedure, with irregular sampling and water samples for phytoplankton monitoring being gathered on other days than samples for toxin analyses of shellfish flesh. *P. seriata* is known for its ability to produce high amounts of DA given the appropriate environmental conditions. Several physical-chemical factors are known to induce DA production in PN. Silicate limitation, light and temperature are well documented as DA inducing factors for *P. seriata*, and presence of copepod grazers is a newly discovered potent inducing factor for DA production [2].

On February 3rd 2016, approximately one month prior to the bloom, large amounts of liquid nitrogen fertilizer were spilled into the environment as a result of thebreakdown of a fertilizer storage silo located on the harbour of Fredericia. The magnitude of this discharge was arguably around 4000

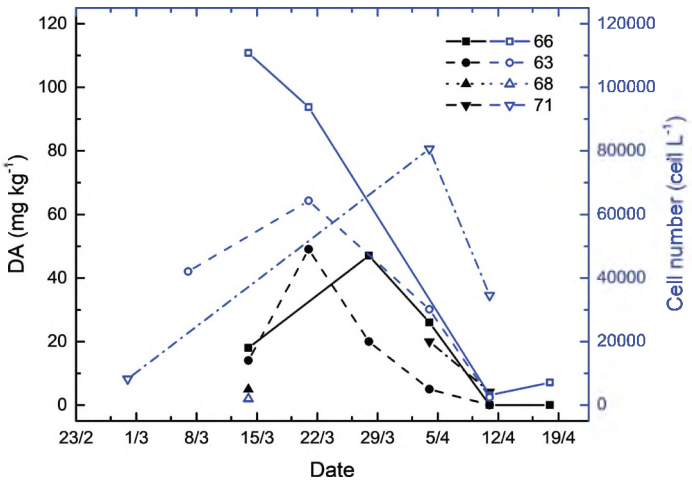

Fig. 2. Concentrations of P. seriata *cells in the water column and of domoic acid content in fresh mussel meat during spring 2016 in four different areas along the east coast of Jutland.*

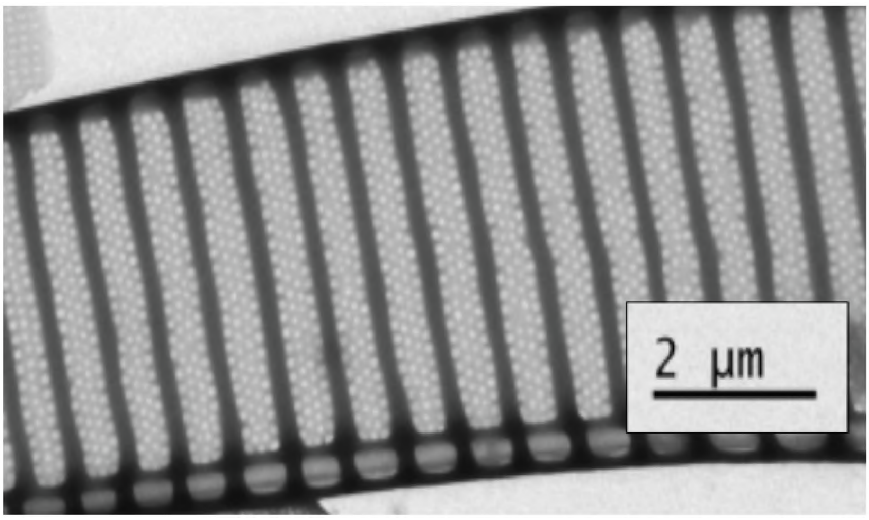

Fig. 3. Pseudo-nitzschia seriata *TEM micrograph of the poroid structure arranged within the striae consisting of two outer rows of larger poroids and one or two inner rows of smaller poroids.*

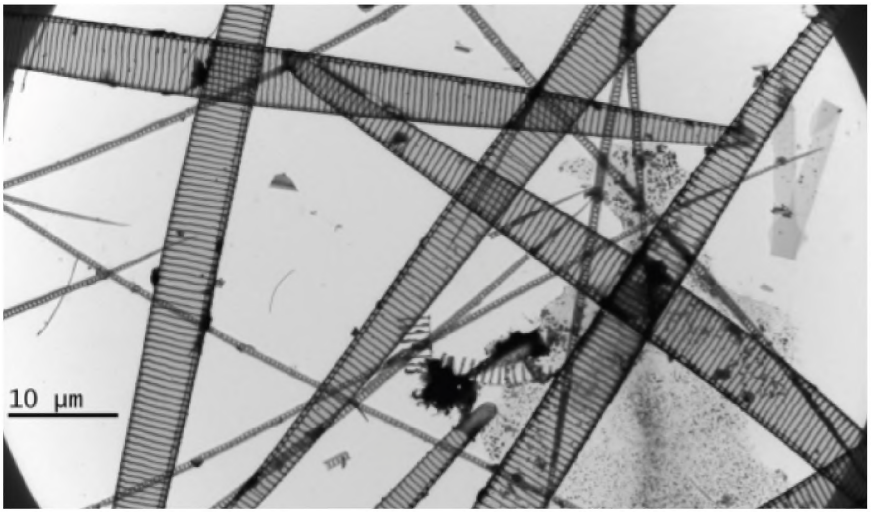

Fig. 4. TEM micrograph of a mixed water sample from the bloom, resembling a culture sample.

tons. The silo contained 9500 tons at the time of the breakdown and thereby passed its maximum capacity by 500 tons [3]. The adjacent areas and especially the waters immediately outside the harbour were heavily affected by the extensive amounts of nitrogen. The discharge consisted of N32 and N16 fertilizer liquids, which is predominantly urea. Organic nitrogen in the form of urea is (as inorganic nitrogen) 100 % bioavailable [4]. The organic molecule in urea is easily separated from the rest by the enzyme urease, splitting ammonium from bicarbonate [5] [6]. Monitoring data showed peaks in nitrate levels immediately after the outlet, revealing more than double the average amount of available nitrate for the area. Phosphorus levels were not increased.

In Denmark, the major part of nitrogen runoff from land happens from October till January. During this period algal growth is limited by light rather than nutrients. From February and further into the summer period, algal growth is controlled mostly by nutrients. The usual pattern is depletion of phosphorous followed by nitrogen depletion.

In spring 2016 in Little Belt (between Jutland and Funen) the discharge of 4000 tons of readily available nitrogen must inarguably have affected the ecosystem in the area. The general concentrations of planktonic algae were unusually high in the following month (data not shown here). Available nitrogen was possibly crucial for *P. seriata* to overtake the diatom community for a period of time and additionally producing the high amounts of DA vectoring up the food web.

Nitrogen repletion has been shown to induce toxin production in other PN species. Different forms of nitrogen can induce DA production in *P. australis* [7], and the most potent DA-inducing N form has been shown to be urea. It is very likely that urea and/or nitrate affected the DA production in *P. seriata*. Studies on *P. australis* show that urea is highly potent and induces the production of DA at least 4-fold compared to nitrate and ammonium treatments. Ammonium doubled the production of DA compared to the control (normal sea levels of nutrients)[7].

Our observations support the hypothesis that high N levels induce DA production in PN. An increase in land-based runoff of N (especially in the form of urea) may increase the frequency and toxicity of toxic blooms. Therefore strict limits of fertilizer usage and spills may be important to avoid washout and runoff of N, potentially causing algal blooms, damaging the environment and mussel harvesting industries.

Acknowledgements

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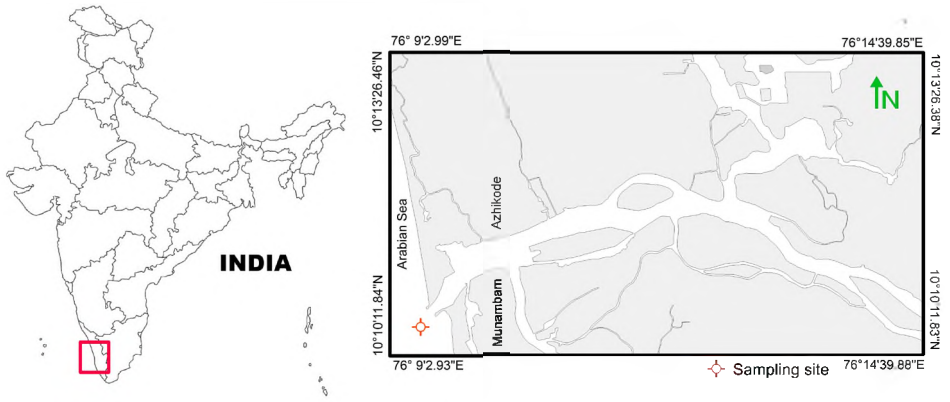
Golden alga *Prymnesium parvum* Carter bloom off Azhikode, southwest India



In September 2009, during the southwest monsoon season, a quasi-monospecific bloom of *Prymnesium parvum* was collected off Azhikode (10º 11' 02" N; 76º 09' 22" E), on the southwest coast of India (Fig. 1). A conspicuous pale brownish surface water discoloration extended through an area about 8-10 nautical miles off the coast. The bloom did not result in foam production or fish mortality and lasted only one day as heavy rain dissipated the cells. This may be the first report of a *P. parvum* bloom from Indian waters (Fig. 2) [1].

At the time of the bloom, a cell maximum of 8 x 10^7cells L^{-1} and chlorophyll *a* and carotenoid concentrations of 13.54 µg L^{-1} and 1.91 µgL^{-1} respectively were observed. The sea surface temperature was 28ºC, salinity 34 psu and pH 8. Nutrient concentrations in the bloom area, determined with standard methods [1] were: total nitrogen 12.6 µmol L^{-1}, phosphate 1.9 µmol L^{-1}, silicate 62 µmol L^{-1} and dissolved oxygen,1.41mg L^{-1}.

Blooms of *P. parvum* associated with faunal mortalities are quite common in temperate seas. Cell densities of 5 x 10^7 cells L^{-1} have been associated with faunal loss [2]. However, in the present bloom event, no fish mortalities were recorded despite the high cell density observed.

In addition to strain variability of *P. parvum* from different geographic areas, nutrients may have played a significant role in the harmless effect of this *P. parvum* bloom. The toxicity of *Prymnesium* is known to increase remarkably under nitrogen and phosphorous stress [3]. Salinity is another factor regulating the toxicity in *Prymnesium*: most toxic blooms of *P. parvum* have been associated with low salinity (3-12.4 psu) conditions, but in the present study, salinity was 34. The factors responsible for the bloom development have yet to be identified. Hence, it could be concluded that, rather than a single factor, multiple environmental conditions, such as temperature, salinity, pH, nutrients, in addition to geographical adaptations, in a favorable range for a particular species, commonly referred to as species-specific adaptations, played a significant role in the development of the bloom event described here.

The bloom forming microalgae *P. parvum* was isolated and cultures successfully established in the Marine Botany Lab, Dept. of Marine Biology, Microbiology& Biochemistry, Cochin University of Science and Technology, to carry out physiological and toxicological studies. Semi-continuous cultures of *P. parvum* were grown in seawater enriched with Walne's medium at a temperature of 22±2ºC, salinity 35psu, pH 8 and an irradiance of 900 lux. The allelopathic effect of *P. parvum* grown in different nutrient conditions (both nutrient sufficient and deficient) was checked against four selected microalgae. Our first results showed that phosphorous limited cultures inhibited growth in cultures of microalgae *Tetraselmis gracilis, Chaetoceros calcitrans, Nanochloropsis salina* and *Isochrysis galbana* (in prep.).

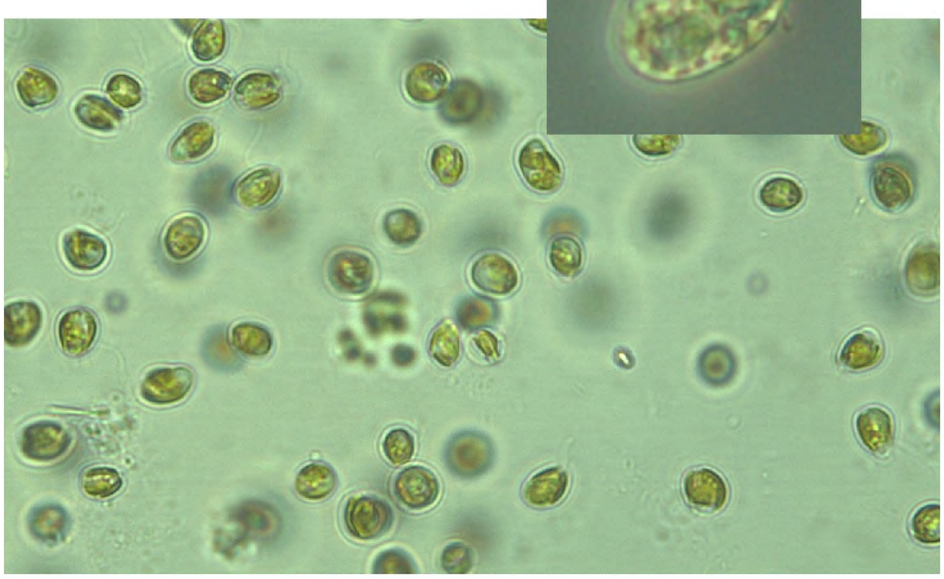


Fig. 2. Micrograph of Prymnesium parvum *established in culture*

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Regional Workshop on Monitoring and Management Strategies for Benthic HABs

In the last decades, important advances in the prevention of the adverse impacts of harmful algal blooms (HABs) have been achieved by surveillance programs that monitor HAB species in the environment as well as toxins in seafood and associated human diseases. Currently, strategies are well defined with specific guidelines to monitor harmful phytoplankton species and control their synthesized biotoxins in marine products and freshwater reservoirs. However, the situation is very different for benthic HAB (BHABs) microalgae such as species belonging to the dinoflagellate genera *Gambierdiscus*, associated with ciguatera fish poisoning, and *Ostreopsis*, whose blooms constitute a potential emergent threat especially in the Mediterranean. CFP remains today the most common non-bacterial food poisoning globally with Small Island Developing States (SIDS) being the most vulnerable. Changes in climate may exacerbate the impacts.

With the objective to identify the main gaps that limit monitoring of BHAB species and their toxins in the most affected areas and define the best approaches to prevent and manage their impacts, a workshop was held during the Monaco's Ocean Week 9-12 April 2018, at the Oceanographic Museum of Monaco. The workshop focused on three major topics: benthic species surveillance, monitoring of toxins in marine resources and, seafood and epidemiology.

The workshop was organized by the International Atomic Energy Agency (IAEA, www.iaea.org) in partnership with RAMOGE (www.ramoge.org), IOC-SCOR GlobalHAB (www.globalhab.info) and NOAA. It was attended by more than 60 participants including scientists and coastal managers from more than 30 countries joining IAEA Technical Cooperation projects from Asia-Pacific (RAS7026), Africa (RAF7014), Latin America and the Caribbean (RLA7022). Participants and experts attended from Algeria, Bangladesh, Brazil, Chile, Colombia, Costa Rica, Cuba, El Salvador, Fiji, France, Guatemala, Indonesia, Italy, Kuwait, Malaysia, Marshall Island, Mauritius, Mexico, Monaco, Nicaragua, Pakistan, Panama, Philippines, Seychelles, Spain, Thailand, Tunisia, United-States, Uruguay, Venezuela, Viet Nam, and from United Nation organizations FAO and WHO (Fig 1).

Before the meeting, surveys were distributed to the participants, to identify the main gaps as well as available tools in their own regions. The outcomes of this survey were used to structure group exercises introduced by experts' lectures. Furthermore, a practical demonstration and analysis of different sampling methods for benthic microalgae were provided. Altogether, the workshop contributed to improve the skills of the participants in establishing a need-based monitoring program for toxic BHABs, in a risk assessment perspective. As a continuation of the workshop a sampling method intercomparison exercise was designed. Artificial substrate kits were distributed among participants to conduct sampling in their areas in parallel to sampling natural substrates (macroalgae). The results will help inform sampling methods and if possible, establish tools for standardized procedures. The exercise is open to the entire international community. The goal of achieving improved assessments of the risks associated with BHABs will help reduce the health, societal and economic impacts, promote sustainable safe seafood, contributing to the achievement of United Nations sustainable development goals (SDGs) worldwide.

For more information or for those interested in participating in the sampling exercise, contact Yasmine Bottein (*M-Y.Bottein@iaea.org*) and Rodolphe Lemée (*rodolphe.lemee@obs-vlfr.fr*)



Fig. 1. Participants in the Regional Workshop on Monitoring and Management Strategies for Benthic HABs, Monaco, 9-12 April 2018

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Workshop on morpho-molecular methods for the study of dinoflagellate cysts

A workshop on techniques for the morphological and molecular identification of cysts from toxic HAB species, led by Drs Kenneth Neil Mertens, Kirsty Smith, Lesley Rhodes and Lincoln MacKenzie, was held recently at the Cawthron Institute in Nelson, New Zealand, 12-14 February 2018. This workshop was supported by the Catalyst:Seeding programme, financed by the Royal Society of New Zealand to establish a new research collaboration between Cawthron and IFREMER (Concarneau, France). Twenty-four researchers participated, with the majority from New Zealand and Australia, but also included people from France, Belgium, Singapore, Ireland, the UK and Canada (Fig1). Due to heavy rains on February 11th, several airplanes had trouble in landing at Nelson and experienced delays, but luckily nearly all participants arrived on time for the start of the workshop.

The workshop started on 12 February with a Māori welcoming ceremony (pōwhiri), which included a speech by Cawthron's CEO Charles Eason and a response by Kenneth (IFREMER) in his Dutch native tongue. The speeches were followed by the singing of traditional Māori songs – most memorable was the impressive falsetto of Dave Clark (Marine Institute, Ireland). The ceremony ended with the hongi (a traditional Māori greeting) – all in all quite a spiritual experience indeed. After getting our feet back on earth, the workshop aims were introduced by Kirsty (Cawthron) and Kenneth, after which participants introduced themselves and their specific research topics, which covered several research fields. Geological applications of cysts were exemplified by Anna Pienkowski, Vera Pospelova, Erica Crouch and Lucia Roncaglia – biological applications by Chris Reid, Gustaaf Hallegraeff, Janet Adamson, Aye Aye Mon, Shereen Lim, Viliami Langi and Tony Bui, and molecular tools by Dave Clarke, Rendy Ruvindy, Henna Savela, Laura Biessy and Xavier Pochon. Cawthron MSc students Enora Jaffrézic, Mailys Picard and Julie Steyen also outlined their research projects on HAB species and cysts. After lunch, morphological techniques for cyst study were explained by Kenneth, and were followed by Vera Pospelova (University of Victoria, Canada) who detailed the application of sediment-traps to study cyst production. Thereafter, Lesley and Kirsty presented molecular techniques used for study of motile stages and cysts. Erica Crouch (GNS, New Zealand) outlined paleontological techniques used for late Quaternary paleocological reconstructions around New Zealand, highlighting the work of her collaborator Joe Prebble. After coffee, Lincoln shared with us how blooms of *Alexandrium pacificum* in New Zealand can be traced using both plankton samples as well as sediment samples. Gustaaf Hallegraeff (University of Tasmania, Australia) emphasized how selective the fossil record is, and highlighted new possibilities using modern molecular techniques. Last but not least, Susie Wood (Cawthron) presented her planned research to study the historical evolution of cyanobacterial and other microbial communities in over 300 lakes across New Zealand. The day ended with a delightful dinner at the Indian Café, where a moderate amount of craft beer was consumed.

The next day included practical demonstrations of both morphological as well as molecular methods (Fig. 2). Lincoln demonstrated the use of primuline staining to enumerate *Alexandrium* cysts in the sediment [1]. Kenneth applied the sodium polytungstate method to isolate living cysts from the sediments for germination experiments [2] and the use of the fluorescent dye Solophenyl Flavine 7GFE500 which avoids fading of fluorescence [3]. The Cawthron laboratories were visited by the participants as well, which were guided by Kirsty and Tim Harwood – everyone was stunned to see the mass production of toxins produced by *Alexandrium pacificum*. After lunch, Kirsty led a hands-on demonstration of three different quantitative PCR experiments that focused on *Alexandrium* and *Azadinium* – the eagerly awaited results showed that everybody did a great job – A for effort! The day ended with a round table discussion about morphological and molecular methods, and highlighted how both methods could be combined, as well with toxin analysis.

After the workshop, several people stayed to visit the Abel Tasman National Park. A water-taxi sped us past fur seals and a rock allegedly split in two by Chuck Norris, to the start of a 12 km hike. The hike was most delightful with impressive views of Tasman Bay even at high temperatures of 27°C, suppressed by tree fern shade along the way. Some pukekos and wekas could be observed, and it was easy to find sand dollars on the beach.

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Fig. 1. Group photo of participants.

Cawthron Summer Scholar Explores Bloom-Forming Freshwater Cyanobacteria

Globally, cyanobacteria blooms in freshwater environments are causing water quality problems and health risks with increasing frequency. New Zealand is no stranger to this, suffering from blooms of toxin-producing cyanobacteria in our lakes (e.g., *Microcystis*) and our rivers (e.g., *Phormidium*). Over the last four decades, this has led to numerous animal deaths (dogs, sheep and cattle) and the closure of recreational swimming sites due to the human risk at certain times of the year. Whilst many advancements in our understanding of cyanobacteria blooms have been made in the past 30-40 years, there are still knowledge gaps surrounding the ecology of bloom-forming cyanobacteria, the triggers for toxin production and risk posed by cyanobacteria blooms in rivers.

Over the summer of 2017/18 the Cawthron Foundation provided the Sir Theodore Rigg scholarship to an undergraduate student to work on research projects associated with bloom-forming cyanobacteria. The scholarship was awarded to Charlotte Tomlinson, a third-year Environmental Science student from the University of Waikato (Hamilton, New Zealand; Fig. 1). Charlotte spent ten weeks at Cawthron Institute working alongside researchers Jonathan Puddick, Konstanze Steiner and Susie Wood on projects related to freshwater cyanobacteria.

Charlotte's enthusiasm and aptitude meant that she worked on three research projects during her scholarship period. For her primary project, Charlotte purified toxic and non-toxic metabolites from cyanobacteria in order to assess how the compounds influenced the growth and photosynthetic activity of non-toxic cyanobacteria from the Cawthron Institute Culture Collection of Micro-algae (CICCM; *http://cultures.cawthron.org.nz/*).

The occurrence of a toxic bloom of *Phormidium* in the Hutt River (Wellington, New Zealand) led to Charlotte taking on a field study to assess whether anatoxins (produced by *Phormidium*) were released into the water and whether any patterns were evident. Going out into the field gave Charlotte a great insight into the diverse range of skills of algal researchers and an appreciation for the importance of work on toxic algae: "There were quite a few people that were interested in what we were doing and the risks posed by toxic algae in the river. "We also noticed a lot of people using the river recreationally, so it is really important to monitor and study what's going on, so that we can help to make it safer for people to use," Charlotte said in a radio interview she took part in during her time at Cawthron (*https://soundcloud.com/cawthroninstitute/kendall-morman-and-charlotte-tomlinson-cawthron-foundation-scholarships*).

Charlotte's third project was a cyanobacteria culturing experiment to evaluate the effect of temperature and light intensity on homoanatoxin-a (HTX) production in *Oscillatoria* strains from the Pasteur Culture Collection of Cyanobacteria (*https://webext.pasteur.fr/cyanobacteria/*). Because one of the *Oscillatoria* strains grew as benthic mats, this meant that 372 individual culturing pottles needed to be prepared, harvested and processed to conduct the experiment under six different culturing conditions. The results from this work have since been used to optimise the production of HTX in the hopes of purifying sufficient quantities for future toxicology work.

Overall, it was a very rewarding experience for all involved in the 2017/18 Cawthron Foundation Summer Scholarship Programme. For the researchers, it provided an opportunity to pass on some of their skills to the next generation of scientists and to inspire them to consider some of the environmental challenges the world faces. Charlotte noted that one of her highlights was being a part of an active science organisation, finding out what other people were working on and assisting others with their research projects.



Fig. 1. Charlotte Tomlinson (Cawthron Foundation Summer Scholar) purifying cyanobacterial toxins for future toxicology work.

Acknowledgements

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Canadian HAB Scientists Hold Workshop to Establish National Priorities and Develop Research Network

A National Harmful Algal Bloom (HAB) workshop was organized and chaired by Dr. Ian Perry at the Institute of Ocean Sciences (IOS), Fisheries and Oceans Canada (DFO), Sidney, British Columbia, July 11-13, 2017. Sixteen workshop participants, representing DFO scientists as well as invited experts from the United States and Canada, discussed Canadian HAB priorities and the development of a network for HAB research in Canada.

Global and national events, including large HAB-related fish kills, HAB impacts on marine mammals and unprecedented domoic-acid-producing HAB events on the Pacific coast, have brought this issue forward as a national research priority. Specific concerns in Canadian marine waters were highlighted, including the impact of HABs as an ecosystem stressor and the negative consequences of HAB events caused by their phycotoxin production and its accumulation in shellfish and the food web. Little is known about HABs in Arctic and sub-Arctic regions of Canada, where climate change is expanding potential areas for such blooms. A concern was noted that low temperatures could result in slow phycotoxin depuration rates in several bivalve species, and this in turn could lead to toxin accumulations and impacts beyond single events. Other particularly vulnerable areas include Marine Protected Areas and aquaculture sites.

Priorities for workshop participants were to expand existing work and strengthen connections to related programs, such as environmental monitoring and invasive species studies, to provide more HAB information for Canada. The potential for linkages between Canadian interests and the priorities of international networks, such as ICES-IOC WGHABD, IOC IPHAB and Global HAB, was also discussed.

A recommendation from workshop participants was to produce a formal CSAS (Canadian Science Advisory Secretariat) research document and review to assess the status of knowledge in Canada, identify knowledge gaps and highlight areas of particular concern for current and future impacts of HABs on ecosystems and resources. The Science Advice on impact of marine HABs on the Canadian ecosystem is scheduled for early 2019.

Membership of the Canadian HAB Working Group (CAN HAB) is currently composed of the workshop participants who have a broad range of expertise including taxonomy, genomic, modelling and remote sensing. However, it is hoped that the network will expand to include additional academic, federal and provincial researchers and managers interested in HAB issues in Canada. The Chair of CAN HAB is Cynthia McKenzie (*Cynthia.mckenzie@dfo-mpo.gc.ca*), a research scientist with DFO based in St. John's, Newfoundland and Labrador. She is the point of contact for more information on this HAB network.

Acknowledgements

We are grateful to Fisheries and Oceans Canada, Science Branch, for financial and technical support for this meeting. The Workshop was hosted by the Institute of Ocean Science, Sydney, BC and we appreciate their hospitality and support.

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Fig. 1. Working Group meeting participants (from left to right): Andrea Locke, Ian Perry, Jennifer Martin, Nicky Haigh, Christine Michel, Emmanuel Devred, Luc Comeau, Vera Trainer, Charlie Trick, Cynthia McKenzie, Caroline Longtin, Amy Tabata, Angelica Pena, Bill Cochlan, Michael Scarratt. Participating by telephone: Elysha Gordon. Members of the working group also include Stephen Bates, Michel Starr and Chris Pearce.

Joint FAO, IAEA, IOC and WHO Technical Meeting for the development of an Inter-Agency Global Ciguatera Strategy

Harmful Algae News has previously brought information on an initiative between the International Atomic Energy Agency (IAEA) Environment Laboratories in Monaco jointly with the Oceanographic Commission (IOC), the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), to coordinate efforts on Ciguatera. To advance the initiative an inter-agency meeting was organized back to back with an IAEA Technical Cooperation Workshop on Monitoring and Management Strategies for Benthic HABs (see separate article in this issue of HAN).

The four agencies met at the IAEA Environment Laboratories, Monaco from April 12-13, 2018 to define key steps to develop and formalize a coordinated strategy to address ciguatera poisoning globally. The strategy covers the following elements: (a) Improving toxic organism detection, monitoring and risk forecasting; (b) Improving toxin detection in the environment and food; and (c) Improving outbreak data collection and human health risk assessments.

The formalized inter-agency agreement will signal to Member States and the user community that the agencies cooperate and thereby raise the awareness on Ciguatera Poisoning and that it also at national level requires interaction and collaboration between different authorities and stakeholders to protect public health and trade against Ciguatera.

We will inform on development of concrete activities in coming issues of Harmful Algae News.

Marie-Yasmine Dechraoui Bottein (IAEA), Henrik Enevoldsen (IOC-UNESCO), Esther Garrido Gamarro (FAO), Angelika Tritscher (WHO)

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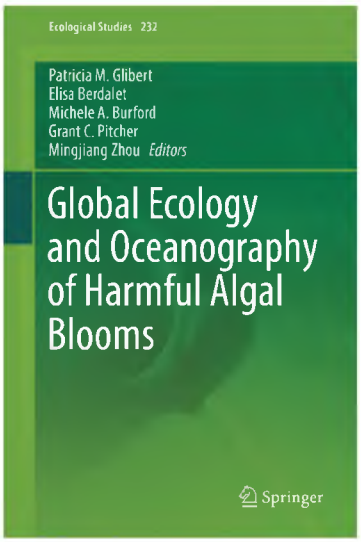
New book



This volume on the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) is aimed at synthesizing the broad range of research and activities that took place during the nearly 2 decades of the international programme bearing this acronym. The central challenge for GEOHAB was to "understand the critical features and mechanisms underlying the population dynamics of HAB species in a variety of oceanographic regimes." GEOHAB fostered research that was multi-faceted, multidisciplinary, international and within an oceanographic context. With contributions from 69 authors from all over the world, this book reflects the global reach of the GEOHAB Programme.

There is no doubt that there are more harmful algal bloom (HAB) events, occurring more often, in more places and lasting longer than in decades past. It is now also well established that many of these events are the result of human activities, primarily through increased nutrient inputs – from land applications, direct sea applications (especially from aquaculture) and from atmospheric deposition, all of which have been increasing and have been altering the proportions and forms of nutrient loads. Species introductions are also occurring in ways and at rates not common in decades past, through ballast water and through transfer of materials in the aquaculture industry. As a result of these human activities, blooms are having more ecological, economic and human health impacts. As stated in the GEOHAB Science Plan (2001), "these occurrences of toxic or harmful microalgae represent a significant and seemingly expanding threat to human health, fishery resources, and marine ecosystems throughout the world."

This volume aims to capture the key focus areas of research under the GEOHAB umbrella. It introduces readers to the overarching framework of GEOHAB, factors contributing to the global expansion of HABs, the complexities of HABs in different habitats, and the forward-looking issues to be tackled by the next generation of GEOHAB, GlobalHAB.

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12th Advanced Phytoplankton Course - APC 12

Identification, Taxonomy, Systematics

Roscoff Biological Station (France) - 19th May to 8th June 2019

APC12 is organized by the Station Biologique de Roscoff together with the Stazione Zoologica Anton Dohrn and the IOC Science and Communication Centre on Harmful Algae.

APC 12 aims to provide participants with in-depth expert knowledge on the identification, classification and phylogeny of marine microalgae (diatoms, dinoflagellates, coccolithophores, other phytoflagellates) by integrating morphological observations with molecular data and new approaches.

The intensive 3-week course will consist of lectures, practical sessions and guest seminars. A diverse collection of preserved and live material will be offered for examination in light and (for selected taxa) electron microscopy. The course will cover: methods and criteria for species identification; up-to-date taxonomic frameworks; morphological and molecular phylogeny; toxic and harmful species; general and specific aspects of phytoplankton biodiversity and biogeography.

Faculty

- **Cedric Berney, Station Biologique de Roscoff, France - Eukaryotes**
- **Nicolas Chomerat, IFREMER, France - Dinoflagellates**
- **Mona Hoppenrath, Senckenberg Am Meer Wilhelmshaven, Germany - Dinoflagellates**
- **Raffaele Siano, IFREMER, France - Dinoflagellates**
- **Marina Montresor, Stazione Zoologica di Napoli, Italy - Dinoflagellates**
- **Jacob Larsen, University of Copenhagen, Denmark - Dinoflagellates**
- **Carina B. Lange, Universidad de Concepcion, Chile - Diatoms**
- **Nina Lundholm, Nat. History Museum, University of Copenhagen, Denmark - Diatoms**
- **Carmelo Tomas, University of South Carolina, Wilmington - Raphidophytes**
- **Diana Sarno, Stazione Zoologica di Napoli, Italy - Diatoms**
- **Kerstin Hoef-Emden, University of Köln, Germany - Cryptophytes**
- **Adriana Zingone, Stazione Zoologica di Napoli, Italy - Chlorophytes**
- **Øjvind Moestrup, University of Copenhagen, Denmark - Chlorophytes**
- **Daniel Vaulot, Station Biologique de Roscoff, France - Chlorophytes**
- **Masanobu Kawachi, NIES, Tsukuba, Japan - Heterokonts**
- **Ian Probert, Station Biologique de Roscoff, France - Haptophytes**
- **Bente Edvardsen, University of Oslo, Norway - Haptophytes**
- **Jeremy Young, University College London, UK - Haptophytes**
- **Christophe Six, Station Biologique de Roscoff, France - Cyanophytes**

Participation is limited to 20 participants with at least a M.Sc. degree (or equivalent), with documented experience and professional interest in phytoplankton identification. The course will be taught in English and a good knowledge of English is therefore required. There will be no registration fee, but participants will have to provide for their travel expenses.

More detailed information are available on the website: https://sites.google.com/view/apc12/home.

Please apply **before September 1, 2018** at https://sites.google.com/view/apc12/applications

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 61:
1 September 2018

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AN IOC NEWSLETTER ON TOXIC ALGAE AND ALGAL BLOOMS

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Red waters in Ría de Vigo (NW Spain)

On June 28, a call to the Oceanographic Center of Vigo (IEO) alerted us that Samil Beach waters were discolored, probably due to a red tide. The authorities sought to identify the causative agent and were evaluating to restrain bathing until the nature of the phenomenon was clarified.

Samil, the most popular urban beach in Vigo, is only a few kilometers away from IEO, so we were able to collect shore samples immediately. The patches, most likely advected by the onset of very mild northwesterly winds, were reddish-brown, forming a strip parallel to the shore and visible from the middle part of the beach to its southern limit, at the mouth of the Lagares River (Fig. 1). Water temperature (18.7 °C) was relatively high for the upwelling-influenced Ría de Vigo in late June. Beachgoers were numerous although Samil was not as crowded as it usually is in the peak season, from mid July to the end of August.

The discolored seawater bands did not resemble the oily orange surface patches of *Noctiluca*, frequently observed in the rías by the end of summer [1]. Based on the previous weekly reports from INTECMAR [2] on increasing densities of *Alexandrium* sp, this became the main suspect, but the responsible species had not been officially reported. First of all, we visited the Samil emergency centre to inform Carlos Vales, coordinator of Vigo beach *Lifeguard and Rescue Services that* the patches were a natural phenomenon and did not pose any serious risk to beachgoers.

Back in the laboratory, cell counts gave density values ranging from 30 to 48 x10^6 cells L^{-1} in shore samples, which looked like a monospecific culture (Fig. 2), accompanied by a few cells of other dinoflagellates species. Densities were a bit lower (10 x10^6 cells L^{-1}) in a sample taken 200 m offshore by a rescue boat. Light microscopy confirmed it was a dinoflagellate of the genus *Alexandrium*. Observations of calcofluor-stained samples under the epifluorescence microscope soon identified the species as *Alexandrium minutum*, a mild PSP agent usually confined to Baiona Bay, a small embayment within the Ria de Vigo. Cytogenetic analyses of the cells confirmed the characteristic low DNA cell quota of *A. minutum* [3]. Additionally, molecular analyses (ITS rDNA phylogeny) of single cells picked from the bloom the next day corroborated the identification. A few days later, laboratory incubations of the patch samples



Fig. 1. PhD student María García-Portela (IEO Vigo) sampling the Alexandrium minutum red tide in Samil Beach, June 28, 2018.

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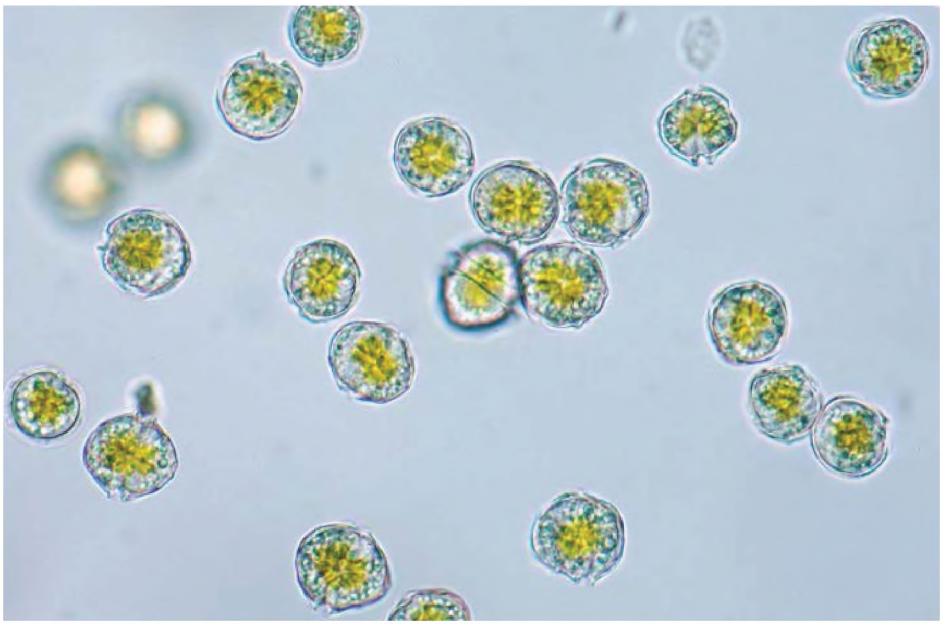


Fig. 2. Alexandrium minutum: light microscopy micrograph of a bloom sample from Samil Beach, June 28 (see also video link #1).

showed the development of parasitoids identified by light microscopy as *Parvilucifera sinerae* [4].

The next day, the red tide was no longer visible by the shoreline, but was entering Ría de Vigo as indicated by aerial photos taken on June 29 afternoon. On Wednesday morning, July 4, we saw the red patches again in the Vigo yacht marina. A passer-by told us these had been visible for three days. The red discoloration seemed more intense off the pier, but it was gradually aggregated in the inner part at low tide, staining the sea surface around the sailing boats. Samples collected that day inside and outside the marina showed densities of ~120 x10^6 and 20 x10^6 cells L^{-1} respectively. In the following days, the red tide became more patchy and widespread in several areas of Vigo harbour, reaching A Lagoa (Teis) at the innermost part of the ría, but patches were also seen in Aldán and Bueu, two embayments of Ría de Pontevedra, and in the Rías Altas (Camariñas) (Fig. 3).

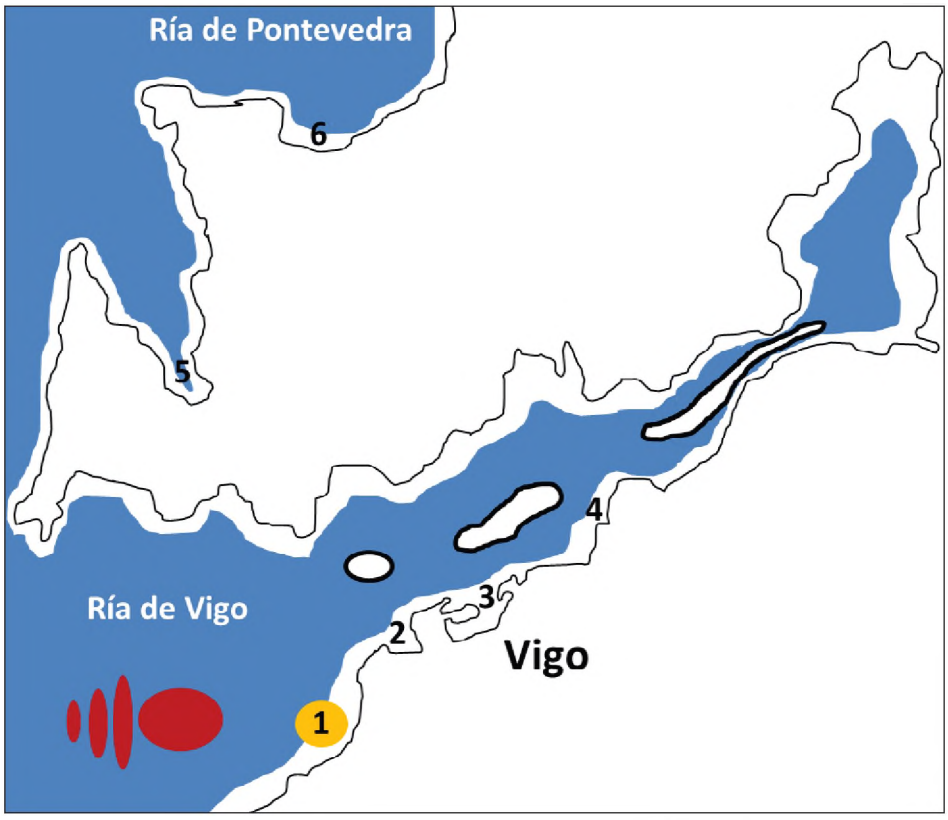


Fig. 3. Locations where A. minutum red tide was reported in the Rías of Vigo and Pontevedra: 1= Samil Beach (June 28), 2-3 = Vigo Marina, 4= A Lagoa Marina, 5= Aldán, 6= Bueu. For comparison, drawings of red tides adapted from Margalef figures [10] showing distribution of patches of other species in the 1950's (red: Mesodinium rubrum, white: Gonyaulax diacantha).

Red tide reports in the Rías Baixas of Vigo and Pontevedra date from as far back as 1918 in the pioneer descriptions of Sobrino [5] concerning *Gonyaulax polyedra* (=*Lingulodinium polyedrum)*. Nevertheless, the 2018 red tide of *A. minutum* must be considered exceptional for two reasons.

First, its toxic nature, because nowadays summer red tides in the Galician Rías Baixas are typically formed by non-toxic organisms, such as *Mesodinium rubrum* and *Noctiluca scintillans* [1,6,7]. Short term (1-2 weeks) blooms of *Pseudo-nitzchia* (ASP) usually aggregate in cryptic thin layers (toxic carpets) at the pycnocline [8], whereas the main toxic events causing long harvesting bans are low biomass (10^2 – 10^4 cells L^{-1}) HABs of *Dinophysis* species (DSP) and occasionally of *Gymnodinium catenatum* (PSP). To our knowledge, the only red tide of *Alexandrium minutum* (in those days called *Gonyaulax tamarensis*) in Galicia was reported in the spring of 1984 in Ría de Ares-Betanzos, Galician Rías Altas [9]. These rías, north of Cape Finisterre have a hydrodynamic regime quite different from the Rías Baixas. Furthermore, *A. minutum* was not included in the list of red tide-forming dinoflagellates – *Gonyaulax diacantha*, *G. polyedra, G. spinifera* – frequent in the days of Margalef's work in Ría de Vigo in the 1950's [10] .

Second, its persistence, because red tides in the Rías Baixas are ephemeral events due to the hydrodynamics: high flushing rates in these "estuaries invaded by the sea" prevent these phenomena from lasting more than a few hours. But in June-July 2018, *A. minutum* patches were observed for a month in different parts of the Ría.

What do we know about Alexandrium minutum and red tides in the Galician Rías Baixas?

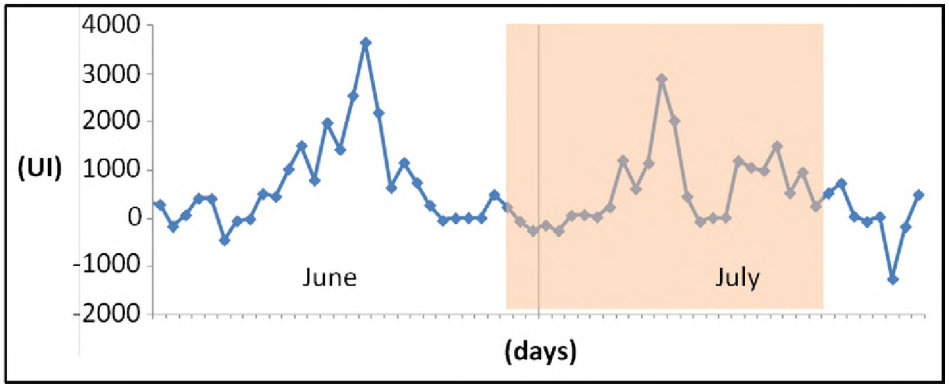
In the last four decades, many scientific studies have focused on the characterization of HAB species present on the Galician coasts, as well as their ecology, life cycles and population dynamics. It is well established that *A. minutum* and *Gymnodinium catenatum* are the main PSP toxin producers in the Galician Rías

Baixas, but they have contrasting "environmental windows of opportunity" and bloom strategies. *G. catenatum* is a late-summer, mid-shelf species and its population dynamics is tightly coupled to the upwelling regime. In contrast, *A. minutum* is a late spring species that thrives in "upwelling shadows", i.e. in semi-enclosed areas protected from the highly dynamic ría-shelf water exchanges. A review of the population dynamics and life cycle of *Alexandrium minutum* concluded that their proliferations occur in sheltered coastal areas in spring-early summer associated with stability and stratification in the water column [11]. On the Galician coast, these relatively calm areas are in estuaries like Baiona Bay (Ría de Vigo) and Ría de Ares (Northern Rías), both with important freshwater inputs. *Alexandrium minutum* blooms there under heavy rain in late spring leading to haline stratification. The same species became a "winner" under the changing, anthropogenically driven, conditions on the Catalan coast, where it forms conspicuous red patches. Multiple marinas there seem to have created the perfect niche for *Alexandrium* to grow, deposit its cysts and bloom again the next year [12].

What were the environmental conditions behind this exceptional bloom?

In the first 11 days of June, extremely heavy rainfall was recorded by the Galician Meteorological service (Meteogalicia, Xunta de Galicia, [13]). In the second half of June, maximum air temperatures in Vigo surpassed by up to 9 °C the average maximum at this time of the year. At the same time, salinity anomalies, weak variable winds and upwelling relaxation favored a strong thermohaline stratification and the maintenance of the bloom (Fig. 4).

The temperature of the water remained high, and reached 20.7 °C on Samil Beach on July 9. It is important to mention here that in the previous autumn 2017, the countryside around Ría de Vigo experienced the most devastating forest fires, with a three times more hectares burnt than the previous record in 2011 [14], and the subsequent tons of eroded soil, rich in nutrients and chelators reaching the ría [15]. All these conditions could have fostered stability and higher nutrient levels in the surface layer, and promoted the outburst of *A. minutum*.



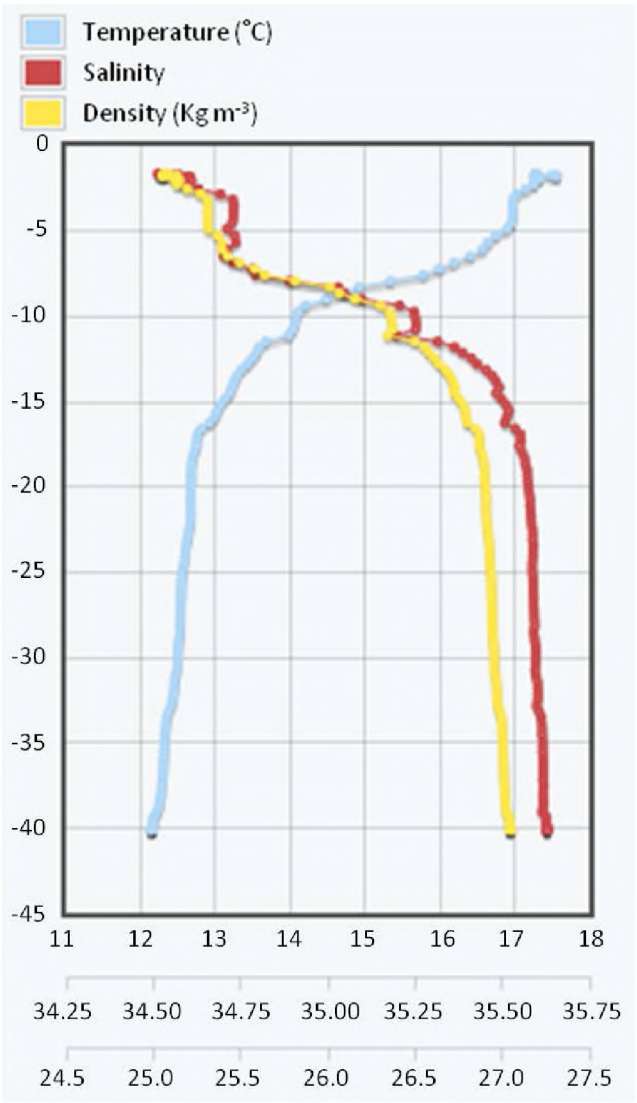


Fig. 4. A: Upwelling indeces before and during the long lasting red tide of Alexandrium minutum in Ria de Vigo (http://www.indicedeafloramiento.ieo.es). B: Vertical profile of temperature, salinity and density from a station in the outer reaches of Ría de Vigo. Alexandrium cell maxima were associated with the warmer and fresher top 3m (adapted from www.intecmar.gal/ctd)

Low salinity values were recorded in surface samples in the Ría de Vigo, but also on the adjacent shelf (due to the river plumes from the Miño and Douro rivers) during the oceanographic cruise of the REMEDIOS project [16], carried out these days on board the *R/V* Ramón Margalef. Researchers from the Universidad de Vigo, IEO, CSIC and IFREMER participated in the cruise, and took advantage of the opportunity to study the proliferation of *A. minutum* in the Vigo and Pontevedra rías. The brownish patches of *Alexandrium minutum* came and went with the tidal currents and

winds, entering and leaving the docks of Vigo almost every day to our knowledge for about three weeks. The last red tide patches were seen on July 22, both in the main entrance of Vigo harbour as well as in Pontevedra (Placeres dock).

Concentrations inside Vigo harbour were typically much higher than outer samples, exceeding 100 x 10^6 cells per liter. The bloom did not smell bad and no respiratory or skin problems were reported. The red tide did not entail risks to the public health. But it is as well to be aware of possible skin reactions in some sensitive people. We bathed in the red tide during sampling without any effect, but in general, discolored seawater should be treated with caution (see video links #2 - 4).

Impact on fauna and shellfish exploitation

Harvesting of raft mussels in Ría de Vigo was banned for several weeks during and just after the bloom due to PSP (GTXs 1-4) toxins above the regulatory limits (800 µg equiv STX kg^{-1}). The event also affected infaunal shellfish (clams, razor-clams) exploitation. In contrast, only a few raft-mussel areas were closed for lipophilic toxins, the most frequent cause of harvesting bans in Galicia, in particular in the neighboring ría of Pontevedra.

Dinoflagellates of the globally distributed genus *Alexandrium* include at least 12 species confirmed as PSP-toxin producers. *Alexandrium catenella,* which causes PSP outbreaks in the antipodes (Alaska, Patagonia) is one of the most lethal representatives. Human poisoning by PSP is linked to the consumption of shellfish, but its effects in the case of massive proliferation can reach the rest of the food chain, affecting marine mammals, fish and birds. Luckily, as mentioned in the Marine Biotoxins FAO report about PSP: *From the point of view of the human being, it is very positive that herring, cod, salmon and other commercial fish species are sensitive to PSP toxins and that, unlike shellfish, they die before the concentrations of toxins in the meat reach dangerous levels. However, some toxins accumulate in the liver and other organs of the fish and endanger other fish, marine mammals and birds, which ingest the whole fish including the viscera* [17].

Based on the effects found in other areas affected by *Alexandrium* PSP events, harmful effects on the marine fauna would have been expected. Nevertheless, to our knowledge, these were apparently limited. An unusual (but not alarming) number of dead mullets (*Mugil cephalus*) were found in Vigo harbour and local fishermen told us "*dead fishes are not common here, it must have been that thing in the water*". Several of these dead specimens, together with other fish and invertebrates caught in Vigo during the bloom were collected by our group in collaboration with fishermen, and brought to the laboratory for further microscopic and toxinological analyses. In addition, an otter apparently agonizing was found with signs of paralysis in its hind legs (Manuel E. Garci, *pers. comm.*). PSP intoxication was suspected, but the next day the animal left and the link with *A. minutum* could not be verified (Faro de Vigo, 14 July 2018). *Alexandrium minutum* strains from Galicia are "mildly toxic. During the 1984 outbreak in the Rías Altas, densities up to 10^6 cells L^{-1} were observed, but maximal toxicity in shellfish was ~400 µg equiv STX 100 g^{-1} [10]. Characterization of *A. minutum* isolates from Ria de Vigo showed a large variability of toxin per cell ranging from a maximum of 18 fmol $cell^{-1}$ to some strains with only traces [18]. Ongoing examination of field samples, *A. minutum* cultures, oceanographic and environmental conditions will help to better characterize this episode and the reasons behind this exceptional bloom.

Acknowledgements

To all those who shared images and information about the red tide: Manuel E. Garci, Carlos Vales, the Subdirectorate Xeral de Gardacostas de Galicia, Xulio Valeiras, Álvaro Roura and Alberto Otero. Special thanks to the VGOHAB and other IEO-Vigo colleagues involved in the analyses, field sampling, culture maintenance and, last but not least, *handling stinking mullets!*

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Video links

i. A. minutum: confocal microscopy https://www.youtube.com/watch?v=f64ElTEGYoI
ii. Samil red tide https://www.youtube.com/watch?v=X69fDU7pMHs
iii. Underwater video footage of the red tide https://www.youtube.com/watch?v=QSwFejcEaE
iv. Submarine footage of red tide https://www.youtube.com/watch?v=z0QISZigHqM

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Mass mortality of sea turtles *Chelonia mydas* in El Salvador

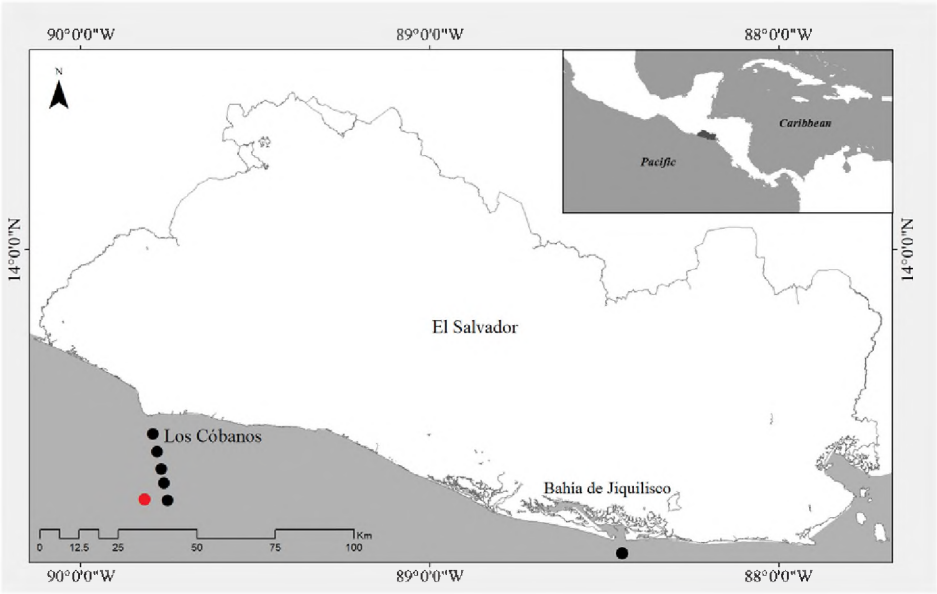

Fig. 1. Map of El Salvador, Pacific coast of Central America, with location of sampling stations and place where dead sea turtles were found in 2017.

During late October and early November 2017, hundreds of sea turtles, mainly green turtles (*Chelonia mydas*) were found dead floating off the coast of Jiquilisco, in western El Salvador coastal waters (Figs. 1,2). Paralytic shellfish toxins (PST) were suspected to be the cause of the mortality since similar events had occurred in El Salvador in 2005 [1], 2010 [2] and 2013 [3], and the mortality was attributed to high levels of PST (associated with blooms of *Pyrodinium bahamense* var *compressa*) found in several turtle tissues.

A total of 25 tissue samples from green turtles (*Chelonia mydas*) from Jiquilisco Bay and Los Cóbanos beach was analyzed (Table 1). The samples were delivered on November 6 and 7, 2017 at the Laboratorio de Toxinas Marinas from the University of El Salvador (LABTOX-UES) by technical staff of the Wildlife Unit, Ministry of Environment and Natural Resources. All samples belonged to dead sea turtles, except blood samples from a living turtle and a dying turtle. PST analyses by the Receptor Binding Assay (RBA), an official AOAC 2011.27 method [4,5], were carried out on November 8, 2017.

Toxic and harmful microalgae were sampled in Los Cóbanos, with the support of technicians from the Ministry of the Environment on November 7, 2017 (Fig. 2). Water samples for quantitative analyses were collected at two depths, along a 15 nm transect perpendicular to the coast; net-haul (20 µm mesh) samples were collected for qualitative analysis. Quantitative analyses were carried out under a inverted microscope (Carl Zeiss, Germany) applying the Utermöhl method. In addition, optical microscopy was performed on the intestinal contents of one specimen of *Chelonia mydas* found 15 nautical miles from Los Cóbanos beach, and on the stomach content of a dead olive ridley turtle (*Lepidochelys olivacea*) that stranded on El Sunzal Beach, department of La Libertad. Table 2 shows the average density of the most abundant microalgae detected in the samples. The diatoms *Dactyliosolen fragilissimus* (110,235 cell L^{-1}) and *Pseudo-nitzschia* spp. (4,033 cells L^{-1}) were the most abundant. All Pseudo-nitzschia species were counted together, given the difficulty in their identification and the existence of both toxic and harmless species within the genus. *Dactyliosolen*



Fig. 2. Collecting dead sea turtles off El Salvador during the mass mortality event, October-November 2017. Source: Ministry of Environment and Natural Resources of El Salvador.

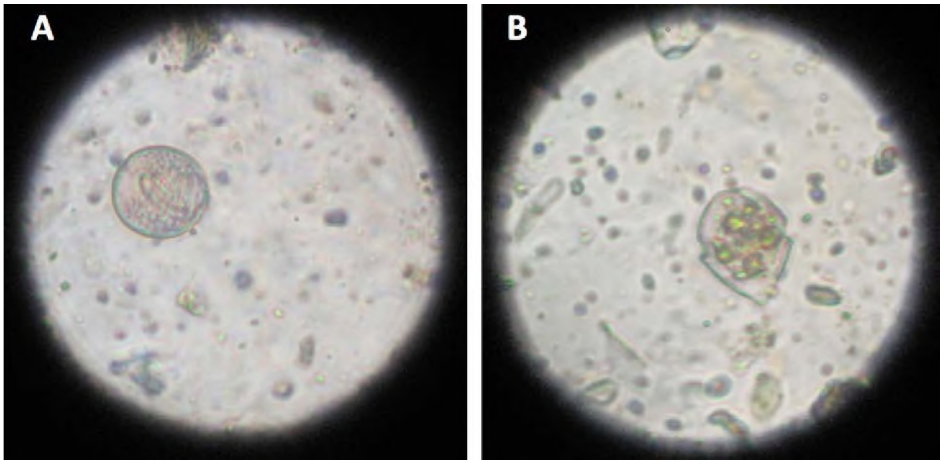

Fig. 3. Micrographs of microalgae found in intestinal and stomach contents of marine turtles. A) Prorocentrum sp. B) Scrippsiella cf trochoidea

fragilissimus was classified as harmless according to the Taxonomic Reference List of Harmful Microalgae of UNESCO. Potentially toxic and noxious species belonging to *Alexandrium* and *Gonyaulax* were also detected, but at cell concentrations far below those associated with noxious events.

Fragments of diatoms and dinoflagellates and whole cells of species such as *Planktoniella sol, Scrippsiella* cf *trochoidea, Prorocentrum* cf *compressum* and *Prorocentrum* sp., but no PST-producing species, were found in the gastrointestinal content of dead sea turtles (Fig. 3). Paralytic shellfish toxins were found in the liver and intestinal content in samples from dead specimens (Table 1). The highest cell concentrations were of species classified as harmless; while potentially toxic and noxious species were not found in cell concentrations high enough to categorise as an episode of a harmful microalgae bloom.

There is no information available in the scientific literature about saxitoxin levels in marine turtles, because most of them are endangered species under protection. The PST levels found in the sea turtle tissues suggest they died from a PSP intoxication, but the causative organisms bloomed elsewhere and escaped detection.

Acknowledgements

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Table 1. Toxicity (µg STX eq./kg) in different tissues from dead sea turtles estimated using the Receptor Binding Assay AOAC 2011.27. <LD = Below the detection limit of the analysis (100 µg STX eq/kg).

Location	Tissue	Number of samples	PST Concentration (µg STX eq./kg)
Jiquilisco Bay	Blood	2	<LD
	Flipper	20	<LD
Los Cóbanos	Intestinal content	1	294.51
	Liver	2	252.87

Table 2. Most abundant phytoplankton species detected in Los Cóbanos on November 7, 2017. [1]According to the Taxonomic Reference List of Harmful Microalgae of UNESCO. [2]Some species are classified as toxic and others as harmless.

Species	Density (cells L^{-1})	Category[1]
Dactyliosolen fragilissimus	110,235	Innocuous
Pseudo-nitzschia spp.[2]	4,033	Potentially toxic/innocuous
Guinardia striata	3,361	Innocuous
Chaetoceros affinis	2,689	Innocuous
Oxytoxum sp	1,344	Innocuous

Unusual winter *Microcystis* spp. bloom in a Chilean oligotrophic lake

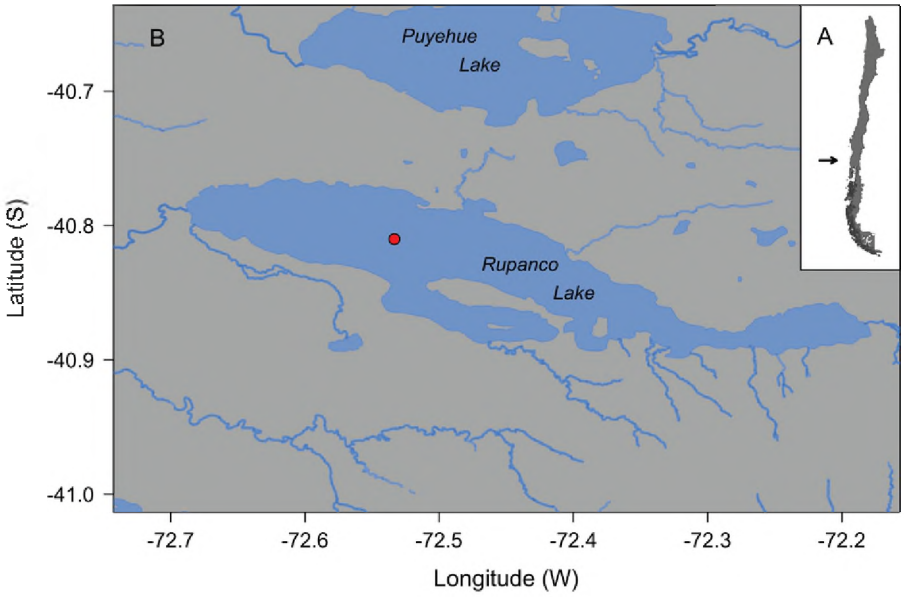

Fig. 1. Location of the study area in A) Southern Chile (arrow) and B) sampling station in Rupanco lake (red point).

Cyanobacteria are oxygenic prokaryotic microorganisms with a wide distribution, in freshwater and saltwater around the world. Blooms are frequently detected in eutrophic water bodies during spring and summer [1]. Recent studies have linked cyanobacterial blooms with global warming and concomitant changes in hydrological conditions [2]. In more than 50% of the cases, the cyanobacterial blooms are toxic [3], containing powerful toxins such as neurotoxins and hepatotoxins [4]. These toxic events have resulted in severe ecosystem impacts, such as massive animal mortalities through ingestion of cyanotoxins, and pose a threat to human health, because they have been associated with liver cancer [5-8].

In Chile, blooms of cyanobacteria have been reported in lakes with high concentrations of nitrogen and phosphorus [9]. In these lakes – located close to the urban centres with high population densities – cyanobacterial blooms have been detected during summer associated with an increase in lake surface temperature (LST). In 1986 a bloom (2.6×10^5 cells L^{-1}) of the cyanobacteria *Gomphosphaeria lacustris* was detected in Chapo Lake, a eutrophic ecosystem located in Southern Chile [10]. Likewise, a toxic bloom of *Microcystis aeruginosa* (3.1×10^6 cells L^{-1}) —a cosmopolitan cyanobacterial species – was observed in summer 2013 in an urban lake in Central Chile [9].

It has been established that high concentrations of nitrogen and phosphorus favour the growth of toxic over non-toxic strains of *Microcystis* [11]. Waters enriched with these nutrients has led to eutrophication and drastic changes in the structure and function of continental aquatic ecosystems [2]. Thus, intense blooms of cyanobacteria are characteristic of eutrophic water bodies [12]. Nevertheless, in the last decade cyanobacteria blooms have also been reported in oligotrophic (nitrogen-limited) lakes of northern Chilean Patagonia during summer.

To evaluate temporary changes of the microalgal communities in Rupanco Lake, an oligotrophic lake in Southern Chile (Fig. 1), monthly samples from March 2016 to February 2018 at five fixed depths (2, 5, 10, 15 and 30 m) were collected at a sampling station in the deepest zone of the lake. This North-Patagonian reservoir is considered a large and deep (maximum depth, 274 m) warm monomictic (summer temperature: 16–22°C) and oligotrophic (dissolved inorganic nitrogen: <0.1–3 mmol L^{-1}; soluble reactive phosphorous: <0.1–0.4 mmol L^{-1}) lake [13].

Phytoplankton analysis recorded a total of 116 microalgae species in 2017. The most abundant species were the diatoms *Synedra acus* (49.6%) and *Tabellaria fenestrata* (7.0%) and the cyanophyceae *Microcystis* spp. (4.9%) and *Nostoc* spp. (4.7%). Intense blooms of *Nostoc* spp. were detected in April and September 2017, with cell maxima of 3.1×10^5 cells L^{-1} and 3.8×10^5 cells L^{-1}, respectively. This last event was associated with a marked water column stratification. At the end of June 2017, an

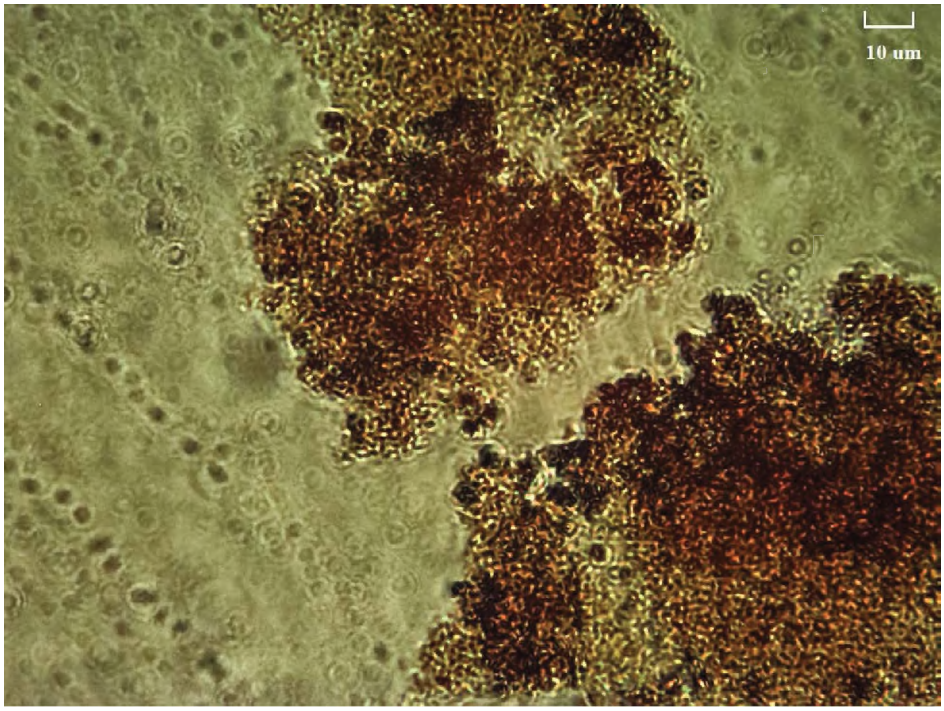

Fig. 2. Micrographs (light microscope 400X) of of Microcystis spp colonies found in Rupanco lake (Southern Chile), during a bloom in June 2017.

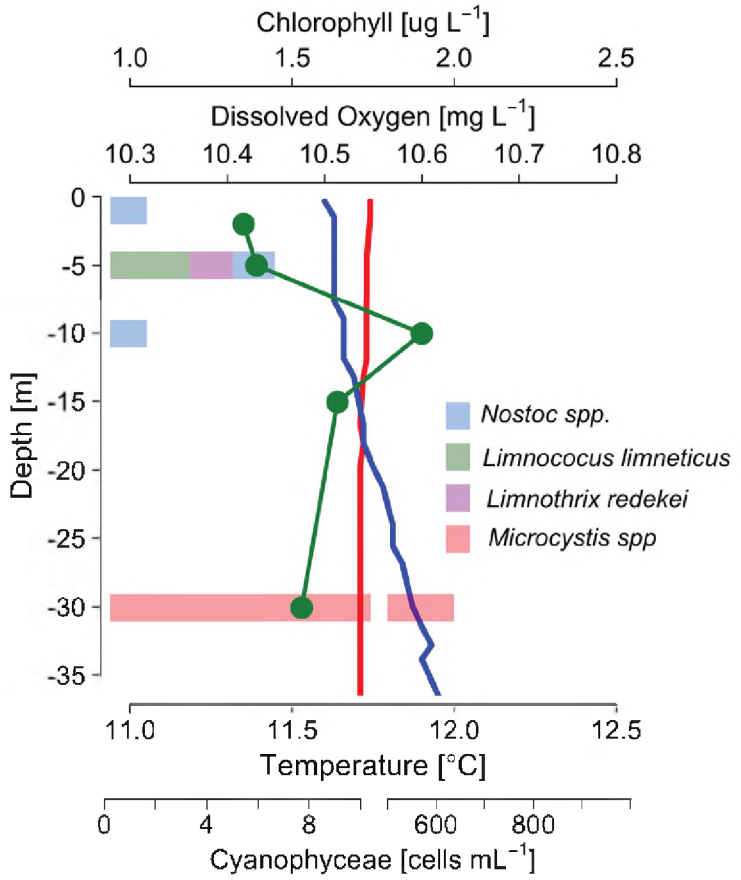

Fig. 3. Vertical distribution of temperature (red), dissolved oxygen (blue), chlorophyll a (green) and Cyanophyceae (bars) recorded during the survey on 23 June 2017. Note that the x-axis (cell densities) is broken.

unusual bloom of *Microcystis spp.* (Fig. 2) —maximum cells density 6.7×10^5 cells L^{-1}— was found at 30 m. (Fig. 3). There were other accompanying Cyanophyceae (*Nostoc* spp., *Limnococcus limneticus and Limnothrix redekei*) but the bloom was overwhelmingly dominated by *Microcystis* spp. (98.3%). The diatom *Tabellaria fenestrata* was present on the top water layer (2m: 77.3%; 5m: 68.4%; 10m: 61.6% y 15m: 89.0%). This event took place when the water column was mixed (higher turbulence), promoted by the high wind velocity (16.9 km/h, daily maxima), intense daily precipitations (51.1 mm) and homogeneous vertical distribution of temperature (11 ± 0.1 °C).

Intense blooms of *Microcystis* spp. (7.3×10^6 cells L^{-1}) comprising 89% of the population have been detected on the surface layers (2m) of this oligotrophic lake during summer [14]. Nevertheless, this is the first record of winter bloom of *Microcystis* spp in a Chilean oligotrophic lake. Thus, the more persistent and intense rainfall mobilized nutrients in the soil and increased the nutrient enrichment of the receiving waters [15, 16], a fact which may have promoted development of this bloom. Considering that the Rupanco Lake is nitrogen and phosphorus limited, this bloom is probably associated with global warming and changes in local hydrological conditions.

Finally, the results here show the need to consider the monitoring of potentially toxic species of cyanophyceae (e.g. *Microcystis* and *Nostoc*) when designing and implementing harmful algal bloom monitoring programmes, and not only in eutrophic continental waters.

Acknowledgements

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First report of the genus *Gambierdiscus* from the Atlantic coast of Guatemala

Gambierdiscus species (Gonyaulacales: Dinophyceae) are toxin-producing marine dinoflagellates causing Ciguatera Fish Poisoning (CFP), known as ciguatera, the most frequently reported seafood illness worldwide [1]. CFP is caused by consuming fish that have accumulated ciguatoxins (CTX), a group of lipid-soluble polyether neurotoxins, in their body, via the food chain [2,3]. Dinoflagellate species of *Gambierdiscus* are usually benthic microalgae associated with coral reefs and macroalgae, and several morphospecies have been reported worldwide.

Recently, Litaker *et al*. [4] presented the global distribution of *Gamberdiscus*, indicating the presence of five species endemic to the Atlantic (*G. belizeanus, G. ruetzleri, G. carolinianus* and ribotipe 1 and 2) with six more endemic to the tropical Pacific (*G. australes, G. pacificus, G. toxicus, G. polynesiensis, G. yasumotoy* and a unique ribotype). Only two species, *G. caribaeus* and *G. carpenteri,* are globally distributed.

To assess the presence of dinoflagellates responsible for CFP, three selected areas off the Atlantic coast of Guatemala (Fig. 1) were monitored during 2016. The first site, Estero Lagarto (15°55.545 / 88°35.576), is a shallow system fully covered by sea grass (*Thalassia testudinum*, and *Acetabularia* sp). The second and third, both coral reef areas, were Cabo Tres Puntas (15°58.128 / 88°33.241), with a high coverage of corals (70%) and a lower proportion (30%) of macroalgae (*Sargassum* sp, *Codium* sp, *Dyctyopteris* sp, and *Padina* sp), and King Fish (15°57.429 / 88°48.320) with a 40% coverage of coral and 60% of macroalgae (the same species as in Cabo Tres Puntas with the additional *Halymenia* sp present).

Dinoflagellates were collected using the macroalgae substrate method [5]. Temperature values ranged between 28.3 and 36.1°C and salinity, between 22.5 and 36.0. Samples were kept in a cooler (~5°C) and brought back to the laboratory without any preservative added. In the laboratory, samples were shaken vigorously to separate the dinoflagellates from the macroalgae. The suspension was passed through mesh sieves ranging between 250 µm and 20 µm. All the material retained on the 20 µm mesh sieve was resuspended in filtered sea water and preserved in acidic Lugol's iodine solution. Samples observed under the light microscope revealed the presence of specimens belonging to the genus *Gambierdiscus.* Morphological features were observed from Scanning Electron Microscopy (SEM) micrographs of the samples at the Centro de Investigación en Estructuras Microscópicas from Universidad de Costa Rica.

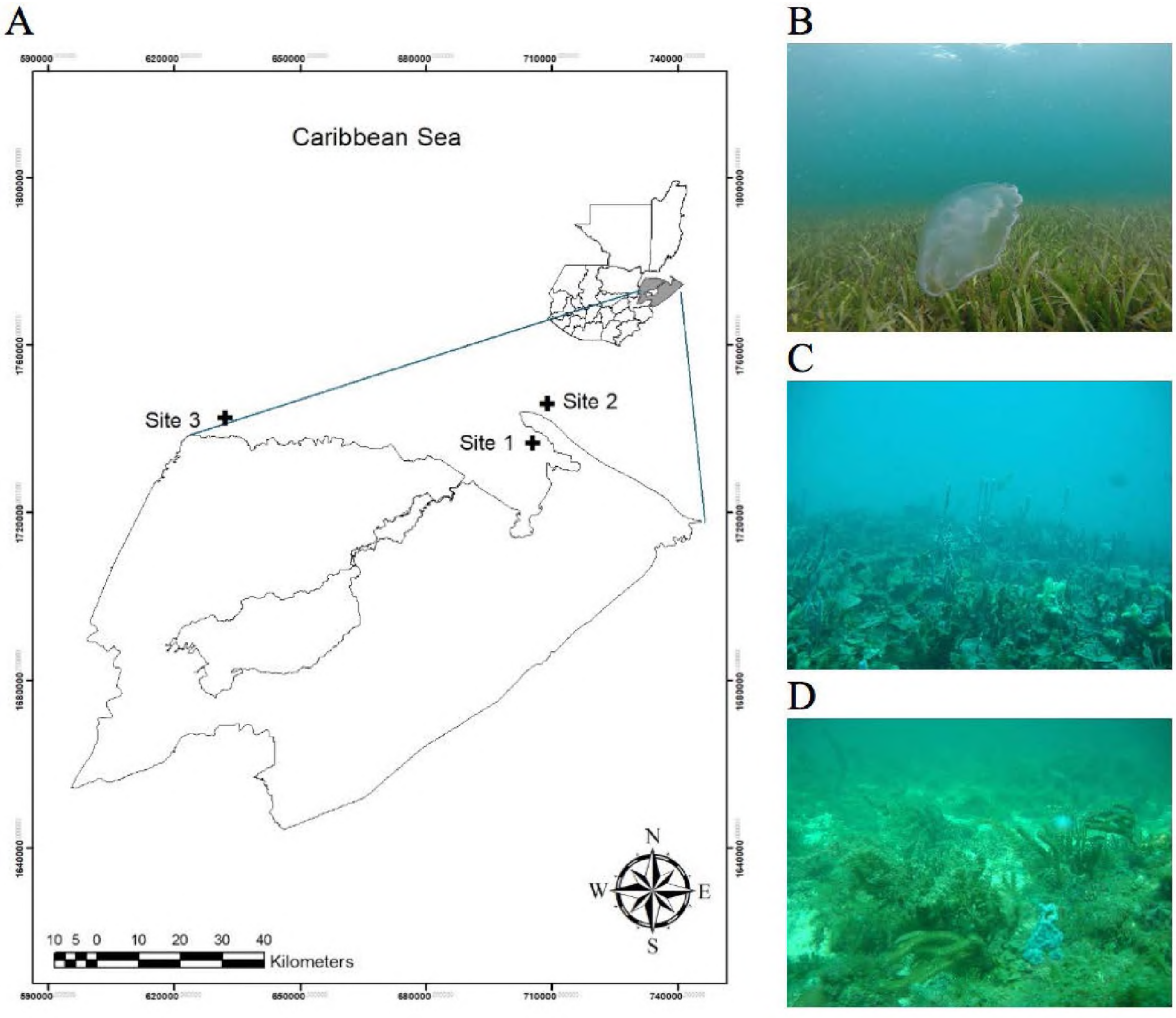

Fig. 1: Benthic dinoflagellate sampling areas on the Atlantic coast of Guatemala: A: Map of Guatemala and location of sampling sites; B: Estero Lagarto (Site 1); C: Cabo Tres Puntas (Site 2); D: King Fish (Site 3).

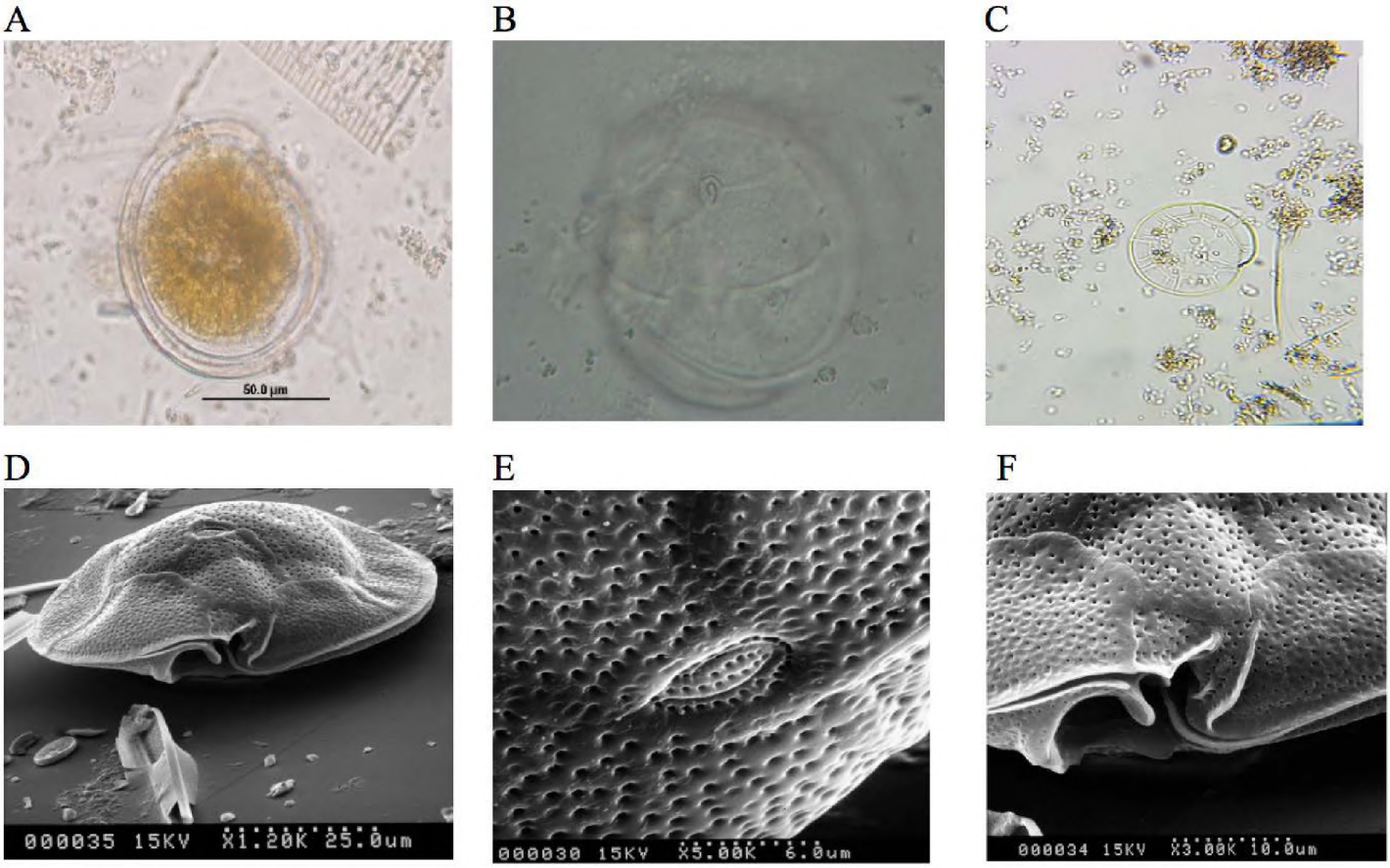

Fig. 2: Micrographs of Gambierdiscus sp. A: Light microscopy (LM), cells showing a globular shape and several golden and brownish chloroplasts; B: LM, apical view of the plates ; C: LM, antapical view; D: Scanning Electron Microscopy (SEM), ventral view; E: SEM, apical pore elongated showing its characteristic fish-hook shape, F: SEM, ventral view of the pattern of the sulcal plates

Gambierdiscus cells were found at all of the sampling sites, even though patterns of abundance varied significantly. In general, the genus *Gambierdiscus* was observed in low abundance at Estero Lagarto in comparison with the other sampling sites. Maximal abundance was observed at King Fish. In addition to *Gambierdiscus*, other epiphytic dinoflagellate genera, such as *Coolia, Ostreopsis,* and *Prorocentrum* were recorded.

Examination by light and SEM microscopy allowed detailed observation of cell morphology and tabulation (Fig. 2). Using light microscopy, several globular golden and brownish chloroplasts could be observed in cells in antapical view. Specimens measured under SEM microscopy had an average width of 68.69 ± 0.76 µm and length of 69.75 ± 5.83 µm. The apical pore was elongated and centrally located, with a length of 6.69 µm and width of 4.46 µm (Fig. 2). Using only morphological characteristics of the *Gambierdiscus* cells in SEM micrographs, it was not possible to determine the precise species. This was mostly due to a limited number of samples examined using SEM, and the incorrect position of the cells when the pictures were taken, a fact which made it difficult to analyse the apical pore complex. We need to continue with further plate morphological analyses and perform molecular sequencing of the *Gambierdiscus* genus specimens to identify them at species level.

This work provides the first report of *Gambierdiscus* species in Guatemala, and improves knowledge on the distribution of this genus in Central America and the Caribbean.

Acknowledgements

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Sampling for benthic dinoflagellates in the Solomon Islands



Fig. 1. Aerial photograph captured with a drone of Lola Island showing the sample collection site (-8.308221, 157.163998).

The distribution of the ciguatera fish poisoning causing dinoflagellate, *Gambierdiscus,* has been documented throughout the Pacific Ocean [1], although very little has been published about Melanesia (western Pacific). While ciguatera is not considered a problem in the Solomon Islands, the indigenous population avoids catching large reef predators, such as parrot fish and coral trout, which could be indicative of an underlying problem. To determine if *Gambierdiscus* was present in the Solomon Islands, sampling was undertaken at four sites across two of the nine provinces between the 15th April and 5th May 2018. The four sites were Lola Island Lagoon (Zipola Habu; -8.308221, 157.163998; Western Province; Figure 1), the Hirokawa Maru (-9.385544, 159.874570; World War II ship wreck; Guadalcanal Province; Figure 2), Kinugawa Maru (-9.378672, 159.870754; World War II ship wreck; Guadalcanal Province), and the coastline and estuarine pond near Honiara (-9.430440, 159.991568; Guadalcanal Province). Samples were collected by shaking macroalgae or disturbing the benthos and trapping the disturbed microalgae in 50 mL Falcon tubes. Germanium dioxide and f/2 medium were added (0.5 mL of each per tube) to suppress the growth of diatoms and encourage dinoflagellate growth respectively.

The analysis included samples collected from a variety of macroalgae (for example, *Actinotrichia* sp. and *Padina* sp.; Figure 3), eel grasses and sediments. An array of marine life was encountered during sampling (Figure 4), although no *Gambierdiscus* was detected at any of the sites by light microscopy. No live cells of the commonly cohabitating dinoflagellate genera *Coolia* or *Ostreopsis* were detected, although dead cells of the latter were noted in the Lola Island sample. However, *Prorocentrum* cf. *lima*, was identified microscopically in the samples from Kinugawa Maru (*Actinotrichia* sp.) and an estuarine pond site near Honiara (*Padina* sp.) and single cells were isolated for culturing. Of the original eight individual cells isolated, four were successfully established and subjected to DNA sequencing. The maximum likelihood (ML) and neighbor-joining (NJ) phylogenetic analyses based on the LSU rDNA D1/D2 revealed that all eight Solomon Islands isolates were *Prorocentrum lima* (Figure 5). The acquired sequences were identical to those of previously reported strains, i.e., Chinese strains 2S1F7 and TIO124, a Mexican strain PL6 and a strain DNS-7 (Figure 5).

Second generation cultures of the *Prorocentrum lima* isolates will be tested for diarrhetic shellfish toxin production (okadaic acid, dinophysistoxin-1 and dinophysistoxin-2) using liquid chromatography-tandem mass spectrometry. A small (~25 µm), unidentified dark brown gymnodinoid dinoflagellate was observed on an *Actinotrichia* sp. at the Kinugawa Maru site, and on a *Padina* sp. in the Honiara pond sites. Also, a cell of *Togula* sp. was isolated from a *Padina* sp. growing in the ponds and is being cultured for further characterisation and toxin analysis.

Cyanobacteria were present at all sites and dominated the samples from the Honiara pond site. Isolates of the genera *Oscillatoria, Phormidium* and *Spirulina* were identified morphologically. Pennate diatoms, in particular, Naviculoid sp., were also present and often dominant in the samples.

Duplicate samples were treated with



Fig. 2. One of the chimnies from the Hirokawa Maru World War II wreck (-9.385544, 159.874570) supporting an array of marine life.

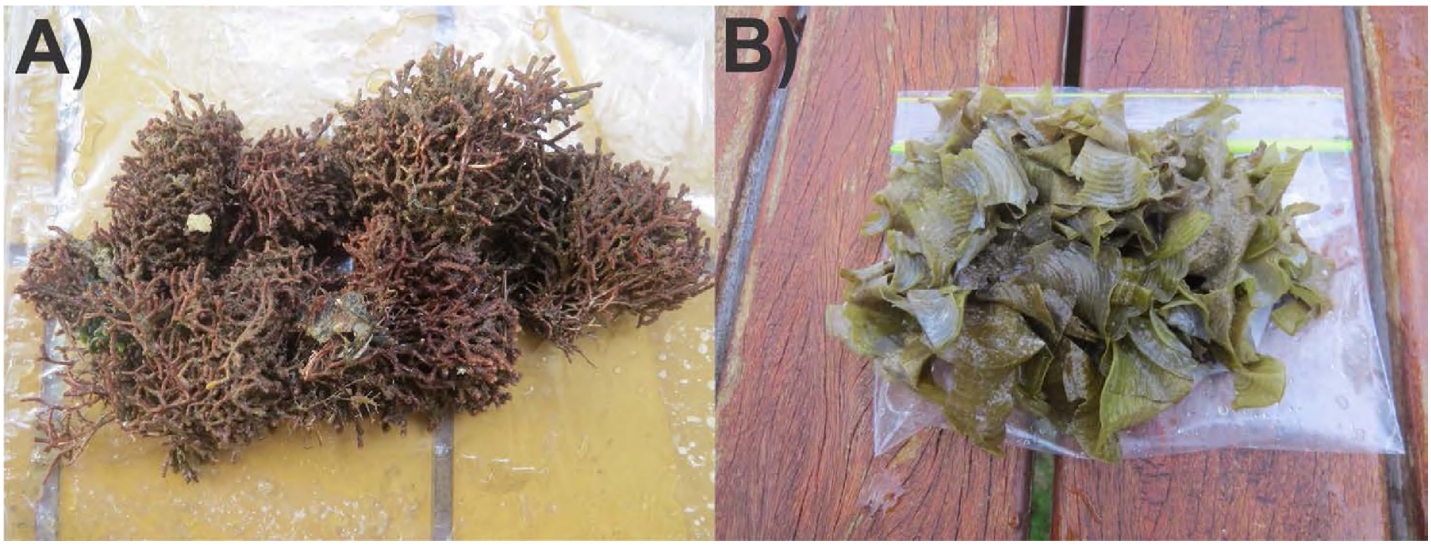

Fig. 3. Macroalgae A) Actinotrichia sp. and B) Padina sp. that were collected during the search for epiphytic microalgae species.



Figure 4. A surprise guest while collecting benthic samples in the Solomon Islands.

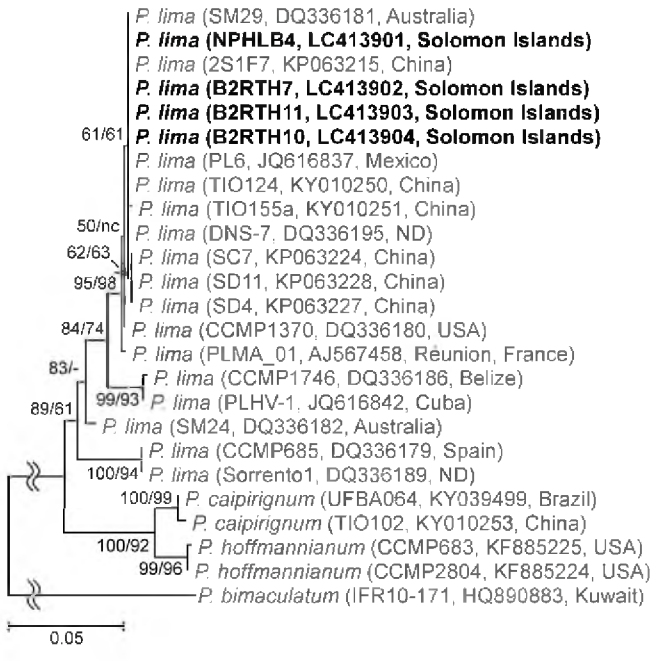

Fig. 5. A ML phylogenetic tree of Prorocentrum lima strains isolated from Solomon Islands based on the LSU rDNA D1/D2 (25 sequences, 630 positions). Sequences obtained in this study are shown in bold and black color. Nodal supports represent bootstrap values obtained in ML/NJ methods. Nodes that were under 50 of bootstrap value or were not present in the NJ tree are shown as hyphen and nc, respectively. Unreported locality where the strain was isolated is shown as ND.

either a preservation solution for nucleic acids [2] to enable next generation sequencing using metabarcoding [3], or Lugol's Iodine for microscopic quantification of the microalgal species present. These analyses will be performed and results published in the future.

Further sampling efforts are planned and will focus on the southern province where anecdotal reports have been received of ciguatera-like intoxication occurring. However, on this occasion no indication of the ciguatera-causing *Gambierdiscus* was found.

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A practical guide to new nomenclature for species within the "*Alexandrium tamarense* species complex"

For several decades, the "*Alexandrium tamarense* species complex" included three morphologically defined species, *A. catenella*, *A. fundyense*, and *A. tamarense* [1]. Worldwide, the group is one of several responsible for paralytic shellfish poisoning, a potentially life-threatening syndrome that occurs following the consumption of shellfish contaminated with paralytic shellfish toxins (saxitoxin and analogs). Morphologically, *Alexandrium catenella* was distinguished by rounded, slightly anterior-posteriorly compressed cells, chain formation, and a 1' plate lacking a ventral pore [1, 2]. *Alexandrium fundyense* and *A. tamarense* shared the same Kofoidian plate tabulation as *A. catenella*, but typically exhibited less compressed cellular morphologies [1, 3]. *Alexandrium tamarense* and *A. fundyense* were distinguished based on the presence of a ventral pore on the 1' plate in *A. tamarense* and its absence in *A. fundyense* [1].

Field and culture studies over the past 20 years often described "*A. tamarense* species complex" cells exhibiting intermediate morphologies when compared to the original descriptions of all three species (see John et al. [4] for a summary). Phylogenetic studies of rDNA gene sequences obtained from "*A. tamarense* complex" isolates fall into one of five distinct ribotype groups. These do not correlate with the original morphologically defined species and were initially designated as Groups I–V [5-8]. The genetic distances among the ribotypes are typical of those separating other dinoflagellate species. Together, these observations indicate that the original species descriptions depicted a series of "morphotypes" shared by various species in the *A. tamarense* complex rather than actual species. Consequently, researchers set out to confirm that the five ribotypes represented separate species (e.g., [5, 8-11]). The most comprehensive of these efforts by John et al. [8] assembled diverse lines of evidence, including detailed morphological analyses, rDNA phylogenies, mating incompatibility assessments, ITS1/5.8S/ITS2 rDNA uncorrected genetic distances, ITS2 complementary base pair changes, saxitoxin production and the presence or absence of a key gene involved in saxitoxin synthesis. These combined data fully support the conclusion that the Group I–V ribotypes are distinct species.

Equally important, the morphological analysis by John et al. [8] showed ***no single morphological trait, or suite of traits,* corroborated the original morphospecies descriptions, nor could any combination of morphological traits distinguish the ribotype groups from one another.** There was simply too much overlap in the morphologies expressed by each of the ribotype groups for morphological characters to prove useful. For example, Group I populations in the entire north Atlantic and north Pacific including Alaska exhibit both "*A. fundyense*" and "*A. tamarense*" morphology. In contrast, those in the eastern Pacific from San Francisco Bay south to Chile, the southeast Atlantic coast of South Africa and many areas in the western Pacific from Australia to Japan, frequently form multi-cell chains, a signature of the original "*A. catenella*" morphological description. These latter Group I populations also display the typical "*A. catenella*" morphology and are indistinguishable from the Group IV populations found in the Western Pacific (South Japan, Korea and China) and the Mediterranean Sea [5, 9, 10, 12].

Based on their analyses, John et al. [8] assigned the following species designations to each ribotype group: *A. fundyense* (Group I), *A. mediterraneum* (Group II), *A. tamarense* (Group III), *A. pacificum* (Group IV) and *A. australiense* (Group V). John et al. [8] took further steps to provide exhaustive morphological and genetic descriptions and submitted holotype and epitype material, as appropriate, to the Herbarium Senckenbergianum (FR) in the Centre of Excellence for Dinophyte Taxonomy (Wilhelmshaven, Germany). Most of these nomenclatural reassignments raised minimal concerns among taxonomists. The primary exception was the designation of the Group I ribotype as *A. fundyense*.

The controversy regarding the Group I designation centers on whether the cells used for the original *A. catenella* description were from Group I or IV, given that populations of both species in the Pacific are known to exhibit the classic "*A. catenella*" morphotype. Molecular analyses of "*A. catenella*" cells collected from the coast of California at Redondo Beach [13] and Monterey Bay [14], south of the type location, indicated that these cells belonged to Group I (Fig. 1). Further, there is no evidence that any species except Group I occurs in all of the Americas [5, 13-17]. Use of species-specific molecular assays in other parts of the world, however, have shown Group I and IV cells matching the *A. catenella* morphotype co-occur (see Fig. 1 and associated references). The lack of any extant type material for molecular testing and the overlapping distributions of Group I and IV cells exhibiting the "*A. catenella*" morphology in other regions creates uncertainty about which ribotype was described originally. Based partially on this uncertainty, John et al. [4] submitted a formal proposal to *Taxon* for rejection of the name *Alexandrium catenella* in favor of *A. fundyense*.

A second reason for using the *A. fundyense* name in lieu of *A. catenella* is that a large volume of literature regarding Group I blooms, especially from the Gulf of Maine, has been published using the name *A. fundyense*. Continued use of *A. fundyense* would cause less disruption to the existing documentation and serve the International Code of Nomenclature (ICN) Article 14.2 goal that states: "Conservation aims at retention of those names that best serve stability of nomenclature". A third reason for rejecting *A. catenella*, and assigning Group I to *A. fundyense* and Group IV to *A. pacificum*, was to provide the scientific community a means of unambiguously distinguishing the Group I and IV cells exhibiting the same *A. catenella* morphotype, especially in regions where they are sympatric.

Based on the known distribution of Group I cells along the coast of North and South America, Fraga et al. [18] submitted a counterproposal to *Taxon* against rejecting the name *A. catenella*. The authors argued that the Group I

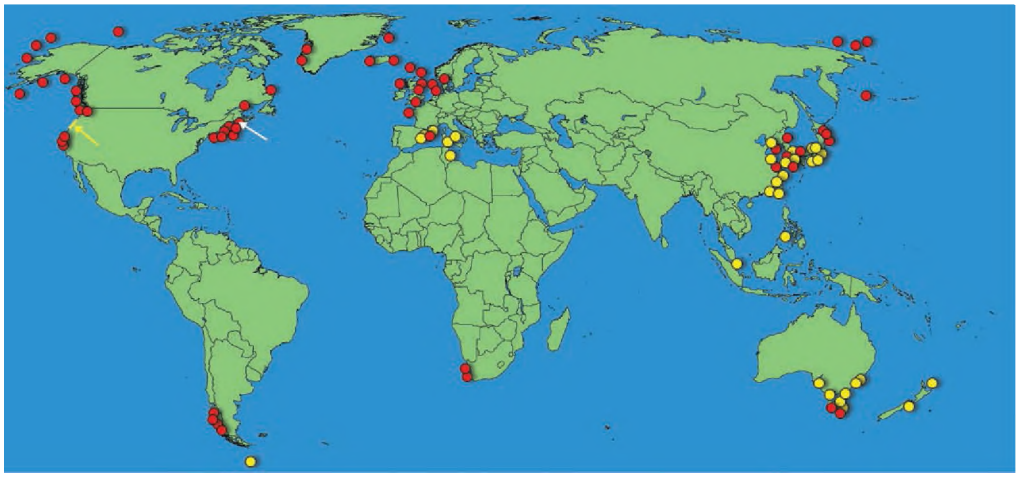

Fig. 1. Known locations where Group I A. catenella (red dots) and Group IV A. pacificum (yellow dots) cells have been collected and confirmed with molecular assays [6-8, 11, 12, 15-17, 20-37]. The yellow line and yellow arrow on the west coast of North America indicates the type locality for A. catenella. The white arrow shows the type locality for A. fundyense in the Bay of Fundy, Canada. The single report of A. catenella in the Mediterranean and A. pacificum in the Drake Passage between South America and Antarctica should be considered provisional until additional isolates were identified in both regions. No isolates of Alexandirum catenella have been obtained for the Mediterranean Sea despite extensive sampling and though Busch et al. [34] detected putative A. catenella sequences from environmental DNA samples collected from Fangar Bay (Catalan coast) using 454 pyrosequencing of LSU rDNA, the reads are so short they cannot be considered as definitive. If A. catenella does occur in the Mediterranean it may represent a recent introduction.

distribution in the type locale was sufficiently established to conclude only Group I cells were used for the original *A. catenella* description (Fig. 1). If true, the ICN rules of priority, in cases where the type material is not in question, clearly dictates retention of the name "*A. catenella*" for Group I because it was published prior to the "*A. fundyense*" Group I description [1, 2].

Nomenclature change proposals such as those of John et al. [4] and Fraga et al. [18] are adjudicated by The ICN Nomenclature Committee for Algae. This committee met to consider the proposals and ruled the name *Gonyaulax catenella* (*Alexandrium catenella*) should not be rejected and that *A. fundyense* and *A. catenella* are conspecific with nomenclatural priority being given to *A. catenella* [19]. For the valid names and synonymy one can refer to the following checklist: *https://www.dinophyta.org/checklists/of-species/alexandrium/*.

The decision of the Nomenclature Committee has the following implications:

1. All publications involving Group I cells published using the name *A. fundyense* should now be considered *A. catenella*.
2. This nomenclatural revision affects the two most widespread toxic species (Group I and IV) within the "*A. tamarense* complex". Resource managers in much of the world need to be aware of this nomenclatural change as it will affect reporting requirements, communication with the public, and use of past literature when developing strategies for dealing with paralytic shellfish poisoning events and regulations.
3. This decision also places significant demands on future researchers as they investigate the literature. For example, with the exception of Chilean and South African research, most of the papers published on "*A. catenella*" before 2015 represent reports of the morphologically indistinguishable species *A. pacificum*. Similarly, future workers will need to recognize the extensive literature published between 1985 and 2017 regarding Group I *A. fundyense* actually refers to *A. catenella*.
4. The formal assignment of the name *A. catenella* to Group I also means this name cannot apply to Group IV (*A. pacificum*) despite Group IV exhibiting morphologies exactly matching the original *A. catenella* description.
5. Ribotype groups II, III and V species should be referred to as *A. mediterraneum*, *A. tamarense* and *A. australiense*, respectively [8].

Example scenarios clarifying how to identify and name "*Alexandrium tamarense* complex" species:

1. You work in a region where co-occurring members of the "*Alexandrium tamarense* species complex" are present. Based on their morphology, you have assigned them the names *A. catenella* and *A. tamarense*. What do you do?
   - You need to sequence barcoding marker regions from the strains, specifically, ribosomal RNA genes or use species-specific molecular assays. Only then can you truly determine the species present, i.e. *A. australiense*, *A. catenella*, *A. mediterraneum*, *A. pacificum*, or *A. tamarense*.
   - A particular case is that from Japan, where species typically identified as *A. catenella* should be called *A. pacificum*, and the species recognized as *A. tamarense* should be designated as *A. catenella.*
2. You are working on an isolate from a culture collection identified as *Alexandrium catenella*. Cells have the typical morphology of *A. catenella* as described by Whedon and Kofoid [2]. When you sequence its ribosomal RNA genes, you discover that it is the *A. pacificum* (Group IV) genotype. What do you do?
   - The isolate should be reclassified as *A. pacificum*.
3. The typical toxic bloom-forming organism that occurs in your region

is well known and has always been known as *Alexandrium fundyense* or *A. tamarense*. It has the Group I genotype. What do you do?
   - You refer to the species as *A. catenella*.
4. Toxic and non-toxic strains with the morphology of *Alexandrium tamarense* coexist in your region. What do you do?
   - Molecular barcoding or sequencing is mandatory.
   - In the case of Scotland, the non-toxic strains correspond to *A. tamarense*, while the toxic ones are *A. catenella.*
   - In the case of the Mediterranean Sea, the non-toxic species *A. tamarense* and *A. mediterraneum* may coexist. The toxic *A. pacificum* also occurs in this region but typically forms chains.
5. Chain forming cells of the "*Alexandrium tamarense* complex" are observed. What do you do?
   - Start with the hypothesis that if it is a high latitude, cold-water area, the species present is *A. catenella*, but if it is a warm temperate area, it is *A. pacificum*, then confirm using molecular assays.
6. Special attention should be given to the non-toxic *A. affine*. Initially, this species was not considered part of the "*Alexandrium tamarense* species complex" because it could be morphologically identified by the position of the anterior attachment pore. However, the anterior attachment pore can be absent in old cells, making it an unreliable morphological character leaving *A. affine* morphologically indistinguishable from the "*Alexandrium tamarense* species complex".

In conclusion, the "*Alexandrium tamarense* complex" currently includes the following five species: *A. catenella* (Group I), *A. mediterraneum* (Group II), *A. tamarense* (Group III), *A. pacificum* (Group IV) and *A. australiense* (Group V). Multiple analyses clearly show these species cannot be distinguished based on morphology. Instead, species-specific molecular assays are required for reliable identification. Vandersea et al. [17] provided an overview of the various assays that can be used for this purpose. It is further recommended that the harmful algae research community work to identify and standardize a set of molecular assays to be used when screening samples for these species. All publications regarding these species should include both the Group I–V ribotype designations and species names. This will ensure unambiguous species identifications and reduce further confusion in the literature. The routine use of multiple, standardized, species-specific molecular assays will also allow better definition of species ranges, avoid any biases regarding which species are present in a region and identify when new introductions have occurred.

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XIII Iberian meeting on harmful algae and biotoxins (REDIBAL)

The XIII Iberian Toxic Algae and Marine Biotoxins Meeting (REDIBAL 2018) was held in Vigo, Spain, from 20 to 22 June, at the *Mar de Vigo* Auditorium. For the first time, REDIBAL was held simultaneously with other marine science conferences (EOF, SIQUIMAR, ISC CDM, Workshop Cíes), seizing the opportunity of the VI International Symposium on Marine Sciences (ISMS) (Fig. 1). The ISMS event is held every two years as a cooperation between the six Spanish universities with Marine Science Degrees (Alicante, Barcelona, Cádiz, Las Palmas de Gran Canaria, Valencia and Vigo) and its celebration in 2018 in Vigo was indeed a great opportunity to recommence REDIBAL meetings, after a five year gap since the XII edition held in Palma de Mallorca in 2013. The local organizing committee was composed by researchers from the IEO-VGO HAB group, and the scientific committee included colleagues from Spanish and Portuguese institutions involved in harmful algae research and/or monitoring programmes (Fig. 2). Full information on the meeting, programme and abstracts can be found at *https://isms.gal/redibal/*

ISMS and the associated events, including REDIBAL, represented an excellent meeting point for students, researchers and companies (e.g. CIFGA, OCEOMIC) involved with marine sciences. All conferences were celebrated in the same facility and ISMS participants were allowed to attend any lecture and activity. Overall, nearly 500 participants registered for the four meetings and 45 communications (oral and poster-type) were submitted to REDIBAL. Plenary speakers were selected for each conference. The invited speaker in REDIBAL, M. Montresor, presented "*The secret life of diatoms: fascinating questions from unicellular microalgae*". That was a review on the ecology and life cycle studies of *Pseudo-nitzschia multistriata* from the Gulf of Naples, with emphasis on the relevance of life-histories in phytoplankton ecology (Fig. 3).

Other activities during the ISMS included a panel discussion about "women and marine science" organized by the Galician Society for the Dissemination of Science (DivulgACCIÓN-AGCCCT). The REDIBAL meeting was closed with a screening of "Red Tides", a documentary financially supported by FECYT (Government of Spain), which reviews the nature of red tides and HABs in Galician waters.

One of the fundamental objectives of REDIBAL meetings is the transfer of knowledge between researchers and students, with the purpose of identifying new HAB-related goals and future challenges. Furthermore, it provides an opportunity to present ongoing work and results conclusions and a suitable platform for students to share their PhD- or Master's Degree theses. With this in mind, specialists coming from different institutions from Spain, Portugal, México and Perú presented their recent studies and debated questions in REDIBAL addressing a number of HAB-related issues. These included toxin detection, harmful algae monitoring, environmental effects on HAB organisms, innovative methodologies related to HABs and biotoxin detection, identification of new organisms, life cycles, etc. Theme Panels were held in three sessions dealing with: i) ciguatera fish poisoning (CFP); ii) toxin detection methods and iii) harmful algae monitoring.

Ciguatera Fish Poisoning (CFP)

Participants at the round table on *A practical approach for the assessment and management of ciguatera risk in Europe* (N. García-Álvarez, ULPGC; A. Gago

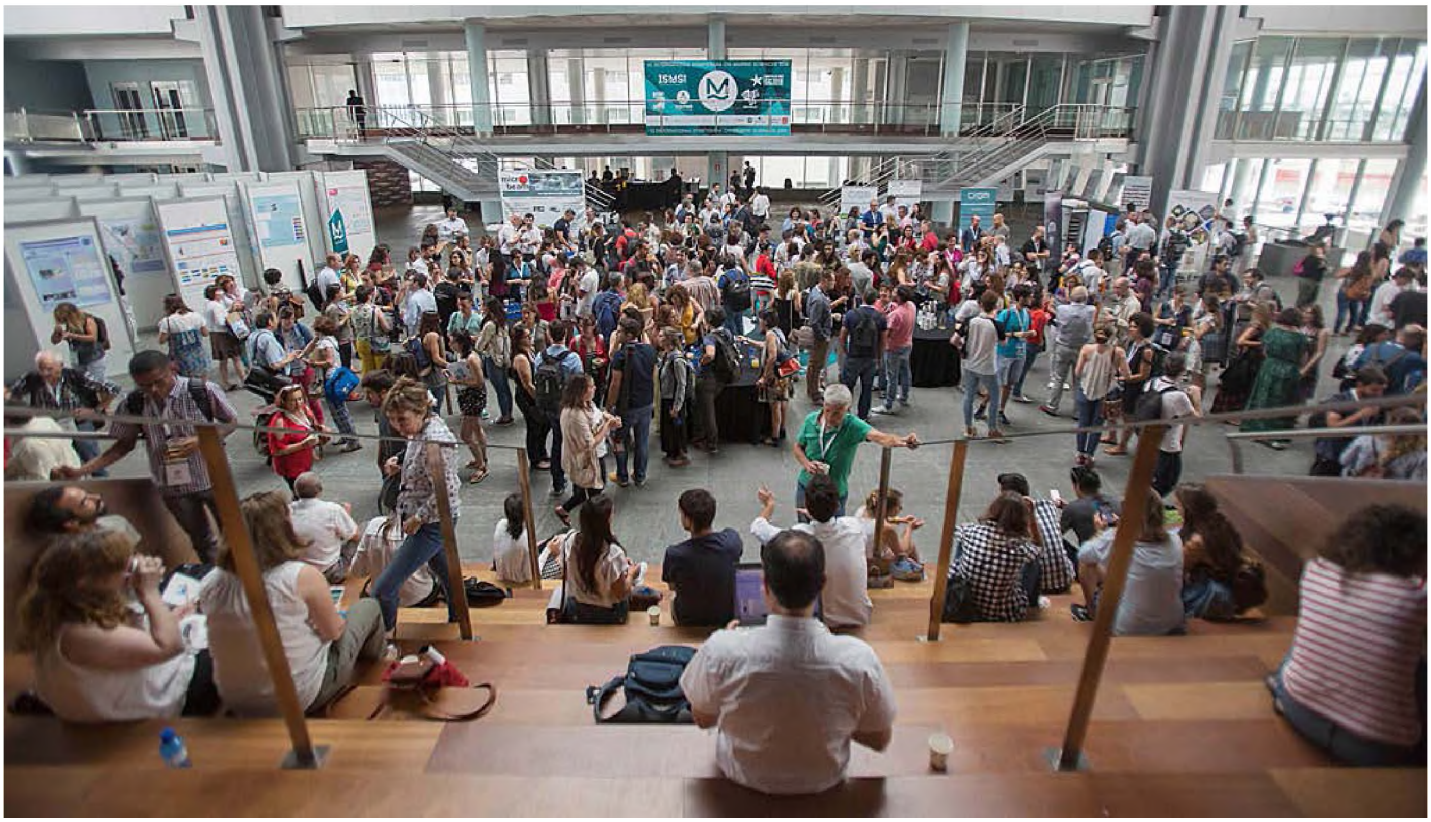

Fig. 1. ISMS meeting, 20-22 June 2018 at the Mar de Vigo Auditorium, Vigo, Spain



Fig. 2. Group photo of participants on the XIII REDIBAL meeting, Vigo, 20-22 June 2018

EURLMB; A. Bilbao, GMR Canarias; P. Reis-Costa, IPMA and J. Diogène, IRTA) highlighted the major challenges involved with sensitivity of instrumental analysis and flagging the lack of standards, the importance of developing efficient extraction procedures, including those for viscera, and the complexity of dealing with CTX derivatives at very low concentrations. The difficulty raised by the lack of *Multiprotocol Label Switching* (MPLs) for CTXs for decision-making in monitoring programmes was described, as well as the necessity to have thorough screening procedures for CTXs. Some areas (Canary Islands and Madeira) have produced lists of fish species and weights above which analysis is compulsory or consumption is prohibited. International cooperation (e.g. EUROCIGUA project), with laboratories providing methods for microalgal identification and toxin analysis, begins to address these issues and to better understand the relationship between *Gambierdiscus* spp, and *Fukuyoa* spp. and the presence of CTXs in fish.

Indeed, the CFP represented a significant number of communications in REDIBAL, focused on current knowledge from the Canary Islands, with updates on toxin analyses on fish (F. Acosta, N. García-Alvarez), benthic dinoflagellate studies in the area including *Gambierdiscus* and *Ostreopsis* (I. Bravo, E. Soler and J. Fernández), as well as the official control programme for CFP, with special focus on scientific, legal and outreach aspects (N. García-Álvarez, A. Bilbao). Moreover, the presence of *Gambierdiscus* in the western Mediterranean Sea was reported for the first time (À. Tudó), although CFP cases have not been observed in the area yet. In addition, studies on the genus *Ostreopsis* in the Catalan and Portuguese coasts were presented by M. Vila and H. I. David.

Biotoxin detection methods

The challenges posed by the analysis of emerging biotoxins in the EU (D. Castro, A. Pequeño) introduced this session. The possible methods that can be used for determination of marine toxins were analyzed also in a second theme panel (P. Reis-Costa, IPMA; M. Campás, IRTA; Á. Moroño, INTECMAR; J. Blanco, CIMA and P. Riobó, IIM-CSIC). Three groups of methods were considered depending on the objectives.

Methods used for monitoring toxicity in shellfish are restricted to those validated and officially accredited and should avoid the use of animals. Currently, in most countries, ASP toxins are quantified by HPLC-DAD, with no noticeable problems. In Spain and Portugal, the regulated lipophilic toxins are monitored by LC-MS/MS, based on the SOP of the EURLMB, in general with satisfactory results. Some drawbacks are derived mostly from the need of intense equipment cleaning and maintenance, which frequently halts the activity of some LC-MSs and consequently, there is a requirement to have one or several spare devices if a high throughput is expected. Matrix effects are also a problem, but it is frequently associated with the maintenance of the LC-MS's. Sample preparation methods, reliable for multi-toxin analysis are required in order to solve, or at least mitigate these problems. No issues have been detected with the validation and accreditation of these methods.

The mouse bioassay is used to quantify PSP toxicity in Spain, but in 2019 it should be replaced by the AOAC Official Method. This method uses pre-column oxidation of the toxins and HPLC analysis with fluorescence detection. The method has been in use in Portugal for some years and it is found to be useful but time consuming and labor intensive. The original method has two parts: 1) the first includes the clean-up of the extracts by solid phase extraction with a C18 column and the analysis after the oxidation with periodate, and 2) the second is performed on any toxin peak detected and requires oxidation of the C18 extract with hydrogen peroxide, fractionation of the extracts through a cationic exchange COOH-SPE column (3 fractions) and the analysis of the C18 extracts and COOH fractions and analysis. In Portugal, this process has been modified to gain effectiveness by adapting it to the known toxin profiles mostly originated by *Gymnodinum catenatum*. During blooms of that species, most toxins could be detected after C18 clean-up and peroxide oxidation, so when the concentration of the main toxins is higher that the legal limit, only a single chromatographic injection is required.

When this is not the case but some toxins are present, fractionation through cation exchange (COOH) is required. Additionally, the extracts obtained after fractionation should be subjected to an acid hydrolysis and re-analyzed in order to indirectly quantify C3 and C4 toxins, for which reference solutions currently do not exist. Obtaining final results in those cases takes a long time and requires a considerable effort, which makes it difficult and inadequate for its use in intensive monitoring systems. Some concerns about this method have been expressed. The main one, that in order to avoid the full process of quantification it is necessary to dynamically adapt the method to the toxin profile in the bivalves determined by the phytoplankton species, and this seems to be a problem for accreditation of the method (which is required for the official monitoring systems). There was a general agreement on the need for

developing an LC-MS/MS or validating an existing one with high throughput, to replace in a near future the current reference method.

Other methods not requiring complex instruments, such as immune-, aptamer- or functional assays, coupled or not to different sensing devices (biosensors), could also be employed, but their generalized use in monitoring systems seems to be difficult as multitoxin reference methods would be required and the running costs (staff and instruments) would not be substantially reduced. When chemical analyses are too complex or/and have a low sensitivity, as is the case for ciguatoxins analysis, some functional assays (neuroblastoma) are being used for monitoring. Their use by other agents, e.g. producers is promising. It would enable them to check some groups of toxins on particular batches of bivalves with a reduced cost in equipment and highly trained personnel. A critical step to foster the use of those methods would be a simplification of the extraction processes which currently are time consuming and difficult to carry out in situ.

For research purposes, different methods are being used and developed, with those based on liquid chromatography coupled to high resolution mass spectrometry probably the most widely used. The methods developed with this technology have an important drawback, which is that they are much less sensitive than those using triple quadrupole mass spectrometers. Biotoxins represented a prominent subject in REDIBAL, ranging from studies covering physiological and genotoxic effects of PSP in mollusks and zooplankton (A. Braga, A. Barreiro) with emphasis on climate change impacts mediated by warming and acidification. In addition, the impact of sexuality in PSP production by *Alexandrium minutum* (P. Riobó), the characterization of new compounds in *Prorocentrum hoffmannianum* (J. Fernández, A. Daranas), atypical OA derivatives in cockles (J. Blanco) and rapid screening of tetrodotoxins (S. Leonardo) were also presented.



Fig. 3. Marina Montresor, invited speaker at the XIII REDIBAL meeting

Harmful Algae Monitoring and Research

There were presentations on historic data series and recent findings (N. Sampedro, M. Fernández, L. Mamán, A. Laza-Martínez and A. del Rocío). The main topic discussed on the theme panel (chaired by M. Fernández, IRTA and L. Mamán, LCRRP) was the application of the new EU regulations on echinoderms and gastropods and how to deal with these changes in relevant harvesting areas. Progress of the EURLMB Working Group on Phytoplankton control on the generation of a "Guide to Monitoring of Toxin-producing Phytoplankton in Production Areas for Bivalve Molluscs" and the need for guidelines for risk assessment of toxins and toxic phytoplankton occurrence were highlighted.

Although harmful dinoflagellate species were the main focus in REDIBAL, other groups (i.e. diatoms) were also dealt with including a couple of studies on the detection of *Pseudo-nitzschia* blooms using artificial intelligence methods (F. Bellas), and mechanisms of orientation in pennate diatoms (G. Basterretxea). Molecular studies included DNA-based assays to detect *Ostreopsis* (A. Toldrá) and the evaluation of different sample treatments for metabarcoding analyses to characterize diversity in coastal sediments (Albert Reñé).

Other topics included the identification of a new non-toxic red tide forming dinoflagellate species (*Gonyaulax undistortata* sp. *nov.*, D. Hernández-Becerril) in México, the relationship between phytoplankton thin layers formation and toxic outbreaks in the Galician Rías (E. Broullón), microscale physical-biological interactions in *Dinophysis* populations (B. Reguera), the effects of turbulence on *Dinophysis* (M. García-Portela), and the host range of a recently described marine parasitoid species (*Parvilucifera corolla*, F. Rodríguez).

Overall, REDIBAL was a fruitful and friendly meeting where colleagues from the Iberian Peninsula (Spain and Portugal) and South America (México and Perú) exchanged their knowledge and debated during three days the current status and perspectives of HAB studies, both at local and global scales. The organizers wish to express their most sincere appreciation to the scientific committee for their help before the meeting (handling abstracts and preparing the scientific agenda), and in general to all participants for their interesting contributions and fruitful discussions arose during the event.

The next edition of REDIBAL, hosted by IPMA, will be held in Lisbon, in 2021.

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ICES-IOC Working Group on Harmful Algal Bloom Dynamics 2018 meeting

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) meeting was hosted by Dr. Margarita Fernandez-Tejedor at IRTA, Sant Carles de le Rapita, Spain from 24-28th April 2018. The 2018 WGHABD report will be available at *http://www.ices.dk/community/groups/Pages/WGHABD.aspx*. WGHABD has nine ToRs in its current programme of work. The main focus of the 2018 meeting was around the WGHABD data in the IOC-ICES-PICES Harmful Algal Event (HAEDAT) database and progress towards generating an ICES Harmful Algal Event Status report. This report will form the ICES contribution to Global HAB Status Report (GHSR) which is currently being produced by the Intergovernmental Oceanographic Commission of UNESCO (IOC).

During the 2017 – 2018, progress with HAEDAT data from the ICES area was presented at a HAB data training week at the IOC office for IODE, Belgium, Sept 2017 and ICES WG Phytoplankton and Microbial Ecology, Aberdeen, UK, 2018. An abstract for an oral presentation on Regional Changes in HAB Distribution in the Atlantic Ocean using HAEDAT data was submitted to the Effects of Climate Change on the World's Oceans in Washington DC conference, June 2018. WGHABD also participated in the Symposium for High Throughput Methods in Marine Time Series, Germany and provided input into a number of activities of the IOC GlobalHAB Scientific Steering Committee.

National reports showed that a variety of HABs continue to cause problems in the ICES area. *Pseudo-nitzschia* and Amnesic Shellfish Toxins continue cause widespread problems on the west coast of the USA and Canada and was once again recorded on the east coast. *Karenia brevis,* brown tides and *Cochlodinium* blooms also caused problems in USA. Shellfish toxins again caused problems in Europe; however in some areas (Ireland) closures were of a shorter duration. There were a number of canine fatalities in the UK resulting from dogs eating starfish and fish that contained high concentrations of PSP toxins that had been washed ashore after a storm. Ciguatoxins were once again detected in fish from the Canary Islands. *Pseudochattonella* were observed in Danish, Swedish and Norwegian waters and cyanobacterial blooms were recorded in the Baltic. Tetrodotoxin (TTX) was again recorded in shellfish from Dutch waters but below the threshold level. Testing of historic shellfish samples in Ireland did not reveal the presence of TTX.

New findings included a review of ongoing projects looking at satellite imagery and generating early warning of HAB events, dynamics of HAB species along the Catalan coast, molecular methodologies, bioanalytical devices for the detection of HAB species and oomycete parasites of *Pseudo-nitzschia*. An update about the OSPAR intermediate assessment and the Marine Strategy Framework Directive (MSFD) was presented. An overview of projects underway to address the emerging risk from Ciguatera Fish Poisoning in Europe, and to investigate analytical methodologies and toxicity of *Gambierdiscus* strains from European waters were presented.

The next meeting of WGHABD will be hosted by Wenche Eikrem at the Natural History Museum in Oslo, Norway from the 2-4 April 2019. WGHABD will continue to work with HAB event data, review how physical, chemical and biological interactions control the dynamics of selected harmful micro-algae; discuss Ciguatera Fish Poisoning (CFP) as an emerging issue in the ICES area and provide an update of CFP incidences, present national reports of harmful algal events, and finally review species specific HAB detection methods and other cutting edge technologies that are now moving from research towards operational use.

WGHABD is open to experts from all IOC Member States. Experts from outside the ICES region interested in joining the WG (at own expense) please contact the WGHABD chair or the IOC.



Fig. 1. Participants on the ICES-IOC Working Group on Harmful Algal Bloom Dynamics, Sant Carles de la Rapita, Tarragona, 24-28th April 2018

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IOC/WESTPAC training workshop on introductory scientific diving for benthic dinoflagellate sampling and processing

A week-long IOC/WESTPAC training workshop was held in Phuket, Thailand from 17-21 September 2018, with the participation of more than 30 young researchers, fishery officers and graduate students from six countries in the WESTPAC region (Fig. 1).

In view of the lack of scientific diving training and limited research capacity for benthic dinoflagellates in the WESTPAC countries, this training workshop aimed to train young scientists and the government authorities in the region with the standard protocols for introductory scientific diving, underwater sampling, sample processing, culturing and identification of marine benthic dinoflagellates.

This workshop was an intensive high-level training held in the WESTPAC region on carefully defined scientific subjects, presented by several tutors of international standing. Trainees and invited lecturers were grouped into two concurrent training programmes, respectively on: (1) introductory scientific diving, and (2) sample processing, identification and culturing techniques for benthic dinoflagellates.

The scientific diving programme was led by Dr Leo Lai CHAN (City University of Hong Kong), Lawrence Long CHAN and their scientific diving team from the Sea Dweller Underwater Academy: Ting HAN, Wing Kin FU, Ki Chun YIP and Walter Ernesto DELLISANTI. This programme was conducted through lectures and both pool and open water training. Dr Leo CHAN delivered lectures on the background and history of scientific diving. He also explained the importance of scientific diving training and its applications in different underwater scientific research. HAN and FU trained the students not only with basic scientific diving techniques such as advance kicking, buoyancy control etc., but also methodologies for collecting biologically relevant data including the use of transects, quadrats, sample bottles and bags, etc. They also trained the students on how to manage risks encountered and perform multitasking missions. YIP and DELLISANTI demonstrated how to use various scientific equipment including 'Coral Watch', 'Coral Finder", Benthic and Epiphytic Toxic Algae (BETA) sampler (Fig. 2), and Coral in Situ Metabolism and Energetics (CISME) (Fig. 3). All students obtained hands on experience with this equipment in both confined and open water environments. At the end of the training workshop, all students were aware that scientific diving systems serve a two-fold purpose: (1) a research support function that assists the diving scientist with specialized underwater equipment, advice, and diver support, and most importantly (2) a risk management function that protects the safety and health of the individual scientist, and the employing organization from excess liability exposure.

"The scientific diving training surely provided me with profound insights on what skills we should have and how to achieve scientific missions effectively," said Lalita Putchim, a researcher from Phuket Marine Biological Center, Thailand,

Another group of trainees focused mainly on marine benthic dinoflagellates, its sampling methods, identification, cell isolation techniques, culture establishment and maintenance. A team of invited lecturers consisting of Drs Po Teen LIM (University of Malaya), Pengbin WANG and Douding LU (Second Institute of Oceanography, State Oceanic Administration of China). This session was conducted through a series of lectures, and hands-on exercises about the application of the single-cell isolation by micropipetting technique, culture media selection and culture preparations.

Lim delivered lectures about HABs in the WESTPAC region, recent emerging HAB related issues and current knowledge gaps in HABs, focusing on ciguatera fish poisoning and the ben-



Fig. 1. IOC/WESTPAC Training course on scientific diving for marine dinoflagellate research, Phuket Marine Biological Center (PMBC), 17-21 September 2018.

thic dinoflagellates. He also shared the sampling methods using artificial substrates as well as his research findings in Malaysian waters. Wang demonstrated the techniques of cell isolation, culture establishment, medium preparation and culture maintenance. Lu, while lecturing on taxonomy of benthic dinoflagellates, emphasized the need to obtain not only morphological data but also molecular evidence to support species delineation.

All participants practiced observing and isolating dinoflagellate cells from the natural substrate samples collected from the nearby shore and coral reefs (Fig. 4). Several genera of harmful dinoflagellates were identified and isolated for culture establishment, including *Ostreopsis*, *Prorocentrum*, *Coolia* and *Amphidinium*. Participants also learned the techniques of medium preparation, setting up culture collection, maintenance and management of culture collection during the practical sessions.

The ensueing plenary discussions culminated in an agreement that participants from China, Indonesia, Malaysia, Thailand, Philippines, and Vietnam would adopt the Benthic HAB artificial substrate sampling method (Tester et al. 2014, Yong et al. 2018) and committed to carrying out comparative studies at the coral reefs and seaweed beds of the respective countries. Participants expect more workshops on ciguatera fish poisoning and benthic dinoflagellates will be organized in the near future in this region to address the emerging issues in seafood safety.

This event was co-organized by the UNESCO/IOC Sub-Commission for the Western Pacific (WESTPAC) and the Phuket Marine Biological Center (PMBC), with generous financial support of the Thai National Commission for UNESCO, and great technical assistance of the City University of Hong Kong (CityU), Sea Dweller Underwater Academy (SDUA), University of Malaya (UM), and the Second Institute of Oceanography (SIO) of the State Oceanic Administration of China.

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Fig. 2. BETA sampler training in open water environment.

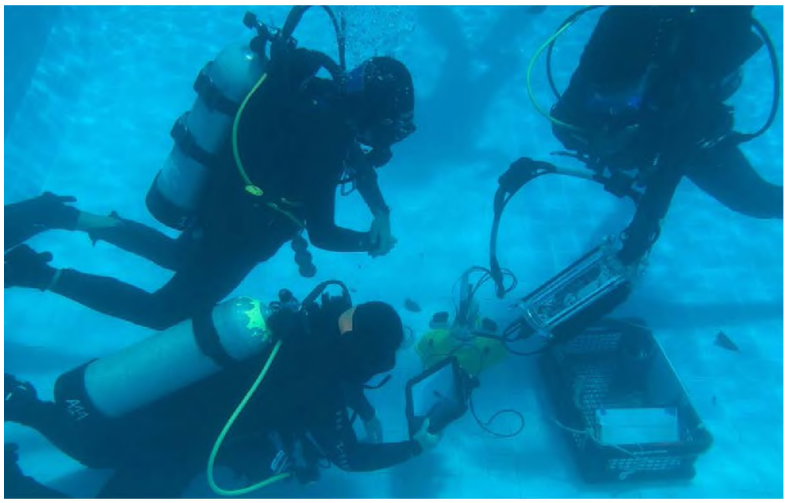

Fig. 3. CISME training in a confine pool.



Fig. 4. Microscopic observation of benthic dinoflagellate cells from samples extracted from seaweed and sediments.

SEAFDEC-MFRD Regional Training Course on Harmful Algal Bloom Monitoring and Studies

A regional training course (TC) on HAB Monitoring and Studies for the Southeast Asian participants was successfully completed from July 8-14, 2018. The TC took place at Bachok Marine Research Station (BMRS), Institute of Ocean and Earth Sciences (IOES), University of Malaya (UM), Kelantan, Malaysia, jointly organized by SEAFDEC-Marine Fisheries Research Department (MFRD), Agri-Food and Veterinary Authority of Singapore (AVA), IOES, UM and IOC/WESTPAC HAB Program. This is part of the continual efforts of SEAFDEC/MFRD on capacity building for HAB monitoring and research in the region. Twenty-one officers from 10 Southeast Asian countries attended the course: Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, Singapore and Vietnam (Fig. 1). This marked the second TC of a series of training courses by SEAFDEC-MFRD.

The training course was structured with the main goal to equip the relevant Southeast Asian fisheries personnel with the knowledge required to implement a systematic HAB monitoring program and to enhance expert capacity in HAB research and development.

In the welcome address of Dr. Po Teen Lim, Head of Bachok Marine Research Station, IOES, UM and the Chair of this TC, he highlighted the seriousness of the impact from HABs and urgency to address the HAB issues in the region. He further emphasized the importance of enhancing capacity building in the effort to minimize the impact of HABs on seafood safety and sustainability of rapid growing mariculture industries. Mr. Soon Eong Yap, Chief of SEAFDEC/MFRD in his opening comments, read by the representative from SEAFDEC/MFRD extended their appreciation to IOES for hosting the event. He stressed that the agency will continue to implement programs to enhance capacity building, in particular relating to post-harvesting technology.

The 7-days training course has been designed to expose participants to several aspects of harmful algae monitoring and investigations, these included experience of microalgae culturing, maintenance, species identification and detection. The TC was divided into two sessions: "Techniques in Culturing and Maintenance of HAB species", and "Basic Techniques in Molecular Characterization and Detection of HAB Species". The course provided extensive hands-on components and in-depth lectures. Participants had the opportunity to work in the laboratory, handling light and fluorescence microscopes, flow cytometry, and molecular instrumentation such as PCR and real-time qPCR.

An introductory lecture was given by Dr. Po Teen Lim on the overview of HABs and increasing trend of HAB events in the Southeast Asian region. He stressed the importance to have precise identification, especially in HABs monitoring, in order to advise mariculture operators on the correct preventive measures.

In this TC, Dr. Mitsunori Iwataki (Asian Natural Environmental Science Center, University of Tokyo) gave comprehensive lectures on the identification and taxonomy of unarmored dinoflagellates and raphidophytes. Prof. Dr. Haifeng Gu (Third Institute of Oceanography, China) presented two lectures on Introduction to Cysts and Azaspiracid producers in the region. The importance of cysts in the life cycles of dinoflagellates and their roles in bloom dynamics was discussed in his first lecture (Fig. 2). Lecture on harmful diatoms, including *Pseudo-nitzschia*, was given by Dr. Hong Chang Lim (Tunku Abdul Rahman University College). He explained the taxonomy requirement on a detailed assessment of the frustule structure under advanced electron microscopy in *Pseudo-nitzschia* identification. The use of the online tool "3i Interactive key and database on *Pseudo-nitzschia*" was demonstrated by Dr. Sing Tung Teng (University Malaysia



Fig. 1. Regional Training Course on Harmful Algal Bloom Monitoring and Studies, BMRS, IOES, University of Malaya, 8-14 July 2018.

Sarawak), who has developed several web-based interactive keys of harmful microalgal species [*http://dmitriev.speciesfile.org/key.asp?key=Bacillariales&lng=En&i=1&keyN=2*].

Following the lectures on specific topics, a series of extensive hands-on and lab activities were conducted. The participants learned various culturing techniques such as culture medium preparation, single-cell isolation (Fig. 3), cell counts for growth rate estimation. Species identification using light and fluorescence microscopy has been demonstrated and hands-on by the participants (Fig. 4). Later the application of flow-cytometry in assessing the changes in population dynamics during a bloom event was introduced by Dr. CP Leaw. Throughout the lab activities, participants managed to identify species from cultured specimens using both Imamura-Fukuyo (IF) and calcoflour white staining.

Molecular detection techniques including genomic DNA isolation, gene amplification by PCR were taught in this TC by Dr. Kieng Soon Hii (UM) and others. Dr. Leaw later demonstrated real-time quantitative PCR (qPCR) for rapid detection and quantification of harmful species. In the following session, a basic tutorial on phylogenetic analyses was delivered by Dr. HC Lim.

A lecture on PSP toxin and the detection methods was delivered by Dr. PT Lim. In this session, an ELISA kit for PSP toxin detection developed by Nissui Pharmaceutical was introduced and demonstrated to participants during the TC.

The TC was ended with a discussion session, where Dr. Iwataki, the Project Leader of IOC/WESTPAC-HAB briefed the participants on the regional activities in the Western Pacific region. He also encouraged participants to strengthen the existing networking and collaboration. He pledged to continue supporting HABs activities (seminars, training courses) through expertise and reference materials in the region. The TC was finally closed by the representative from SEAFDEC, Mr. Yihang Ong, expressing his sincere thanks to IOES, IOC WESTPAC for making the TC a success.

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Fig. 2. Question and answer session with the facilitators, Prof. Dr. Haifeng Gu and Dr. Chui Pin Leaw.



Fig. 3. Participants actively involved in single-cell isolation.



Fig. 4. Participants were exposed to various microscopic techniques in species identification; a participant from Thailand using a fluorescence microscope.

XVIII International Conference on Harmful Algae

Dear participants,
As chairman of ICHA 2018, and on behalf of the scientific and international advisory committees, I am pleased to present you the abstract book for ICHA 2018 (*www.icha2018.com*).

We are delighted to have a rich set of 621 contributions from 64 countries for which I thank all authors. This edition should be an interesting event with over 700 participants. The program contains 9 plenary and 254 oral presentations in 45 parallel sessions, as well as 45 ignite and 358 poster presentations. While the large interest is probably partly due to the two previous ICHA conferences taking place in the Southern hemisphere, we believe that it is also a testimony to a persistent research effort on harmful algae, a continuing and, in many places, growing problem for society. In line with the design of our French research network GdR PHYCOTOX and other international efforts, e.g. GlobalHAB, we have attempted to include all aspects from the causes of micro-algae and cyanobacteria to their impacts on both society and ecosystems; hence our subtitle FROM ECOSYSTEMS TO SOCIO-ECOSYSTEMS.

While the choice of topics is a continuation from the previous conferences, I would like to take the opportunity to thank all the scientific committee members who accompanied me with a lot of enthusiasm in designing the conference, especially those studying impacts. The scientific committee consisted of 45 scientists from the GdR PHYCOTOX and the GIS CYANO research networks and supported the organisation by formulating the large set of topics and by suggesting plenary speakers and international session chairs. The international session chairs, as well as members of the ISSHA Council conference-subcommittee, also helped the scientific committee in the evaluation of submissions. We went through two rounds of evaluation (one blind and one with affiliations known). Criteria for selection as oral or poster presentation were based on scientific novelty, societal interest of the contribution, gender and geographical balance and publication status. We also made every effort to balance contributions from experienced and young scientists as much as possible in the oral and ignite sessions.
I would also like to very heartily thank our 30 sponsors whose participation also testifies toward the societal importance of HAB-research. In particular, the support of ISSHA, NOAA, SCOR, IAEA and GdR PHYCOTOX has helped many (ca. 60 young) scientists to attend, and 137 student contributions will be evaluated for the Maureen Keller Best Student awards.I hope you all draw inspiration from this compilation – enjoy the read !

Philipp Hess,
Chair of the ICHA 2018, Nantes, France



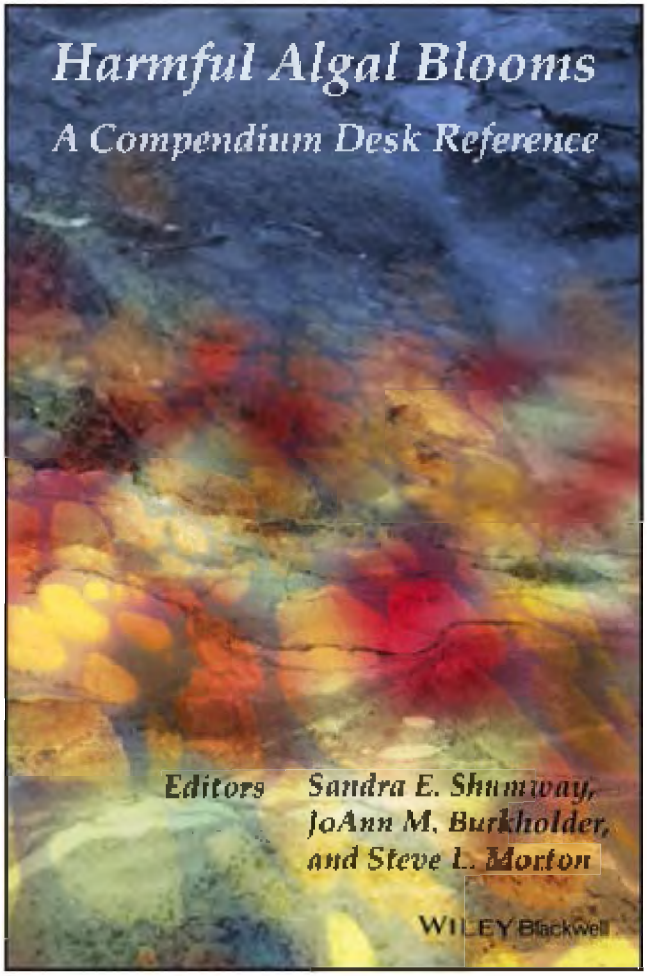
New books

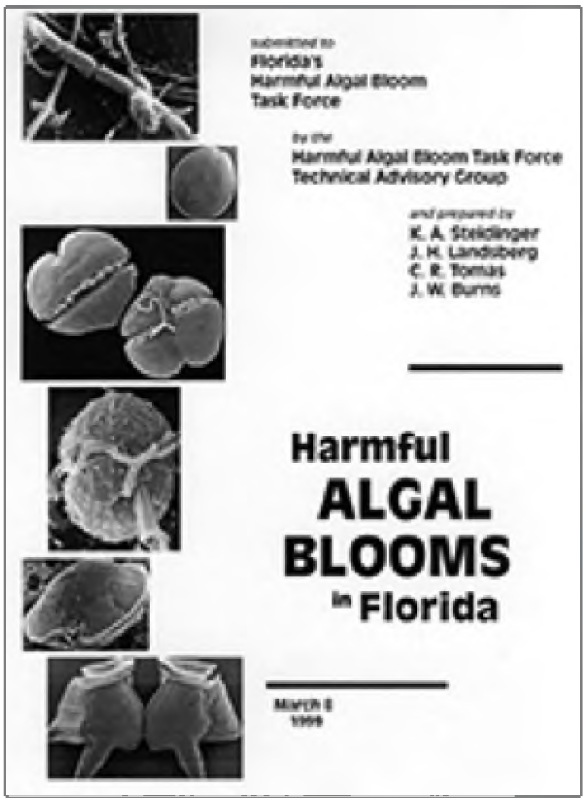
Harmful Algal Blooms: A Compendium Desk Reference

Shumway,Burkholder & Morton (Eds)
This book provides basic information on harmful algal blooms (HAB) and references for individuals in need of technical information when faced with unexpected or unknown harmful algal events. Chapters in this volume will provide readers with information on causes of HAB, successful management and monitoring programs, control, prevention, and mitigation strategies, economic consequences of HAB, associated risks to human health, impacts of HAB on food webs and ecosystems, and detailed information on the most common HAB species.

A valuable resource to managers, newcomers to the field, those who do not have easy or affordable access to scientific literature, and individuals who simply do not know where to begin searching for the information needed, especially when faced with novel and unexpected HAB events.

More information on: *https://www.wiley.com/en-us/Harmful+Algal+Blooms%3A+A+Compendium+Desk+Reference-p-9781118994658*





Guide to the Identification of Harmful Microalgae in the Gulf of Mexico

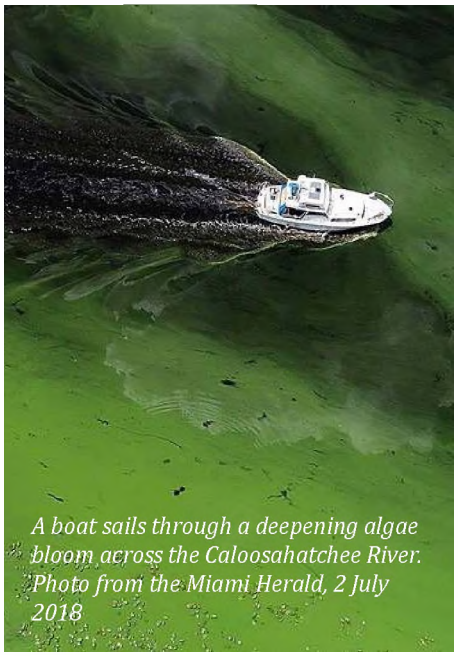
Steidinger, Landsberg, Furnas & Burns
This guide was developed for analysts and managers world-wide involved in marine HAB monitoring, assessment and forecasting. The Guide is the result of two initial grants from the Environmental Protection Agency's Gulf of Mexico Program and with cooperation from the University of South Florida, Florida Institute of Oceanography, Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute, Universidad Autónoma Metropolitana, Unidad Iztapalapa Ciudad de México and private funds.

It is available via a free download from: *http://myfwc.com/research/redtide/research/scientific-products/*.

Devasting *Karenia* and cyanobacterial blooms in Florida, USA

On the Gulf Coast of Florida, USA, there is an ongoing HAB attributed to *Karenia brevis.* Depending on the concentration of cells, the water can look red or brown, particularly from the air. Waves crashing on shore may look reddish or muddy. Brevetoxin, produced by *K. brevis*, has killed ocean wildlife, including fish and sea turtles. Brevetoxin has become incorporated into sea breezes and caused respiratory irritation in beach visitors.

Florida is also experiencing a large freshwater cyanobacteria bloom in Lake Okeechobee that is affecting the St. Lucie and Caloosahatchee Rivers. Exposure to gases, such as hydrogen sulfide and methane, emitted as the blooms senescence have been associated with adverse health effects in people. There have been reports of animal poisonings from exposure to cyanobacterial toxins.



A boat sails through a deepening algae bloom across the Caloosahatchee River. Photo from the Miami Herald, 2 July 2018

Cyanobacterial blooms in Florida:
http://www.floridahealth.gov/environmental-health/aquatic-toxins/cyanobacteria.html
http://myfwc.com/research/redtide/general/cyanobacteria/

Current information about *K. brevis* blooms from the Florida Wildlife Commission: *https://floridadep.gov/Algal-Bloom; https://oceanservice.noaa.gov/news/redtide-florida/*

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Deadline
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The 18th International Conference for Harmful Algae

Twenty five years after the 6th *International Conference on Toxic Marine Phytoplankton* (6th ICHA, 1993), experts on harmful algal blooms and phycotoxins met again in Nantes, the beautiful capital of the Loire-Atlantique, to participate in the *18th International Conference on Harmful Algae* convened by ISSHA. The conference was hosted by IFREMER, France from 21th to 26th October 2018. The local organizing committee was chaired by **Philipp Hess**, with the support of the French PHYCOTOX society.

As in 1993, this event was held in the Conference Centre at the Cité des Congrés, a few minutes' walk from the Château des Ducs de Bretagne, a major landmark in the city. For HAB experts, particularly those interested in *Dinophysis*, Brittanny and the Loire-Atlantique region with the Loire and Vilaine river estuaries, is famous for being a hot spot of *Dinophysis* and DSP outbreaks in western Europe. That was the focus of Patrick Lassus, the local organizer of the HAB conference in 1993, when he coined the term "*Dinophysis acuminata* complex" to label a continuum of cell morphotypes difficult to ascribe to *D. acuminata* or to *D. sacculus*, recently reviewed in the monograph by Lassus et al. (Fig. 1). Participants were welcomed on Sunday evening with an ice-breaker at La Cité (Fig. 2), and got a taste of the local gastronomy and traditions during the Bretonne Party on Tuesday night (Fig. 3). They were taken again on board a boat along the Erdre River for the Conference Dinner, this time at the Château de la Poterie.

The 18th ICHA, had the largest participation of all ICHA's to date with 709 delegates from 64 countries registered. This exceeded the record from the 10th ICHA in St Pete's Beach, Florida, 2002. Gone are the old days when all of the oral contributions were given in plenary sessions. At the 18th ICHA, delegates had the program, downloaded onto their mobile phones, and selected their presentation of choice from two or even three parallel sessions. The theme of the 18th ICHA conference was "From Ecosystems to Socio-Ecosystems". Delegates were kept busy over the five days with three parallel sessions containing 45 ignite talks, 255 oral presentations and 358 poster presentations. This was a lot of hard work for the local organizers (Fig. 4) and the Scientific Steering Committee. In the background, IS-



Fig. 1. Drs Philipp Hess (left) and Patrick Lassus (right), from IFREMER, Nantes, local organizers of the 18th ICHA (2018) and 6th ICHA (1993) respectively, signing copies of their monograph (see book on the right) during the conference in Nantes, 21-26 October 2018.

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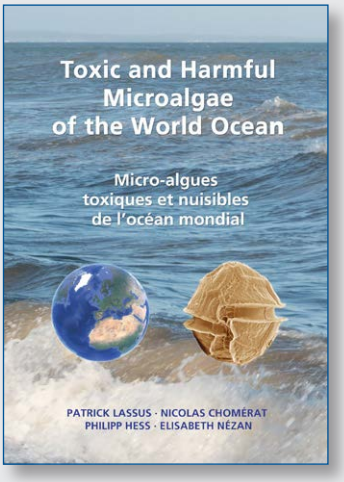




Fig. 2. Ice-breaking at "La Cité", Nantes, during the 18th ICHA, 2018



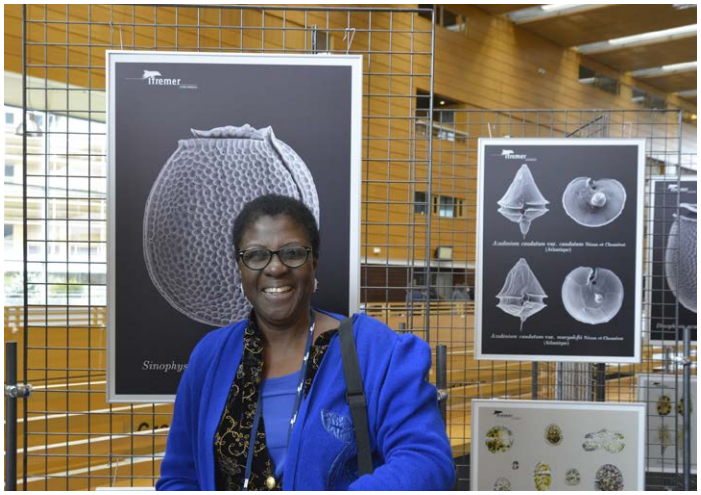
Fig. 3. Live Folk music for the participants during the conference "Bretonne Party"

SHA society members were kept very busy, fully engaged with the evaluation of student presentations to choose the candidates for the Maureen Keller awards, the election of Yasumoto lifetime achievement awards, voting for future conference venues, new ISSHA committee elections and the popular ISSHA auction (see ISSHA's Corner).

Philipp Hess, IFREMER, Nantes, France, Eileen Bresnan, Marine Scotland, UK & Beatriz Reguera, IEO, Spain



Fig. 4. Philipp Hess (Chair), with the French Local Organizing Committee, receiving an ovation from the conference participants during the Closing Ceremony



















Scientific and social scenes from participants during the 18th ICHA, Nantes, 2018.

Scientific Highlights of the 18th International Conference on Harmful Algae

Plenary talks and Yasumoto awards

Each morning and afternoon session began with plenary speakers who covered a number of topics including: climate change impacts on inland and coastal eutrophication (Anna M. Michalek), chemical studies on toxic cyanobacteria (William Gerwick), 21st century alternative methods for 21st century safety sciences (Thomas Hartung), parasites to control HABs (Laure Guillou), trait based frameworks for understanding and predicting HABs (Elena Lichtman), cyanobacteria in Danish and Florida lakes and their response to nutrient loading reduction, biomanipulation and climate warming (Erik Jeppesen) and ciguatera risk assessment and management (Mireille Chinain). The two 2016 Yasumoto award presentations, "An emerging chemical ecology paradigm" (Allan Cembella) and "The evolution of algal toxin measurement science" (Mike Quillam) were presented on the last day.

Taxonomy

Taxonomy presentations covered a range of topics. The discovery of two classes of non-photosynthetic cyanobacteria, Melainabacteria and Sericytochromatia, using genomic data (Nico Salmaso) suggests a later evolution of photosynthetic cyanobacteria than previously considered. Efforts to establish a classification of photosynthetic cyanobacteria based of genomic data (Muriel Gugger) suggest changes to the present classification, e.g the presence of only one *Microcystis* species. Cécile Bernard reported on the diversity of cyanobacteria from an extreme environment analogous to marine paleoenvironments. A new exciting dinoflagellate belonging to the Kareniaceae was described, with differing pigment composition and containing an eyespot which is mainly seen in freshwater dinoflagellates (Kazuya Takahashi). A major fish kill in Malaysia in 2016 was studied by Chui Pin Leaw, who found it to be caused by a hitherto undescribed *Chattonella* species differing genetically from *Chattonella* species already recorded. Masao Adachi found considerable diversity in *Prorocentrum* from Japanese waters. Among the 244 strains, which all were found to be diarrhetic shellfish toxin producers, two new species were recorded and a very high genetic diversity was observed within *P. lima*.

Carlos Eduardo Tibrica reported on the evolution of a semiautomated imaged-based identification program for *Gambierdiscus*. Sirje Sildeveer and Haifeng Gu presented detailed studies expanding current knowledge on the genetic diversity of *Alexandrium ostenfeldii* and *Azadinium poporum*, respectively. Advances in understanding the confusing taxonomy of *Ostreopsis* were presented by Helena David and Olga Carnicer. *Ostreopsis* cf. *siamensis* was suggested to be renamed *O. rinoi*, and the "true" *O. siamensis* was suggested to belong to an unnamed clade comprising strains from the Thai type locality. Furthermore, two taxa of *Ostreopsis* were reported from Galapagos waters for the first time.

Ecology

Presentations covered the impact of herbivorous predation on domoic acid production in *Pseudo-nitzschia* (Nina Lundholm) and interspecific variability was demonstrated by the different migratory behaviour of *Dinophysis* in the Ria de Vigo (Beatriz Reguera). A trait based approach was applied to metabarcoding data to investigate protist functional diversity across size-fractionated coastal planktonic communities (Pierre Ramond).

New investigative approaches to address ecological questions were presented. The *Within Outlying Mean index* (WitOMI) approach was used to investigate the niches of harmful algae using the REPHY monitoring data from France (Karasiewicz), while a Gaussian mixture was used to investigate the phenology of phytoplankton blooms in the Southern Bight of the North Sea (Lefebvre).

Alexis Fisher presented information from a study in Massachusetts on how dormancy length in *Alexandrium catenella* cysts is modulated by temperature, with dormancy shorter with colder treatments. Dormancy of cysts at 6°C, the average bottom water temperature of the Gulf of Maine, was a full year. Raffaele Siano used metabolomic analyses to investigate differences in phosphate utilization on cysts germinated from sediments up to 20 years old and compared with those from present day. The results of this study suggest an intra-specific variability of the phosphate utilization between phenotypes of dinoflagellate of different ages in Brittany.

Leonardo Guzmán showed that the exceptional blooms of *Alexandrium catenella* in Chile during summer 2018 were highly influenced by a particular suite of coastal hydrographic conditions, which led to southeast winds induced northwards physical transport of cells from northern Aysén to the southeast of Chiloé Island and their export off the fjords. These particular coastal oceanographic conditions were influenced by large scale climatic patterns.

Omics

The polyketide synthase (PKS) pathway in dinoflagellates was investigated (Allen Place) showing them to have two parallel pathways for making fatty acids and polyketides. Results showed the presence of a transcript does not predict formation of the protein, and homology to a model system homolog does not provide a lot of information about whether the toxin will be present in dinoflagellates.

Shaun McKinnie, John Brunson and Sara Hardardóttir presented studies investigating the synthesis pathway of domoic acid (DA). Benjamin Kramer looked at transcriptome and physiology of *Dolichospermum* in response to N and P addition.

Sylke Wohlrab investigated *Alexandrium ostenfeldii* populations in two different locations, the established population from the Baltic Sea, and a small creek in the Netherlands where the population is recently established. Results showed traits under stabilizing selection are enriched in genes that have adaptive evolution. Neutral traits show lower adaptation/evolution in genes. Trait variability in the Baltic Sea is driven by gene variants, while plasticity is more common in the Netherlands. The different adaptation strategies between the populations could potentially be due to evolutionary age.

Maria Immacolata Ferrante investigated the *Pseudo-nitzschia multistriata* sexual cycle and found the gene that determines the mating type in this pennate diatom. *Pseudo-nitzschia* is found globally in the Tara Oceans dataset and the genes expressed during the sexual phase can track this important but overlooked event.

Cyanobacteria

Advanced technologies for the early warning of cyanoHABs including optical detection and drones are an exciting and important field that will continue to significantly aid traditional monitoring. (Jean-François Humbert, Yi Hong; Chris Bolch; Dianne Greenfield; Bengt Karlson; Sara Imran Khan). Long term reductions in N and P has led to the mitigation of cyanoHABs (Eric Jeppesen) and changes in N, P, and CO_2 can alter toxin production (Dedmer Van de Waal, Benjamin Kramer; Declan Maxwell). Cyanobacteria can be harmful to human health (Zorigto Namsaraev; Louise Vanysacker; Bojan Sedmak; Barbara Kubickova; Lenka Šindlerová), but they can also be a source of novel pharmaceuticals. (William Gerwick). The field of Omics continues to mature, facilitating new discoveries and advancing our understanding of cyanoHAB physiology, interactions, genetic diversity and opening the door for new genera to be discovered. (Anusuya Willis; Barbara Weisbrod; Todd Miller; Yingzhong Tang; Gorenka Bojadzija; Cécile Bernard; Maxime Georges Des Aulnois; Benjamin Marie; Leonardo Cerasino; Yves Terrat; Jean-François Briand; Jennifer Jankowiak; Fatma Zohra Guellati; Alexandre Campos). An emerging and important 'Omics' is socioeconomics (Chloé L'Ecuyer-Sauvageau; Olokotum Mark).

Toxins

Nathalie Arnich presented an ANSES Opinion on Acute and Chronic Toxicity of BMAA. BMAA is neurotoxic but the causal link between exposure to BMAA and the occurrence of ALS is not proven, in the current state of knowledge. Tim Harwood presented a method for simultaneous monitoring of maitotoxin and miguatoxins in algae and fish extracts, resolving a difficult analytical challenge via tandem mass spectrometry. Mark Poli presented a novel intraperitoneal and aerosol administration in rats to investigate the toxicity and pathophysiology of palytoxin congeners.

Ecophysiology and cellular biology of harmful algae and cyanobacteria

Angela Falciatore explored the molecular basis of responses to light in marine diatoms and found that the functional diversification of the light-harvesting complex family specialized in photoprotection (LHCXs) allows diatoms to respond to a highly variable ocean environment. Yingzhong Tang demonstrated that *Karenia mikimotoi* can produce sexual, thin walled, resting cysts which are widely distributed in the East China Sea. Irradiance seems not to have a significant effect on growth rate and toxin production for *Prymnesium parvum* in culture while nutrient limitation increases prymnesin content (Nikola Medić). Anusuya Willis observed a high variation in growth rate and toxin content between strains of *Raphidiopsis raciborskii* and this variability is also observed at a genomic level. The concentration of domoic acid in cells of *Pseudo-nitzschia australis* can be predicted from the cell size by a Gaussian model which shows an increase in domoic acid production when *Pseudo-nitzschia australis* cells become gametangia (Aurore Sauvey). Martin Pfannkuchen examined different mechanisms for competition for nutrients in harmful algae from the Adriatic Sea. Stefano Accoroni showed *Ostreopsis* blooms in inorganic phosphorus limited environments may be maintained by the use organic phosphorous through alkaline phosphatase activity. A review of the autoecology of bloom forming algae from the Gulf of California showed it is necessary to account for the interactions between species when investigating phytoplankton dynamics, for example *Gymnodinium catenatum* can be consumed by *Noctiluca scintillans* and lysed in presence of *Chattonella marina*. A variety of cyanobacterial toxins may co-occur at the same time in Green Bay, USA which presents difficulties with the water quality control. The detection of the anatoxin-a and saxitoxin genes is not always coincident with the presence of these toxins (Todd Miller). In Lake Taihu (China), *Microcystis aeruginosa* gene expression patterns are mainly linked to conductivity and nutrient availability (Xainming Tang). The photosynthetic activity of a toxic strain of *Microcystis aeruginosa* is decreased by metabolites produced by *Daphnia magna* but this was not observed to the same extent in the case of the nontoxic strain of *M. aeruginosa* (Gorenka Bojadzija).

Parasites

Reviewing important concepts in parasitology in order to answer the question "Can we use parasites to control HABs?" reveals that a better understanding about the dynamics of both host and parasites is needed (Laure Guillou). Caution should be taken when interpreting results obtained from network analysis of high-throughput sequencing as correlation between host and parasites interactions does not necessarily imply causality (Catharina Alves-de-Souza)

Accumulation and biotransformation of toxins

Pedro Reis Costa investigated transformation of algal toxins in the food web from Portugese waters. Cephalopods found to be intoxicated with DA showed long term retention of the toxins. Ev-

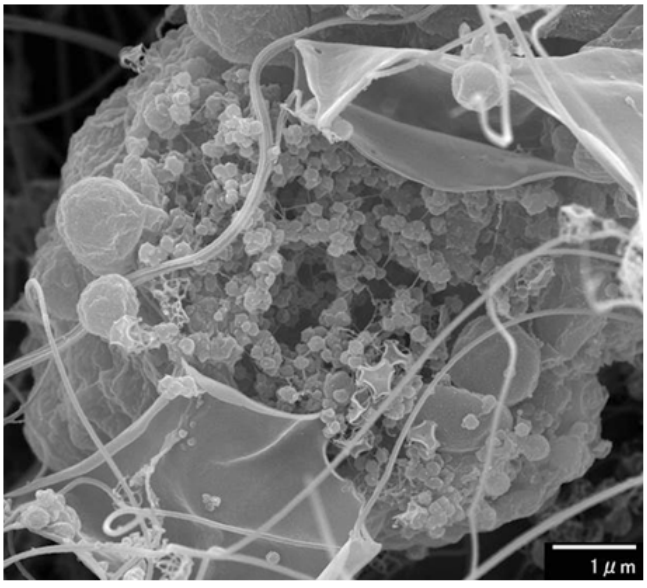


Fig. 1. Numerous virus particles produced in the cytoplasm of a bloom-forming dinoflagellate H. circularisquama. Photo courtesy of Takano et al from Viruses 10 (10): E554 (2018).

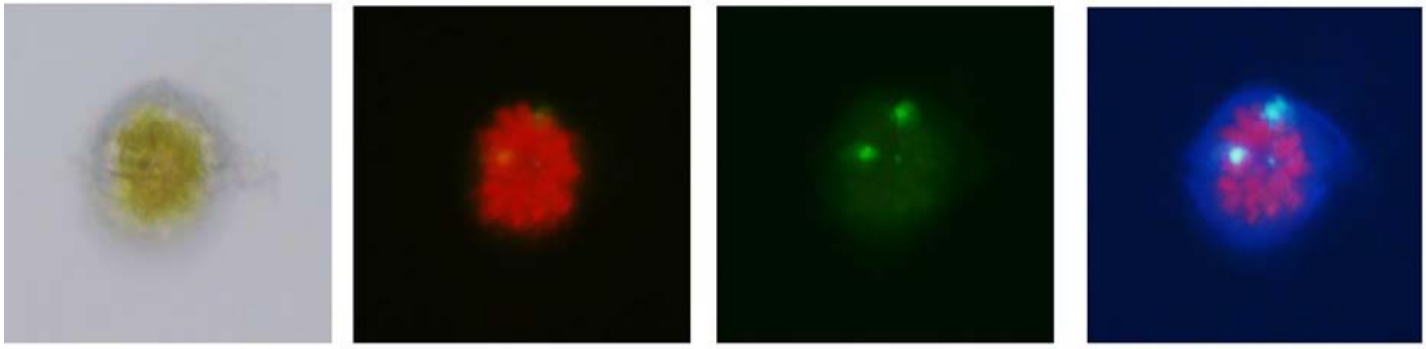


Fig. 2. Extracellular vesicles in Alexandrium minutum. From left to right: Vegetative cell under light microscopy; epifluorescence microscopy showing the cell chloroplasts in red; vesicles in green due to lipid stain PKH67, and composite of the epifluorescence images, theca of the cell is in blue, chloroplasts are shown in red, stained vesicles are shown in light blue. Photo courtesy of Esther Garcés

idence of PSP toxin profile changes were observed during trophic transfer events. Top predators showed dissimilar profiles to source algae and initially contaminated oganisms.

Aifeng Li presented data from outbreaks of PSP in China 2016-2017. *Mytilus galloprovincialis* converted some toxins into M toxin analogues within 7 days of contamination but these comprised a relatively small proportion of total toxins present and were transformed in the hepatopancreas of mussels and scallops. M toxins are hypothesised to have toxicities similar to C toxin analogues. M toxins also found in trace levels within *Alexandrium pacificum.* M toxin quantification is tentative as standards are not available.

John Kristoffer Andres presented data from green lipped mussels which are the primary PST vector in the Philippines. During experimental exposure a cycle of PST uptake and depuration was observed. Within the first 24 hours toxins were primarily detected in the mantle, later the highest concentrations appeared within the digestive tract. Variability across treatments in both uptake and depuration was high. During the course of the study a conversion of toxins from GTX1/4 to GTX 2/3 was observed.

Biological oceanography and limnology of HABs

This interesting session contained many presentations using observations of the distribution of HAB organisms together with observations and models of physical oceanography. These different factors all contribute to improved understanding of HAB dynamics. Investigations presented include cyanobacteria blooms in the Baltic, the USA and Brazil (Norbert Wasmund, Fabiola Martins), *Phaeocystis* in the English channel (Arnaud Louchart), *Dinophysis* in Sweden, Spain, Scotland and Ireland (Bengt Karlson, Paul Dees, Robin Raine), *Azadinium* and AZA in Ireland (Stephan Mcgirr), *Pseudo-nitzschia* in Chile and the USA (coast of California) (Maximo Frangopulos, Holly Bowers), *Alexandrium* in Chile and in the USA (Oscar Espinoza-Gonzalez, Leonardo Guzman, Mike Brosnahan), *Karenia brevis* in the Gulf of Mexico (Richard Stumpf), dinoflagellate cysts in Morocco (Chaira Karima), *Microcystis* and microcystins in a New Zealand lake (Barbara Weisbrod). Presentations focusing on modelling and prediction used Individual Based Model combined with observations based on satellite ocean colour to examine *Karenia brevis* in the Gulf of Mexico (Darren Henrichs), Operational tools in Galicia, Spain (Yolanda Pazos), *Dinophysis* in the Shetland Islands (Paul Dees). Novel automated methods were used in a detailed study of *Pseudo-nitzschia* in Monterey Bay (Holly Bowers), high temporal resolution studies of HAB using the Imaging Flow Cytobot in the Baltic, the Skagerrak and in the USA (Bengt Karlson, Kaisa Kraft), machine learning and imaging (Guillaume Wacquet), high biomass blooms studied using multi-wavelength fluorometry, PAM and Fast Repetition Rate Fluorometry (Katharine Perri) and enhancement of water temperature by dense dinoflagellate blooms (Mary Ruiz-de la Torre).

Major events and long term time series

Presentations dealt with time series up to 30 years long from France (Catherine Belin), the North Sea (Callum Whyte), Canada (Nicky Haigh), Chile (Benjamin Suarez-Isla) and Asia (Satoshi Nagai) with a number of presentations from fresh waters including canine fatalities in a Berlin lake (Jutta Fastner), toxic benthic cyanobacteria from France (Isidora Echenique-Subiabre) and a review of an outbreak of Haff disease in Russia (Zorigto Namsaraev).

A variety presentations utilising new technologies to investigate HABs included looking at qPCR methods to screen for cyanotoxins (Mark Van Asten), a combination of satellite algorithms and biogeochemical Argo floats to investigate green *Noctiluca* blooms in the Arabian Sea (Vera Trainer) and a microscopy and next generation sequencing approach to investigate waste water treatment (Arash Zamaydi).

Ciguatera and Benthic HABs

A wide ranging and diverse suite of oral and poster presentations relating to benthic HABs and ciguatera were presented over three sessions. A range of topics were addressed covering toxin extraction improvements (Mika Nagae) to CTX detection kits (Takeshi Tsumuraya), to the description of two new *Gambierdiscus* species. Food web toxin transfer paths e.g. in marine invertebrates (Taiana Darius) and the influence of habitat complexity in species composition (Po Teen Lim) are important new areas of research. New efforts to raise awareness of ciguatoxin poisoning among medics in French Polynesia will serve as a model for other endemic areas (Mélanie Tranchet).

Socio Economics

From fishermen's statements on the devastation of whole communities in the Pacific Northwest of the US (Stephanie Moore) to a trip through the Cold



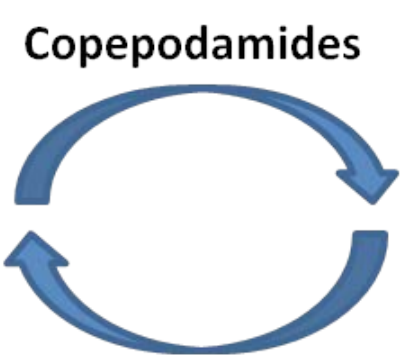




Fig. 3. Co-evolutionary arms race. Diatoms sense presence of copepods via presence of chemical cues (copepodamides), and respond by producing domoic acid which then affect the copepods (reduced escape response). Photo courtesy of Nina Lundholm.

War (Pat Tester) the socio economics session was as diverse as the algal genera causing the problems. Four of the six presentations made were focused on water and resource management and one included citizens monitoring efforts to provide data from African lakes (Mark Olokotum). Cyanobacteria blooms factored heavily in the choices citizens of Quebec made for the quality of their recreational areas (Chloé L'Ecuyer-Sauvageau). Most believed that the government was responsible for the resolution of cyanobacteria blooms. Water quality alert systems using satellite data went beyond the static economic loss to determine the number of fishing/shellfishing bans, mapping the risk of closures from October to December along the French Coast. This provides fishermen the opportunity to relocate to different fishing areas or change gear for another fishery (Pascal Raux).

Global HABs and Climate Change

Research towards predicting the impact of climate change on HABs has progressed from single factor growth experiments (e.g. temperature, pCO_2) with limited HAB strains, to paying due attention to genetic population diversity (Wayne Litaker) and even work with sediment revived culture strains of different ages (Raffaele Siano). High pCO_2 increases cellular toxicity in many HABs, e.g., *Karlodinium* (Nayani Vidyarathna), but a sound working hypothesis of causative mechanism is still lacking. Field work is focusing on learning from extreme events- ENSO 2015-16 "hot blob" and Chile salmon mortality; 2017 US summer cyano blooms (Gregory Boyer), heatwaves, climate and ciguatera habitat hot spots. Modelling efforts are increasing in complexity also focusing on changes in habitat suitability (Anke Kremp), precipitation and nutrient delivery, and even including the role of mixotrophy (Patricia Glibert). Solid global HAB data bases are gradually building up using HAEDAT and OBIS platforms, and notably the efforts by PICES, ICES, FANSA, IAEA working groups. HAB time series analysis approaches are becoming more sophisticated (Chris Gobler, Catherine Belin). Not surprisingly, no single answer is emerging as to whether HABs are /will be increasing or decreasing (Gustaaf Hallegraeff), but this should be examined on a species-by-species, case-by-case, site-by-site basis.





Fig. 4. A. Student researcher placing a battery-powered personal air sampler underneath a lifeguard chair, connected to a filter house attached to the top. B. Stainless steel filter house, connected to a diaphragm membrane vacuum filtration pump hidden inside a beach cabin at the Belgian coast (Ostend). Courtesy of Maarten De Rijcke.

There were six presentations that were flagged by many during the conference.

Yoshihito Takano gave a presentation on the viral infection of *Heterocapsa circularisquama* by a HcDNAV virus. Visualisation of these HcDNAV virions using field emission scanning electron microscopy showed the virions to have a characteristic vertex lump. Most virions were observed to be absorbed on the tranverse groove of the *Heterocapsa* host cells. The presence of a potential viroplasm could be observed beside the host nucleus 11 hours post infection which enlarged with time (Fig. 1). A video showing the dissection of the *Heterocapsa* cell to remove the nucleus earned a spontaneous round of applause from the audience. Sequencing of the HcDNAV virus is in progress.

Esther Garcés presented an investigation into extracellular vesicles (EV)



Fig. 5. IAEA Technical Cooperation project: the Caribbean team having a coordination meeting with the boss.

produced by *Alexandrium minutum* and demonstrated that these EVs were produced in laboratory cultures and *in situ* bloom conditions. The EVs appeared adhered to the cell surface of *A. minutum* cells during incubation experiments but it was not clear if they incorporated them into their cells (Fig. 2). There is ongoing work to identify the composition and functions of the EVs.

Nina Lundholm showed how copepodimides, polar lipids produced by copepods, induced DA production in *Pseudo-nitzschia seriata* cells. All herbivorous copepods tested induced toxin production, whereas carnivorous copepods did not, supporting the theory that the toxin production in diatoms evolved as an inducible defence. However not all diatom strains tested were induced to produce DA by the presence of copepodimides. The escape response of two arctic copepods was reduced after eating DA containing diatoms but the two species were not affected in the same way (Fig. 3).

Pat Tester presented a history of STX and the Cold War which began when a contract for clams containing STX and a shipping receipt to the US Army's Biological Warfare Laboratories at Camp Detrick in the 1950s was unearthed while scanning in 'lost' PSP data from Alaska. STX was being trialled to replace the L-cyanide pill issued to agents during WWII and a dollar with a pin impregnated with STX was issued to Gary Powers who was shot down over Russia in 1960 and exchanged at the 'Bridge of Spies' in Berlin.

Maarten De Rijcke developed a marine aerosol reference tank (MART) which allowed the concentration of toxins in sea spray aerosols (SSAs) to be quantified (Fig. 4). This facilitated the detection and quantification of OA, DTX1 and hYTX in the coast of the Southern North Sea.

Thomas Larsen presented an elegant study examining the chemical diversity of prymnesins by sequencing different strains of *Prymnesin parvum*. This showed that while individual strains produce exclusively A, B, or C-type prymnesins, the chemodiversity was great with more than 100 analogues detected. There was a random geographic distribution of A, B and C prymnesins across the globe however correlation between geno- and chemotype in microalgae was shown for the first time.

The review of the conference science would not have been possible without the valuable help of an army of reviewers:

- Elisa Berdalet & Esther Garcés, ICM (CSIC), Barcelona, Spain,
- Dave Clarke, Marine Institute, Galway, Ireland
- Tim Davis, Bowling Green State University, USA
- Margarita Fernandez-Tejedor, IRTA, Tarragona, Spain
- Chris Gobler, Stony Brook University, USA
- Maarten De Rijcke, Flanders Marine Institute, Belgium
- Gustaaf Hallegraeff, University of Tasmania, Australia
- Bengt Karlson, SMHI, Sweden
- Raphael Kudela, University of California, USA
- Adam Lewis, CEFAS, UK
- Maud Lemoine, IFREMER, France
- Nina Lundholm, Natural History Museum of Denmark, Denmark
- Chris Miles, National Research Council, Canada
- Pat Tester, Oceantester, USA
- Tim Wyatt, Borreiros, Spain
- Adriana Zingone, SZN, Naples, Italy

Eileen Bresnan, Marine Scotland, UK
& Beatriz Reguera, IEO, Spain

Global HABs, Global HAB Status Reporting, and Climate Change

HABs and climate change attracted a record of 139 abstracts at the Nantes Conference. Research towards predicting the impact of climate change has progressed from single factor growth experiments (e.g. temperature, pCO_2) with limited HAB strains, to paying due attention to genetic population diversity (*Alexandrium catenella* in Alaska; **Wayne Litaker**) and novel metabolomic approaches with sediment revived culture strains of different ages of *Alexandrium* vs *Scrippsiella* (**Raffaele Siano)**. High pCO_2 increases cellular toxicity in many HABs (e.g. *Karlodinium*;Vidyarathna and *Alexandrium catenella;* **Máximo Frangópulos**), but a sound working hypothesis of causative mechanism is still lacking.

Field work is focusing on learning lessons from extreme events such as the ENSO 2015-16 driven *Pseudo-nitzschia* west coast US "hot blob", 2016 Chilean record *Pseudochattonella* associated salmon mortality, and 2017 US massive summer cyanobacterial blooms in the Finger Lakes (**Greg Boyer**). Other climate lessons are being derived from studying heatwaves, and identifying climate change hotspots and ciguatera hot spots.

Modelling efforts are increasing in complexity also taking into account changes in habitat suitability (for *Alexandrium ostenfeldii*; **Anke Kremp**), precipitation and nutrient delivery, and even now including the role of mixotrophy (for *Karlodinium* vs *Prorocentrum*; **Patricia Glibert**). Increasing temperatures facilitate earlier and longer persisting *Margalefidinium* (*Cochlodinium) polykrikoides* blooms in the US and Korea, and earlier and large *Microcystis* blooms in Lake Erie (**Chris Gobler**).

Solid global HAB data bases are gradually building up using HAEDAT and OBIS platforms, aided by the efforts by PICES, ICES, FANSA and IAEA working groups. HAB time series analyses are becoming more sophisticated and datasets are becoming ever longer and more robust (22 years in Uruguy, **Silvia Méndez**; 30 years for the French REPHY network, **Catherine Belin**; 33 years in Philippines, **Aletta Yñiguez**; 40 years in northern Adriatic, **Martin Pfannkuchen**).

The first series of contributions resulting from the *Global HAB Status Report (GHSR)* initiative were presented in Nantes. This effort aims to settle questions on the probability of change in HAB frequencies, intensities, and range resulting from environmental changes at local and global scales. Regional HAB status reports were presented for the Mediterranean (**Adriana Zingone**), the North Atlantic (**Eileen Bresnan**), coastal waters of Ghana (**Denutsui Dzifa**), Canada (**Cynthia McKenzie**), east coast US (**Elizabeth Fensin**), Australia (**Gustaaf Hallegraeff**), New Zealand (**Enora Jaffrezic**), Latin America and the Caribbean (**Silvia Méndez** and **Inés Sunesen**].

Different regions are dominated by different seafood toxins: PSP in North America, DSP in Northern Europe, NSP in Central America (Florida), CFP in Oceania and Australia (Fig.1). Some HAB problems flare up (*Noctiluca* on the rise in Australia) and then dissipate (*Pyrodinium* in Philippines, **Aletta Yñiguez**; *Cochlodinium* in southern Korea in 2016, **Seung Ho Baeck**).

It is important to emphasize that available information in HAEDAT or OBIS on individual events varies greatly from event to event or country to country. Therefore there is no direct proportionality between recorded events and toxicity in a given region. Areas with numerous recorded HAEDAT occurrences but with efficient monitoring and management programmes (as raised in HAN 58), may have low risk of intoxications, whereas rare events in other areas may cause severe problems and represent significant health risk. HAEDAT data sets represent the compound result of HAB events as well as how human society responds to them via changes in monitoring and regulatory approaches. The incidence of ASP closures in Scotland thus was effectively reduced post 2005 by an amendment to the EU shellfish hygiene directive (EU854/2004)

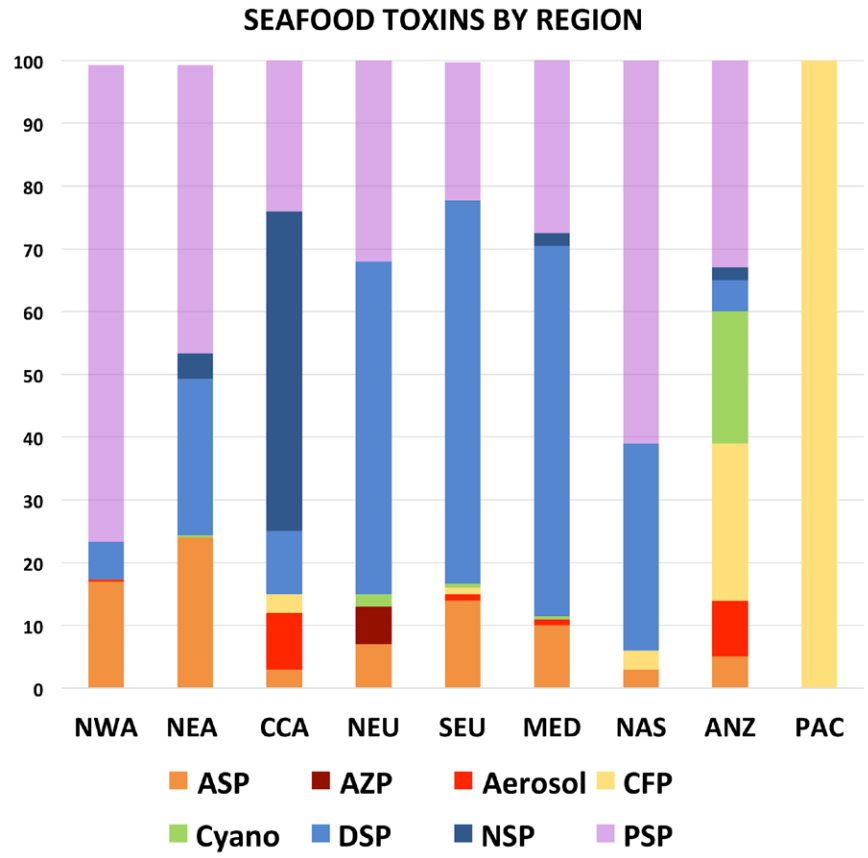


Fig. 1. Relative abundance of different seafood toxin syndromes in different geographic regions (North West America NWA; North East America NEA; Caribbean Central America CCA; North East Europe NEU; South East Europe SEA; Mediterranean MED; North Asia NAS; Australia New Zealand ANZ; Oceania/ Tropical Pacific (PAC). Based on an analysis of 5774 HAEDAT events recorded as of 1/3/2018. Data compiled by G .Hallegraeff & L. Schweibold.

Continued on p. 11

Barcodes and long term changes in HAB distributions

Thal-ass-ee-oh-CY-ra,Key-TOSS-er-us,Pro-toe-pear-a-DIN-ee-um, Sir-A-she-um... this might be a parody of a (very short) genetic barcode or it might be gibberish. It is in fact a sample from a list of phytoplankton genera on flashcards designed to help children pronounce Latin names. Fun for the kids, perhaps. Useful? It may lead to some idiosyncratic spelling habits. As Roman poet Horace famously wrote, *Nihil est ab omni parte beatum* (there are no unmixed blessings). But let's return to barcodes.

DNA barcoding uses short standardized DNA regions, commonly parts of genes which code for ribosomal RNAs like 18S rDNA, both to identify species which bear such names and the range of functional diversity. Today it is easy to scoop terabases of meta-genomic data out of the oceans (**Cedrić Bernay**, O-243). Barcoding now provides a rapid tool to survey harmful species (**Kieng Soon Hii**, P-048; **Patricia Paredes-Banda**, P-056; **Ana Baricević**, P-186), to define species boundaries and taxonomic diversity in general, and to delineate phylogenies. It is not yet fully foolproof, and the taxonomic resolution which can be achieved varies between groups and geographical regions. A more challenging application is to find ecological meaning, to look inside the black box of the microbial community, but functional roles based on genomics are slowly emerging (**Pierre Ramond**, P-063).

The Humboldt Current bathes the coasts of Chile and Peru and forms the eastern limb of the South Pacific subtropical gyre (Fig. 1). Off Ecuador it turns west and becomes the South Equatorial Current. The East Australian (EAC) Current forms the western limb of this gyre. The circuit is completed by the West Wind Drift (Antarctic Circumpolar Current), which is shared with the Indian Ocean and South Atlantic gyres. Variations in the intensity of these currents on decadal and longer time scales are now well established [1-2], associated with changes in wind forcing, more precisely with a poleward shift of westerlies in the Southern Hemisphere [3-5]. Winds have increased since the 1950s off Peru, with a decadal signal superimposed on the longer term trend (there is also an ENSO signal). Such variations are associated with fluctuations in the yields of fisheries, partly mediated through changes in primary production and subsequent recruitment, the match-mismatch hypothesis [6]).

The EAC has two branches. One branch turns east along the Tasman front towards New Zealand, the other flows southwards along the Australian coast. Intensification of the latter since the 1940s has caused it to flow farther south (7), and it now casts eddies to the east of Tasmania. Some faunal elements from further north have colonized coastal waters in eastern Australia. These include the invasive shore crab *Carcinus maenas*, and the native sea urchin *Centrostephanus rodgersii* which has created urchin barrens. Heavy fishing of lobsters, *Jasus edwardsii*, which eat urchins may have augmented the barrens. Some plankters too have extended their ranges, in the EAC [8, 9]. Range expansions (and contractions) reflect the dynamics of population pressures and local extinctions. For HAB enthusiasts, one of the most significant aspects of these variations must be the timing of phytoplankton growth, which over a four decade period (1940s to 1980s) off Tasmania (Maria Island) varied by several months [6].

Some changes here may not be entirely due to intensification of the EAC; a short list of other possible causative agents includes introductions, eutrophication, aquaculture, and more intense exploration. Rare or cryptic species may still resist discovery. Some of these may act concurrently. The number of possible permutations of n such agents is $n!/(n-k)!$, where k is the number we eliminate from consideration. So even if we consider that two of the five agents mentioned above are implausible, we still have $5!/(5-2)! = 20$ permutations, already a lengthy agenda. Bolch & Salas [10] tackled these difficulties on the basis of rDNA barcodes and other evidence, and concluded that three HAB species in the region are introductions (*Gymnodinium catenatum*, *Alexandrium catenella*, *A.* '*tamarensis*'). There are of course doubts, due in part to a longstanding habbological tradition which maintains *Alexandrium* taxonomy in a permanent state of flux (**Sirje Sildever**, O-126; **Tomohiro Nishimura**, P-107; **Silke Wohlrab**, O-152), and also to problem with algorithms, also in flux. At least one of Bolch's isolates (ATBB01) has been given a new name, *A australiense* [11]. The agenda lengthens.

There have been frequent suggestions in the decades following a PSP outbreak in Chile in 1972, that the *Alexandrium catenella* responsible had by the 1990s extended its range about 10° of latitude northwards, from Magallanes to Aysén and Chiloé. But cyst data and genetics do not support the hypothesis of an expansion on this time scale. Sediments in Bahía Quellón in 43° S contain cysts dating back to 1929 [12]. Regional rDNA variations of Chilean isolates are larger than expected, and rather indicate recolonization [13], in other words the plankton sampling and genetic analyses would appear to provide evidence

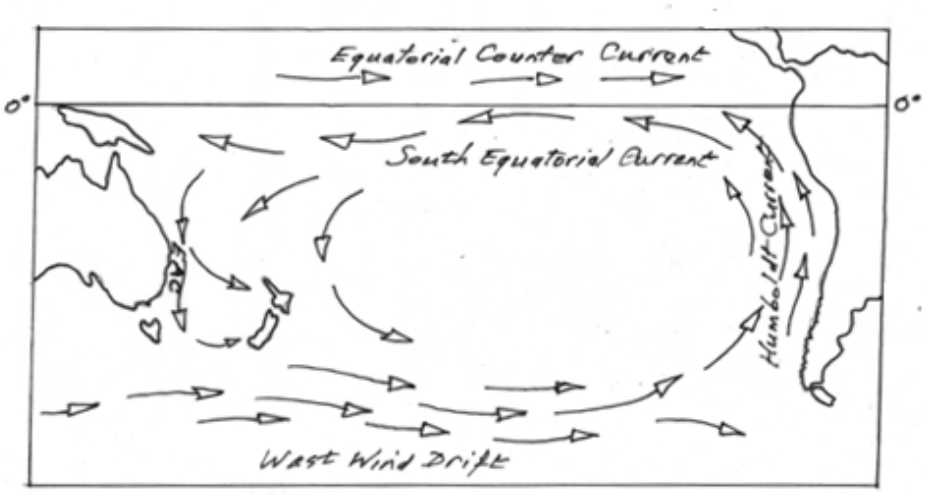


Fig. 1. Sketch of South Pacific Gyre, EAC = East Australian Current

of a highly dynamic biogeographical boundary. Taxonomic uncertainties exist in Chilean waters too, and more recently (2006) PSP was detected as far north as Bahía Mejillones in 23° S.

Pre-instrumental proxies indicate climate trends on secular and longer time scales. Luminescent bands in *Porites* corals show lower calcification rates due to lower salinity, and thus serve as a proxy for runoff onto the Great Barrier Reef [14]. Similarly, diatom abundance in lake varves in central Chile are proxies for rainfall there; these time series are about four and six centuries long respectively, and are largely coherent [14-15]. It is well known that as time series lengthen variance increases. The decadal changes identified in abundance and range of different *Alexandrium* species, as well as other phytoplankton, whether exotic or homegrown, may eventually appear, as longer records are recovered, as no more than the tip of the proverbial iceberg. Biogeographical patterns of phytoplankton and their taxonomic diversity seem to be more dynamic than hitherto expected. Barcoding is one of the best tools we have to construct detailed records of these long term changes.

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facilitating the shucking of scallops and sale of the adductor muscle.(Fig. 2).

Not surprisingly, no single answer is emerging as to whether HABs are / will be increasing or decreasing, but this should be examined on a species-by-species, case-by-case, and site-by-site basis. A special issue of the journal Harmful Algae devoted to the GHSR initiative is being prepared for completion by late 2019.

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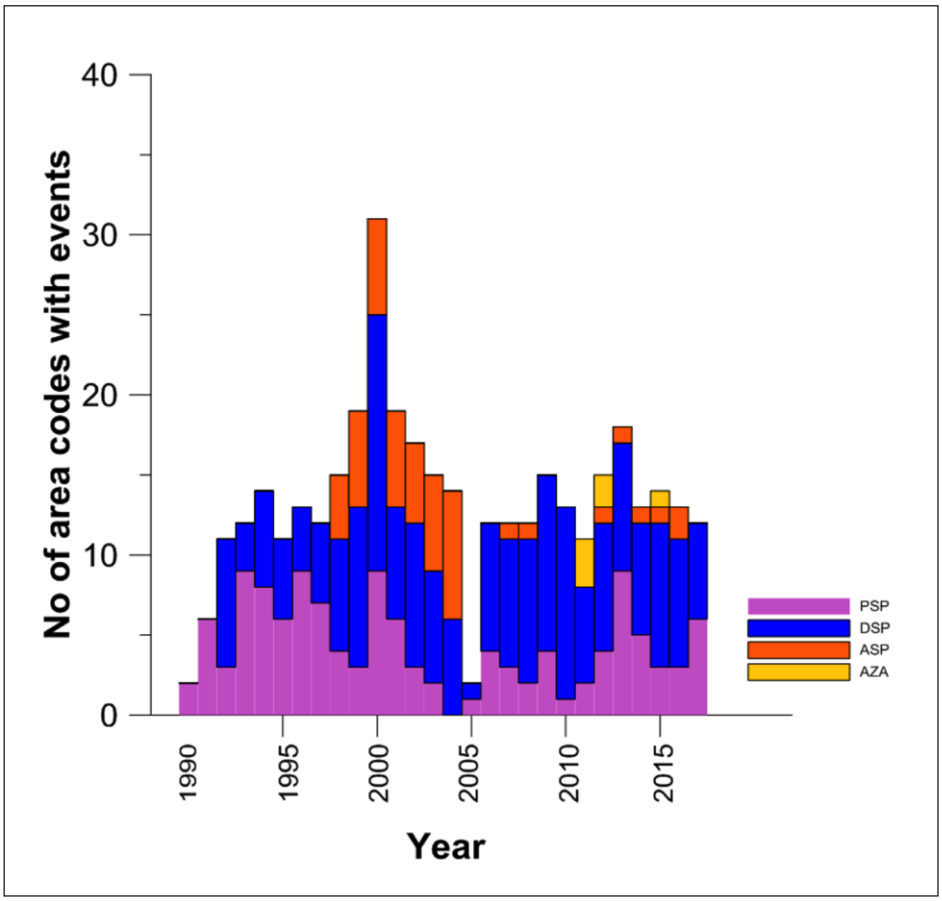

Fig. 2. Changes in the incidence of different seafood toxin syndromes in the United Kingdom between 1990 and 2017, based on the number of area codes with closure events per year. Such data sets represent the compound result of HAB events as well as changes in monitoring and regulatory approaches. Routine DSP monitoring started in 1992, ASP in 1998, AZA in 2011, while the data gap in 2005 reflects reduced monitoring in Scotland. PSP closures were more prominent in the 1990s but completely absent in 2004. DSP was most prominent in 2000, while reduced ASP post 2005 resulted from a change in monitoring strategy for Pecten maximus to end product testing by the shellfish processors, that facilitates shucking and sale of the adductor muscle. Data compiled by E. Bresnan.

Unprecedented bloom of the cyanobacteria *Aphanizomenon* in a coastal bay of El Salvador

Cyanobacteria bloom in marine, freshwater and estuarine ecosystems [1]. It is widely recognized that increased nutrient inputs in waterbodies may enhance cyanobacterial growth, resulting in harmful algal blooms [2-3]. In estuarine systems, blooms are enhanced by factors such as excessive nutrient loading, increased surface water temperature, persistent water column stratification, long water residence time, organic matter enrichment, and hydrodynamic and salinity anomalies [4].

Several cyanobacterial species produce toxins, such as microcystins, nodularins, cylindrospermopsin, anatoxin-a, saxitoxins, LPS, aplysiatoxins, and lyngbyatoxins, which can affect humans and animals. [5]. In El Salvador, cyanobacterial blooms have only been reported in freshwater ecosystems, and blooms in estuarine environments are more commonly caused by diatoms or dinoflagellates.

In February 2019, green water discoloration, possibly caused by an intense microalgal bloom, was reported in western Bahía de Jiquilisco, particularly around La Pirraya island (Fig. 1A-B; Supplementary material 1). According to the locals, this potential bloom had started on February 16th and dead clams belonging to the genus *Anadara* were found along the coast of La Pirraya Island during the bloom period (Supplementary material 2). Concern about the impacts from this bloom increased when four piglets, less than one year old, showed signs of dizziness, disorientation and pupil dilation after eating these dead clams washed ashore La Pirraya island at low tide. These piglets subsequently died as they were not able to walk or swim when the tide rose (Supplementary material 3).

In response to this event, five sites in Bahía de Jiquilisco (Fig. 1A) were sampled on 19th by LABTOX-UES staff using a Van Dorn bottle and 20 µm phytoplankton net. Phytoplankton species were quantified using the Utermöhl method or a Sedgewick-Rafter chamber (depending on cell abundance). An additional water sample from February 18th sampled by staff from the Ministry of the Environment and Natural Resources (MARN) offshore from La Pirraya island was also available for analysis.

Samples from February 18th revealed the presence of a high density population (127 x 10^3 colonies L^{-1}) of the cyanobacteria *Aphanizomenon* (Fig. 2). Colonies were counted since cells were moderately disintegrated and difficult to indentify individually. This species of *Aphanizomenon* presented filaments forming fascicle-like colonies that ranged from 99 to 698 µm in length and 46 to 257 µm in width.

Water temperature ranged from 30.4 to 31.1°C, pH from 7.0 to 8.1 and Secchi disk depth from 2.0 to 2.3m.

Aphanizomenon is one of the most common bloom-forming cyanobacteria reported to form blooms in brackish environments [3]and more recently anthropogenic modifications of aquatic environments. However, this genus has not been previously reported in the brackish environments of El Salvador. The genera *Dolichospermum, Pseudanabaena, Lyngbya,* and *Oscillatoria* have been recorded in Bahía de Jiquilisco, [8]. This event is the first first report of an *Aphanizomenon* bloom in the brackish environments of El Salvador.

Despite of the high concentration of *Aphanizomenon* in the sample of February 18th, the genus was not detected

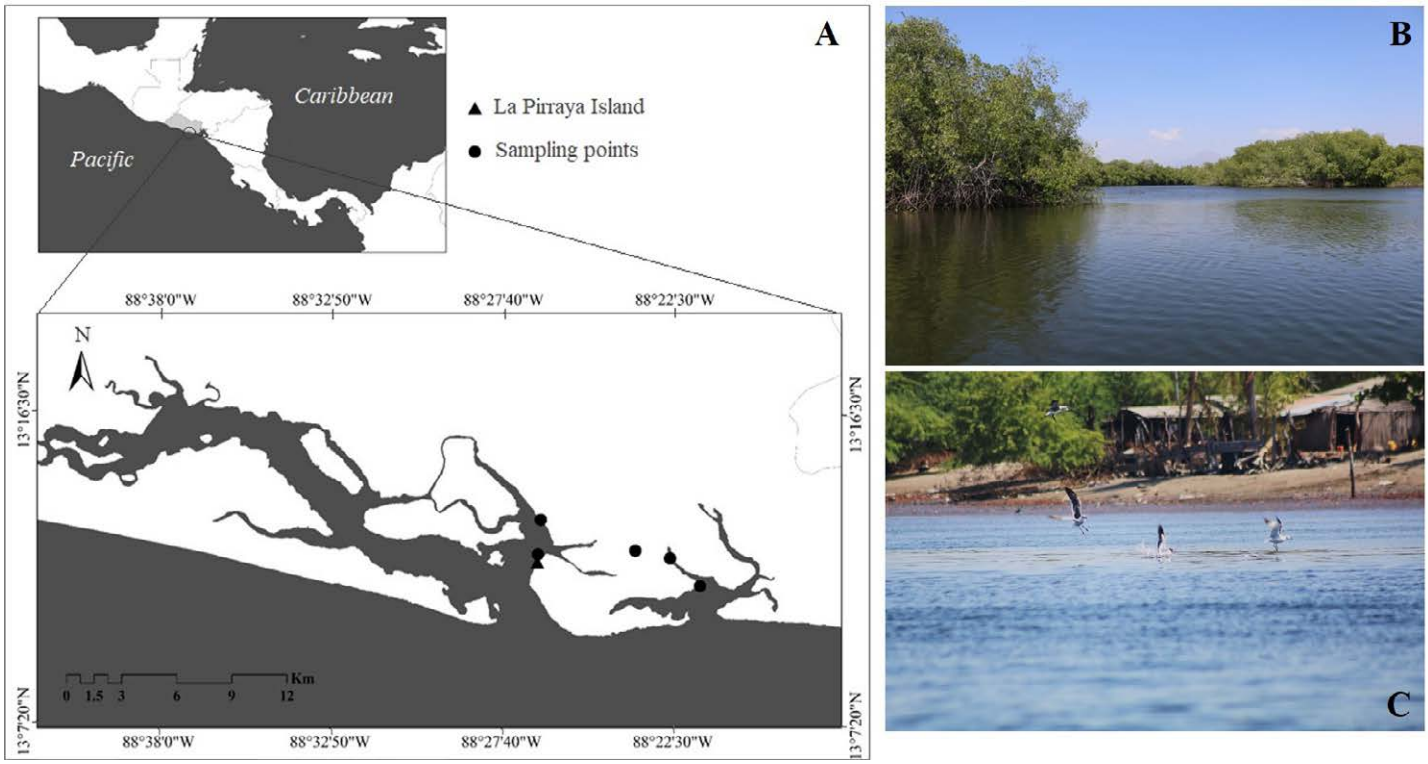

Fig. 1. A. Location of La Pirraya Island and sampling point in Bahía de Jiquilisco, El Salvador. B. Sampling location in Bahía de Jiquilisco. C. Shore of La Pirraya Island, where four pigs died.



Fig. 2. Aphanizomenon fascicles found in Bahía de Jiquilisco observed using an inverted microscope.

Table 1. Most abundant phytoplankton genera and species found in Bahía de Jiquilisco,February 19th, 2019.

Taxa	Cell abundance (cells L^{-1})
Coscinodiscus spp.	54,429
Skeletonema costatum	44,363
Pseudo-nitzschia spp.	18,148
Prorocentrum micans	672
Tripos fusus	672
Prorocentrum c.f. *compressum*	336
Tripos furca	336
Dinophysis caudata	20

in the samples collected during February 19th; suggesting the bloom lasted around three days. Table 1 shows the most abundant phytoplankton genera and species found at the five sampling sites on February 19th. These species were found in similar abundances in previous reports from this area.

It is most likely that the spring high tides that occurred during the night of February 18th washed away the bloom, since local inhabitants reported that after high tide they no longer saw water discoloration. Locals of La Pirraya also expressed they had never seen such type of bloom in the area before.

Although toxicity was not measured in tissues from the dead piglets, it is suspected that the *Aphanizomenon* bloom was linked to their intoxication, since this genus has been reported to produce toxins such as cylindrospermopsin, anatoxin-a, and saxitoxins than can cause hepatic and neurologic effects on mammals [5, 9]. Although four piglets died, there were several other pigs (1.5 years old) nearby that ate dead molluscs but did not die, and signs of dizziness and disorientation they had exhibited disappeared with time.

This event highlights the need to monitor cyanobacteria blooms in El Salvador and the toxins produced by species within this group, since the current national capability to study these blooms is low.

Acknowledgements

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Supplementary material

This material was provided by NGO ProCosta and can be accessed in the following links:
https://drive.google.com/drive/folders/1HLgwp_d9JNhzQQYedVsQVCWOoYQONvn4?usp=sharing

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Dinoflagellate toxins recorded during an extensive coastal bloom in northern Chile

Harmful algal blooms (HABs), commonly known as red tides, are primarily caused by the accelerated growth of phytoplankton due to a variety of oceanographic factors. Dinoflagellates and diatoms are often the phytoplankton groups associated with HABs [1-2]. In the present study we investigated a coastal bloom occurring along the city of Antofagasta, located on Atacama Desert, Chile. This particular bloom began in late November 2018 and continued into February 2019. The bloom extended more than 30 kilometers from the coast to the open ocean and over 600 kilometers along the coastline from the northernmost Chilean city to the south of Antofagasta. This bloom expanded even more in 2019, to approximately 1,500 kilometers in length, reaching the coast of Peru near Arequipa city. A NASA WorldView platform was established to monitor chlorophyll *a* fluorescence on a daily basis from December 2018. Chlorophyll *a* values estimated by this platform remained over 10 mg/m^3 until the beginning of March 2019 (Fig. 1). This is one of the most extensive and persistent algal blooms in Chilean history. Microalgal blooms of this scale may have sporadically occurred in this region in the past, but their magnitude has never been investigated using satellite images of chlorophyll fluorescence. Here we studied this event and established a set of HAB monitoring methodologies to understand microalgal dynamics in this region.

Approximately 100 phytoplankton species are capable of producing toxins. Some of these toxins are harmful to humans, even resulting in fatal outcomes in developing nations (e.g. South Africa, Madagascar and Comoros, among others) [3, 4]. The causes of the different poisoning cases can be attributed to a variety of microorganisms, toxins (derivatives and analogs), and even vector organisms concentrating these compounds (e.g. puffer fish) [3, 5-6].

We collected the seawater samples from five different stations, using a boat along the coast of Antofagasta in December 2018, namely CAP, SR, PUERTO, NR, and 2ED. These samples were concentrated in two steps: First, we towed an adapted conical 20 µm mesh for approximately 100 meters from a moving boat. Secondly, the concentrated volume was again filtered in the laboratory through a borosilicate MGA grade membrane with a 1.6 µm pore size (Munktell Ahlstrom). It should be noted that 30 mL of unfiltered water instead of 60 mL were filtered through borosilicate membrane in the case of station 2ED. Lastly, toxins were extracted from the borosilicate membrane filters with methanol, the sample extracts filtered through a PVDF membrane, pore size 0.2 µm, and the filtrates analyzed by HPLC/MS/MS. (Table 1).

Prorocentrum micans was the dominant species in all stations. Other species observed belonged to the genera *Tripos, Peridinium, Dinophysis, Protoperidinium,* and *Gymnodinium* (Fig. 2). Preliminary observations confirmed the presence of four of the five most reported genera of toxin producing dinoflagellates (Fig. 2, images 7-11) [3]. Interestingly, *Dictyocha* sp., a rare species in this region, was observed in some samples. High biomass blooms of this genus may be harmful to fish due to development of anoxic conditions (Fig. 2, image 3). It has been shown that *Dictyocha* spp. produce toxins, but they have not been fully characterized [7].

These dinoflagellate genera observed are in accordance with the different toxins recorded. Species within the *Dinophysis* and *Protoceratium* genera are known to be producers of pectonoxins (PTXs) and yessotoxins (YTXs)

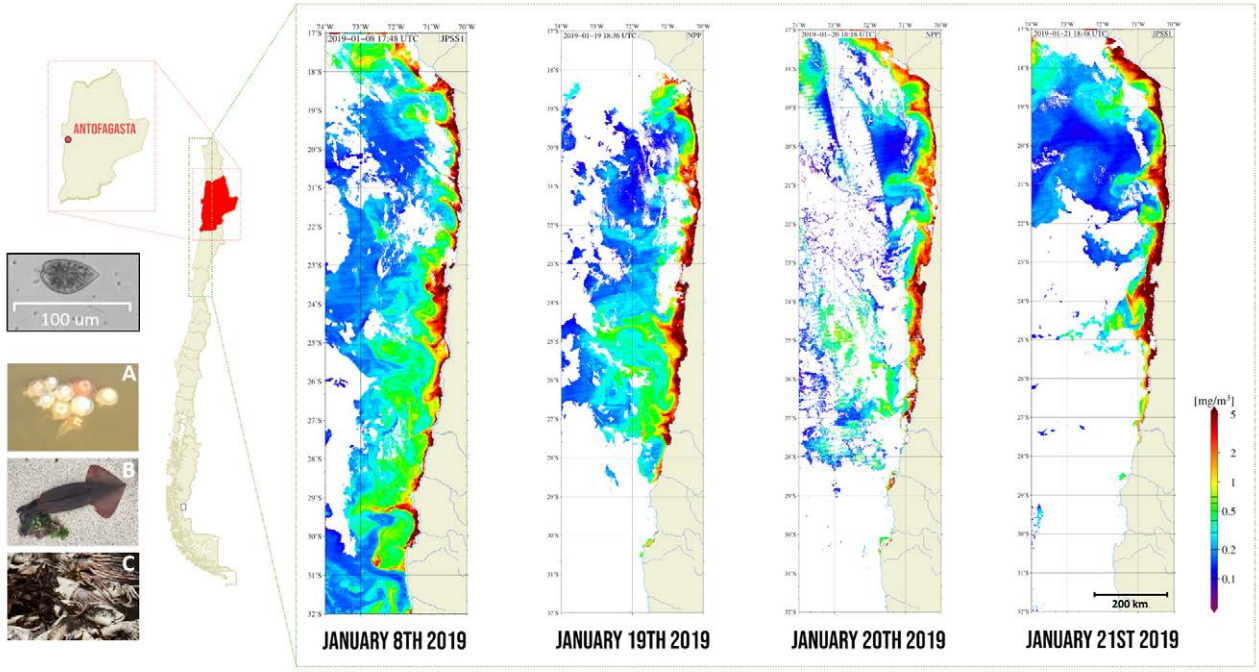

Fig. 1. Chlorophyll 'a' estimates along the northern Chilean coast captured by satellite images. Region of Antofagasta is pointed by the red dotted square and Antofagasta city is shown by the red dot. The cell image shows Prorocentrum sp., the main bloom species of this HAB event. Image A shows some dead jellyfish observed at sampling points and image B shows squid from a massive squid mortality in Bahía Inglesa (coastal bay close to latitude 27° S). Satellite images were taken from Joint Polar Satellite System (JPSS) and Suomi National Polar-orbiting Partnership (NPP) satellites imagery viewer.

Table 1. Toxin concentration per sample. Toxins screened included PTX1 (pectenotoxin-1), PTX2 (pectenotoxin-2) and YTX (yessotoxin). DA (domoic acid), SPX1 (spirolide-1), GYM (gymnodimine), OA (okadaic acid), DTX1 (dinophysistoxin-1), DTX2 (dinophysistoxin-2), AZA1 (azaspiracid-1), AZA2 (azaspiracid-2), AZA3 (azaspiracid-3), 45-OH-YTX (45-hydroxy-YTX), 45-OH-homo-YTX (45-hydroxy-homo-YTX) were analyzed with no detectable amounts recorded. Each result is given in pg per cell. ND refers to "not detectable".

Sample	Coordinates (Latitude/Longitude)	Cell count (cells/mL)	PTX1 (pg/cell)	PTX2 (pg/cell)	YTX (pg/cell)
3CAP	23° 40' 53.2757" S / 70° 25' 19.6918" W	3,252,500	0,00194	0,00950	0,00336
SR	23° 40' 04.8082" S / 70° 24' 39.9169" W	3,102,500	0,00082	0,00328	0,00240
PUERTO	23° 39' 07.6038" S / 70° 24' 34.7150" W	2,667,500	0,00153	0,00406	0,00026
NR	23° 39' 16.6949" S / 70° 24' 28.8831" W	3,482,500	0,00102	0,00606	0,00588
2ED	23° 41' 18.8641" S / 70° 25' 44.1511" W	2,547,500	0,00008	0,00446	ND

respectively. Dinoflagellate strains from the bloom samples were also isolated and established in axenic culures. Their 8S ribosomal DNA was analyzed using conventional Sanger sequencing techniques.

The toxicity of PTXs, YTXs and analogs to humans has yet to be confirmed however these lipophilic toxins may cause cellular death, apoptosis and other damages in mouse neuroblastoma cell tests. PTXs and DSP (diarrhetic shellfish poisoning) toxins are both produced by *Dinophysis* species and often co-occur in shellfish samples. The frequent co-occurrence of DSP with PSP (paralytic shellfish poisoning) toxins in Chile, presents additional complications to the application of standard toxin detection methods [10-13].

Previous research on shellfish (bivalves, gastropods) toxins has been carried out mainly in southern Chile. Further studies should be developed, especially on rapid-detection methods, to focus on local seafood products in northern Chile. Little is known about the toxin profiles and toxicity of shellfish in this region.. This lack of information presents a potential threat to seafood exports from Antofagasta and other high production areas in northern Chile [14].

Acknowledgements

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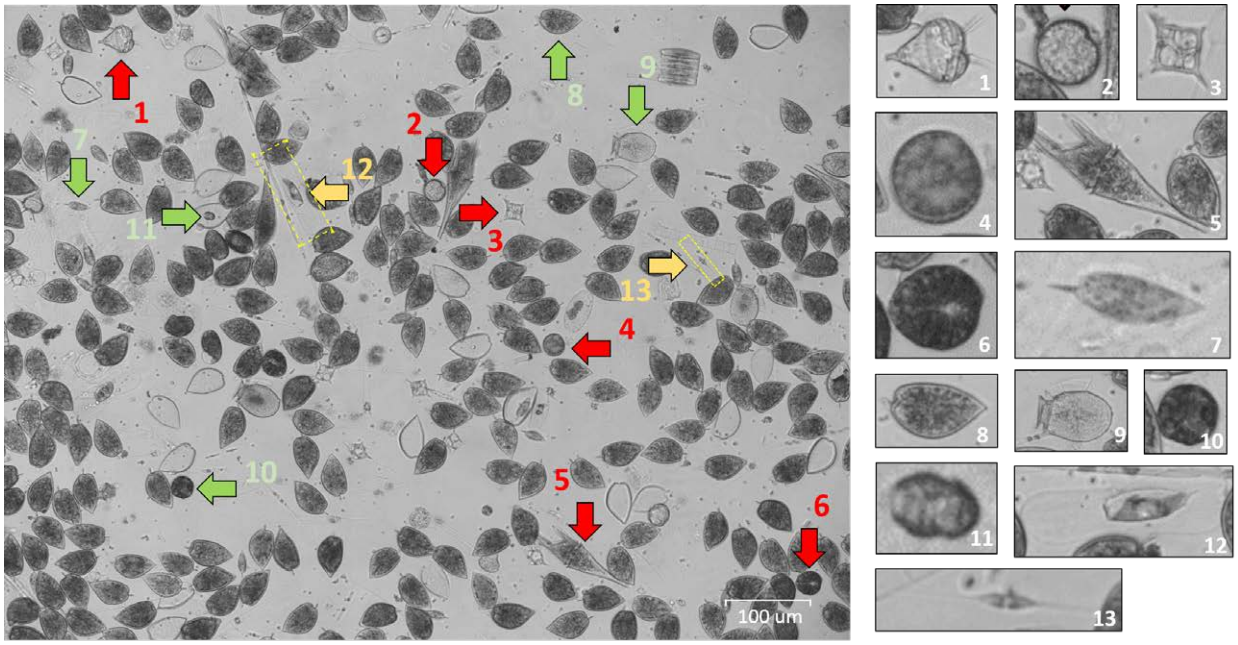

Fig. 2. Clear field microscopy image of a sample collected from station "PUERTO" during the bloom in Antofagasta in December 2018. The image shows a variety of dinoflagellates. All green arrows flag a number of different organisms groups 1. Protoperidinium cf steinii; 2. Prorocentrum sp. 3. Dictyocha; 4. Thecate dinoflagellate (possibly apical view of Peridinium sp..; 5. Tripos furca; 6. Possibly Gambierdiscus sp.; 7. Prorocentrum triestinum, 8. Prorocentrum micans.; 9. Dinophysis acuminata complex. 10. Thecate dinoflagellate (possibly apical view of Alexandrium sp.; 11. Gymnodinium sp.; 12. Entomoneis sp.; 13. Cylindrotheca sp. The yellow arrows indicate two diatoms observed in the sample, and the red arrows indicate the remaining dinoflagellates

HABs in Paradise revisited

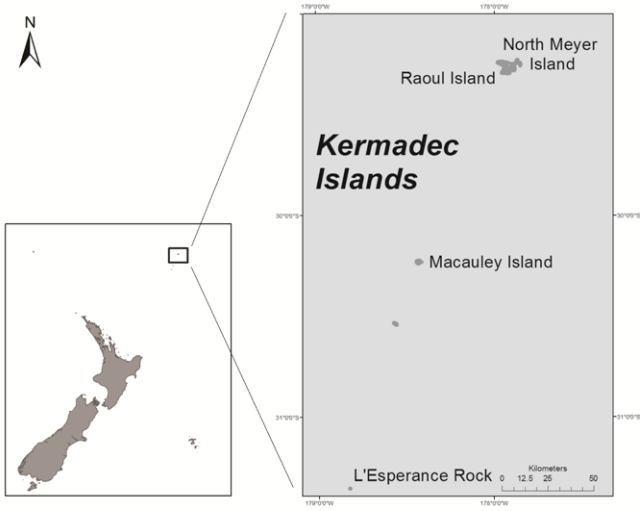

Fig. 1. Map of Rangitāhua/Kermadec Islands.

Over 700 kilometres to the northeast of New Zealand are the largely uninhabited Rangitāhua/Kermadec Islands (Fig. 1). The islands fall within New Zealand's exclusive economic zone and several recent expeditions have resulted in the identification of dinoflagellate genera, particularly epiphytic, harmful algae bloom (HAB) species, collected from macroalgae at depths of up to 28 m. The first expedition to collect epiphytic dinoflagellate samples was to Boat Cove, Raoul Island, in 2013, and the results were reported in a previous Harmful Algae News article [1]. The first *Gambierdiscus* species identified were from North Meyer Island, from an expedition in 2014 [2-4]. Species included *G. australes*, a novel *Gambierdiscus* species, and possibly *G. polynesiensis.* The identification of *G. polynesiensis*, however, was by morphology alone and as cultures failed to thrive and were not able to be confirmed by DNA sequencing. Maitotoxin (MTX)-1 and MTX-3 were produced by *G. australes* and MTX-3 by the unknown species as determined by liquid chromatography-tandem-mass spectrometry (LC-MS/MS; Table 1) [5,6]. The novel species, which was collected from non-geniculate coralline turfs, was characterised as *G. honu* [7] (Fig. 2). This species has also been reported from Rarotonga, Cook Islands. No MTX-1 or algal ciguatoxins (CTX-3B, CTX-3C, CTX-4A, CTX-4B) were detected by LC-MS/MS analyses, although MTX-3 was detected in extracts of *G. honu* from all sites. Interestingly, extracts of *G. honu* (CAWD242) were toxic by intraperitoneal injection to mice, but much less toxic by the oral route [7,8]. The differences in toxicity of extracts from known MTX-producers suggest the probability of an uncharacterised compound being produced and this is being investigated.



Fig. 2. Scanning electron micrograph of Gambierdiscus honu, isolated from samples collected from macroalgae in North Meyer Island, Rangitāhua/Kermadec Islands. Bar = 20µm.

Subsequent published reports summarised the dominant dinoflagellate genera detected at Raoul Island, North Meyer Island, Macauley Island and L'Esperance Rock and the presence of *Gambierdiscus*, *Coolia, Prorocentrum, Ostreopsis* and *Amphidinium* was noted [2-6]. An isolate of *G. polynesiensis* from Macauley Island, identification confirmed by DNA sequencing, was tested for ciguatoxins but, unusually, did not produce those toxins, although it did produce MTX-3 [5]. It is noted, however, that the ciguatoxin production may have been below the limit of detection of the method, rather than a non-producer.

The *Ostreopsis* species isolated from the Kermadec Islands has yet to be described but was genetically identical to *Ostreopsis* sp. 3 [9]. One isolate, from the 2013 expedition to Raoul Island, produced detectable concentrations of a palytoxin-like compound (0.013 pg palytoxin equivalents per cell) [2] whereas another isolate of the same species from a later expedition to North Meyer Island was non-toxic [3].

The most recent expedition was in March 2018 on the HMNZS Canterbury. Samples collected from macroalgae (Fig. 3) were mainly from *Dictyocha* sp. and resulted in many isolates of *Gambierdiscus* and the successful culture of individual cells of *Coolia malayensis*, *Ostreopsis* sp. 3 [10] and *Prorocentrum lima.* The *C. malayensis* isolate is yet to be analysed for MTXs but isolates from the Cook Islands and New Zealand (CAWD154 and CAWD175) do produce MTX-3 (unpublished data).

The only *Gambierdiscus* species isolated on this occasion was *G. australes* (species determination by DNA sequencing) and it was also the dominant micro-alga in all samples. Analyses of ciguatoxins and MTX-1 and MTX-3 (LC-MS/MS) of the four isolates indicated varying production rates of MTX-1 and MTX-3 per isolate.

The *P. lima* isolate (maintained in the CICCM as CAWD283) produced 14 pg/cell total okadaic acid and 0.5 pg/cell total dinophysistoxin-1 (DTX-1). A dinophysistoxin isomer was also detected by LC-MS/MS analysis. The LSU rDNA D1/D2 sequence of this strain was identical to those of previously reported strains, i.e., Chinese strains 2S1F7 and TIO124, a Mexican strain PL6 and strain DNS-7.

As more research is undertaken it has become clear that the *Gambierdiscus* species that have been found in the Pacific region are wide-spread and that toxin production by *Gambierdiscus* is variable within species. Isolates of *G. australes* have produced from 1 – 36 pg/cell of MTX-1 [3]. The best MTX-1 and MTX-3 producer from the 2018 trip, CAWD282, has been lodged in the Cawthron Institute Culture Collection of Microalgae to underpin future research and production of certified reference materials. Non-toxic *G. polynesiensis* strains, such as CAWD254 from Macauley Island, will enable further molecular research into toxin producing genes, for example using a comparative transcriptomic approach in which transcriptomes of species with contrasting toxin profiles are compared [11].

Acknowledgements

Thanks to Janet Adamson for technical support and to the curators of the

Table 1. Dinoflagellate species identified in epiphytic samples collected from Rangitāhua/Kermadec Islands, 2013-2018.

Species	CICCM+ CAWD Code	Sampling site	Macro-algal host	GenBank codes	Toxins	Reference
Amphidinium carterae	(N: #1 Amph)	North Meyer Is	Mixed rhodophytes	KY069056	NT	[3]
*A.*cf. *massartii*	222	Raoul Is	″	KM259619	NT	[2]
Coolia malayensis	(N: Mac#4)	Macauley Is	Non-geniculate coralline	MF109031	Not tested	[5]
*Gambierdiscus australes**	244	North Meyer Is	Mixed rhodophytes	KY069059	MTX-1, -3	[3,8]
″ ″	255	Macauley Is	″	MF109033	MTX-1, -3	[5]
″ ″	256	″	″	MF109034	MTX-1, -3	[5]
G. honu	242	North Meyer Is	″	KY062662	MTX-3	[8]
G. polynesiensis	254	Macauley Is	Non-geniculate coralline	MF109032	MTX-3[a]	[5]
Ostreopsis sp. 3	221	Raoul Is	Mixed rhodophytes	KY197854	Palytoxin[b]	[2,5]
″ ″	241	North Meyer Is	″	KY069060	Negative for palytoxin	[3]
″ ″	272	Macauley Is	″	NE	NT	Not published
Prorocentrum cf. *emarginatum*	228	Raoul Is	″	MF109035	NT	[2]
P. hoffmanianum	(N: Prorocentrum #1)	″	″	KM360089	NT	[3]
P. lima	283	″	*Dictyocha* sp	LC422235	OA, DTX-1	this study

+CICCM: Cawthron Institute Culture Collection of Microalgae. *Many more isolates of *G. australes* have been reported but are not entered in GenBank and are not included in this table [5]. MTX: maitotoxin; CTX: ciguatoxins; N: not maintained in CICCM (original code name given); NT: non-toxic; NE: not entered in GenBank, identification by qPCR assay; [a]: no CTX produced; [b]: Palytoxin-like compounds.

Cawthron Institute Culture Collection of Microalgae, Krystyna Ponikla and Sarah Challenger. Thanks also to Libby Liggins and J. David Aguirre from Massey University who collected additional algae during the HMNZS Canterbury trip. Funding for research and the collection was from the NZ Ministry for Business, Innovation and Employment (Seafood Safety programme, Contract No. CAWX1801; National Databases contract No. CAWX0902) and a Japan Society for the Promotion of Science (JSPS) Overseas Research Fellowship.

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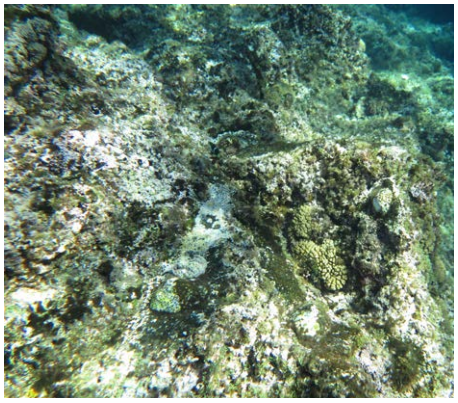





Fig. 3. A. Shallow water reef, west side of North Meyer Island (image by Tom Trnski); B. Typical reef cover where macroalgae are abundant; C. Sargassum at Havre Rock where large, dense macroalgae grow in shallow waters (images B and C by Crispin Middleton, NIWA, New Zealand).

Epiphytic dinoflagellates from Niue, South Pacific Ocean

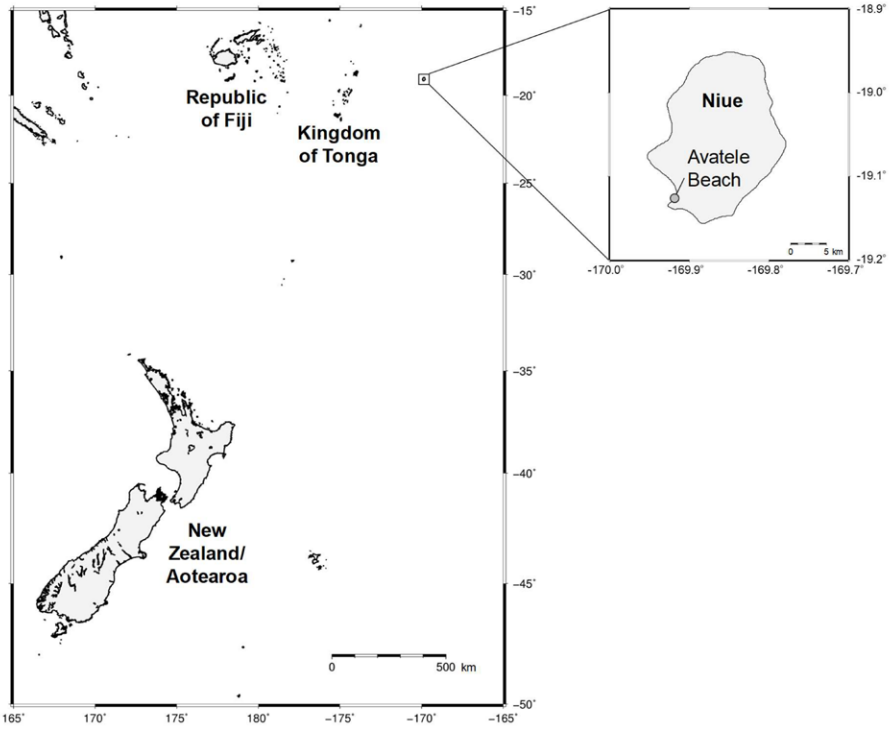

Fig. 1. Map of the South West Pacific Ocean with enlarged map of Niue included, showing sampling site at Avatele Beach.

Niue is a tropical island nation in the southern Pacific region, lying between the Kingdom of Tonga and the Cook Islands (Fig. 1). It is considered one of the smallest countries in the world (with a population of less than 2000) and yet is one of the largest raised coral atolls. Tourism is increasing, more than doubling from <3000 to 7000 in the decade between 2003 and 2013 [1]. Reef fish are an important source of protein, with the total annual reef finfish catch meeting approximately three quarters of the consumption needs of Niue's total island population [1].

Ciguatera fish poisoning (CFP) is caused by toxins produced by *Gambierdiscus* species and has been previously reported for travellers to Niue who have eaten ciguatoxin contaminated reef fish. In order to determine if *Gambierdiscus* species were present, samples were collected at low tide on 18th September 2018 at Avatele Beach (lat: 19°07'36.1"S; long: 169°54'49.3"W). The method of collection has been described previously [2]. Macroalgae, in this case the segmented and calcareous chlorophyte *Halimeda* sp., were brushed to release micro-algae and the resultant debris trapped in 50 ml plastic tubes or were shaken directly into the tubes already containing local seawater. Two drops of germanium dioxide were added to each tube to inhibit diatom growth. Samples were transported back to Cawthron Institute under a quarantine permit and isolates were cultured (f2/seawater, 1:3) to enable DNA sequencing and toxin testing [2].

Gambierdiscus was identified to genus level, but only one cell was observed, and it failed to thrive when isolated. However, isolates of *Ostreopsis, Prorocentrum, Coolia* and *Amphidinium* were successfully cultured. Analysis of DNA sequencing data confirmed the presence of *Ostreopsis* sp. 3 [3], *C. malayensis, Prorocentrum hoffmannianum* and *Amphidinium operculatum.* The *Ostreopsis* sp. 3 isolate was negative for palytoxin-like compounds as determined by LC-MS/MS [4]. One isolate has been submitted to the Cawthron Institute Culture Collection of Micro-algae (CICCM). The *C. malayensis* isolate was tested for maitotoxins (MTX) but was negative, although MTX-3 has been reported for other *C. malayensis* isolates [5].

Regarding CFP, an advisory from the International Association for Medical Assistance to Travellers (IAMAT) [6] suggests avoidance of reef fish over 2.7 kg and avoidance of the liver, intestine, head and roe of smaller reef fish. *Gambierdiscus* sp. was reported in Niue in 2008, although no molecular confirmation of species was able to be carried out at that time [7] and 0.4 incidences of CFP per 1000 people was reported in 1990 [8]. A reference was made to fluctuating CFPs and to a local hotspot at the Alofi Wharf being recorded in 2002 [7]. While *Gambierdiscus* was recorded from this study it was not identified to species level. However, it is hoped that further samples will be collected in the future to determine the exact source of the illnesses.

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Thanks to Janet Adamson (technical support) and the curators of the Cawthron Institute Culture Collection of Microalgae, Krystyna Ponikla and Sarah Challenger. Funding for research and the collection was from the NZ Ministry for Business, Innovation and Employment (Seafood Safety programme, Contract No. CAWX1801; National Databases contract No. CAWX0902) and a Japan Society for the Promotion of Science (JSPS) Overseas Research Fellowship.

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Fig. 2. Sampling site at Avatele Beach (left) and coastal view (right), Niue.

12th International Conference on Molluscan Shellfish Safety

The Federal Commission for the Protection against Sanitary Risks (COFEPRIS), Mexico, is pleased to invite you to attend the 12th International Conference on Molluscan Shellfish Safety (ICMSS 2019) in

Ensenada, Baja California, Mexico

September 9 – 13 20019

icmss2019@cofepris.gob.mx

The ICMSS is an initiative of researchers from various international institutions, including the Food and Agriculture Organization of the United Nations (FAO), related to the safety of molluscan shellfish. It seeks to be a forum for the diffusion and discussion of advances and trends in this subject. Reknown experts from around the world will take part in the conference. Students, researchers, health authorities, producers of molluscan shellfish and interested public in this subject are welcome.

The main topics to be discussed during the conference will be:

- Sanitary control programs for molluscan shellfish.
- Sanitary classification of harvest areas.
- Pathogenic organisms for humans in molluscan shellfish
- Climate change and its effect on the safety of molluscan shellfish.
- Toxic phytoplankton and marine toxin.

For more general information of the event, please consult this *link*.

Deadline to submit abstracts for the ICMSS 2019, *July 5*

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics 2019 Meeting

The International Council for the Exploration of the Sea (ICES) - Intergovernmental Oceanographic Commission of UNESCO (IOC) Working Group on Harmful Algal Bloom Dynamics (WGHABD) met from the 2nd – 4th April 2019. The meeting was hosted by Dr. Wenche Eikrem at NIVA in Oslo, Norway.

Working group members continued to update the IOC-ICES-PICES Harmful Algal Event Database (HAEDAT) during 2018 and production of the ICES Harmful Algal Event status report is underway. This ICES report will form the contribution from the North Atlantic area to the IOC Global HAB Status Report. A summary of the HAEDAT data from the North Atlantic area and changes in the regional distribution of harmful algal events over the last 20 years will be made at the ICES Annual Science Conference, 9th – 12th September, Gothenburg, Sweden.

National reports revealed that HABs continued to cause a variety of problems in the ICES area during 2018. Examples of some extreme events reported included impacts from a *Karenia brevis* bloom in Gulf of Mexico. This bloom was very prolonged along the western coast of the Florida panhandle and was associated with extensive mortalities of fish, marine mammals and seabirds, as well as human respiratory irritation and negative impacts on tourism. Cyanobacteria in the rivers flowing from Lake Okeechobee also impacted waterways in the west and east of Florida resulting in extensive scums and water discolourations. Cyanobacteria caused problems in Polish coastal waters, with an exceptional bloom resulting in the closure of many tourist beaches during the summer of 2018. In Denmark an extensive bloom of *Lepidodinium* cf. *chlorophorum* was observed in western coastal waters, resulting in green water discolourations. The first closure of shellfish harvesting areas in Sweden due to high concentrations of azaspiracids was enforced during 2018. Closures of shellfish harvesting areas as a result of paralytic shellfish toxins associated with *Gymnodinium catenatum* were reported from Portugal and Spain. In contrast, some countries experienced fewer impacts from HABs. For example no closures of shellfish harvesting areas were enforced due of paralytic shellfish toxins in Ireland, the south west of the UK and France during 2018.



Participants in the ICES-IOC WGHABD 2019 meeting

New findings presented included an update of the use of satellite imaging to identify and assess the risk from HABs using examples from ongoing projects (PRIMROSE, SHELLeye, S-3 EUROHAB), investigations in the Netherlands into the occurrence of tetrodotoxin (TTX) in shellfish, the use of molecular methods to identify HAB species using qPCR methodologies (Ireland) and metabarcoding (Norway), investigations into the occurrence of *Nodularia spumigena* blooms in Oslofjord using sediment cores, a review of *Dinophysis* and diarrhetic shellfish toxins in Irish shellfish from 2005 to 2017 and preliminary analysis of historic phytoplankton and toxin data from Scotland. Phytoplankton images from the live stream of the Imaging Flow Cytobot in Hong Kong were also viewed.

Progress on work underway in Europe addressing issues associated ciguatera fish poisoning as part of the Eurocigua and MIMAR projects was reported. Details of a workshop, now fully subscribed, on real time quantitiative PCR to develop climate services for monitoring harmful algae blooms in their marine environment (co-sponsored by the Co-Clime project) hosted by the Alfred Wegner Institute in Bremerhaven in October 2019 was presented. As WGHABD is co-sponsored by the IOC, the WG was invited to complete an informal survey to inform the development of the Science Plan for the UN Decade of Ocean Science for Sustainable Development. More information about UN Decade can be found at *https://en.unesco.org/ocean-decade*.

Studies underway on Polar HAB species included results from investigations from Greenland and Iceland. Preliminary results were presented from new projects focused on polar HABs e.g. TaXMarc (Norway), Arctic PRIZE (UK) and a dedicated cruise planned to the Arctic in 2020 (Sweden). As a result of the growing interest in polar science to identify and understand impacts from climate change, this term of reference will be carried over to next year's meeting to allow further results to be presented.

The next WGHABD meeting, 2 – 4th March 2020, will be hosted by Dr. Hanne Mazur from Institute of Oceanography in Sopot, Poland. The meeting will be held jointly with the ICES -IOC Working Group on Ballast and Other Ship Vectors (WG BOSV) and ICES WG Introduction and Transfer of Marine Organisms (WG ITMO) with Wednesday 3rd March being a shared day between the three working groups. During the 2020 meeting WGHABD will also present national reports and new findings, review progress with new technologies to identify HABs particularly in operational settings, update and discuss future work with HAB event data, provide updates on incidences of Ciguatera Fish Poisoning (CFP) and associated efforts on research and prevention and present any relevant updates relating to HABs

Continued on next page

Intergovernmental Panel convenes to set priorities for international cooperation on mitigating the effects of Harmful Algae

The Fourteenth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB) was held at UNESCO Headquarters, Paris, from 24th to 26th April 2019.

The Panel reviewed actions completed during the intersessional period and set priorities for 2020-2021. The focus for 2020-2021 will be on capacity building; facilitating further development of early detection, warning and forecasting of harmful algal events; continuing the development of a periodic Global Harmful Algal Bloom Status Report; implementing a Global UN Inter-Agency Ciguatera Strategy for improved research and management; guidance on harmful algae and desalination of seawater; international coordination of biotoxin monitoring, management and regulations; guidance on algal taxonomy; and on better understanding of the relationship between harmful algae and fish kills.

The Panel also reviewed activities at a regional level taking into account differences in support for the various groups and networks and affiliation to regional IOC subsidiary bodies.

Dr. Joe Silke (Ireland) was elected as Chair and Dr. Alexandra Silva (Portugal) was elected as Vice-Chair.

The Executive Summary, Decisions and Recommendations are available *here.*

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Participants in the XIV IPHAB meeting, UNESCO, Paris

Continued from p. 20

and the EU Marine Strategy Framework Directive.

WGHABD welcomes new members at any time and is open to experts from all IOC Member States. HABs are a global problem and experts from outside the ICES region interested in joining the WG (at own expense) are particularly welcome. WGHABD will be setting terms of reference for a new three year reporting cycle at the 2020 meeting. Now is your chance to get involved. For further information please contact the WGHABD chair or the IOC.

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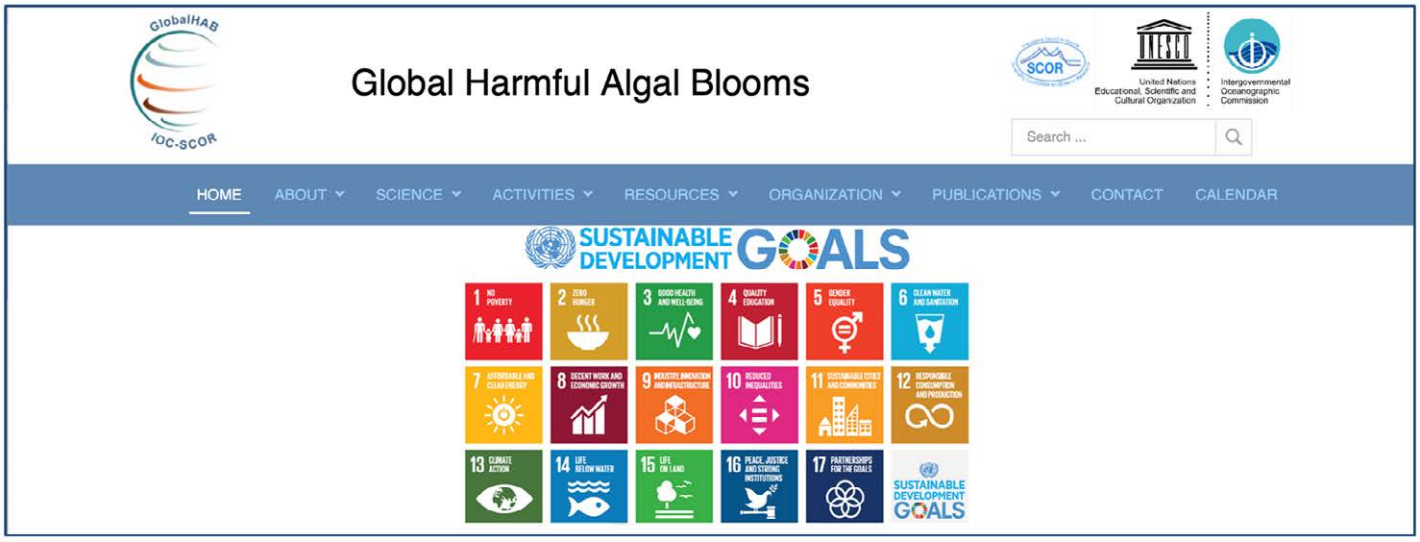
GlobalHAB and the UN Decade of Ocean Science for Sustainable Development (2021-2030)



GlobalHAB webpage (www.globalhab.info)

On 1st January 2016, the 17 UN Sustainable Development Goals (SDGs) of the 2030 Agenda for Sustainable Development (adopted by world leaders in September 2015 at an historic UN Summit) officially came into force. Over the next fifteen years, with these new Goals that universally apply to all, countries will mobilize efforts to end all forms of poverty, fight inequalities and tackle climate change, while ensuring that no one is left behind. The United Nations has proclaimed a Decade of Ocean Science for Sustainable Development (2021-2030) to support efforts to reverse the cycle of decline in ocean health. It aims to gather ocean stakeholders worldwide behind a common framework that will ensure ocean science can fully support countries in creating improved conditions for sustainable development of the Ocean. The Decade will provide a 'once in a lifetime' opportunity to create a new foundation, across the science-policy interface, to strengthen the management of the ocean. The Sustainable Development Goals are a call for action by all countries – poor, rich and middle-income – to promote prosperity while protecting the planet.

How can GlobalHAB contribute to these important UN goals?

In the broader picture GlobalHAB contributes to improved management of HABs as an ocean hazard through improved preparedness and early warning systems contributing to UN Sustainable Development Goal 11, target 11.5 and Priority 4 and Global target 7 of the Sendai Framework on Disaster Risk Reduction (UNISDR) 2015-2030.

GlobalHAB is contributing to these goals because it was born as a bottom-up initiative from the international community to coordinate research on HABs. The GlobalHAB Science and Implementation Plan integrated the most relevant objectives and pressures identified by the researchers to progress understanding, prediction and if possible, mitigation of the negative impacts of HABs on the environment, human health and wellbeing. To address these objectives some activities were listed in the GlobalHAB Plan and are already on going in different countries and regions, being led by national and international research teams and supported by different funding sources. **GlobalHAB can endorse all the research that contributes to the implementation of the GlobalHAB Plan, particularly initiatives facilitating international coordination.** Information on the procedure and application process for endorsement is available in the GlobalHAB webpage (*http://www.globalhab.info/about-us/get-involved*).

Why seek endorsement from GlobalHAB?

GlobalHAB provides an international structure for planning and coordination of international scientific activities relating to HABs. In addition, GlobalHAB provides a framework for national and regional projects to (1) coordinate their activities and, (2) participate in coordinated research, observation, and modelling initiatives focused on HABs. The GlobalHAB Scientific Steering Committee (SSC) may endorse projects which have already been funded, project proposals prior to submission for funding, or even letters of intent. The primary criterion for endorsement is applicability to any of the GlobalHAB Science Plan objectives. Endorsement from GlobalHAB can support justification and resources for international standardization and intercalibrations of sampling methods, observations, modelling, and toxin quantification worldwide. It can assist researchers and agencies to secure funding via letters of support and endorsement for research proposals and initiatives; support communication of information about HABs to managers, policymakers and the public. It can; facilitate the establishment of links with other relevant international programmes and related projects also endorsed by GlobalHAB; and disseminate project information through newsletters, websites and other communication mechanisms.

GlobalHAB APPLICATION FORM
FOR ENDORSEMENT OF ACTIVITIES

To be completed in English and send to the Chair of the GlobalHAB SSC.
For further guidance consult the Chair and/or Vice-chair of the GlobalHAB SSC.

Date:

1. PROJECT TITLE:

Planned duration of activity, from : to:

2. APPLICANT(S):
Name and title:
Address:
Tel/Fax:
E-mail:
Home page URL: (if applicable)
Other **key persons** (name, title and institution):

3. PROJECT DESCRIPTION:
Attach brief summary or abstract. One page is sufficient; no more than five pages please.

Project web site (if applicable):

Data management contact (web site and/or contact person, if applicable):

4. BENEFITS FROM GlobalHAB:
Please comment on a) how the activity could benefit from endorsement by GlobalHAB, and b) how the SSC might assist the activity.

5. CONTRIBUTION TO THE GlobalHAB SCIENTIFIC OBJECTIVES AND TOPICS
Indicate how the project contributes to the Implementation of the Objectives of GlobalHAB.

6. LINKAGES WITH OTHER PROGRAMMES:
Is the project part of a National Programme? Yes: ___No: ___ If yes, give title:

Is the activity part of, coordinated with, or affiliated with, other international/regional programs? Yes: ___ No. ___ If yes, give program title:

7. FUNDING
Has funding been obtained? Yes: No: (Prospective) source(s):

8. APPENDICES
Feel free to attach any additional materials if relevant.



GlobalHAB International Coordinated Activities

Some international coordinated activities listed in the Science and Implementation Plan are being led by the GlobalHAB SSC because their implementation depends on international coordination and funding. The SSC has assumed this responsibility for their implementation, with the ultimate aim to serve **the international community**, which **is invited to participate**. Activities include:

June 10-11, 2019

Initiated within GEOHAB (lead by Grant Pitcher and Raphael M. Kudela), GlobalHAB included fostering research on the potential links between ocean deoxygenation and HABs through interaction with IOC GO2NE (Global Ocean Oxygen Network, *http://www.unesco.org/new/en/natural-sciences/ioc-oceans/sections-and-programmes/ocean-sciences/global-ocean-oxygen-network/*). A joint GO2NE - GlobalHAB workshop is being organized in Paris, 2019 to identify potential research on this topic, to coincide with the next GO2NE workshop (*http://hab.ioc-unesco.org/index.php?option=com_oe&task=viewEventRecord&eventID=2469*).

Scientists interested in the topic can contact Grant Pitcher (*GrantP@nda.agric.za*) and Raphael M. Kudela (*kudela@ucsc.edu*).

October 17-19, 2019

An international workshop, "*Evaluating, reducing and mitigating the cost of harmful algal blooms: a compendium of case studies*", which will be held in Victoria, British Columbia, Canada as part of the Annual Meeting of the North Pacific Marine Science Organization (PICES). The workshop co-convenors are Drs Vera Trainer (USA), Keith Davidson (UK) and Kazumi Wakita (Japan) and it is jointly sponsored by GlobalHAB (SCOR and IOC), PICES, NOWPAP, ISSHA, NOAA, FAO and private companies. The goal of the 2.5-day workshop is to bring together international experts in economics, social sciences, and the study of harmful algal blooms (HABs) to develop a compendium of case studies to guide future research on the economic and social costs of HABs. The intent is that this compendium will identify priorities and unify methods for future collaborative assessments of HAB impacts.

More information can be found at: *https://meetings.pices.int/meetings/annual/2019/PICES/Program*. Scientists interested in the topic can contact Vera Trainer (*vera.l.trainer@noaa.gov*).

March 2018 to October 2019

Elisa Berdalet representing GlobalHAB at the CLEFSA project activities *"Emerging threats on human health in Europe due to climate change".* CLEFSA is a project of the European Food Safety Agency (EFSA) that explores the risks of food intoxication in future climate change scenarios. CLEFSA included aquatic biotoxins in the European landscape. E. Berdalet is collaborating in the elaboration of Reports and documents through online communication and particular meetings.

Scientists interested in the topic can contact Elisa Berdalet (*berdalet@icm.csic.es*).

Fall 2019

Manual for water managers on mitigation of cyanobacterial HABs elaborated by Michele Burford and Chris Gobler. This manual will be an easy to understand document for drinking and recreational water managers on managing cyanobacterial HABs and will be available both in print and on web.

Fall 2019

A fish-killing HABs workshop is planned, co-funded by GlobalHAB, IOC UNESCO and the government of Chile. This workshop is coordinated by Leonardo Guzmán and the IOC/IPHAB Task Team on Fish-killing algae. The workshop will include: a) plenary lectures by invited experts to provide a synthesis of current state-of-knowledge and focus direction in furthering the understanding of fish-killing HABs, b) presentation of high impact case studies from Chile and other areas; c) participation of Chilian national scientists, monitoring and regulatory agencies and the local industries; d) laboratory demonstrations (if feasible). The outcomes of the workshop will be published as a book.

Scientists interested in the topic can contact Leonardo Guzmán (*leonardo.guzman@ifop.cl*) and Henrik Enevoldsen (*h.enevoldsen@bio.ku.dk*).

May 2020

Planning is underway for a workshop on "*Modelling and prediction of harmful algal blooms, from event response to multi-decadal projections*" to be held in Glasgow, UK. The organising committee consists of Neil Banas, David McKee, Bingzhang Chen, Paul Udom (University of Strathclyde), Bengt Karlson (Swedish Meteorological and Hydrological Institute), Keith Davidson, Dmitri Aleynik (Scottish Association of Marine Science), Clarissa Anderson (Scripps / SCCOOS), Dennis McGillicuddy (Woods Hole Oceanographic Institution), Beatrix Siemering (Marine Institute, Galway), and is also coordinating with Katja Fennel and Marion Gehlen, chairs of the Marine Ecosystem Analysis and Prediction Task Team (MEAP-TT) of the GODAE OceanView programme.

We hope to secure enough funds to invite a substantial number of early-career and developing-world scientists. A programme of summer-school-like tutorials will be woven into conference-style presentations and discussions. The draft programme is organised into four parts:

- Exploring the diversity of HAB modelling approaches
- Emerging technologies and platforms to support HAB monitoring
- Linking models, observations, and stakeholder needs
- Scaling up: the global impact of global change on HABs

Scientists interested in the topic can contact Neil Banas (*neil.banas@strath.ac.uk*). We would especially appreciate hearing from potential partners outside North America and Europe.

Summer 2020

Symposium on automated in situ observations of plankton. In recent years novel in situ instrumentation has been developed for automated high frequency HAB detection in near real time. Instruments for observing grazers, e.g. microzooplankton and multicellular zooplankton are also becoming available commercially. These instruments are now being adopted in research and also in monitoring programmes. The aim of the mini-symposium is to bring together experts on, and users of, in automated in situ imaging systems, novel sampling equipment etc. to present methods, recent results and to share experiences. Another aim is to carry out a comparison of results when analysing plankton communities quantitatively. Young scientists are one target group of the symposium. After the main symposium a young scientist's data workshop for data processing and report/article writing is planned. The symposium is planned on summer 2020, pending the results of several applications for funds, besides some potential support from GlobalHAB. Scientists interested in the topic can contact Bengt Karlson (*Bengt.Karlson@smhi.se*).

GlobalHAB Scientific Steering Committee members

Elisa Berdalet, Institute of Marine Sciences, CSIC, Spain, Chair
Raphael Kudela, University of California, Santa Cruz, USA, Vice-chair
Neil S. Banas, University of Strathclyde, United Kingdom
Michele Burford, Griffith University, Australia
Christopher J. Gobler, Stony Brook University, USA
Bengt Karlson, Swedish Meteorological and Hydrological Institute, Sweden
Po Teen Lim, University of Malaya, Kuala Lumpur, Malaysia
Lincoln Mackenzie, Cawthron Institute, New Zealand
Marina Montresor, Stazione Zoologica Anton Dohrn, Italy
Kedong Yin, Sun Yat-Sen (Zhongshan) University, China

Eileen Bresnan, Marine Scotland Science, United Kingdom, ICES representative
Keith Davidson, The Scottish Association for Marine Science, United Kingdom, Ex-officio
Vera L. Trainer, National Oceanic and Atmospheric Administration, USA, ISSHA and PICES representative
Joe Silke, Marine Institute, Ireland, IPHAB representative

Henrik Enevoldsen, IOC UNESCO/ IOC Science and Communication Centre on Harmful Algae at the University of Copenhagen, Denmark
Ed Urban, Scientific Committee on Oceanic Research, USA

Do you have initiatives that contribute to the implementation of the GlobalHAB Scientific Programme with an international coordination perspectives?

Contact GlobalHAB @ *http://www.globalhab.info/contact*

ISSHA's Corner

The International Society for the Study of Harmful Algae (ISSHA) convened the 18th International Conference on Harmful Algae (ICHA 18), held in Nantes, France, from 21th to 26th October 2018. The ISSHA Council had two opportunistic meetings: the first before the conference on Sunday 21st October and the second after the Conference, on Saturday 27th October in addition to the organization of the General Assembly.

Young Students Session

Following the successful experience of the first S *tudent Speed Networking* mini-course, held on Sunday October 9th, before ICHA 2016 in Brazil [1], a new call for a similar activity was published on the ISSHA conference website. Mentors for the *Young Investigators Workshop* this year were: **Stephanie Moore** and **Kathi Lefebvre**, from NOAA; **Mike Parsons**, Florida Gulf Coast University; **Tim Davis**, Bowling Green State University and **Harry Nelson**, Industry-FlowCam. Thirty five participants were selected from over 100 applications. The group was composed of a mixture of students, post docs, and early career scientists. The Young Investigators Workshop allowed students and senior scientists to interact and practice brief sales pitches about their research (Fig. 1).

Travel Awards

There were 74 applications for travel awards from countries all over the world to the ISSHA Travel Award Committee. Sponsors for the awards included the Scientific Committee for Ocean Research (SCOR), the National Oceanic and Atmospheric Administration (NOAA), the International Atomic Energy Agency (IAEA), the French Groupe de Recherche GdR-PHYCOTOX and ISSHA itself. A total of 41 students (11 from US, 6 from Europe, 11 from Latin American, 3 from Oceania and 6 from Africa) received travel support and 19 French PhD students and postdocs had their registration waived. Once more, the lively ISSHA Auction raised funds for the next conference generation of students (Fig. 2).



Fig. 1. The Young Students Session, ICHA 2018. Teachers (top panel) from left to right: Stephanie Moore and Kathi Lefebvre; Mike Parsons; Tim Davis and Harry Nelson. Bottom: Young students and mentors.

General Assembly, Council Elections and Venue for the 20th ICHA 2022

The ISSHA General Assembly was held on Monday 22nd October from 18.30 to 20.00, followed by a generous drink and snacks party preceding the traditional ISSHA Auction led for the third time by **Robin Raine**.

Christine Band Schmidt (Mexico) presented ongoing preparations and logistic information for those planning to attend the 19th ISSHA to be held in La Paz, Baja California Sur, México (see forthcoming events, p. 31) in October 2020. The bid from Japan presented by **Ichiro Imai** (Japan) to host the 20th ICHA (2022) in Hiroshima was chosenr the conference venue in 4 years time (Fig. 3).

The 11th ISSHA General Assembly was opened by President **Vera Trainer** and minutes from the previous assembly were approved. ISSHA members had voted on-line to confirm Council officials willing to continue another term. Others had to step down after years serving the Society or changing positions in their jobs and new candidates had to be chosen. Results of the elections were given by ISSHA Secretary. Officials stepping down were Vice-president **Gustaaf Hallegraeff** (Australia), ISSHA Executive Secretary, **Anke Kremp** (Finland), and treasurer, **Henrik Enevoldsen** (Denmark) who accepted to continue his service as council member. Council members **Karen Lebret** (Sweden), **Lincoln McKenzie** (New Zealand) and **Luis Proença** (Brazil) also stepped down. The President thanked all of them in the name



Fig. 2. Scenes from the ISSHA Auction during the 18th ICHA in Nantes, 2018



Fig. 3. Happy Japanese delegates after learning their bid to host the 20th ICHA (2022) was successful.



Fig. 4: Eminent ISSHA members being nominated Honorary Members during the 18th Conference. From left to right: Marta Estrada, Linda Medlin, Pat Tester, Robin Raine and Tim Wyatt.

of ISSHA for their years of service, in particular Gustaaf Hallegraeff, council and executive member since 2006, and strongly committed to the Publication and Dissemination Committee through the edition of conference proceedings and other website sections (trail-blazers, HAB art gallery and others), and L. MacKenzie and L. Proença for hosting the 16th ICHA (New Zealand) and 17th ICHA (Brazil) respectively. The new ISSHA Executive and Council for the 2018-2020 period is (new members in bold):

Executive
President: Vera Trainer (USA)
Vice-Presidents:
Wayne Litaker (USA)
Po Teen Lim (Malaysia)
Secretary**: Elin Lindehoff** (Sweden)
Treasurer : **Nina Lundholm** (Denmark)
Past-president: Beatriz Reguera (Spain)

Council Members
Ana Amorin (Portugal)
Aurelia Tubaro (Italy)
Christine Band-Schmidt (Mexico)
Esther Garcés (Spain)
Ian Jenkinson (China, France)
Ingrid Sassenhagen (France, Germany)
Keith Davidson (United Kingdom)
Henrik Enevoldsen (Denmark)
Luis Mafra (Brazil)
Shauna Murray (Australia)
Marta Estrada (Spain)
Philipp Hess (France)
Ichiro Imai (Japan)
Dedmer B. Van de Wall (Netherlands)
Steffaney Wood (Finland)

In addition, the Society established the "honorary member" figure, to include ISSHA members with outstanding contributions to HAB science and society and a retirement age who will be welcome to future conferences with a considerable discount in their registration fees.

Honorary members, **Marta Estrada**, **Linda Medlin**, **Pat Tester**, **Robin Raine** and **Tim Wyatt** were called to the stage during the Closing Ceremony and synopses of their biographies presented (Fig. 4).

The profiles of these honorary members can be found at *https://issha.org/publications-resources/hab-trailblazers/* and those of the ISSHA officers and council members at *http://www.issha.org/Society/ISSHA-Council-Members*

ISSHA Achievement Awards

Maureen Keller Student Presentation Awards

Best student oral presentation was awarded to **Marc Long** from University of Wollongong (UOW, Australia) and Université de Bretagne Occidentale (UBO, France) for his contribution, *A multi-trait approach reveals the physiologic impacts of Cu on Alexandrium minutum and their exudates*.

Marc obtained his Master's degree in Marine Biology in 2015. In parallel with his university studies, Marc has been working on phytoplankton physiology and chemical ecology since 2011 in the laboratory of marine environmental studies (LEMAR, France) under the supervision of Dr. Hélène Hégaret and Dr. Philippe Soudant. During his studies, he also undertook research projects at the University of Wollongong (Australia) under the supervision of Professor D. Jolley and at the National Oceanic and Atmospheric Administration (NOAA,



Marc Long
(Best Oral Presentation)

Northeast Fisheries Science Center, United States) in Milford Connecticut, studying phytoplankton physiology with Dr. Gary H. Wikfors.

The work presented at ICHA2018 was part of his Ph.D. research, revealing that toxic concentrations of Cu increased the allelochemical potency of A. minutum and that the release was related to physiological stress. Overall, this research highlighted a complex interplay between allelochemical interactions and abiotic factors.

Marc is currently submitting his Ph.D. thesis as a collaboration between the University of Wollongong (UOW, Australia) and the Université de Bretagne Occidentale (UBO, France). During his Ph.D. project, Marc characterized the allelochemical interactions between the toxic dinoflagellate *A. minutum* and the diatom *Chaetoceros muelleri*. His main interests are the biologic interactions, chemical ecology and microalgal physiology.

Oral Presentation, Honorable Mention was awarded to **Juan Fernández Zabala**'s: *Are macroalgae a reliable method for the quantification of BHABs*, co-authored with Emilio Soler-Onís, Ana Amorim and Fernando Tuya. Juan was born in the Basque Country, Spain. He studied Biology and Biochemistry at the University of Navarra, taking his last year at the University of Tromsø, Norway. During this year, Juan was involved in a project which aimed at the production of biosurfactants from marine hydrocarbon-degrading bacteria at the Norwegian College of Fishery Science. In 2013, he moved back to Spain where he obtained his master´s degree in biotechnology at the Autonomous University of Madrid (UAM). The following year, Juan moved to the Canary Islands where he has been living for the past four years. During this time, he has been working at the Spanish Bank of Algae (BEA) as a flow-cytometry technician and participating in monitoring campaigns in the Canary Islands for BHAB risk assessment. Currently he is working on his PhD project in the framework of the European project MIMAR, supervised by Emilio Soler Onis, Ana Amorim and Rogelio Herrera at the University of Las Palmas de Gran Canaria (ULPGC). The main topic of his research is the ecology and bloom dynamics of BHAB dinoflagellates in the Macaronesian region. To address this, several methodologies are being developed to optimize sampling and cell collection and to study the distribution (both horizontal and vertical) of BHAB populations and how different biotic and abiotic factors contribute to this distribution. He is also interested in taxonomy of benthic dinoflagellates using both molecular and morphological approaches.



Océane Reale
(Best Poster Presentation)

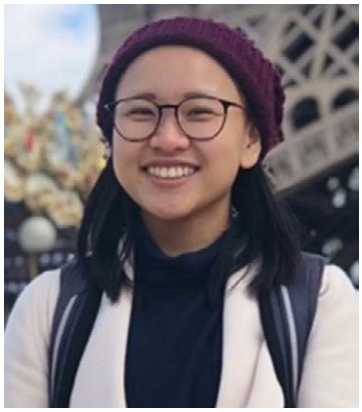
Océane Reale, *Best Poster Presentation,* a PhD Student at ANSES, Laboratory of Fougères (France) in Toxicology in vitro of Food Contaminants, received the award for her poster *In vitro toxicological assessment of lipophilic phycotoxins on Enteric Glial Cells*, co-authored by Valerie Fessard and Antoine Huguet. She is studying in the science agronomy engineering school (AgroCampus Ouest, Rennes, France) and specializing in Biotechnology for Health. She had the opportunity to do a 6-month internship in Montréal (Canada) in 2013-2014 in Biochemistry at the University of Quebec where she became fascinated with the world of research and experimentation. For her last internship and after her engineer diploma, she worked in the pharmaceutical industry at Servier (Biologie Servier, Gidy, France) in Preclinical Toxicology in vitro department as a scientific research associate. She worked on the development of 3D HepaRG cell models, a new tool for the evaluation of toxicity of new drugs. Captivated by the toxicology universe, she had the chance to work at ANSES (the French Agency for Ffood, Environment and Occupational Health and Safety) for a European Food Safety Authority project in genotoxicology evaluating the toxicity of two mycotoxins (Enniatin B and Beauvericin) using a mouse model.



Juan Fernández Zabala (Oral Presentation, Honorable Mention)
facing La Graciosa Island, his sampling area in northern Lanzarote, Canary Island

She continued her research in toxicology with a thesis project on the development of new cell models of the intestinal barrier (tri-culture and co-culture with epithelial cells, goblet cells and glial enteric cells), a tool for a best evaluation of toxicity of 6 phycotoxins. This thesis is supervised by Valerie Fessard and Antoine Huguet at ANSES Fougères.

Wai Mun Lum, *Poster Presentation, Honorable Mention,* from University of Tokyo, Japan, received the Maureen Keller award for the poster titled *Phylogenetic and morphological comparisons of Chattonella spp. collected from Southeast Asia* co-authored by Kazuya Takahashi, Garry Benico, Hong Chang Lim, Po Teen Lim, Chui Pin Leaw, Rhodora Azanza, Elsa Furio, Sandric Chee Yew Leong, Thaithaworn Lirdwitayaprasit, Hikmah Thoha and Mitsunori Iwataki. Born in Malaysia, she pursued Bachelor (with Honors) of Marine Science at the University of Malaysia, Sabah, Malaysia. Aiming to develop biosensors or chemical sensors for shellfish poisoning, she conducted her bachelor graduation research project entitled "Nanosensor based formaldehyde detection of narrow-barred Spanish mackerel", under the supervision of Dr. Md. Shafiquzzaman Siddiquee. However, she realized that extensive understanding of these harmful algae was necessary before developing such sensors. Thus, she is currently pursuing her Master's degree at the University of Tokyo, Japan, supervised by Dr. Mitsunori Iwataki, with the support of a Japanese Government (Monbukagakusho: MEXT) Scholarship. Through collaboration with several laboratories in Southeast Asia, she is studying the taxonomy of harmful raphidophytes species of the genus *Chattonella* as well as other marine harmful dinoflagellates in that region.



Wai Mun Lum
(Poster Presentation, Honorable Mention)

Yasumoto Lifetime Achievement Award

The ***Yasumoto Lifetime Achievement Award* 2016** was given to **Beatriz Reguera** for outstanding contributions to the biology and population dynamics of shellfish toxin-producing microalgae, in particular *Dinophysis* species (Fig. 5). These are low biomass HABs that even at densities of a few hundreds per litre, contaminate shellfish with diarrhetic shellfish poisons and cause lengthy shellfish harvesting closures in Atlantic coastal waters in Europe. B. Reguera's objective has been to obtain knowledge of species-specific differences (niche descriptions), mixotrophic behaviour and the physical-biological interactions driving the initiation, development and decline of *Dinophysis* populations in upwelling- influenced and stratified systems. Her work has informed conceptual models and contributed to the parameterization of biological processes for the development of predictive models in the framework of operational oceanography. Mainly based on field observations and laboratory incubations she explained the polymorphic life cycles and behaviour of *Dinophysis* species, showing that different small morphotypes labeled as separate taxons were but different stages of larger-sized *Dinophysis* species. Her research contributed to species-specific design of field sampling strategies to estimate physiological parameters of low-density *Dinophysis* populations in situ.

Beatriz has been a tireless mentor, promoter and communicator of HAB research by serving on numerous committees and panels. She hosted the highly successful 8th ICHA conference in Vigo in 1997, since 1992 served as the Spanish delegate to IPHAB which she chaired from 2002-2006, served two terms as ISSHA President (2008-2014), was member of the SCOR-IOC GEOHAB Scientific Steering Committee (2002-2006), chaired the ICES-IOC Working Group on HAB Dynamics (Chair 1992-1996), and currently is chief editor of the IOC-UNESCO newsletter *Harmful Algae News*

A short biography of her can be found at the *HAB Trail Blazers* section on the ISSHA website *https://issha.org/publications-resources/hab-trailblazers/beatrizreguera/*



Fig. 5. Beatriz Reguera receiving her award from Prof Yasumoto himself

Anna Godhe - In Memoriam

Anna Godhe passed away on April 4th 2019 after a strenuous fight with cancer.

Her untimely passing represents a big loss for the scientific community. We all remember her true passion for research, her important contributions to marine phytoplankton ecology and her open mind. She was always available to share knowledge and discuss with colleagues and students the fascinating mysteries of the life of unicellular organisms. Anna was a very positive person, a dedicated mentor of PhD students and post docs, and an excellent scientist.



Anna started her scientific career investigating the diversity and distribution patterns of dinoflagellate resting cysts along the Swedish coast. These studies provided an account of the different phytoplankton species that produce benthic resting stages in these coastal waters, explored the link between resting stage abundance and distribution and different environmental factors and provided the first evidence that many diatom and dinoflagellate resting stages are capable of long term (up to decades) survival in anoxic deep sediments. She tested the influence of benthic versus planktonic inocula on the development and composition of diatom communities with microcosm experiments demonstrating the importance of benthic resting stages and providing evidence for distinct species-specific life strategies.

Anna provided several valuable contributions on the use of molecular tools for the identification and quantification of phytoplankton species in the natural environment. The focus was the identification of harmful species, of resting stages in the sediments, and of cryptic species of the marine genus *Skeletonema*.

In recent years, the scientific interest of Anna has focussed on the marine diatom *Skeletonema marinoi*, which became a model for genetic and genomic studies. Microsatellite markers were used to investigate the population genetic structure of this diatom at various spatial and temporal scales along the Swedish coast and in the Baltic Sea, in collaboration with various colleagues. The in-depth knowledge of the genetic profile of *S. marinoi*, together with the results of laboratory investigations on the intraspecific variability in physiological responses at different environmental conditions, set the premises for establishing this centric diatom as a model species for genomic research and for studies addressing the evolution and adaptations in unicellular microalgae. Anna promoted the whole genome sequencing of *Skeletonema marinoi* within the CEMEB consortium at the University of Gothenburg. The genome sequencing of this species and the establishment of various genomic tools that are in progress will allow investigation of the molecular bases of the biology and adaptive mechanisms of this species. Reviving natural populations from their dormant phase buried in core sediments offer a unique setting to study evolution in action in microalgae over a time scale that is not affordable with laboratory experimentation.

A part of Anna's research activity has been carried out at the University of Mangalore (India) within a collaboration with the University of Gothenburg. This collaboration allowed to develop her favourite research themes in a different environmental setting, the tropical oligotrophic Arabian Sea.

Finally, it is with heavy hearts but in the spirit of Anna, that we add a personal note about Anna as a colleague and friend. The words we think about when we think about Anna are: enthusiastic, genuinely interested, open-minded, courageous, strong, ready to try new ways, excited of venturing into new territory, bridges disciplines, great collaborator, prefers complementing rather than competition, takes on challenges, not discouraged by failure, positive attitude, sees the potential rather than the problems, has opinions, limitless energy, and finally, an endlessly supportive and loyal friend.

Marina Montresor, Stazione Zoologica Anthon Dorn, Napoli, Italy
Anke Kremp, Leibniz Institute for Baltic Sea Research, Warnemünde, Germany
Karin Rengefors, University of Lund, Sweden



ICHA 2020

19th ICHA October 11-16, 2020. La Paz, B.C.S. Mexico ***Save the date!***

Registration will open during 2019. ISSHA members can register at special rates!

Participants wishing to receive the ISSHA member rate for conference registration must join ISSHA or renew their memberships prior to the 12th of June, 2020.

The 19th International Conference on Harmful Algae will take place from the 11th to the 16th of October 2020 at the Conference Center of La Paz, BCS, Mexico, being the 2nd ICHA conference held in Latin America. As the last conferences, the 2020 conference will include topics related with the understanding of the causes, evolution and impacts of harmful microalgae and cyanobacteria. We are planning an enjoyable meeting where scientists can present their research, share their ideas, establish new collaborations, and connect the science on harmful algae with the beneficiaries of this research.

Our reasons for holding this meeting are based on the recurrent presence of Harmful Algal Blooms that have affected the economy and public health in various coastal regions and inland waters of Mexico. These events have been associated with the mortality of marine organisms such has free-living and farmed fish, dolphins, turtles and seabirds; as well as the presence of diverse phycotoxins in clams, oysters and mussels, which put people´s health and lives at risk, reason for which it is necessary to establish sanitary closures for periods of weeks or months. Undoubtedly, this is a problem requiring collaboration efforts to reduce the adverse effects of these natural phenomena.

La Paz is an ideal city for the meeting where many academic institutes are found. It is a small, secure and quiet city, with beautiful surroundings, which is visited by many international tourists and academics year round. The average temperature in October is between 21 and 34 °C.

For some countries, a visa may be required. Please check visa regulations well in advance with your local Mexican Consulate for official instructions on the specific visa regulations and application procedures.

As the major host of the conference, ISSHA will support the event with various activities: Travel awards to students and post-docs, several achievement awards, and ISSHA auction.

For more detailed information concerning the conference, please consult our web page *www.icha2020.com*

Looking forward to seeing you in La Paz, Mexico!

Dra. Christine J. Band Schmidt
On behalf of the Local Organizing Committee
IPN-CICIMAR

icha.mex2020@gmail.com

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 63:
September 1st 2019

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Harmful Algal Blooms in a Changing Climate

In September 2019 the Intergovernmental Panel on Climate Change (IPCC) approved and accepted the *Special Report on the Ocean and Cryosphere in a Changing Climate* (SROCC) at its 51st Session [1]. This report was notable for many reasons, but of particular note was that an IPCC report identified "increased frequency in coastal areas since the 1980s of harmful algal blooms, attributed to both climatic and non-climatic drivers, with *high confidence*", meaning that experts agreed that there was medium to high agreement and robust evidence for this statement. In contrast the last major IPCC report published in 2014 (AR5 Chapters 5-6), just 5 years ago, reported limited evidence and low confidence for future climate change effects on HABs. In the most recent report there was also high confidence that trends in HABs were associated with ocean warming, marine heatwaves, oxygen loss, eutrophication and pollution, and high confidence that these events have had negative impacts on food security, tourism, local economies, and human health. Vulnerability to HAB events was linked to areas without sustained monitoring programs and early warning systems (medium confidence).

For some this statement from the IPCC may seem premature given that linking climate change and HABs has previously been identified as a "formidable predictive challenge" [2], and there are nearly as many questions as answers regarding climate interactions with HAB events [3,4]. Despite these questions the overall scientific consensus has shifted rapidly in the last 5-10 years.

The SROCC report cites several specific examples of climate-induced changes in HAB events, including range expansion of warm-water organisms such as *Gambierdiscus,* contraction of cold-water species, detection of novel phycotoxins and toxic species, and regional increases in frequency and intensity of toxic blooms, such as the *Pseudonitzschia australis* event in the eastern Pacific during 2015 and the record HAB events in Chile during 2016 caused by *Pseudochattonella* cf. *verruculosa* and *Alexandrium catenella*. Further evidence was documented from experimental studies linking multiple drivers to enhanced HAB activity, such as the synergistic effects of increasing CO_2 and temperature.

It is important to note that the SROCC report clearly identified the combined effects of both climate change (e.g. rising temperatures, decreasing pH) and global change attributed to human activity (e.g. eutrophication and pollution) as important drivers. SROCC identified the greatest risk for estuarine organisms because HABs are stimulated by riverine nutrient loads and exacerbated by warming and lower dissolved oxygen and pH associated with these environments. This inevitably leads to HAB-related risks for coastal biodiversity and ecosystem services.

SROCC also noted that there is considerable variability in HAB responses at a local and regional scale. It was suggested that regional variations could be explained by spatial differences in climate drivers such as temperature, water column stratification, ocean acidification, precipitation and extreme weather events, as well as by non-climatic drivers such as eutrophication and pollution.

SROCC makes several key points that provide a roadmap for ongoing efforts. First, HAB occurrence, toxicity, and risk are projected to increase with warming and rising CO_2. Second, increasing likelihood of occurrences of HABs under climate change elevates associated risks to fisheries, aquaculture and tourism as well as public health. Third, there is *limited evidence* that re-

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New Initiative on Fish-Killing Algal Blooms

An "Advanced International Colloquium and Technical Workshop on Fish-Killing HABs" under the auspices of IOC-IPHAB and GlobalHAB, and with the support of the government of Chile through CORFO and collaboration of CREAN-IFOP, was held in Puerto Varas, Chile, 8 – 11 October 2019. Ten invited participants (D.M. Anderson, A.D. Cembella (WG chair, apologies), O. Espinoza, L. Guzman, G.M. Hallegraeff, P.J. Hansen (acting chair), H. Hegaret, T.O. Larsen, J. Mardones, M. Iwataki, L. MacKenzie) reviewed the state-of-knowledge on fish-killing algal blooms (Fig. 1).

The socio-economic impacts from fish-killing microalgal blooms are much greater on a global scale than those from HAB species causing seafood biotoxin accumulation and contamination. Examples are the 1973 *Chattonella marina* bloom in the Seto Inland Sea, Japan (estimated US$71M loss to sea bream aquaculture), the 1988 *Prymnesium polylepis* bloom in the European Kattegat with widescale marine ecosystem impacts, and the 2015/16 *Pseudochattonella verruculosa* bloom in Chile (US$800M loss to salmon aquaculture). Highly potent fish-killers include the globally distributed, taxonomically unrelated flagellate groups *Alexandrium, Margalefidinium* (*Cochlodinium)*, *Chattonella*, *Heterosigma*, *Pseudochattonella*/*Vicicitus*, *Karenia*, *Karlodinium*, *Prymnesium*/*Chrysochromulina* which all produce lytic compounds that irreparably damage the sensitive gill tissues of fish which then die from suffocation. With perhaps a single exception (*Karenia brevis* in Florida), none of these fish-killing algal blooms have ever caused human health problems from seafood consumption, which partially explains the limited attention these HABs previously have attracted. Except for recent advances with *Prymnesium* (prymnesins) and *Karlodinium* (karlotoxins), the precise mechanisms of how such microalgae kill finfish remain poorly understood. *Pseudochattonella* is particularly poorly understood, with high ichthyotoxicity in nature but limited potency observed in culture studies. While some species are always ichthyotoxic, others such as *Heterosigma* and *Alexandrium catenella* cause problems only under special environmental conditions. Progress in this field has been hindered by the use of widely different bioassay systems (*Artemia*, blood and fish gill RT1 cell lines, embryos, juvenile and adult fish) and lack of analytical chemical methods and reference materials to quantify and characterize so-called "ichthyotoxins". Furthermore these compounds cause problems in extremely low concentrations dissolved in an aqueous medium, as opposed to the well known toxins PST, DST, AST, NST bioaccumulating in high concentrations in seafood. Essentially similar ichthyotoxins also adversely impact shellfish, shellfish hatcheries and aquariums that draw in water from bloom affected areas. Climate change is adding to the unpredictability of such rapid fish killing blooms and puts further pressure on seafood security for an ever increasing human population. Fish-killing HABs can be compared to disastrous forest fires, and not doing anything is no longer deemed acceptable. Prevention, prediction and monitoring are no longer sufficient, and we actively need to pursue strategies to stop the blooms, for example by means of clay flocculation of algal biomass and/or mopping up of ichthyotoxins.

The working group reviewed our knowledge of algal taxonomy, toxin chemistry, modes of action, fish-killing mechanisms, bioassays, broader ecosystem impacts, role of climate change, and options for prevention, control and mitigation. A well-attended open conference, accessible via webinar, and a visit to a local salmon farm formed part of the workshop. A follow-up session on fish-killing HABs is next scheduled for ICHA 2020 in Mexico, and a dedicated journal issue on this topic is being planned.

Some of the presentations are available on the link below; *https://www.facebook.com/1871967802832922/videos/1254975338037553/*.

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Invited speakers and hosts of the "Advanced International Colloquium and Technical Workshop on Fish-Killing HABs". Puerto Varas, Chile October 8-11, 2019. Photo courtesy of Oscar Espinosa, IFOP.

IOC-SCOR GlobalHAB Workshop: Evaluating, Reducing and Mitigating the Cost of Harmful Algal Blooms: a Compendium of Case Studies

Over the last two decades, several reports have compiled what is known about the economic impacts of harmful algal blooms (HABs) [1-4]. Although these reports attempted to gather comprehensive economic impact data, both the type and amount of data available were limited. One past study estimated the cost of HABs in the European Union at $800 Million [2] but most of that cost was extrapolated for a very few HAB organisms. Furthermore, most countries have neither conducted economic analyses of HABs nor collected data that can be used to generate reliable quantitative estimates of net economic losses and economic impacts. The lack of data, appropriate and standardized protocols, and the dearth of peer-reviewed studies hampers efforts to quantify the societal costs of increasingly frequent, intense and long-lasting HAB events and to help evaluate the cost of various strategies being developed for HAB prevention, control, and mitigation.

To strategize how specific economic studies can be used to assess the economic impacts of HAB and mitigate their risks, a joint GlobalHAB-PICES workshop was held on 17-19 October, 2019, prior to the annual meeting of the North Pacific Marine Science Organization (PICES). During this 2.5-day workshop, over 48 international experts on economics and the science of HABs from the USA, Japan, Australia, Canada, Korea, the UAE, China, France, Norway, Chile, Scotland, Spain, and the UK discussed a compendium of case studies that highlight the economic impacts of HABs on farmed salmon and shellfish and on wild-caught, reef-based fisheries.

Workshop discussion topics included the net impacts of HABs, their costs, and coastal resilience to HABs worldwide. Plenary lectures included worldwide examples of wild fisheries, recreational fisheries and aquaculture losses. The five case studies were: 1. US west coast *Pseudo-nitzschia* and impacts on shellfish and marine mammals; 2. Korea Margalefidinium (=*Cochlodinium) polykridoides* including impacts on wild and aquacultured fish kills; 3. Ciguatera fish poisoning; 4. Fish aquaculture including examples from the E.U., Canada, Chile; and 5. Shellfish aquaculture losses.

The economic impact of HABs is recognized to be large, although currently poorly quantified in many of the world's coastal areas. The losses faced by insurers are huge. At the workshop, a representative from a reinsurance company specified that 45% of insurance claims are now from HABs. In fact, it was stated that the loss due to HABs is larger than any storm that insurers have ever faced. In the Republic of Korea, an insurer has already collapsed due to frequent and enormous losses of aquaculture fish due to HABs.



Participants at the GLOBALHAB Workshop: Evaluating, Reducing and Mitigating the Cost of Harmful Algal Blooms, at the Victoria, British Columbia, Convention Center from 17-19 October 2019.

Several examples of HAB-related losses and loss mitigation were discussed at the workshop in detail. A HAB incident in northern Norway alone resulted in the loss of 14 thousand tons of Atlantic salmon in May 2019, resulting in a total loss of at least 330 million USD, including insured losses of 45 million USD, underinsured values and deductibles of 40 million USD, losses of future salmon sales at 160 million USD, cleanup costs at 30-40 million USD, and loss of taxes and unemployment benefits at 50 million USD. In Brittany, France, the Laboratoire d'Economie et de Management de Nantes-Atlantique (LEMNA), University of Nantes, is conducting a detailed estimation of the impacts of shellfish trade bans caused by HABs. Researchers at LEMNA are creating a database documenting these trade bans from 2004 through 2018 at shellfish harvesting areas in four French departments (Finistère, Morbihan, Loire-Atlantique and Vendée). These four areas encompass about 700 shellfish farms representing 37,600 tonnes of products with an estimated value €141 million, i.e. 20% of the national shellfish harvest.

Finally, breakout groups discussed strategies for mitigation including the value of information from better or more refined forecasts. Questions addressed included: Can contingency planning reduce loss? How do we open areas more quickly, how do we make closures shorter, and what is the value of information from better forecasts? What is the cost benefit analysis of monitoring programs? How much should be spent on monitoring? For insurance purposes, how do we reduce the cost of HABs?

The huge HAB-related losses to industry, consumers and governments illustrate the need for insurers, aquaculturists, public health professionals, economists, and HAB scientists to work together to estimate the cost of HAB events relative to the costs of mitigation and management. Studies of economic and social losses and their impacts need to be planned and teams need to be formed prior to HAB events to ensure that they are comprehensively studied. Toward this goal, the workshop further helped to establish greater connections between economists, industry scientists, and HAB researchers. Participants plan to refine and publish case studies to help guide future research and management priorities. A series of white papers are being prepared to document the workshop goals, the five case study examples, and summary recommendations for the future. These white papers will be published on the GlobalHAB and PICES websites. A summary of this work will be published in a peer-reviewed paper, providing several examples that can be used to steer future studies on the economic impact of HABs.

The workshop was co-sponsored by GlobalHAB and PICES with the support of the Scientific Committee on Ocean Research (*SCOR*), the International Society for the Study of Harmful Algae (*ISSHA*), Northwest Pacific Action Plan Coastal Environmental Assessment Regional Activity Centre (*NOWPAP CEARAC*), *Greig Seafood Ltd.*, the Intergovernmental Oceanographic Commission of *UNESCO*, and *AXA XL Reinsurance*.

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duction of non-climatic anthropogenic stressors can reduce the risk of HABs without also mitigating climate change. Finally, risks will be greatest in poorly monitored areas, and poleward distributional shifts of HAB organisms may exacerbate our ability to monitor HAB organisms and impacts.

Wells et al [3] identified two central goals to improve our understanding of HABs and climate change. First, to obtain compelling evidence that climate change has altered HAB distribution, prevalence, and character. Second, to develop the theoretical, experimental, and empirical evidence for these changes. SROCC argues that there is clear consensus for the former, and that the latter, combined with a renewed emphasis on documenting the efficacy of mitigation strategies, would increase our ability to predict how HABs will respond to climate change and to respond appropriately. As has been stated numerous times in the past we require a renewed commitment to global observation systems through efforts such as the Global Ocean Observing System and GlobalHAB so as to quantitatively monitor the multiple interacting factors shaping our ocean's future [2-5].

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Massive fish mortality in Teluk Bahang, Penang, Malaysia caused by a hypoxia-inducing algal bloom

Fish kill events due to algal blooms have been increased dramatically over the past decades. Several massive fish kill events have been reported in Malaysia [1-5]. Among the incidents reported, some are caused by toxins (ichthyotoxic, hemolytic, cytotoxic) released by harmful microalgae while others are due to high biomass blooms leading to dissolved oxygen depletion (hypoxia and anoxia)[1-5].

On August 11, 2019, a massive fish kill event was reported in Teluk Bahang, Penang, Malaysia (Fig. 1a). The local farmers claimed losses of over 50 tons of caged fish, with an estimated worth of USD 190,000 (Fig. 1b). Abnormal fish behaviors were observed by the fish farmers, as they noticed the caged fish jumped aggressively the night before the fish kill event occurred.

A rapid response field sampling study was conducted in the area (August 13-14, 2019) to investigate the cause of this fish kill event. Water samples were collected from four stations, St1 (near the river mouth), St2 (fish cage area – 0.5 km away from shoreline), St3 (2.1 km away from shoreline), and St4 (4.3 km away from shoreline) (Fig. 2). Water temperatures ranged from 30.1 – 30.7 °C, salinity from 30.8 – 31.6, while pH was relatively low, in the range of 7.42 – 7.69.

Dissolved oxygen (DO) levels in the water column were depleted; the lowest DO levels were observed at St 2 (fish cage area), with surface values as low as 2.47 mg/L, and 0.94 mg/L at the bottom (4 m depth) (Fig. 2b). These values were much lower than the recommended minimum DO requirement for tropical marine fish, i.e. 5 mg/L (75% saturation). A 24-h monitoring of DO levels revealed that surface (~1 m) values dropped drastically at night from ~8 mg/L to 1.93 mg/L, but rose steadily in the morning. Hypoxia occurs and conditions become unsuitable for any mariculture activity when dissolved oxygen levels fall below 2 mg/L [6].

Macronutrient concentrations at the study sites were determined spectrophotometrically. The highest nitrate and silicate concentrations, 25 µM and 21 µM, respectively, were observed at St2. The Chl-*a* (53.6 µg/L) maximum was also observed at this station. Microscopic phytoplankton analysis showed a high abundance of *Proboscia* sp., *Rhizosolenia* sp., *Chaetoceros* spp., *Guinardia* sp., *Coscinodiscus* spp., and *Leptocylindrus* sp. *Coscinodiscus* sp. was the dominant genus in all stations, with a maximum of 2.5×10^4 cells/L (Fig. 2c). Fish-killing dinoflagellates and raphidophytes were not observed at the samples. Most likely, excessive nutrient levels were one of the drivers inducing massive phytoplankton blooms in this area. The bloom-induced hypoxia reported here had a severe negative impact on the local aquaculture.

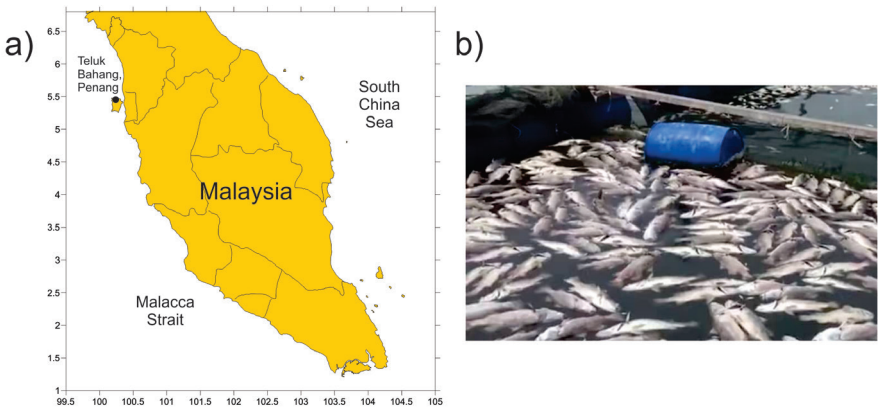

Fig. 1. (a) Map of Peninsular Malaysia showing the location of Teluk Bahang, Penang. (b) Fish kill phenomenon in a fish farm at Teluk Bahang (photo credit to [5]).

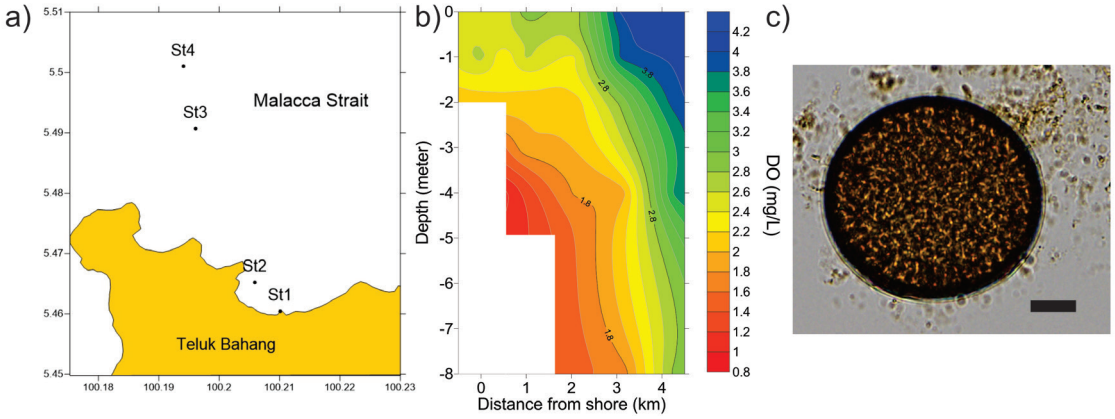

Fig. 2. (a) Sampling stations. (b) Dissolved oxygen levels (mg/L) in the study site. (c) Light micrograph of Coscinodiscus sp., scale bar = 20 µM.

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Blooms of the potentially harmful raphidophyte *Chattonella antiqua* and the occurrence of the epiphytic dinoflagellate *Ostreopsis* cf. *ovata* in the coastal waters of Alexandria, Egypt

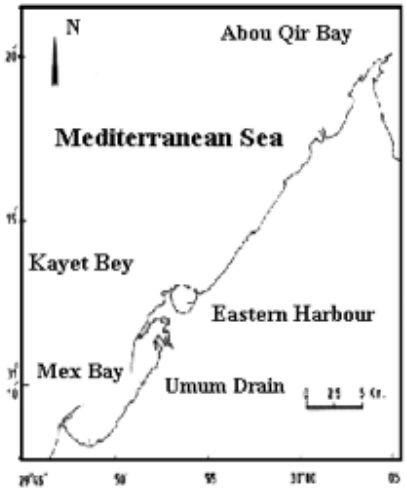

Fig. 1. Location of sampling stations

This article reports blooms of two harmful microalgal species, a raphidophyte and a dinoflagellate, at two localities, El Dekhila and Abou Qir, at the western and eastern sides of Alexandria coastal waters, south Mediterranean (Fig. 1).

Blooms of *Chattonella antiqua* (Y. Hada) C.Ono 1980

Chattonella antiqua is a unicellular, biflagellated raphidophyte frequently found in the plankton of temperate nutrient-rich coastal waters under a wide range of environmental conditions [1-3]. Members of the *Chattonella* genus can form harmful algal blooms (HABs) that cause massive mortalities of wild and cultivated fish via the generation of reactive oxygen species (ROS, e.g. superoxide). These ROS are responsible for gill tissue injury and mucus production that kill fish [1-2,4]. *Chattonella antiqua,* was also found to produce brevetoxins [5]. These microalgae are the focus in monitoring studies in some coastal marine areas. Blooms of this genus are usually accompanied by golden-brown seawater discoloration due to their high content of fucoxanthins [3,6].

The El Dekhila area is part of Mex Bay, western Alexandria. This is a highly eutrophic marine basin due to the huge amounts of domestic sewage, industrial wastewater, and agricultural run-off from different land based sources (estimated as 7-8×10^6 m^3 per day), discharged directly into the sea, or indirectly from Lake Mariout. The area, considered ecologically, economically and socially important, is an extremely variable system. High inputs of nutrients and organic matter of anthropogenic origin may create favourable conditions for the development of recurrent massive harmful algal blooms causing fish and invertebrate mortality [7]. Blooms of *C. antiqua* have been previously described in Alexandria waters [1], and its presence as a member of the local phytoplankton community was reported several decades ago [7].

Cell densities of *C. antiqua* (Fig. 2) in surface waters of the El Dekhila area during the summer of 2019 reached up to 16.7 × 10^6 cells l^{-1}, causing reddish water discoloration. The bloom appeared associated with high temperatures, intermediate salinity and extremely high nutrient loads (Table 1). The observed physico-chemical conditions are not very different from those observed previously associated with the occurrence of red tides in Alexandria waters. Among the co-occurring phytoplankton species, *Chaetoceros compressus* (4.1x10^6 cell l^{-1}), *Skeletonema costatum* (0.28x10^6 cell l^{-1}), and *Leptocylindrus minimus* (0.16x10^6 cell l^{-1}) thrived during these bloom conditions. This is the second report of massive *C. antiqua* blooms in Alexandria. During the 2006 bloom [1], a few cells of *Heterosigma,* which later became a red tide bloom species in the area, were observed [7]. Due to their tolerance to a wide range of environmental conditions, it may be assumed that blooms of raphidophytes in Alexandria waters are more common than has hitherto been documented in the literature.

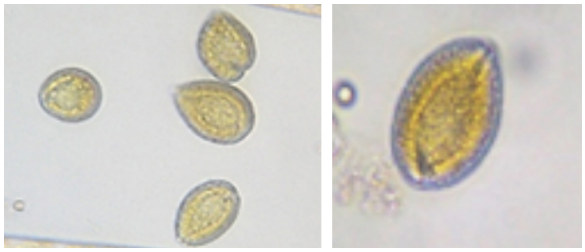

Fig. 2. Micrographs of Chattonella antiqua (Y.Hada) C.Ono

Currently the Egyptian government is implementing a substantial initiative to improve the water quality of Lake Mariout. Reduction of nutrient loads would be one of the best strategies to mitigate blooms of the genus *Chattonella* and other potentially harmful algal species in Mex Bay.

Epiphytic *Ostreopsis* cf *ovata*

In the last few decades there have been an increasing number of reports worldwide detailing the geographical expansion and impacts of epiphytic microalgal species belonging to the genus *Ostreopsis* in temperate areas [8,9]. *Ostreopsis* cf *ovata* is known to produce toxins and its toxicity is associated with the presence of palytoxin analogues [10]. In the Mediterranean Sea, neurotoxic effects due to ingestion of seafood contaminated with *Ostreopsis*-related toxins transferred through the food web have not yet been reported. So far, blooms of certain *Ostreopsis* species are implicated only in mild respiratory, skin and eye irritation [11]. A review of the existing literature over the last three decades on harmful red tide blooms in Alexandria coastal waters has been performed and focuses on planktonic bloom forming species [12]. Information on the ecology of potentially harmful epiphytic dinoflagellates from Alexandria coastal waters is extremely poor. Planktonic stages of *Ostreopsis* cf. *ovata* were recorded in the study area in July 2007, but maximal density reached (1980 cells l^{-1}) was well below the alert values [13].

The relationship between environmental factors and the abundance of epiphytic *O.* cf *ovata* cells in Abou Qri was investigated. This is a highly exposed rocky area in eastern Alexandria.

Table 1. Seawater physical and chemical parameters during the summer 2019 Chattonella bloom

			mg O_2/L		Nutrient concentration µM				
°C	Salinity	pH	DO	COD	PO_4	SiO_4	NO_2	NO_3	NH_4
35.00	17.8	8.64	6.3	22.5	13.35	58	12.6	20.6	45.2

Table 2. Physical and chemical properties of seawater in the study area

Parameter	°C	Salinity	pH	DO	DO%	NO_2	NO_3	NH_4	TN	SiO_4	PO_4
May	25,1	37,2	8,37	10,4	61,5	3	11,02	2,5	16,52	0,12	0,66
July	28,37	35,43	8,59	9,35	39,2	1,2	8,34	2,8	12,34	5,64	5,44
October	22,34	36,6	8,11	2,65	139,2	1,5	10,1	1,2	12,8	0,62	1
February	17,63	35,8	8,65	3,58	163,1	2,34	7,8	2,2	12,34	0,45	0,39
Mean	23,36	36,26	8,43	6,50	100,75	2,01	9,32	2,18	13,50	1,71	1,87
S.D.	4,54	0,80	0,24	3,94	59,71	0,82	1,50	0,69	2,02	2,63	2,39

Table 3. The abundance of Ostreopsis cf ovata attached to Chlorophyta, Rhodophyta and Phaeophyta macroalgae.

May	St. I		St. II	
Macroalgae	**Cell g fw^{-1}**	**%**	**Cell g fw^{-1}**	**%**
Chlorophyta				
Total	401.06	63.84	325.50	66.50
Mean	133.69		108.50	
SD	102.06		88.71	
Rhodophyta				
Total	227.19	10.15	164.00	9.35
Mean	75.73		54.67	
SD	64.76		46.28	
Phaeophyta				
Total	10.80	0.09	9.35	1.90
Mean	5.40		3.10	
Standard Dev	0.57		0.49	

July	St. I		St. II	
Macroalgae	**Cell g fw^{-1}**	**%**	**Cell g fw^{-1}**	**%**
Chlorophyta				
Total	60.49	9.63	38.10	7.78
Mean	20.16		12.70	
SD	24.11		17.07	
Rhodophyta				
Total	12.77	2.03	8.70	1.78
Mean	6.39		4.35	
SD	4.08		3.26	

October	St. I		St. II	
Macroalgae	**Cell g fw^{-1}**	**%**	**Cell g fw^{-1}**	**%**
Rhodophyta				
Total	7.4	10.41	1.1	1.55
Mean	2.47		0.37	
SD	3.67		0.46	

February	St. I		St. II	
Macroalgae	**Cell g fw^{-1}**	**%**	**Cell g fw^{-1}**	**%**
Rhodophyta				
Total	1.06	5.65	0.78	2.89
Mean	0.35		0.26	
SD	0.10		0.11	

Measurements of physical and chemical parameters at the sampling sites are given in Table 2. Salinity values didn't show a particular seasonal variation, and pH values were very stable. Dissolved oxygen levels in May and July were 3.5-3.9 times greater than the minimum in October. Nitrite concentrations exhibited the typical summer minimum, and it was highest in May. Nitrate concentrations were always high, and represented > 60% of the total estimated nitrogenous resources. Ammonia concentrations were often <3 µM, and reached a minimum in October. Silicate values were the lowest in May, with a maximum of 5.6 µM in July. Phosphate trends followed that of silicate.

Abundance on the thalli of 14 different macroalgae species was estimated during a full year sampling. Analysis of variance showed significant differences in abundance between seasons and macroalgal hosts. Water temperature and nutrients (NO_2^-, NO_3^- and PO_4^{3-}) seemed to be the major factors affecting the abundance of *Ostreopsis* cf *ovata*. Salinity variations exhibit a weak, insignificant influence on the abundance of specimens attached to Chlorophyta spp., and a negligible effect for those on Rhodophyta spp. (Table 3).

The *Ostreopsis* maximum (Fig. 3) was found in summer (July) associated with the highest water temperature (28.4 °C), while its minimum was recorded in winter (February) at a much lower temperature (17.6 °C). Temperature is a key factor determining the *Ostreopsis* blooms, but on site-specific bases, its dynamics and distribution in summer is driven by a combination of light intensity, wave activity, etc. Allelopathy has also been suggested among the biotic factors crucial to *Ostreopsis* occurrence [14].

Although *O*. cf *ovata* was found on all of the examined macroalgae, its abundance was ranked between Chlorophyta > Rhodophyta > Phaeophyta. The highest abundance was observed between spring and autumn with the peak on chlorophycean species in July, followed by a sharp decline in winter with the minimum in February (Table 3). The results showed relevant differences in the abundance of *O*. cf *ovata* depending on the macroalgal host species. The Chlorophytes *Ulva linza*, followed by *U. fasciata* and *U. compressa* seemed particularly suitable to support relatively high abundances of *Ostreopsis* during May and July; abundance was relatively less on *U. lactuca* in October. Our results suggest Chlorophytes might be an optimal macroalgal substrate for epiphytic *Ostreopsis* in the study area. Conversely, previous studies in the Mediterranean showed that the Ulvaceae constituted the substrate of least preference. Some factors, such as the sampling protocol and seasonality of the macroalgae, may explain these discrepancies. As for the Rhodophyta, the maximum abundance was found on *Corallina (=Ellisolandia) officinalis* in July and October. The occurrence of Phaeophyta species was restricted to May and July, and among them, *Padina pavonia* contained the highest abundances.

In summary, *Ostreopsis* cf *ovata* may pose an additional threat to public health and the economy of Alexandria. Temperature, PO_4 and NO_3 seemed to be the main factors influencing its abundance. *Ostreopsis* cf. *ovata* had the ability to attach to diverse macroalgae species, with preference for Chlorophyta. Since *O*. cf. *ovata* cells are loosely attached to the substrata and can be easily removed and re-suspended in the water column, future studies about their relation with wave dynamics should be addressed. To our knowledge, this is the first ecological study of epiphytic forms of this species during a full annual cycle in the SE

Continued on page 16

First records of *Gambierdiscus excentricus* and *Ostreopsis lenticularis* in the Cape Verde Archipelago (Macaronesia, Central Eastern Atlanctic)

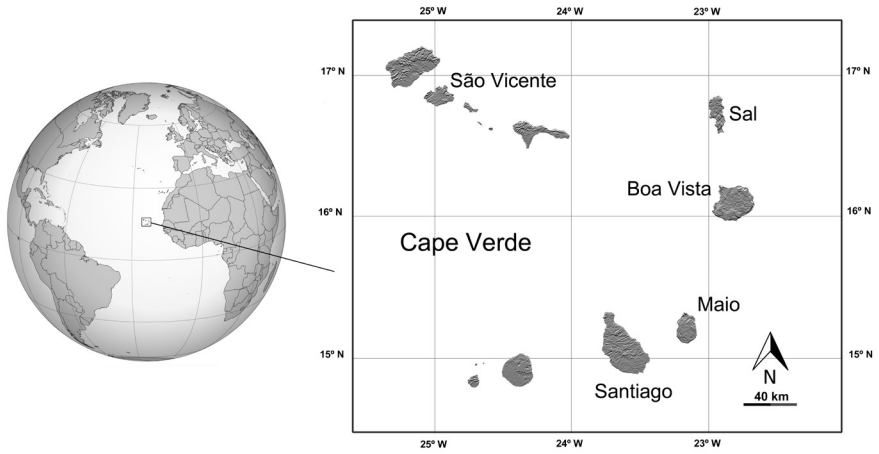

Fig. 1. Map of Cape Verde archipelago (Macaronesia Region).

Harmful algal blooms (HAB) species frequently recorded in tropical latitudes are apparently increasing their distribution in temperate areas [1]. This expansion is linked both to naturally occurring changes, such as global warming, as well as anthropogenic causes such as ship ballast water transport, nutrient loading, marine habitat deterioration and expanding aquaculture [2]. These changes are allowing the establishment of HAB species in areas where they were previously unknown. In some areas, this has been well studied, whereas in others the only evidence of the presence of these species is the harmful impact they have had on marine organisms, the environment and human health.

Macaronesia is a name used to designate several archipelagos of volcanic origin in the Atlantic off the south west coast of Europe and north west of Africa; the Azores and Madeira (Portugal), the Canary Islands (Spain) and Cape Verde. There are no reports on the occurrence of ciguatoxins (CTXs) and maiotoxins (MTXs)-producing dinoflagellates in Cape Verde.

Currently, the only information available on microalgae in the Cape Verde Islands was published by Silva in 1956 from material collected in Boa Vista Island in 1948 [3]. Silva's study included the first report of a presumed *Gambierdiscus* species named as *Goniodoma* sp. Fraga and Rodríguez described a new benthic dinoflagellate species, *Gambierdiscus silvae*, in the Canary Islands and suggested that probably corresponded to Silva's original description of *Gonodioma* sp. [4].

The objectives of the project MIMAR (MAC/4.6d/066), funded by the European Union included the monitoring, control and mitigation of the observed changes in the marine ecosystems from Macaronesia. Within the framework of this project, different islands have been sampled within the Cape Verde Archipelago and since 2017 an exhaustive inventory of microalgae species with particular interest on potentially harmful species is being carried out. Sampling has been performed in 32 coastal sites on five Islands: São Vicente, Sal, Maio, Santiago and Boa Vista (Fig.1). Sampling sites included a variety of intertidal and subtidal marine habitats ranging from 1 to 40 meters depth. Habitats included sandy and rocky substrates, macroalgal beds and coral beds (Fig. 2). Benthic microalgae species, both from macroalgae and from artificial substrates were sampled by snorkelling and by SCUBA diving. Water temperature during the sampling period ranged from 24 to 27 °C. Cells were examined with scanning electron microscopy (SEM) (JEOL JSM-6380 LV) and with light microscopy/fluorescence microscopy (LEICA DM6000 B). Cells were stained with fluorescent stain calcofluor for the examination of their plate tabulation. A total of 140 microalgae species were identified. Diatoms were the most numerous group with 108 species. *Gambierdiscus excentricus* Fraga, *Ostreopsis lenticularis* Y. Fukuyo and *Ostreopsis* cf. *ovata* Y. Fukuyo were identified amongst the dinoflagellates.

Gambierdiscus excentricus had an anteroposteriorly compressed lenticular shape. Measurement ranges



Fig. 2. Macroalgae and corals habitat at Baia das Gatas, São Vicente.

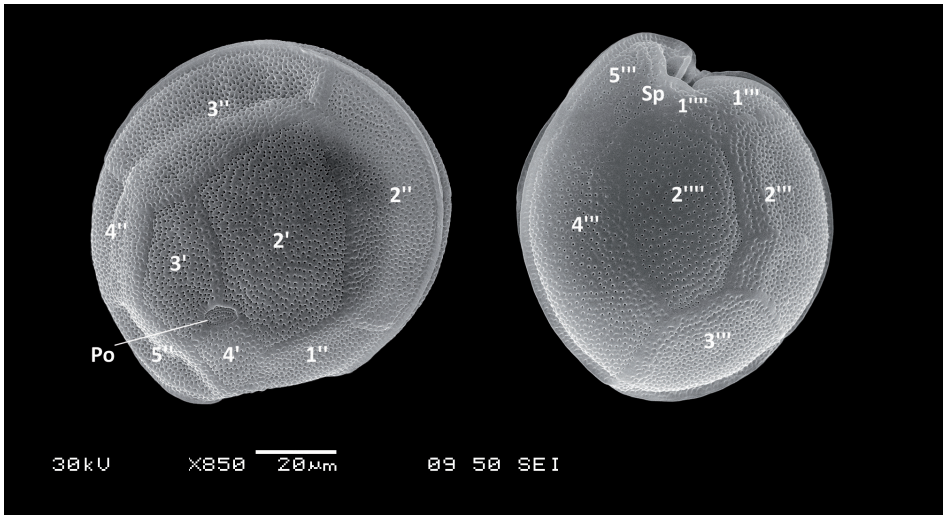

Fig. 3. Gambierdiscus excentricus. Scanning electron micrographs, apical and ventral views

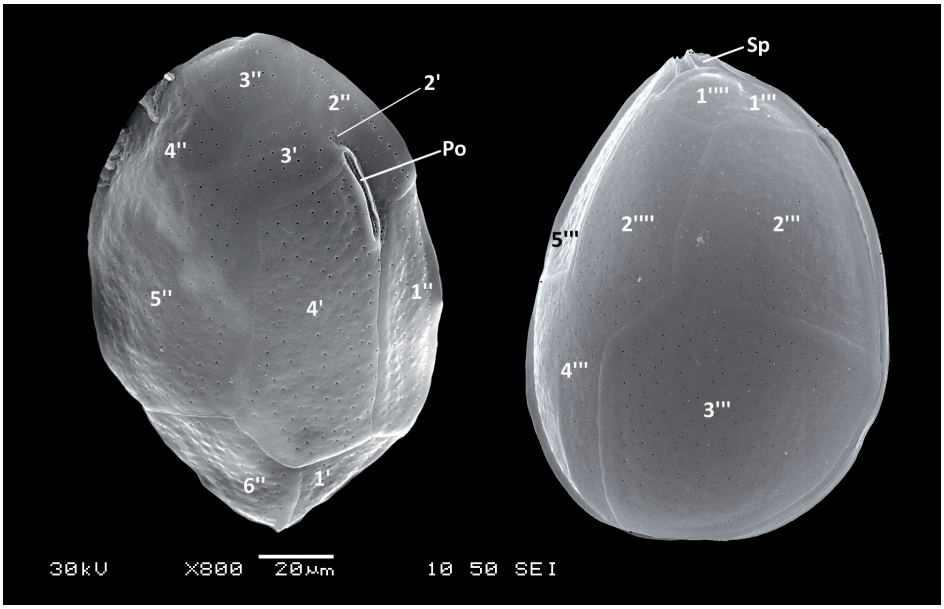

Fig. 4. Ostreopsis lenticularis. Scanning electron micrographs, apical and ventral views

were: depth (D) 84-110 µm, width (W) 70-105 µm, and length 35-40 µm. The thecal plate formula was: Po 4' 6'' 6c ?s 5'' 1p 2''''. Thecal plates were smooth with fine round to oval pores. Apical pore plate Po was oval with a fishhook-shaped slit and was ventrally displaced. First apical plate, 1', was very small. Ratio between plates 2'/3' and 2'/4' suture lengths ranged from 2 to -2.5. Plates 1' and 6'' were very small and facing the posterior part of the cell due to the torsion of the flagellar area which formed a hollow. From this hollow, two flagella emerged, the longitudinal one being perpendicularly projected. The Sp was in the hypotheca, out of the sulcus. The 2'''' plate was about twice as long as wide (Fig. 3).

Ostreopsis lenticularis Y. Fukuyo cells were broadly oval in apical and antapical views, lenticular-shaped, biconvex, and flattened, with the cingulum straight in lateral view. Measurements ranges were: depth (D) 60-105 µm, width (W) 53-74 µm and the DV/W ratio 1.13-1.42 (mean 1.27). The thecal plate formula was: Po 4' 6'' 6c 4?s 5''' 2''''. The apical pore plate Po was long and narrow, slightly curved with the outline of the cell. The fourth apical plate (4') was elongated and hexagonal, located mostly on the left side of the cell. The second apical plate (2') was narrow and elongated, and located below the APC, extending dorsally to the Po plate, and reaching about the mid-position of the 3' plate. Plates 5'' were pentagonal and reached half of the DW axis (Fig. 4). The thecal surface was smooth and plates presented numerous pores of two types (Fig. 5). Large pores and small pores visible with the light microscope with epifluorescence and with SEM. Analyses of the LSU rDNA D1-D3 corroborated previous identifications of *Gambierdiscus* and *Ostreopsis* species. The analyses of samples from 32 sampling sites showed that *O. lenticularis* was widely distributed in Sal, Sao Vicente and Boa Vista but was not observed in Maio and Santiago. *Ostreopsis lenticularis* cell densities ranged from 50 per sample to 1 x 10^6 cells per 100 cm^{-2}. *G. excentricus* was distributed in all the islands visited except for Maio. *Gambierdiscus excentricus* concentrations ranged from a few cells per sample to 150 cells per 100 cm^{-2}.

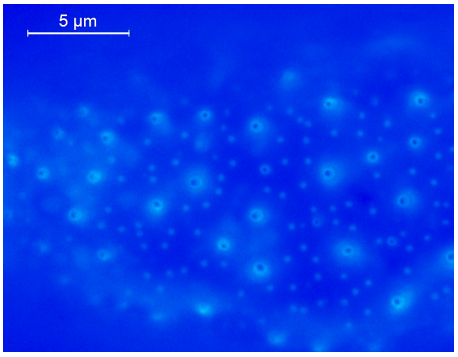

Fig. 5. Ostreopsis lenticularis. Epifluorescence micrograph showing detail of the thecal surface with two kinds of thecal pores.

Potentially toxic species of the genera *Gambierdiscus* and *Ostreopsis* are reported for the first time in the Cape Verde Islands. The presence of these species would explain the ciguatera cases that have been previously documented for this archipelago.

Acknowledgements

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Microcystis bloom in Saladito river, central-southern Cuba

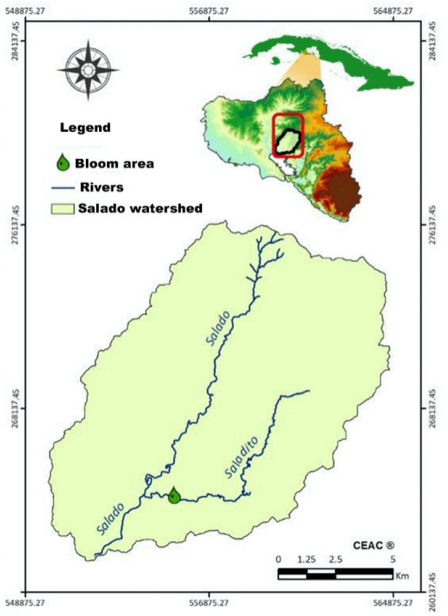

Fig. 1. Map showing the cyanobacterial bloom area in Saladito River, central-southern Cuba.

"Water blooms" or simply "blooms" in freshwater reservoirs are mass accumulations of planktonic microalgae or cyanobacteria. Water blooms ("Wasserblüte") were defined as floating agglomerations in the water surface composed mostly by some blue-green algae (cyanobacteria) [1]. In freshwater systems, these blooms are planktonic communities in which cyanobacteria are dominant. Other microalgal group species, such as the green alga *Botryoccus braunii* Kützing (Trebouxiophyceae) [2], may co-occur with the cyanobacteria, but always as a lesser part of the community. The occurrence of toxic cyanobacterial blooms poses a threat to human health and environmental quality [3, 4].

The unicellular genus *Microcystis,* a producer of microcystins, is one of the most important bloom-forming cyanobacterial genera worldwide, particularly in stagnant freshwater bodies with increasing eutrophication [4]. Komárek [5], reported *Microcystis,* represented by 6 species, as the most frequent bloom-forming genus in stagnant freshwater reservoirs in Cuba, but the central part of the country was not included in his studies. Comas et al. [6] reported blooms from reservoirs in the Cienfuegos province were mainly formed by *M. panniformis* Komárek et al. and *M. cf. smithii* Komárek et Anagnostidis. Blooms composed by cyanobacterial filamentous genera were also recorded from the same area [7].

The microalgal and cyanobacterial flora of Cuban lotic systems (rivers) is practically unknown and cyanobacterial blooms have not been reported in these systems. During the routine monitoring program for validation of SWAT (Soil and Water Assessment Tool) model, a dense cyanobacterial bloom was observed (on June 14th, 2019), in the lower part of the Saladito river (22°16' 43'' N; 80° 22' 53''W), Cienfuegos province, Central-southern Cuba. The Saladito River is 17.2 km long with an average depth of 1.24 m. Its waters are used for irrigation and aquaculture [8]. The river flows into the Salado river, which in turn flows into Cienfuegos bay, one of the most important natural resources of the region (Fig. 1). Green water discoloration extended for about 1 km (Fig.2). The June 2019 bloom was formed by a *Microcystis* species with a cell density of 6 x 10^4 cells mL^{-1}. Low densities of pennate diatoms were also present as accompanying species. No deleterious effects of the bloom on freshwater fauna were observed, but it represented a potential risk to agriculture and aquaculture in the area. Environmental factors favorable for the occurrence of this bloom included high irradiance and water temperature (32.4 °C) during summer. Salinity was 0.26, total solids in suspension 50 mg L^{-1}, dissolved oxygen 17.30 mg L^{-1} and pH 8.53. The bloom may have also been favored by droughts at the beginning of the rainy period (May-October). These droughts lead to increased stability of the water column and nutrient concentrations.

Taxonomic classification of chroococalean cyanobacteria is difficult. The genus *Microcystis* is not an exception. Species identification has been based on a few morphological features (shape of colonies, structure of mucilaginous

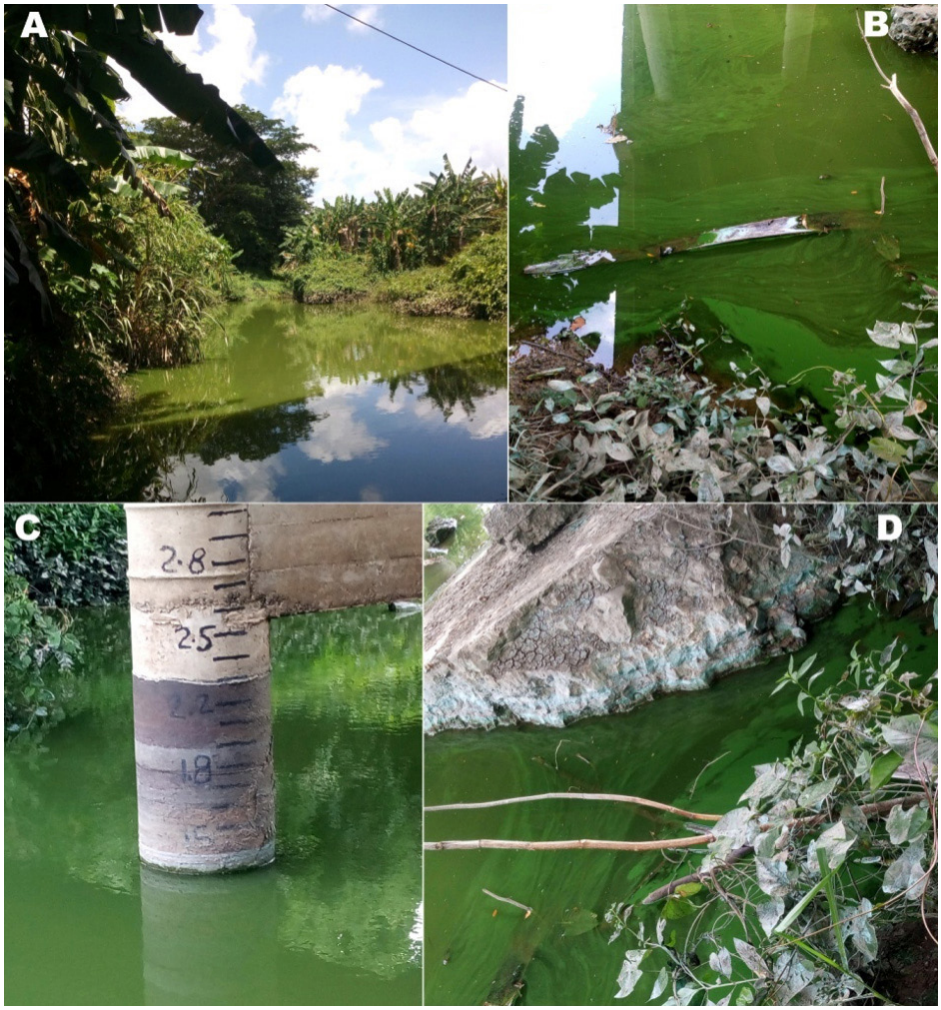

Fig. 2. Microcystis bloom in the Saladito River. A. Bloom and some dried riverine vegetation. B-D. Intense green water discolorations.

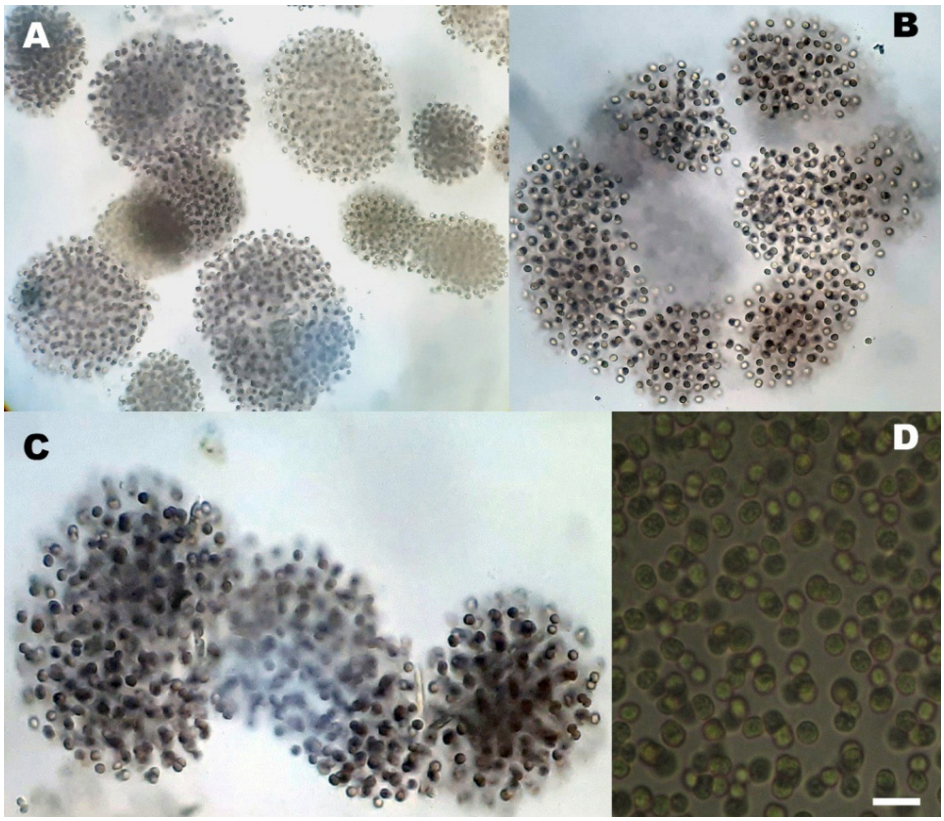

Fig. 3. Microcystis sp. A-C. Colonies. D. Details of cells inside a colony (Scale bar = 10 µm).

envelope, cell size, presence of aerotopes, etc). These characteristics show a great variability and often the features of several populations overlap the limited morphospecies criteria. In addition, some populations often present local varibility. The genus Microcystis has a clear genotype delimitation, but many of its intrageneric units are not yet strongly supported [9]. Several *Microcystis* morphospecies, with similar morphology and ecology and classified as the traditional species, occur in different regions of the world. Their accurate identification is useful and necessary for applied hydrobiology and ecological investigations.

Cuban specimens belong to these populations whose characteristics overlap the limited morphospecies criteria. Colonies are spherical, spheroidal to irregularly elongated, flat or twisted, sometimes more or less hollow, with densely agglomerated cells, mostly without holes or seldom with small holes; solitary or after fragmentation of a colony, 2-4 new colonies are formed, remaining into a single mucilaginous envelope. Mucilage is colourless, slimy, with margins diffused, relatively wide, sometimes overlapping cells. Cells are spherical, 3-6 µm in diameter, pale blue-green, with aerotopes. Solitary cells in colony were not observed (Fig. 3).

According to the morphology of the solitary colonies (spheroidal, without holes, 5.6 µm in diameter) the Cuban specimen in this study resembles those of *M. flos-aquae* (Wittrock) Kirchner ex Forti, but composed colonies are not present. The typical *M. flos-aquae* occurs in temperate zones, and is not very common here. Tropical populations under this name are considered problematic [9]. The pantropical *M. panniformis* Komárek et al. forms similar solitary colonies in young stages, composed colonies are not formed and the mucilage margins of colonies are not overlapping the cells, which are smaller (2.5-5 µm in diameter). *M. aeruginosa* f. *flos-aquae* (Wittrock) Elenkin (*M. flos-aquae*) was frequently found as the dominant species in Cuban cyanobacterial blooms [5]; however these populations belong to *M. panniformis* [10]. By the form and structure of the colonies, our specimens could be related also to *M. novacekii* (Komárek) Compere. This tropical species, very rare in warmer areas of temperate zones, forms spherical solitary colonies during its early stages; typical composed colonies are formed in older stages. The mucilage is wide, with delimited margins, rarely diffuse, homogeneous or slightly concentrically lamellated. Cells are spherical, 2.4-6 µm in diameter, densely concentrated in the center of the colony; a few solitary cells may appear in the mucilage. In our populations the typical solitary cells in mucilage were not observed, neither the concentrically lamellated margins. It is possible that the Cuban specimens could be identified as *M. panniformis* or *M. novacekii*, but further studies about the whole life cycle will be necessary for a reliable identification of this morphotype.

Acknowledgements

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Citizen Science Oceanography in the Strait of Georgia, Canada – an overview of five years of operations

The Citizen Science Oceanography Program for the coastal waters of British Columbia (BC), Canada was proposed by Dr. Eddy Carmack, Fisheries and Oceans Canada (DFO). Carmack envisioned a 'mosquito fleet' of private boats collecting oceanographic data simultaneously throughout the Strait of Georgia (SoG). He said "[using] this citizen science approach, you can get into the shallow waters, you can get up the bays and estuaries. You can get around rocks. You get that quasi-three-dimensional perspective, almost four-dimensional". Such a program was funded in 2015 by the Pacific Salmon Foundation (PSF) and supported by the Oceans and Networks Canada (ONC) and DFO.

Keen volunteers from local communities were trained and their boats equipped with the necessary equipment (Fig. 1), and they have made oceanographic measurements and collected samples at ~80 defined locations on the same day approximately every two weeks between February and October, 2015-2019 resulting in complete coverage of the SoG. Oceanographic conditions were measured with CTDs (conductivity, temperature, depth) and water samples were taken for nutrient, phytoplankton, and zooplankton analysis. Samples were analyzed by skilled technicians at DFO, University of Victoria, and PSF. The data collected allows the examination of spatial and temporal variations in the physical and chemical oceanography, and plankton ecology throughout the entire Strait. This information has provided a unique high-resolution data series of the physical and lower trophic state of the SoG: this resolution is crucial for coastal waters as significant changes happen on a fine temporal scale in these areas, and only high frequency sampling can capture it.

Harmful algae monitoring is part of the Citizen Science Program. Some algae cause visible, high cell concentration blooms (that can be harmful or non-harmful) while some algae can cause



Fig. 1. Citizen Scientists sampling in the Strait of Georgia.



Fig. 2. Blooms of Noctiluca scintillans in: Salt Spring Island (top), May 2, 2018 (photo by Michael Bahrey); Nelson Island (bottom left), July 25, 2019 (photo by Ted Woodard) and mixed with an Heterosigma akashiwo bloom, Kuper Island (bottom right), May 22, 2018 (photo by Michael Bahrey)

harm (*e.g.* fish kills, shellfish poisoning) at very low concentrations. In the latter case, they are still called blooms because of their effects. These types of blooms can be invisible to the naked eye and only *in-situ* sampling can detect them. During five years of observations, the heaviest blooms were bright orange-red blooms of *Noctiluca scintillans* in 2018 and 2019 (Fig. 2) and the bright turquoise blooms of coccolithophorids in 2016 (Fig. 3). These blooms were visually striking and attracted a lot of public attention and news coverage. Other blooms, visually less prominent but potentially highly significant to marine life included blooms of: *Heterosigma akashiwo*, *Pseudo-nitzschia* spp. (Fig. 4), *Gonyaulax* spp., *Rhizosolenia setigera*, and *Dictyocha* spp. Phytoplankton time series obtained through the Citizen Science Oceanography Program showed clear seasonal and spatial patterns of some species. Investigation of the relationship between environmental factors and blooms will improve our understanding of regional bloom dynamics.

Citizen scientists sample areas close to where they live and to which they have emotional connections. Giving them the ability to measure their own waters is empowering. Carmack says that "if you look at bang for buck, value for money and engagement of people, and drawing kids into the game, it should be on every coast in the world". ONC states that "historically, oceanographic data collection relied on specialty research vessels and highly trained scientists in limited locations. These new tools reduce the high cost of sporadic data collection, increasing the geographic range of quality measurements." Spencer Taft, Tsleil-Waututh Nation adds that Citizen Science monitoring of the SoG "will allow us to precisely track changes as the waters are affected by climate change, development and restoration activities". October 2019 marks the end of the fifth year that the Citizen Science Oceanography Program has been carried out in the SoG: PSF hopes to raise funds to continue this program in 2020.

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Fig. 3. Bloom of Coccolithophorids, Maple Bay, 2016 (photo by Will Duguid)

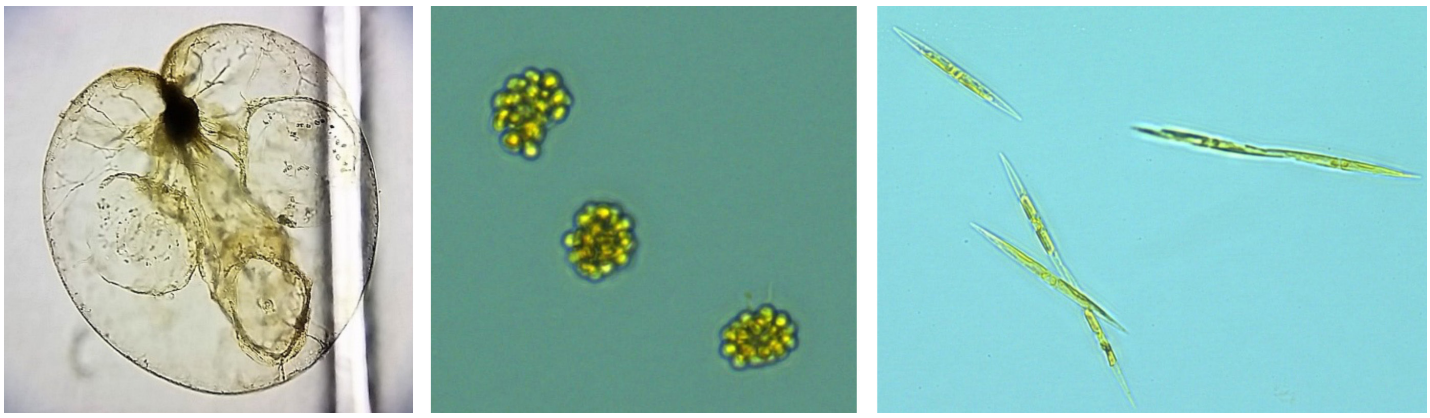

Fig. 4. Citizen Science samples: left, Noctiluca scintillans; middle, Heterosigma akashiwo; right, Pseudo-nitzschia spp.

Multicoloured algal blooms in the NW Adriatic during 2018

The northern Adriatic is characterized by shallow waters (mean depth about 35 m), a weak bathymetric gradient along the main axis and a high riverine input on the western side, affecting both the circulation regime and the trophic status. As a consequence, the productivity of the western coastal waters of the North Adriatic is markedly higher compared to the general oligotrophic conditions of the Mediterranean Sea. The spatial distribution and the seasonal variability of phytoplankton communities in the Adriatic Sea are mainly driven by major river discharge (and therefore by the rainfall regime), in combination with the stratification/mixing condition [1].

Since the last decade, the irregularity of meteorological events has markedly altered the seasonal cycle of phytoplankton. During rainy years, the very intense and sudden rainfall has caused anomalous blooms throughout the year [2]. This was observed during 2018, when several intense anomalous blooms occurred along the Marche coast (northern Adriatic Sea) associated with water discoloration. Here we have summarized the major events that occurred.

In April an exceptional bloom of the diatom *Chaetoceros socialis* (Fig 1) reaching cell densities of 10^7 cells l^{-1} caused an intense brown water discoloration along the Ancona coasts. Blooms of that species are not common, although they have been reported in the past. No cases of anoxia or mortality of benthic organisms were reported.

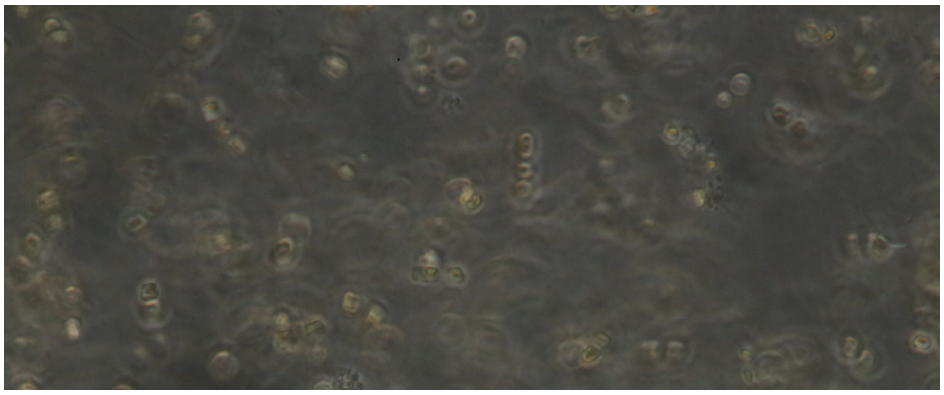

Fig. 1. Chaetoceros socialis intricate colonies.



Fig. 2 Greenish waters due to Vicicitus globosus bloom.



Fig. 3. Brick-red water discoloration from a Fibrocapsa japonica bloom.

In May, an unusual green coloration extended for several kilometres offshore, causing concern among bathers and yachtsmen. Microscopy analysis revealed that the causative organism of this bloom was the dictyochophycean *Vicicitus globosus* (formerly *Chattonella globosa*, raphidophytes, Fig 2). The presence of *V. globosus* in this area was reported only once in 2012 [3], and although this species is indicated as potential fish killer, fish mortalities were not observed.

In July in the coastal waters north of Ancona, an intense bloom of the raphidophyte *Fibrocapsa japonica* was observed (Fig 3), causing a brick-red discoloration. The first report of a *F. japonica* bloom in the Adriatic Sea dates back to 1997 in the Marche coast [4] and a bloom generically ascribed to raphidophyceans was reported in the southern part of Emilia Romagna coast even in 1994 [5]. *F. japonica* blooms reach cell densities of 10^7 cells l^{-1} and have occurred almost each year in July – August, both in Marche and Emilia Romagna coasts, typically extending from the shoreline to 300 m offshore and showing an intensification of colour in the early afternoon. Except for a detriment to tourism and recreational activities due to the intense water discoloration, fish kills have been observed only once in front of the Ancona port, but autopsies revealed that the cause of death was not due to haemolytic toxins, but to asphyxia [6].

In August, a greenish- brown water discoloration was observed due to an intense proliferation of an unidentified gymnodinioid (Fig. 4), and in November an impressive green tide due to a mixed diatom community and *Prorocentrum cordatum* (Fig. 5) extended from the shoreline to several kilometres offshore.

Such events have been related to the intense and irregular rainfalls that occurred during 2018, causing sudden massive releases of nutrient-rich



Fig. 4. A bloom of an unidentified gymnodinioid caused a brown-greenish discoloration.



Fig. 6. Ostreopsis cf. ovata bloom causing bleaching of macroalgal thalli.

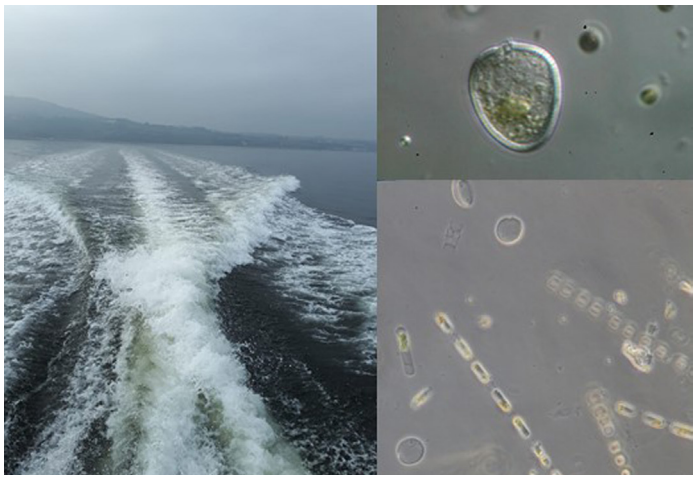

Fig. 5. Green colored waters from a mixed bloom of diatoms and Prorocentrum cordatum.

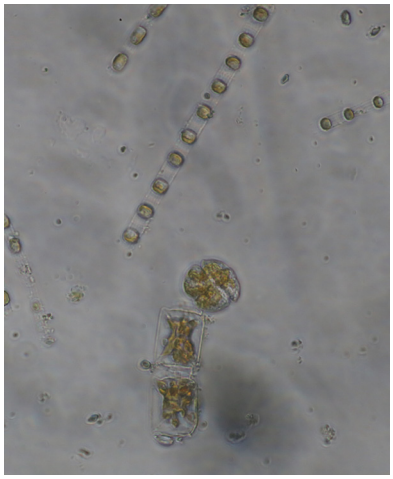

Fig. 7. Field sample showing Takayama tasmanica and diatoms.

freshwater that enhance phytoplankton blooms.

Benthic substrata hosted summer-autumn blooms of the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata*. This species has regularly occurred along the rocky coast of the Conero Riviera since 2006, reaching abundances among the highest in the entire Mediterranean Sea (order of magnitude of 10^4 cells cm^{-2}, 10^6 cells g^{-1} fw) [7]. In 2018, an *Ostreopsis* bloom started at the beginning of August, reaching the maximum values at mid-September (Fig._6). No evident noxious effects on human health were observed but impacts on benthic organisms were detected.

Finally, in December, a gymnodinioid considered toxic to marine fauna i.e. *Takayama tasmanica*, identified through microscopy and molecular analyses (Fig. 7), was reported for the first time in the area, although in non-blooming cell concentrations.

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A bloom of *Prorocentrum triestinum* in the Hossegor Marine Lake (France)

Phytoplankton communities in the Hossegor marine lake (Southern French Atlantic coast, Fig. 1) have been monthly monitored since 1997 to protect human health (REPHY network: monitoring of toxin producing species which may contaminate cultivated oysters) and environmental (EU Water Framework Directive (WFD) network) purposes.

This shallow, eutrophic marine lake which is subject to opportunistic green algae blooms is fed by a narrow canal that receives water from the ocean and two small rivers. Water temperature ranges between 10 and 25° C and salinity between 20 and 35. In this lake, the phytoplanktonic communities are usually dominated by marine species (*i.e. Leptocylindrus minimus, Dactyliosolen fragilissimus, Cylindrotheca closterium*) whilst blooms of freshwater species (Cyanobacteria: *Planktothrix agardhii*) may occur during periods of high river discharge.

In August 2019, following the observation of a brown coloured water phenomenon, analysis of water samples revealed high abundances (about 6 million cells per liter) of *Prorocentrum triestinum* (class *Dinophyceae*, order *Prorocentrales*, Fig. 2). This was the first observation of this species in this lake. Since then, its presence has been quite sporadic due to the high tidal-induced renewal of water within the lake.

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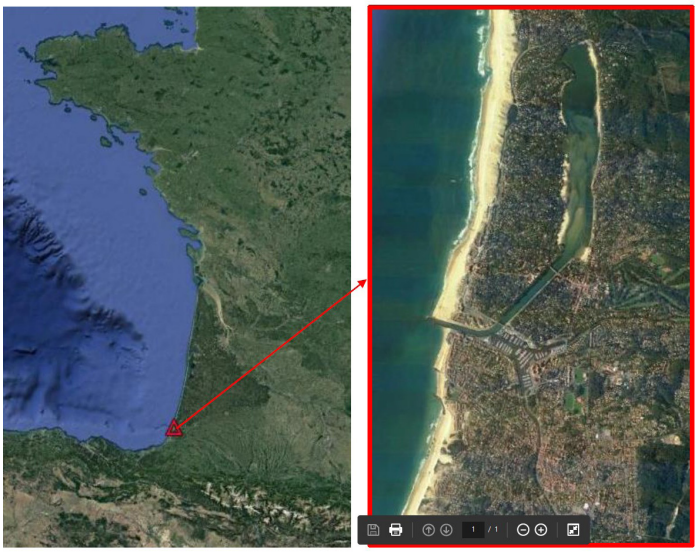

Fig. 1. Study area - Hossegor marine lake

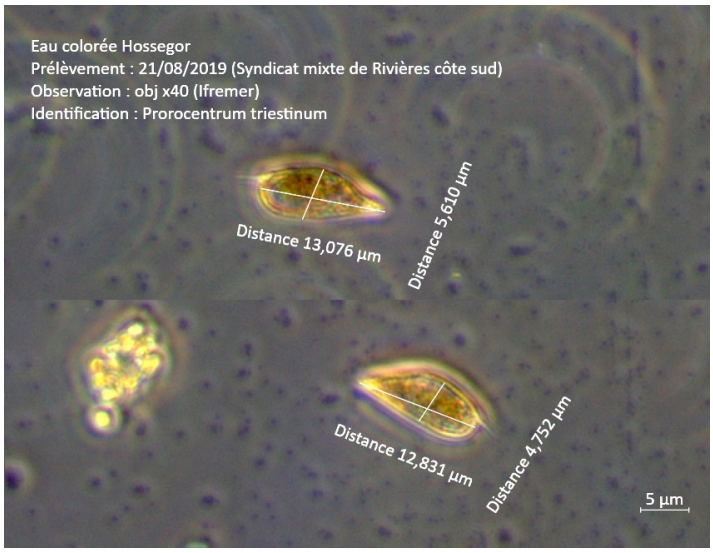

Fig. 2. Prorocentrum triestinum (fixed with neutral Lugol's solution) in a lake water sample collected during August 2019.

Continued from page 7

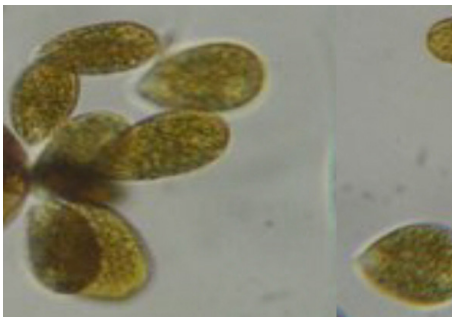

Fig. 3. Micrographs of Ostreopsis cf. ovata

Mediterranean Sea. Results of this study may contribute to a better understanding of *Ostreopsis* ecology and population dynamics and its trends in Alexandria waters. In addition, it provides the basis for an effective bloom management and mitigation of its effects.

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The ICES Annual Science Conference 2019

The International Council for the Exploration of the Sea (ICES) annual science conference took place in Gothenburg, Sweden, 9-12th Sept 2019 with 738 participants from 38 countries attending. The conference opened with a lively panel discussion around sustainable management of the sea particularly in relation to the UN sustainable development goals. Daily keynote presentations included 'The future of ¬fish and its role in securing food for a 9-billion world' by Manual Barange, Food and Agriculture Organization of the United Nations (FAO), 'Climate-driven redistribution of ocean life and its implications for society' by Greta Pecl from the Centre for Marine Socioecology (CMS), Australia and 'Re-examining physical-biological linkages in a changing ocean: what will we need to know by 2030? 'by Cisco Werner, NOAA Fisheries, USA. These presentations can be viewed online at *https://www.youtube.com/playlist?list=PL4gXA2v1PWKOwlHZKXe7ClpaVmAX4lnuM*.

Eighteen science theme sessions addressed a variety of topics including oceanography, fisheries, aquaculture and man's interaction with the ocean. Theme session J focused on 'HABs and Jellyfish- Impacts on ecosystems and ecosystems services'. The session was convened by Eileen Bresnan (ICES-IOC WG Harmful Algal Bloom Dynamics), Sophie Pitois (ICES WG Zooplankton Ecology) and Mike Rust (ICES Aquaculture steering group) as a first step to begin a dialogue between the three different work strands within ICES. HAB presentations focussed on reviews of HAB monitoring and impact data collected over the last two decades with presentations from the North Atlantic Area, Irish coastal waters, and the Kattegat-Skagerrak/the Baltic Sea. In contrast to HABs, routine monitoring for jellyfish is not established in many areas. A new method to use fish surveys to collect jellyfish data was presented and molecular methods which showed them to be a component of the diet of forage fish in the Celtic Seas and Western Channel. Modelling methods were used by both HAB and jellyfish scientists to look at bloom advection and development with a view to providing a service for the aquaculture industry. Challenges for offshore aquaculture, the distribution of benthic dinoflagellates in Sweden, impacts from filamentous algae on herring larvae in the Baltic were the focus of poster presentations. Conveners welcome new members to the working groups and can be contacted via the email addresses below.

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In Memoriam – Professor Tufan Koray

It is with a heavy heart we announce that Professor Tufan Koray, internationally recognized phytoplankton taxonomist and ecologist, died on March 9, 2019, after a short illness, in Hamburg, Germany.

Dr. Koray was an exceptionally kind and thoughtful person, known for his love of nature, art, music, and animals. He was able to build a distinguished career focused on the taxonomy and ecology of plankton, especially toxic/harmful microalgae species, as well as pollution in marine ecosystems and fish farms. Overcoming several setbacks, he became the pioneer and founder of modern phytoplankton taxonomy in his country, making a significant contribution to the scientific knowledge in this region.

Dr. Koray was born 1954 in Turkey, received his PhD in 1985 from Ege University, Izmir, Turkey, and was a faculty member at the same university until



2016. After his retirement, he moved to Hamburg, Germany, where he spent his last years pursuing his passion of art and music.

As well as being a very thorough and knowledgeable reviewer of the scientific literature, Dr. Koray was the editor, associate editor/board member for several journals in Turkey, such as *Ege Journal of Fisheries and Aquatic Sciences and Turkish Journal of Botany*. He and his team also published *The Microplanktonic Atlas of the Turkish Seas* in 2007. He was a member of ASLO, and a contributor to ERMS, FAO/UNEP-MAP, SCIENCEnet, IOC-UNESCO Harmful Algal Bloom Programme, and the International Scientific Community Expertise Center for Taxonomic Identification.

Dr. Koray was an outstanding scientist and committed teacher and mentor, and he has certainly left his mark on our world. The scientific community will miss him.

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11th Irish Shellfish Safety Workshop

At the 11th Shellfish Safety Workshop held in the Radisson Blu Hotel Athlone, Ireland, Joe Silke, Director of Marine Environment and Food Safety Services at the Marine Institute said, "Ireland's Shellfish Safety Monitoring Programme ensures that shellfish placed on the market meet the highest standards of food safety. This workshop enables scientists and regulators to exchange information and discuss the latest research, advances in technology, and forecast any issues for the industry, to help ensure Ireland continues to offer high-quality products."

More than 90 shellfish producers and processors, scientists, researchers, agencies and stakeholder representatives recently attended the Shellfish Safety Workshop to discuss the latest advances in shellfish safety in Ireland.

The workshop, held on 8th October, was hosted by the Marine Institute and co-sponsors Food Safety Authority of Ireland, Sea Fisheries Protection Authority and Bord Iascaigh Mhara. The event offered an opportunity to exchange information on the latest research and information on the cause and control of shellfish products harvested and farmed around Ireland's coast.

Speakers included Dr Conor Graham, GMIT Marine and Freshwater Research Centre on the development of the world's first scientific-based shellfish traceability tool. This unique tool used trace elemental fingerprinting of shellfish soft tissues and shells to identify the harvest location of blue mussels and scallops with 100% success, including mussels reared from two sites located just 6km apart within the one bay.

Other speakers included Dr Monika Dhanji Rapkova, Centre for Environment, Fisheries and Aquaculture Science on the learnings on regulated and emerging biotoxins in British shellfish. Dr Eileen Bresnan, Marine Scotland Science presented a talk on the regional distribution of harmful algal events in North Atlantic Area. Dave Clarke, Marine Institute also talked about the insights and perspectives on monitoring algal and biotoxin events in Irish coastal waters from the past 20 years.

Micheál O'Mahony of the Sea Fisheries Protection Authority presented on the recently published European baseline survey of norovirus in oysters, while Dr Sinéad Keaveney, Marine Institute discussed the survey in the Irish context.

There were also a series of flash presentations from representatives of the Marine Institute, Bord Iascaigh Mhara, Sea Fisheries Protection Authority, Food Safety Authority of Ireland, Dublin City University, Sligo Institute of Technology and Health Services Executive.

The proceedings of the workshop are currently being compiled for publication in the coming weeks and will be available for download from the Marine Institute's Open Access Repository.

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Fig. 1. Participants at the 11th Irish Shellfish Safety Workshop



Fig. 2. Oyster Farm. Photo courtesy of Fionn O'Fearghail, Marine Institute

Marine Institute

The Marine Institute is the state agency responsible for marine research, technology development and innovation in Ireland. The Marine Institute provides government, public agencies and the maritime industry with a range of scientific, advisory and economic development services that inform policy-making, regulation and the sustainable management and growth of Ireland's marine resources.

Foras na Mara
Marine Institute



Fig. 3. Micheál O'Mahony of the Sea Fisheries Protection Authority

The 33rd annual meeting of the Australasian Society for Phycology and Aquatic Botany (ASPAB)

The 33rd annual ASPAB meeting was held at NIWA's Greta Point site in Wellington, New Zealand on 11-13 November 2019. This year most presentations were on macroalgae although in the past microalgae and HABs have been a hot topic. This year's HAB presentations were by Rossella Nicolai (MSc student, Victoria University of Wellington, Cawthron Institute), who presented 'Investigating the active export of microcystins in freshwater cyanobacteria', and Dr Tomohiro Nishimura (JSPS Overseas Research Fellow, Cawthron Institute), who spoke on the 'Diversity, diarrhetic shellfish toxin production, and distribution of benthic dinoflagellates *Prorocentrum lima* complex and *P. caipirignum* in Japan' (Nishimura et al. 2019). The meeting draws together algal researchers from across Australia and New Zealand and is a great opportunity for students to present their work.

ASPAB was established in 1980 and its aims are to promote, develop and assist the study of, or an interest in, phycology (the study of macro- and micro-algae such as seaweeds and phytoplankton) and aquatic botany (the study of aquatic plants) within Australasia and elsewhere (see *https://www.aspab.org/*).

The next meeting, the 34th annual ASPAB meeting, will be held in Australia in 2020. The date and venue will be announced on the website. Marine and freshwater algae and aquatic plants researchers and students from within Australasia and from overseas are welcome to attend.

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Participants at the 33rd ASPAB meeting, Wellington, New Zealand

Forthcoming events

Call for abstracts - ICHA 2020



The Organizing Committee is pleased to announce the call for abstracts and pre-registration for the 19th International Conference on Harmful Algal Blooms to be held from the 11th to the 16th of October 2020 in La Paz, Baja California Sur, Mexico. La Paz is a small, secure and vibrant maritime city where many scientific institutions are situated – an ideal city for the meeting!

The organizing committee invites the submission of abstracts within a broad range of topics related to the study of harmful algae from basic studies to future applications. For the full list of topics, please visit the conference website (*icha2020.com/Secciones/contenido/33*). The deadline for submitting an abstract for a poster, speed-talk or oral presentation is the 12th of April 2020. All abstracts must be submitted at the conference website (*icha2020.com/Secciones/contenido/35*).

Participants wishing to receive the ISSHA member rate for the conference registration must join ISSHA or renew their memberships prior to 12th of June 2020.

A visa may be required for participants from some countries. Please check visa regulations well in advance with your local Mexican Embassy or Consulate for official instructions on the specific visa regulations and application procedures.

As the main host of the conference, ISSHA will support student travel awards, achievement awards, and the popular ISSHA auction!

Modeling and Prediction of Harmful Algal Blooms: Emerging Methods, Regional Problem-solving, and Patterns of Global Change

IOC-SCOR GlobalHAB and EuroMarine are pleased to announce this international workshop to be held in Glasgow, Scotland, 18-21 May 2020.

The typical HAB is a regional- or local-scale phenomenon, a "perfect storm" of environmental conditions, ocean transport and mixing patterns, and microbial ecology. Because of this complexity, prediction of HABs is a grand challenge that requires multidisciplinary dialogue among physical scientists, biologists, computer modellers, and technologists, as well as community stakeholders and the government and industry end-users of prediction systems.

This 3.5-day workshop will combine oral and poster presentations, round-table discussions, and tutorials in order to:

- Increase awareness of the range of modelling and observational tools that are in our community toolbox (or should be);
- Help the HAB community speak with one voice regarding climate-change impacts on the global ocean; and
- Help scientists and technologists develop creative approaches to meeting the needs of coastal communities, governments, and industry worldwide.

Hands-on tutorials will include "Getting started with…" sessions on remote-sensing data, machine-learning approaches, global climate model output, and dynamical plankton models, with presenters including Clarissa Anderson, (SCCOOS/Scripps, USA), Raphael Kudela (UC Santa Cruz, USA), Charles Stock (NOAA GFDL, USA), and Androniki Tamvakis (University of the Aegean, Greece).

Abstract submission is open through **15 Dec 2019** at ***habmodelworkshop.sccoos.org***, for presentations and facilitated discussions in the areas of:

1. Regional problem-solving: linking models, observations, and stakeholder needs
2. Emerging approaches and technologies: physical and ecological model methods and observational capacities that open up new directions in HAB prediction
3. Global patterns and global change: links between HABs and environmental drivers at large spatial scales and on long time horizons
4. Scalable solutions: applications of global models, remote sensing, and other communal resources to predicting HABs and managing their impacts in data- and resource-poor systems

We particularly invite scientists outside North America and Europe, and early-career scientists in general, to propose discussion topics and get involved in facilitating the meeting. **Funding is available to cover travel costs** for a subset of participants, with the goal of increasing international and career-stage diversity as much as possible.



12th International Conference on Modern and Fossil Dinoflagellates

The Canarian HABs Observatory (OCH) hosts the 12th edition of the International Conference on Modern and Fossil Dinoflagellates (DINO12), to be held from 13th to 17th July 2020 at the Alfredo Kraus Auditorium in Las Canteras beach, Las Palmas de Gran Canaria, Spain. The International Conference on Modern and Fossil Dinoflagellates (DINO) has been held on a continuous basis at different places of the world since 1978.

DINO12 will have global warming as an overarching theme in the context of dinoflagellates and their cysts. As usual, the conference will gather biologists working with modern dinoflagellates and geologists working with fossil dinoflagellates.

For further information visit: *www.dino12conference.com*

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 64:
March 1st 2020

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Harmful Algae News

An IOC Newsletter on Toxic Algae and Algal Blooms

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

The IOC Taxonomic Reference List of Harmful Microalgae

The creation of the *IOC Taxonomic Reference List of Harmful Microalgae* was first discussed 23 years ago at the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms in Vigo, Spain, 30 June-2 July 1997. It was decided to establish a *Task Team on Algal Taxonomy* in order to create an agreed reference list of harmful algal species as well as the correct citation of the author(s), date of valid publication, and a list of synonyms. The focus of the list was to be on toxin producing HAB species. Further suggestions for the list were made during the HAB 2000 Conference in Hobart, Tasmania, February 2000, and the list has subsequently been discussed at the biannual meetings of the IOC Intergovernmental Panel on Harmful Algal Blooms (IOC IPHAB) in Paris.

At recent IOC IPHAB meetings it was suggested that information about the list should be presented at regular intervals in *Harmful Algae News* and at the international HAB conferences. This is currently under consideration. The background to the taxonomic list is given here. The list was created because of the confusion that existed at the time about the proper names for HAB species and also the correct author citations. The years following the creation of the list have not resulted in a general agreement regarding the names to be used for some HAB species (nor for many other plant or animal species), and with the wisdom of hindsight this was probably a somewhat naïve aim. Research on HAB species has proceeded rapidly in many parts of the world, but the use of more and more sophisticated research tools has not always resulted in more clarity. Presently molecular methods are used as a tool for phylogenetic information and for classification. However, this has not led to a universally accepted taxonomy. Some journals have accepted articles in which the evidence presented did not stand up to closer scrutiny. Also, the old problem of splitters and lumpers remains, with little resolution in sight. Splitters prefer to divide taxa into smaller units, from the highest level (class or phylum or even higher) to the lowest (species level or below). Each taxonomic unit therefore contains only closely or very closely related taxa. The number of taxa therefore increases, sometimes drastically, and the picture becomes more complex. Lumpers prefer to work with larger taxonomic units and aim to create a simpler, but still correct system. In fact the term 'correct' is also under debate. Must all taxonomic units be monophyletic or are paraphyletic units sometimes to be preferred? There is no simple answer. Classification is a subjective exercise, and has always been. It is therefore not always easy for the members of the Task Team to decide on 'correct' names. Before going into more specific problems, a brief overview is presented.

The list presently contains 178 species. Dinoflagellates are the most numerous with 93 species, more than half the total number of species in the list. The cyanobacteria follow in second place with 37 species and the diatoms rank third, with 29 species. The remaining groups are small and contain less than 10 species: haptophytes (8 species), raphidophyceans (6 species), dictyocho- and pelagophyceans (5 species in total). While the list initially contained only marine species, problems associated with cyanobacteria (blue-green algae) continue to grow in many parts of the world and it was therefore decided some time ago to also include this group in the list.

At the IOC IPHAB meeting in Paris in 2019 more emphasis on cyanobacteria was requested and Catarina Churro from Portugal was suggested as a new thematic editor, a post she subsequently accepted. The list of cyanobacteria can be expected to grow further, how-

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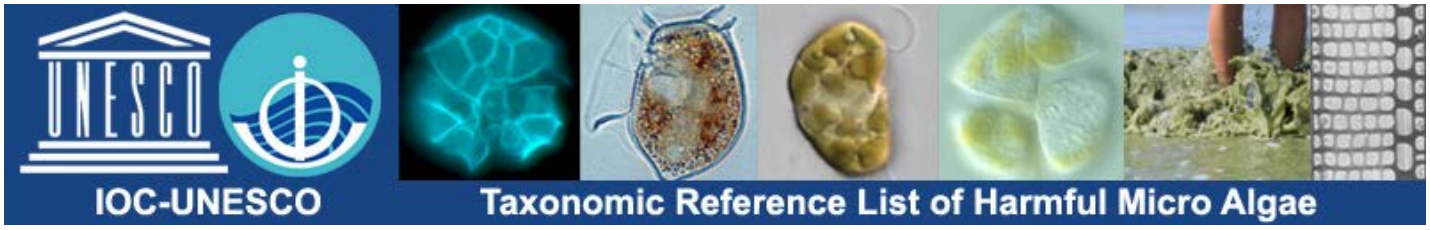

ever the information presented in publications is not always easy to interpret. Toxic blooms often contain several species and studies on individual species are therefore required to determine the species responsible for the toxin production. In addition, species are sometimes difficult to identify and the species concept in cyanobacteria is a challenge.

Nomenclatural Problems

There are four specific problems facing the Task Team:

Problem 1. The *Alexandrium* problem

Alexandrium is one of the commonest used generic names for toxin-producing HAB dinoflagellate species, but there was initial disagreement about the name to be used for 'the *tamarense* group' and its allies: *Alexandrium* Halim, *Protogonyaulax* F.J.R. Taylor, *Gessnerium* Halim or *Gonyaulax* Diesing. An important step was made towards avoiding further confusion at the HAB Conference in Lund, Sweden, in 1988 when the taxonomic expert, Prof. Enrique Balech from Argentina, was invited to discuss and give his opinion on the taxonomy of the group. After a detailed presentation, Balech concluded that all species should be included in the genus *Alexandrium* Halim. This resulted in heated discussion from/in the audience but objections raised were rejected by Prof. Balech. The chairman of the session, Prof. Karen Steidinger, finally concluded the discussion by asking all persons in the audience to agree on using only *Alexandrium* in the future. This proposal was adopted and the name *Alexandrium* has subsequently been used by scientists working on HABs.

However in 2019 it was discovered that a species of the poorly known genus *Centrodinium* nested within the genus *Alexandrium* in the molecular trees provided by Li et al [1]. Species belonging to both genera formed a monophyletic group. As *Centrodinium* was described by Kofoid in 1907 [2] but *Alexandrium* by Halim only in 1960, the old name *Centrodinium* takes priority if the two genera are to be merged.

As changes of well-known names are a source of confusion, the 'botanical' code establishes that well-known names of particular importance may be conserved: 'Conservation aims at retention of those names that best serve stability of nomenclature' (International Code of Nomenclature for Algae, Fungi and Plants 2018, Art. 14, 2). The case of *Alexandrium* versus *Centrodinium* is such a case. A formal proposal to conserve the name *Alexandrium* instead of *Centrodinium* is now being prepared to be submitted to the *General Committee of the International Code of Nomenclature for Algae, Fungi and Plants*. Subsequently splitting of *Alexandrium* was suggested [3]. An article containing arguments against this move has been submitted by Mertens et al. (pers. comm.). Until the problems have been settled, the IOC List of Harmful Algae will continue to use the name *Alexandrium* in the context of the 1988 decision.

Problem 2. The *Cochlodinium polykrikoides* problem

One of the most harmful fish killers has been referred to as *Cochlodinium polykrikoides* since its description from Puerto Rico in 1961. Huge fish kills have occurred in East Asia as a result of blooms of this species and South Korea has been particularly badly impacted. The species name was recently changed to *Margalefidinium polykrikoides* (Margalef) F. Gómez, Richlen & Anderson 2017 [4]. To change the name of well-known species always causes confusion and misunderstandings, and the problem in this case is as follows. A genus is nomenclaturally anchored to its type species and the type species of *Cochlodinium* is *Cochlodinium strangulatum* (F. Schütt) F. Schütt 1896. Like many other unarmoured (naked) dinoflagellates, *Cochlodinium* is and has always been a heterogenous genus. It comprises both chloroplast-containing and chloroplast-lacking species, and molecular data have shown, not surprisingly, that the chloroplast-containing species *Cochlodinium polykrikoides* Margalef is not closely related to the heterotrophic, chloroplast-lacking type species *C. strangulatum*. They should therefore not be retained in the same genus, and the transfer of *C. polykrikoides* to the new genus *Margalefidinium* is a logical proposal. Due to the economic importance of this species, conservation of the name *Cochlodinium polykrikoides* would cause much less confusion. Conservation can be attained by formally proposing to the *General Committee of the International Code of Nomenclature for Algae, Fungi and Plants* that the genus *Cochlodinium* be conserved with *C. polykrikoides* as type species, to replace *C. strangulatum*. A proposal to that effect is now being prepared and, if accepted, will conserve the name *Cochlodinium polykrikoides*. The species *C. strangulatum* will then need to be given a new generic name but given the fact that it is a little-known species, a new generic name for this species will cause little confusion. A species from the Far East related to *Cochlodinium polykrikoides* is *Cochlodinium catenatum* Okamura described from Tokyo Bay [5]. If the proposal to conserve the genus *Cochlodinium* with *Cochlodinium polykrikoides* as type is accepted, *C. catenatum* will again be the correct name of that species.

Problem 3. *Prorocentrum rhathymum* and *P. mexicanum*

Gómez et al [4] have recently claimed that the species *Prorocentrum rhathymum* Osorio-Tafall and *P. mexicanum* A.R. Loeblich, Sherley & R.J. Schmidt are synonyms. The question of possible synonymy goes back to Steidinger in 1983 [6] who claimed, four years after *P. mexicanum* was described by Loeblich et al [7], that the two taxa were synonyms. Faust [8] went on to state, incorrectly, that Loeblich et al, when describing the new species *P. rhathymum*,

considered this to be identical to the previously described *P. mexicanum* [9]. This statement makes little sense, and Loeblich et al in fact do not mention *P. mexicanum* in their article. Faust [8] illustrated material which resembled *P. rhathymum* using the radical arrangement of some of the trichocyst pores on the two large amphiesma plates, while no such arrangement was described in *P. mexicanum* by Osorio-Tafall [9]. The amphiesma surface in Faust's [8] beautiful SEMs is rugose, in contrast to both *P. mexicanum* and *P. rhathymum*, and Faust's material was subsequently given the name *Prorocentrum steidingerae* by Gómez et al. [4]. However, Faust [8] mentions that her material was sometimes smooth (as *P. mexicanum*), sometimes rugose, thus removing a main distinguishing character of *P. steidingerae*. Faust's rugose cells resemble *P. rhathymum* in the radial arrangement of some of the trichocyst pores (as in another benthic species, *P. lima*). If we accept Faust's statement that cells are sometimes rugose, sometimes smooth, then *P. steidingerae* is a synonym of *P. rhathymum*. Osorio-Tafall's *Prorocentrum mexicanum* is a separate species, which lacks the radiating arrangement of trichocyst pores.

Present evidence indicates that *P. rhathymum* is a benthic species which occurs in sediments or attached to macroalgae and floating detritus ([8] and references herein) while *P. mexicanum* appears to be planktonic. Cells of *Prorocentrum rhathymum* from Malaysia produce okadaic acid (cells documented by an excellent micrograph in Caillaud et al. [10]) as does material from Florida (not illustrated but genetically identical to the Malaysian cells: [11]). There appears to be no reliable information on the toxic potential of *P. mexicanum*.

Problem 4. The *Ceratium* question: *Ceratium*, *Neoceratium* or *Tripos*?

One of the editors asked me some time ago about which of the names *Ceratium*, *Neoceratium* and *Tripos*, should be used in monitoring programmes? *Ceratium* species are only harmful in large numbers when they may cause low levels of oxygen in the water. However since many HAB people are involved in monitoring of marine phytoplankton in general, I will address this question, which is relatively straightforward and which may serve as an example of the splitting concept.

It has been known for a long time that freshwater and marine species of *Ceratium* fall into two different groups which differ in the number of cingular plates: 4 cingular plates in the marine species [2], 5 plates in the freshwater species [12]. It was therefore no great surprise when molecular sequencing found the species to be distributed into the same two groups. Gómez et al [13] accordingly suggested splitting the genus into two, one comprising the freshwater species and the other the marine species. As the type species is from freshwater the name *Ceratium* was retained for the freshwater species while the marine species were given the name *Neoceratium* gen. nov. [13]. However this last name was against the rules of the international code of nomenclature and therefore illegitimate [14]. New generic names must not be created if a name for the same group of species exists already, and this is the case for *Ceratium*. Thus the many new combinations of names based on *Neoceratium* created by Gómez et al [13] are not to be used. Among the generic names applied to species of the *Ceratium* group, the oldest available name for the marine species is the soon 200-year-old *Tripos* Bory 1823. When this had been established, the marine species, varieties and forms of *Ceratium* were transferred to *Tripos* [15].

Then to the question: should the name *Tripos* from now on be applied to all marine species of *Ceratium*? The answer is that it depends on whether one prefers to split the genus *Ceratium* into two genera, or to keep the species in one genus. Both solutions are acceptable. *Ceratium sensu lato* containing both the freshwater and the marine species is monophyletic. *Ceratium sensu stricto*, containing only the freshwater species, and *Tripos* containing the marine species only, are also (both) monophyletic. A simple solution is therefore to retain the name *Ceratium* for all the species, freshwater and marine, but to group them into subgenera, one comprising the freshwater species, the other the marine species. This solution has been applied to other large groups of algae (e.g. *Chaetoceros*) and to many vascular plants. All species then retain the old generic name *Ceratium* Schrank 1793.

Acknowledgements

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Massive salmon mortalities during a *Chrysochromulina leadbeateri* bloom in Northern Norway

From mid May to mid June 2019, fish farmers along the coast of Nordland and Troms, northern Norway, experienced sudden mortalities of caged salmon [1] (Fig. 1). These mortalities were assumed to be due to a bloom of the haptophyte *Chrysochromulina leadbeateri*. Fish death was relatively sudden with gill damage frequently observed. Little or no changes were seen in the pathology of the internal organs.

Around seven and a half million farmed salmon died valued at 800 million Norwegian kroner – NOK (80 million €). This is the most extensive fish mortality event associated with a HAB recorded in Norway to date.

The first reports of fish mortalities came from Astafjorden in Troms and Ofotfjorden in Nordland. The *C. leadbeateri* bloom then moved further north to Vestfjorden and Troms during the following weeks (Fig. 2). A maximum cell density of 45 x 10^6 cells L^{-1} was recorded. *Chrysochromulina leadbeateri*, was reported to have caused fish-kills in the same area before. In the second half of May 1991, *C. leadbeateri* bloomed in Vestfjorden and surrounding areas [3,4]. The total loss then was 742 tons of salmon with an estimated value of 22.5 million NOK (3.5 mill US dollars), a substantial loss considering the size of the fish farming industry at the time. A smaller bloom of *C. leadbeateri* caused fish-kills in the same area in 2008.

One of the first extensive fish kill events in Norway was caused by a bloom of *Chrysochromulina polylepis* (now *Prymnesium polylepis*) in 1988. That bloom occurred in Skagerrak and Kattegat along the coast of Norway, Sweden and Denmark in May and June. Both farmed and wild fish were killed as well as a wide range of benthic fauna. Since then more than 30 different species of *Chrysochromulina* have been recorded in Norwegian coastal waters, some new to science. A result of these events was the formation of the Norwegian algal monitoring program (*www.imr.algeinfo.no*).

Questions remain as to why this alga bloomed again in this area, and why it became so toxic. It is still unknown which toxins are produced by *C. leadbeateri* and the conditions which promote toxicity. However, previous research on *Prymnesium polylepis* indicates that the toxicity increases during nitrogen-sufficient but phosphorus- limiting conditions. The toxicity may reduce grazing by protozoans and zooplankton, allowing rapid growth and bloom formation of these potentially toxic haptophytes [4]. To fully understand what caused these blooms and promoted toxicity of *C. leadbeateri* in 2019 more research is needed. Monoalgal strains of *C. leadbeateri* have been established at the University of Oslo from the blooms in 1991 and in 2019 and are currently being used in experiments to improve our knowledge on bloom drivers to implement mitigation practises for fish kills in the future [5] (Fig. 3).



Fig. 1. Boxes of dead salmon are transported to the company Northern Lights Salmon land base in Sør-Troms. Photo Northern Light Salmon.

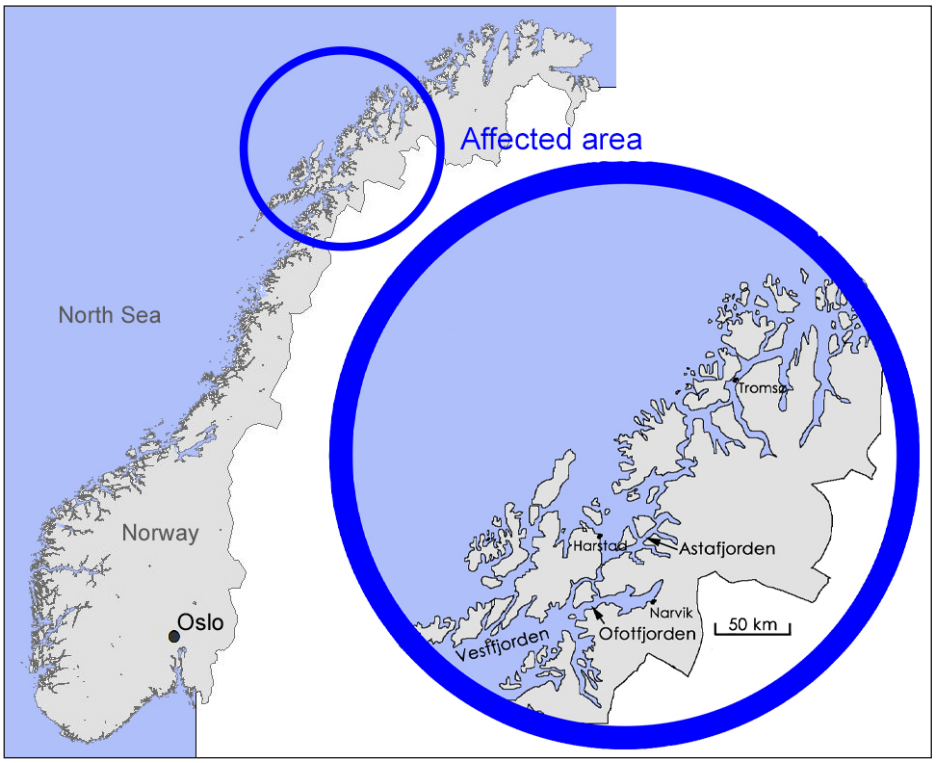

Fig. 2. Map of Norway showing the area where the Chrysochromulina leadbeateri bloom occurred causing massive fish kills in May-June 2019

Fish kill in numbers [2]

- 13 000 ton fish
- 7.5 mill salmon
- 80 mill EUR
- 14 companies

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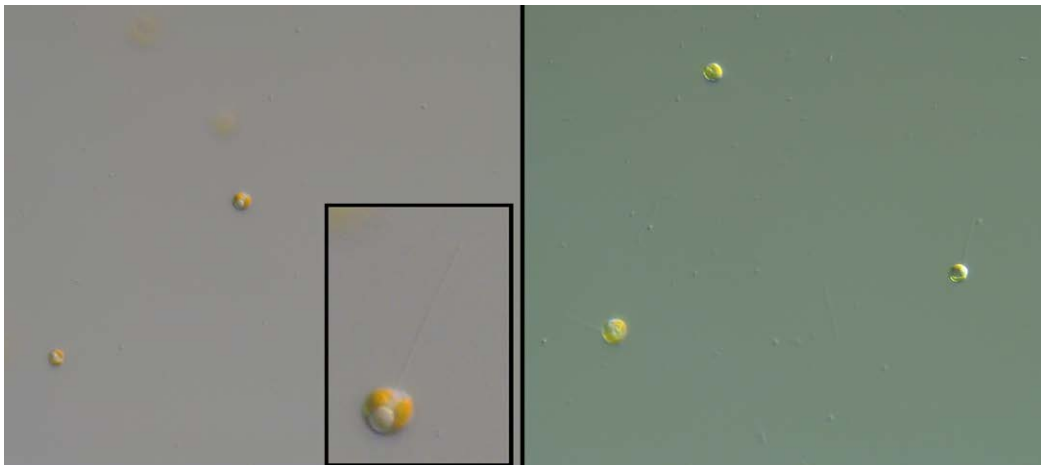

Fig. 3. Chrysochromulina leadbeatericells seen under differential interference contrast (DIC) (left) and phase contrast (right) light microscopy. They are tiny microflagellates, 5-8 µm in diameter, with two flagella, a haptonema and two chloroplasts. Photo: Bente Edvardsen

Mass mortality of marine invertebrates associated with the presence of yessotoxins in northern Chile

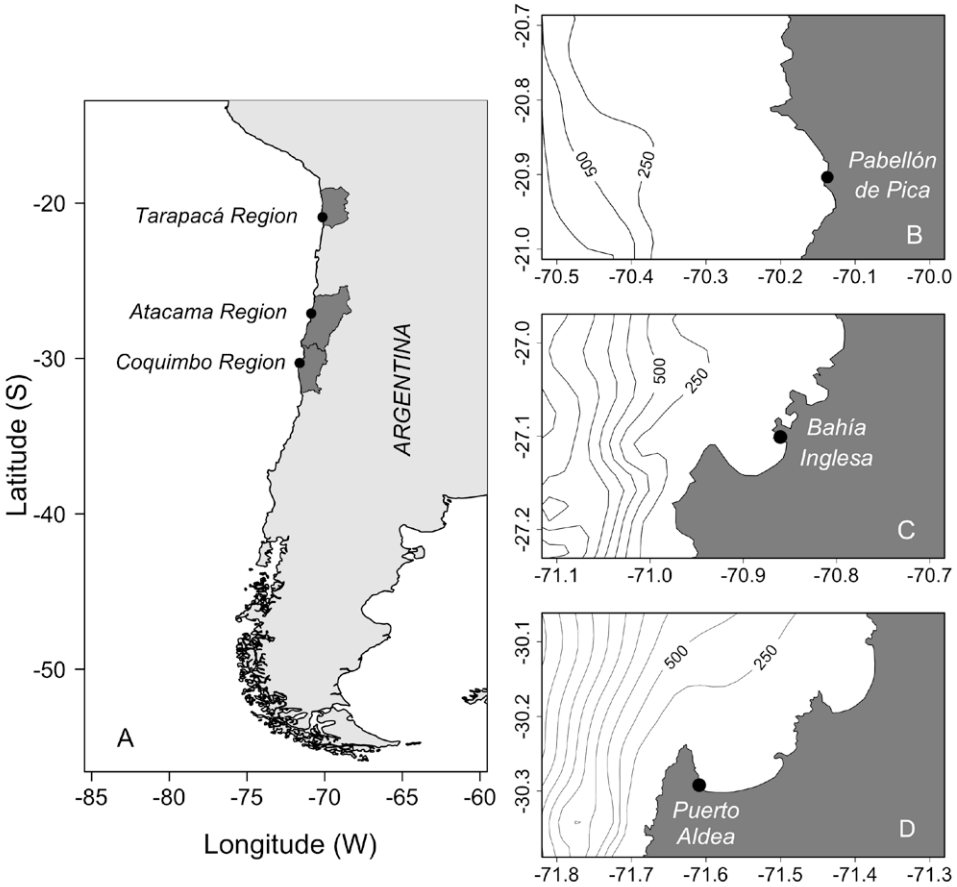

Fig. 1. Map of the study area showing A) Chilean coast; B) Pabellón de Pica, Tarapacá Region C) Bahía Inglesa, Atacama Region; D) Puerto Aldea, Coquimbo Region

During the austral summer of 2019, mass mortalities of marine invertebrates were detected at different locations throughout an extensive geographic area (up to 1000 km coastline) along the northern Chilean coast (Fig. 1). On January 24th, massive strandings of starfish (*Stichaster striatus*), red sea urchins (*Loxechinus albus*), and clams (*Ameghinomya antiqua*) were detected in Pabellón de Pica, Tarapacá Region (Fig. 2A, B). Unfortunately, accurate information about the total stranded biomass of each species was not obtained. The estimated density of sea urchins was between 15 and 20 individuals m^2. Toxin analyses of digestive tissue samples of the stranded specimens carried out by Liquid Chromatography- High Resolution Mass Spectrometry (LC-HRMS) revealed the presence of yessotoxins (YTX) with concentrations ranging between 0.1 and 0.4 mg YTX kg^{-1} (Table 1).

Two weeks later, on February 10-11th, a mass mortality of Humboldt squid (*Dosidicus gigas*) was detected in Bahía Inglesa, Atacama Region (Fig. 2C, D). During this episode, thousands of dying or dead squid were washed ashore or appeared floating near the shore of one of the most visited beaches in northern Chile. At the end of this event, the density of beached squid on the sand was estimated to be between 45 and 50 individual m^{-2} (approximately 15 tons). The health risk posed by the contaminated dead animals led the Ministry of Health to carry out a social media campaign to prevent their consumption. In addition, the City Council organized the beaches to be cleaned, to mitigate the negative impact on tourism caused by the decaying animals. LC-HRMS analyses of pooled samples of the viscera from individuals collected on February 11th revealed the presence of YTX with a concentration of 0.42 mg YTX kg^{-1}.

By the end of summer, on March 31th, a new mass mortality event of squid *D. gigas* was detected at the artisanal fishing harbor of Puerto Aldea, Coquimbo (Fig. 2E, F). During this event which took place during the night and early morning, thousands of individuals were found dead or dying, mainly lying on the surface of harbor facilities and, to a lesser extent, on nearby beaches. A total of 130 tons of stranded biomass was quickly collected and sold by fishermen to fish processing plants and the local market. However, in order to guarantee seafood safety the health authorities demanded an analysis of the squid in all of the fishing plants involved and withdrawal of the squid being sold in the local market. LC-HRMS analyses of the viscera from pooled samples revealed the presence of YTX with a concentration of 0.12 mg YTX kg^{-1}.

Table 1. Concentration of yessotoxin detected in different marine invertebrate species during the mass mortality events detected along the northern Chilean coast.

Date	Species	Location	Type of sample	mg YTX kg^{-1}
01/24/19	*Stichaster striatus*	Pabellón de Pica	Digestive tissues	0.10
01/24/19	*Loxechinus albus*	Pabellón de Pica	Digestive tissues	0.11
01/24/19	*Ameghinomya antiqua*	Pabellón de Pica	Digestive gland	0.45
02/11/19	*Dosidicus gigas*	Bahía Inglesa	Visceral mass	0.42
31/03/19	*Dosidicus gigas*	Puerto Aldea	Visceral mass	0.12



Fig. 2. A-B) Stranded specimens of the red sea urchin Loxechinus albus on the beach at Pabellón de Pica, Tarapacá Region; C) Specimens of the Humboldt squid Dosidicus gigas on the beach at Bahía Inglesa, Atacama Region; D) Control of the Chilean army to avoid commercialization of stranded organisms; E-F) Squid Dosidicus gigas on the beach at Puerto Aldea, Coquimbo Region.

The relationship between YTX and mass mortality events of invertebrates has been reported in other geographical areas around the world. In 2011, a mortality event associated with the presence of the dinoflagellate *Gonyaulax spinifera* and low levels of YTX (<0.1 mg kg^{-1}) was reported in Sonoma County, California affecting different marine invertebrates such as the red sea urchin (*Strongylocentrotus franciscanus*), the purple sea urchin (*S. purpuratus*), the starfish (*Pisaster ochraceous*) and the abalone (*Haliotis rufescens*) [1-2]. Recently, in 2017 Pitcher et al [3] reported a mass mortality of 250 tons of the abalone (*H. midae*) in different aquaculture farms along the South African coast associated with a bloom of *Gonyaulax spinifera*. During this episode, the toxin profile in the digestive gland was dominated by homo-YTX, 45-hydroxy-YTX, and a minor contribution of YTX, with average concentrations of 0.73; 0.21 and 0.09 mg kg^{-1}, respectively. The gill tissues, with an average concentration of 1.1 mg kg^{-1} (homo-YTX), 0.33 mg kg^{-1} (45-hydroxy-YTX) and 0.11 mg kg^{-1} (YTX), was the most contaminated organ. It is worth noting that the YTX concentrations found during these episodes were very similar to those reported from Northern Chile. These coincidences suggest that yessotoxins may have been the main cause of marine invertebrate mortalities. More research is needed to determine the mechanism of action and the toxin effects on tissues and cells of the main affected species. Finally, there is a need to establish an educational plan to protect the public and avoid the consumption and commercialization of potentially toxic marine invertebrates.

Acknowledgements

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First Report of *Heterocapsa minima* (Dinophyceae) from Aotearoa/ New Zealand

Small planktonic armoured dinoflagellates within the genus *Heterocapsa* are currently represented by 20 species with some having a world-wide distribution [1-2]. Blooms of some *Heterocapsa* species have been associated with fish kills due to oxygen deficiency or oversaturation. Toxicity by *H. circularisquama* has also been reported, with blooms causing tremendous damage to bivalve species [3-4]). *Heterocapsa triquetra* is one of the most common red tide species around the world [1]. Recently, Tillmann et al. [2] proposed a new species, *H. steinii*, for a taxon that has been known as *H. triquetra* [5]. It is therefore important to elucidate the diversity and distribution of *Heterocapsa* species and to understand their potential adverse impacts in coastal areas.

In Aotearoa/New Zealand, a nearshore red-tide bloom of a *Heterocapsa* species at Pukehina Beach, Bay of Plenty on 10 December 2003 was associated with swimmers complaining of skin irritations [6] (Site 3 in Fig. 1). Previous studies identified and reported four *Heterocapsa* species from New Zealand based on the results of morphological characterisations of clonal strains (Sites 1, 2, and 3 in Fig. 1) [7]: *H.* cf. *circularisquama, H. illdefina*, and *H. niei* from the subtropical zone [6,8] and *H. steinii* (previously *H. triquetra*) from the temperate zone [7]. However, the diversity of *Heterocapsa* species in the temperate zone of New Zealand has not been well elucidated.

In the present study, seawater samples were collected from two sampling sites from the South Island and three sampling sites from Stewart Island of New Zealand which are within the temperate zone (Fig. 1): Site 4 (Wedge Point, Marlborough; -41.2578, 174.0116) on 12 December 2018, Site 5 (Akaroa, Canterbury; -43.8048, 172.9653) on 18 October 2018, Sites 6–8 [Site 6 (Halfmoon Bay; -46.8968, 168.1305), Site 7 (Golden Bay Wharf; -46.9041, 168.1216), and Site 8 (Big Glory Bay; -46.9793, 168.1099), Stewart Island, Southland] on 02 September 2018. The samples were collected by hose sampling at 0–15 m depths at Site 4 and from surface seawater at Sites 5–8. *Heterocapsa* cells were observed under an inverted light microscope (LM), isolated in f/2 medium to establish clonal strains, and incubated at 18 °C and 90 µmol photons $m^{-2}s^{-1}$ under a 12:12 L/D cycle. A total of 16 clonal strains were established: one from Site 4, four from Site 5, and three from Site 6 (Halfmoon Bay), three from Site 7 (Golden Bay Wharf), and five from Site 8 (Big Glory Bay) in Stewart Island. The cells of the strain from Site 4 were smaller than those from Sites 5–8 (data not shown). The cells of strains from Sites 5–8 showed the same morphology under LM (data not shown). Therefore, three representative strains were selected for molecular phylogenetic identifications: strains CAWD302 (Site 4), CAk01H (Site 5), and SSB03H (Site 8). Genomic DNA of the three strains was extracted, and the D1/D3 region of the large subunit ribosomal RNA gene (LSU rDNA) was amplified and sequenced as previously described [9]. Molecular phylogenetic analyses were conducted using maximum likelihood (ML) and neighbour-joining (NJ) methods.

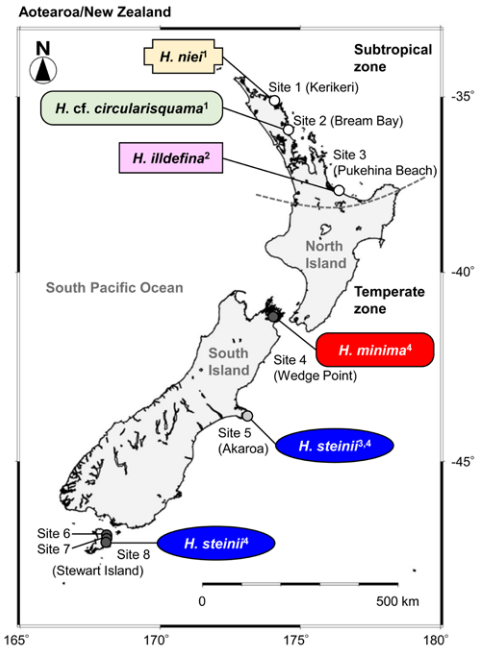

Fig. 1. Summary of the geographic distribution of Heterocapsa species in coastal areas of New Zealand. Circles represent the sampling sites of clonal strains (white: previous studies, grey: previous and present studies, dark grey: the present study). [1][8], [2][6], [3][7], [4]The present study. [1,2] Strains of H. niei and H. illdefina were identified by electron microscope examination of thecal plate structure and scale morphology. A strain of H. cf. circularisquama was identified by electron microscope examination of thecal plate structure alone. [3]A strain of H. steinii was identified by light microscope examination alone. [4]Strains of H. minima and H. steinii were identified by ribosomal RNA gene sequencing. Site 1: Kerikeri, Northland. Site 2: Bream Bay, Northland. Site 3: Pukehina Beach, Bay of Plenty. Site 4: Wedge Point, Marlborough. Site 5: Akaroa, Canterbury. Sites 6–8: Site 6, Halfmoon Bay; Site 7, Golden Bay Wharf; Site 8, Big Glory Bay; Stewart Island, Southland. The potential boundary between the subtropical and temperate zones is reported by National Institute of Water and Atmospheric Research [15].

The phylogenetic analyses revealed that two strains, CAk01H and SSB03H, belonged to the species *H. steinii* (Fig. 2). The sequences of these two strains were identical to those of strain UTKG7 (GenBank accession numbers: MF423357 and MF423361) that corresponds to the type material of this species. Strain CAWD302 belonged to species *H. minima* (Fig. 2). The sequence of CAWD302 was identical to those of strains JK2 and HMMJ1604 (GenBank accession numbers: KF031312 and MK483261, respectively).

Previous studies in New Zealand have reported three *Heterocapsa* species (*H.* cf. *circularisquama, H. illdefina*, and *H. niei*) from the subtropical zone [6,8]. The present study has revealed presence of two additional species: *H. minima* from Site 4 and *H. steinii* from Sites 5 and 8 in the temperate zone. Using molecular phylogenetic methods, the present study supports the previous finding of *H. steinii* (previously *H. triquetra*), identified by morphology as described above, from Site 5 [7] (Fig. 2). The present study is the first report of *H. minima* from New Zealand. Furthermore, it indicates that *Heterocapsa* species composition may be different in the subtropical and temperate zones of New Zealand (Fig. 2).

Regarding the distribution of *H. minima*, this species was first described by morphological characterization without molecular phylogenetic data from the Celtic Sea in the temperate North Atlantic Ocean in 1989 [10]. In 2001, abundance data of *H. minima* were reported in the fixed samples collected from the Bay of Biscay, North Spain in

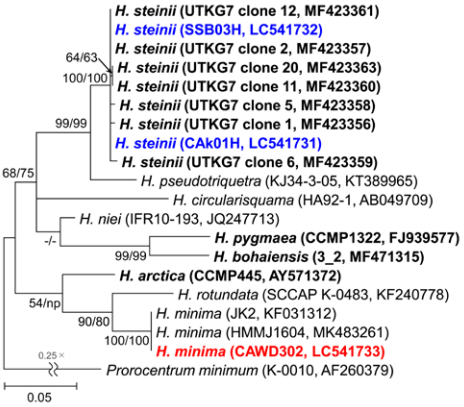

Fig. 2. Maximum likelihood (ML) molecular phylogenetic tree of Heterocapsa minima and H. steinii strains isolated from coastal areas of New Zealand based on the LSU rDNA D1/D2 sequences (20 sequences, 700 positions). The alignment site corresponded to the 69–768 bp site of a sequence from H. steinii strain UTKG7 (clone 1, MF423356). The ML and neighbour joining (NJ) phylogenies were analysed using the best-fit models, GTR+G+I and TN93+G models, respectivelywith bootstrap analyses performed with 1,000 replicates. Sequences obtained in the present study are shown in blue and red font. Sequences corresponding to the type material of each species are shown in bold font. Nodal support represents ML/NJ bootstrap values. Nodal supports that were under 50 are shown as hyphens. Nodes that were not present in the NJ tree are labelled as np.

the temperate North Atlantic. However, details of morphological and molecular characterisations were not reported [11]. In 2009, *H. minima* strain JK2 was established from Bantry Bay, Southwest Ireland in the temperate North Atlantic, potentially representing the same water mass as the type locality of *H. minima* [10], and identified by a combination of morphological and molecular phylogenetic analyses [12]. Recently, in 2016, a *H. minima* strain HMMJ1604, was established and identified by molecular and morphological analyses from Mijo Port, South Sea of Korea in the temperate North Pacific. This finding was the first report of this species from the Pacific [13]. Therefore, the present study is the second record of *H. minima* from the Pacific region and suggests that this species is widely distributed in the temperate zone of the Atlantic and Pacific. To the best of our knowledge, the present study is the first record of this species from the Southern Hemisphere.

In previous studies reporting the occurrence and abundance of *H. minima* cells [10-11,13], it was stated that this species occurred throughout the year and was widely distributed in the Celtic Sea and Korean waters [10,13]. Because the cell morphologies of *Heterocapsa* spp. are superficially similar in size, shape and swimming mode to other small dinoflagellates (e.g., *Azadinium* and *Biecheleria*) under LM observation, it is difficult to differentiate them and high magnification microscopy or molecular tools are required [14,7]. Therefore, the determination of accurate cell concentrations of these taxa from routine samples used for phytoplankton monitoring is difficult. For example, the New Zealand Marine Phytoplankton Monitoring Programme reports these cell types as "cf. *Azadinium* spp." (= *Azadinium*-like species) to provide a conservative report of potentially toxic species [9]. In the present study, the *H. minima* strain CAWD302 was isolated from a seawater sample collected at Site 4 on 12 December 2018, from which a cell density of 6,000 cells/L of "cf. *Azadinium* spp." was reported. In addition to *H. minima*, two clonal strains of *Biecheleria* spp. strains MB11 and CAWD304 (GenBank accession numbers: LC542926 and LC542925, respectively) were also isolated from the samples collected at Site 4 on 5 and 12, December 2018, respectively. To resolve this issue for *H. minima*, Lee et al. [13] developed a species-specific quantitative PCR (qPCR) method to detect and quantify cells from the Korean seawater samples. To reveal the accurate distribution of *H. minima*, applying rDNA sequencing and/or qPCR to isolated strains or seawater samples collected from the other climate zones (e.g., tropical, subtropical, and/or subboreal zones), as well as other temperate areas is required. Additionally, toxin and/or toxicity assessments are also needed to further elucidate the potential impacts of this species.

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Yellow-green tides could become a recurrent event along the Ligurian coast (Italy)

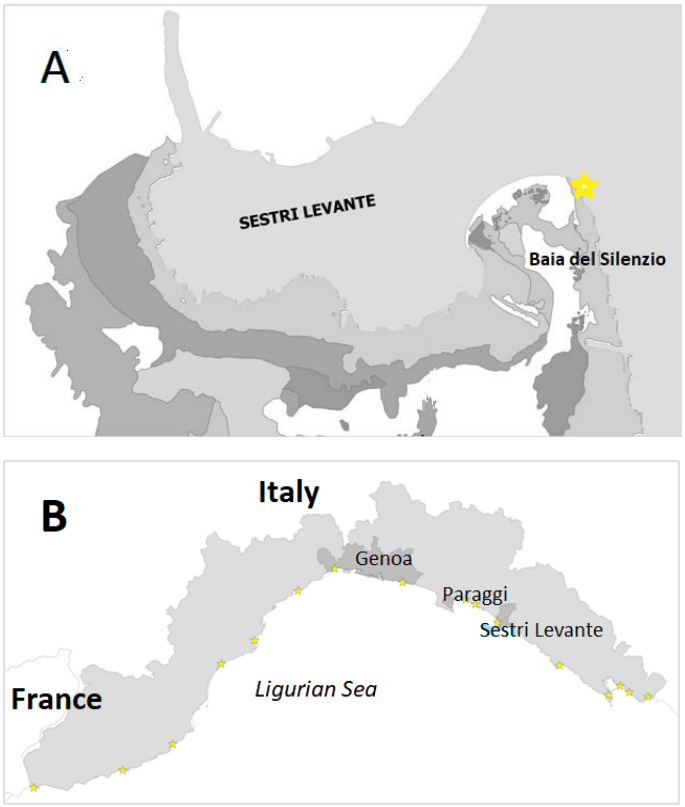

Fig. 1. A) Sestri Levante and Baia del Silenzio bay, characterized by shallow water and mixed rocky-sandy seabed containing the macrophyte, Posidonia oceanica (dark grey) and carpet-like "matte" (light grey) habitat. B) Liguria Region and the sites (yellow stars) included in the harmful algal bloom monitoring program

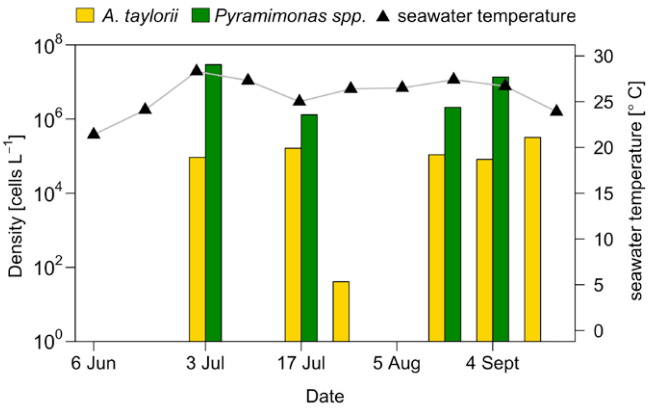

Fig. 2. Cell densities of Alexandrium taylorii and Pyramimonas spp. and seawater temperature (°C) from Sestri Levante Bay (Genoa, Italy), summer 2019.

Alexandrium blooms are becoming an increasing phenomenon in the Mediterranean Sea. These blooms mainly occur in semi-enclosed areas exposed to anthropogenic pressures from aquaculture, tourism and nutrient enrichment [1]. *Alexandrium taylorii* Balech is a potentially toxic dinoflagellate that causes a yellow-green water discoloration. Some published studies suggest that four western Mediterranean areas are affected by blooms of this species: the Catalan Coast (NE Spain), the Balearic Islands, northern Sardinia and Sicily (Vulcano Island, Italy) [2].

During the summer of 2019, blooms of *A. taylorii* impacted the eastern Ligurian coast (north-western Italy) causing significant long lasting yellow-green water discolorations. These were especially noticeable along the Sestri Levante area (Fig. 1) causing concern from both the tourism industry and local authorities.

The first bloom of *A. taylorii* in Ligurian coastal waters, with a maximum density of 1.4 x10^6 cells L^{-1}, was reported by the Regional Environmental Protection Agency of Liguria (ARPAL) in the summer of 2010. Although samples from this bloom did not test positive for algal toxins, since then ARPAL have increased monitoring efforts in compliance with the European Bathing Water Directive. Every year, from June to September, ARPAL collects and analyses samples of both seawater and macrophytes according to the Italian monitoring protocols for harmful algal blooms [3].

Significant *A. taylorii* blooms have been reported during the summers of 2012 (up to 6.0 x 10^4 cells L^{-1}), 2013 (> 3.0 x 10^6 cells L^{-1}), 2016 (5.0 x 10^4 cells L^{-1}) and 2019 (> 3.0 x 10^5 cells L^{-1}) (Fig.2). This suggests an increased frequency in the occurrence of *A. taylorii* blooms, especially on the eastern Ligurian coast. These blooms often co-occur with high cell densities of *Pyramimonas* spp., a nano-phytoplankton taxon belonging to the *Chlorophyta* group that may cause "bright green tides".

During the summer of 2019, additional water discoloration events at different sites along the eastern Ligurian coast (e.g. Paraggi, S. Margherita Ligure) with more than 6.5 x 10^4 cells L^{-1} were recorded by the local sanitary agency and Genoa University. A study was performed isolating that causative organisms into laboratory culture to confirm species identification by molecular analysis, thecal plate morphology using epifluorescence microscopy, and toxin analysis. Unfortunately, it was not possible to maintain the algal culture for the toxin characterization of the strain.

In general, *A. taylorii* is considered a high biomass HAB species that negatively impacts the use of coastal waters for recreational purposes. This species also has the potential to produce Paralytic Shellfish Toxins (PSTs) and other unknown harmful compounds [4]. To date, there have never

Continued on page 12

First report of a high biomass bloom of *Peridinium quadridentatum* (F. Stein) Gert Hansen from the tropical Cochin estuary – SW coast of India

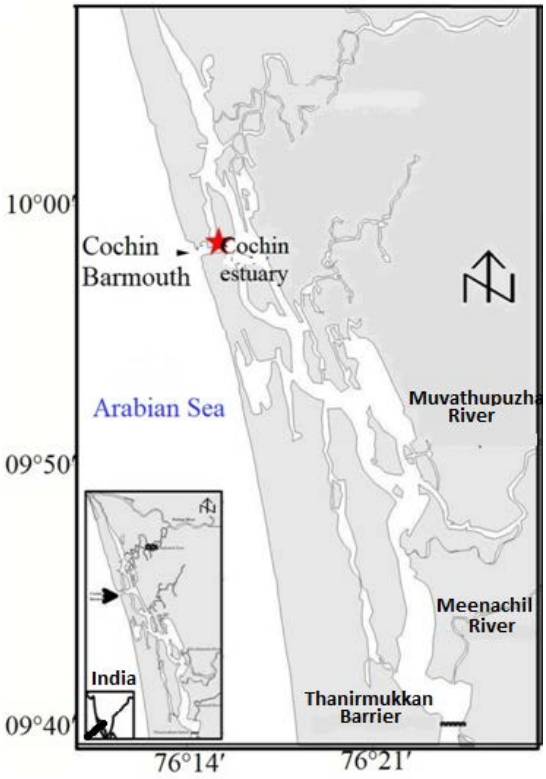

Fig. 1. Map showing the area of P. quadridentatum bloom in Cochin estuary, southwest coast of India

Peridinium quadridentatum (F. Stein) Gert Hansen is a cosmopolitan dinoflagellate which can bloom under eutrophic conditions [1-3]. It was first reported as *Heterocapsa quadridentata* from Fiji in the tropical South Pacific [4] and later renamed as *Peridinium quinquecorne*, based on morphological features such as the presence of one complete cell and one incomplete cell (hypotheca), both bearing four spines emerging from the hypotheca [5]. Only recently, Hansen [6] suggested these two species names as synonyms and proposed a new name *Peridinium quadridentatum*; but still it is commonly known under the name of *P. quinquecorne*.

P. quadridentatum is considered as non-toxic harmful dinoflagellate as it leads to reduced dissolved oxygen, higher turbidity, lower planktonic species richness and discolouration of water [7-9]. Even though *P. quadridentatum* has been reported from different parts of the world, unil now there have been no records of blooms or harmful effects of this species in Indian waters. The study reports the first bloom of *P. quadridentatum* along the tropical waters of Cochin estuary (Southwest coast of India).

Cochin backwater is a productive estuarine system with a high plankton diversity. It is highly influenced by the monsoon run off and inputs from anthropogenic activities which results in a dynamic change in the overall functioning of the system between seasons [10,11]. In November 2018, a sudden discolouration of water was observed near the Marine Science Jetty (Lat. 09º57'51''N; Long. 76º16'56'' E) in Cochin estuary (Fig.1). Phytoplankton samples were collected from the bloom area by filtering ~50 liters of surface water through 20µm mesh bolting silk and analysed using a Leica DM2000 microscope. Hydrobiological parameters such as temperature, salinity and dissolved oxygen were measured immediately and dissolved nutrients (nitrate and silicate) were estimated according to standard protocols. Chlorophyll a was measured spectrophotometrically using a Hitachi U-2900 UV/Visible spectrophotometer following the acetone extraction method.

Microscopic examination of samples collected from the bloom event revealed that the discoloration was caused by the dinoflagellate *P. quadridentatum* (Fig. 2). The cells were solitary, small in size and little longer than wide. The cell was overall ovoid in shape and divided by a cingulum in the middle. The epitheca was conical with pointed apex and the hypotheca was round in shape with four antapical spines, which var *Peridinium quadridentatum* (F. Stein) Gert Hansen is a cosmopolitan dinoflagellate which can bloom under eutrophic conditions [1-3]. It was first reported as *Heterocapsa quadridentata* from Fiji in the tropical South Pacific [4] and later renamed as *Peridinium quinquecorne*, based on morphological features such as the presence of one complete cell and one incomplete cell (hypotheca), both bearing four spines emerging from the hypotheca [5]. Only recently, Hansen [6] suggested these two species names as synonyms and proposed a new name *Peridinium quadridentatum*; but still it is commonly known under the name of *P. quinquecorne*.

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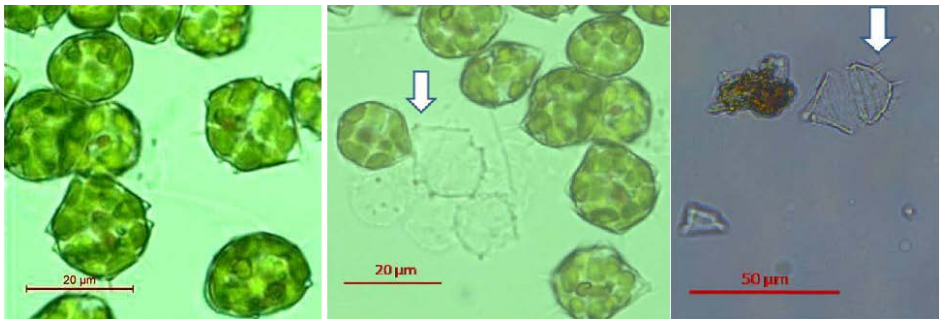

Fig. 2. Microphotographs of Peridinium quadridentatum bloom

Table 1. Physico-chemical parameters of P. quadridentatum bloom area in Cochin Estuary

Parameters	
Water Temperature (°C)	28
Salinity (psu)	20
Dissolved Oxygen (ml L^{-1})	2.01
Nitrate (µmol L^{-1})	10.6
Silicate (µmol L^{-1})	23.3
Phosphate (µmol L^{-1})	1.3
Chlorophyll a (mg m^{-3})	27.5

higher turbidity, lower planktonic species richness and discolouration of water [7-9]. Even though *P. quadridentatum* has been reported from different parts of the world, unil now there have been no records of blooms or harmful effects of this species in Indian waters. The study reports the first bloom of *P. quadridentatum* along the tropical waters of Cochin estuary (Southwest coast of India).

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During the bloom event, the surface chlorophyll a concentration was relatively high (27.5 mg m^{-3}) and *P. quadridentatum* contributed more than 70% of the overall phytoplankton population with a cell density 6.2 x 10^4 cells L^{-1}. As well as *P. quadridentatum*, the dinoflagellate community included *Noctiluca scintillans, Tripos muelleri* and *Protoperidinium* sp. *Skeletonema costatum* and *Nitzschia sigma* were the dominant diatom species.

The *P. quadridentatum* bloom occurred when physical and chemical conditions during the bloom revealed the surface water temperature was 28°C, salinity 20 psu, and nitrate and silicate concentrations were 10.6 µmol L^{-1} and 23.3 µmol L^{-1} respectively. A low dissolved oxygen concentration (2.01 mg L^{-1}) was recorded during the bloom event, but there were no reports of fish mortalities from the bloom area.

This observation is the first bloom report of *P. quadridentatum* along Cochin estuary and further investigation on the bloom dynamics is required. This information will be useful in planning for potential HAB events along Cochin estuary.

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Continued from page 10

been any reports of toxicity associated with Mediterranean strains of *A. taylorii* [5]. For these reasons, further studies will be planned to better understand the potential risks associated with this microalga and its co-occurrence with other phytoplankton species along the Ligurian coast.

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Are mesophotic seamounts reservoirs for potentially toxic dinoflagellates associated with Ciguatera poisoning? A case study from the SW Indian ocean (expédition La Pérouse, 2019)

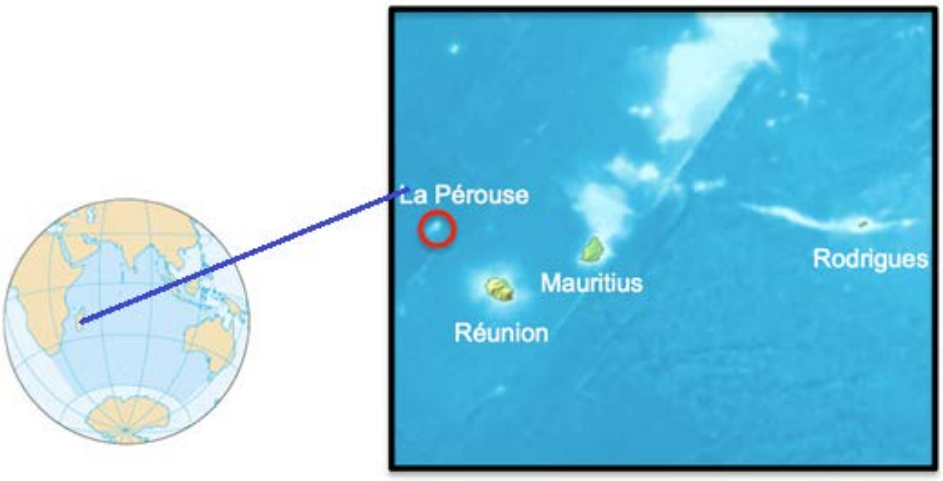

Fig. 1. Location map of La Pérouse seamount off La Réunion.

In the western Indian Ocean, a Ciguatera Fish Poisoning (CFP) hotspot is located in the coastal waters of the Mascarene Islands (Mauritius, Rodrigues, Réunion) and the offshore fishing banks of the Mauritius-Seychelles ridge, seamounts near Réunion and Mauritius (Fig. 1).

The size of the La Pérouse seamount, 160 km northwest of Réunion Island on the oceanic floor, is comparable to that of Mont Blanc in the Alps. La Pérouse is one of the rare shallow (-60m) seamounts located in the region. The plateau comprises mainly fossil limestone plaques covered with macrophytes, sponges, corals (few), and sand and rubble zones (see back page photo).

In 2005, an expedition organized by ARVAM at La Pérouse seamount (also known by local fishermen as 90 miles bank) collected carnivorous fish containing I-CTXs for analysis, as well as samples of sediment and macroalgae which subsequently facilitated the identification of *Gambierdiscus* spp in the area.

During October-November 2019, a diving and scientific expedition (La Pérouse 2019) was organized to generate a first description of the habitats and associated fauna and flora on the seamount based on a High Quality (HQ) photographic inventory. Although few samples for geological and biological analyses were taken, the focus on macroalgal biodiversity and sediments meant it was possible to isolate the microalgae assemblage from these samples.

A preliminary inventory from these samples collected at depths between -60 & -120m, revealed the presence of the main genera observed in typical CFP habitats: *Gambierdiscus, Prorocentrum, Coolia* but also athecate taxa such as *Amphidinium* and gymnodinoid dinoflagellates (Fig. 2), and thecate taxa such as *Cabra, Sinophysis, Bysmatrum* and scrippsielloids. Interestingly, among potentially toxic taxa, *Gambierdiscus* spp were clearly predominant in the samples (approx. 95% of isolated cells) while *Ostreopsis* spp and *Fukuyoa* spp were very rare. In order to give a precise identification and better evaluation of the diversity, preserved cells from approx. 35-40 morphotaxa have been isolated individually in order to allow SEM observations and molecular sequencing of LSU rDNA from single-cells in the coming months.

This study will provide a significant contribution for better knowledge of habitat preferences and vertical distribution of CFP sources. While CFP is traditionally associated with coral reefs near the ocean surface where sunlight conditions are optimal for growth and toxin production, in this case only a low concentration of light penetrates to the depths encountered at La Pérouse seamount, considered in the mesophotic zone. Only blue light reaches the benthic community here and literature about the influence of these conditions on toxin production by primary progenitors involved in seafood poisonings is scarce.

This study will also inform results of "source & sink" mechanisms that allow harmful species to spread at regional scales from these seamounts to coastal ecosystems.

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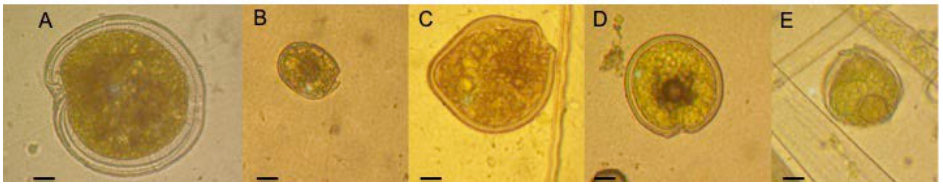

Fig. 2. Benthic dinoflagellates from La Pérouse seamount. (A) Gambierdiscus sp. (B) Amphidinium sp. (C) Prorocentrum sp. (D) Prorocentrum sp. (E) Coolia sp. Scale bars, 10 µm.

Using Machine Learning to Observe Abundance Patterns of the Dinoflagellate *Noctiluca scintillans* in the Western English Channel

Noctiluca scintillans is a Harmful Algal Bloom (HAB) species with a wide geographic distribution. It frequently blooms and causes negative impacts on marine ecosystems [1], although this species is not thought to be a toxin producer. As a result of their high biomass bloom-forming abilities, *N. scintillans* can potentially cause anoxic conditions and the generation of high levels of ammonia leading to localised die-offs of higher trophic levels [2].

We aim to develop a rapid detection of this species from flow cell-sorted images of plankton off the south-west coast of the UK. To that end, we have trained a Random Forest (RF) algorithm to i) rapidly and accurately quantify the abundance of *Noctiluca* in seawater samples of mixed plankton communities, ii) aid the examination of temporal patterns in *Noctiluca* abundance in the long-term, and iii) create a monitoring tool to show when cell counts approach bloom levels.

Seawater samples taken between March and December 2018 at the L4 autonomous buoy in the Western English Channel were processed using dynamic imaging particle analysis (DIPA) performed using FlowCam (Fluid Imaging Technologies, USA). All particles ranging in size from 380 to 1500 µm were automatically imaged and enumerated by VisualSpreadsheet (the software accompanying FlowCam).

A RF algorithm was then trained and tested on 32 particle features (for example, length, width, area, transparency etc. as measured by VisualSpreadsheet) in a mixed plankton community of 246,000 total particles; 167,000 of these particles had been manually identified as *Noctiluca* cells to give a "True Count" against which to test the model. One of the challenges of training the RF model was to encompass the ontogenetic changes seen in *Noctiluca* cells over their development and so training sets were compiled from cell samples taken on random dates throughout the year. Fig. 1 shows examples of some of the morphological and textural variety of *Noctiluca* that our RF model is tasked to classify.

The resulting model was then deployed on the 2018 dataset to estimate *Noctiluca* abundance. RF model abundance and True Count were compared using linear regression with a significance level = 0.05. A strong positive relationship was found between the RF abundance and True Count, with the RF model almost exactly matching the True Count ($p < 0.001$, $R2 = 1.000$). It must be noted that the model is limited by FlowCam's ability to identify individual particles in a sample which is likely not as accurate as manual counting with microscopy [3]

A total of 33 plankton samples were collected between March and December 2018 comprising 1,008,084 particles in total, 540,499 of which were manually identified as *Noctiluca* from the FlowCam images. To observe the temporal pattern of *Noctiluca* abundance, the RF model was deployed on the 2018 datasets and compared with total particle counts (Fig. 2).

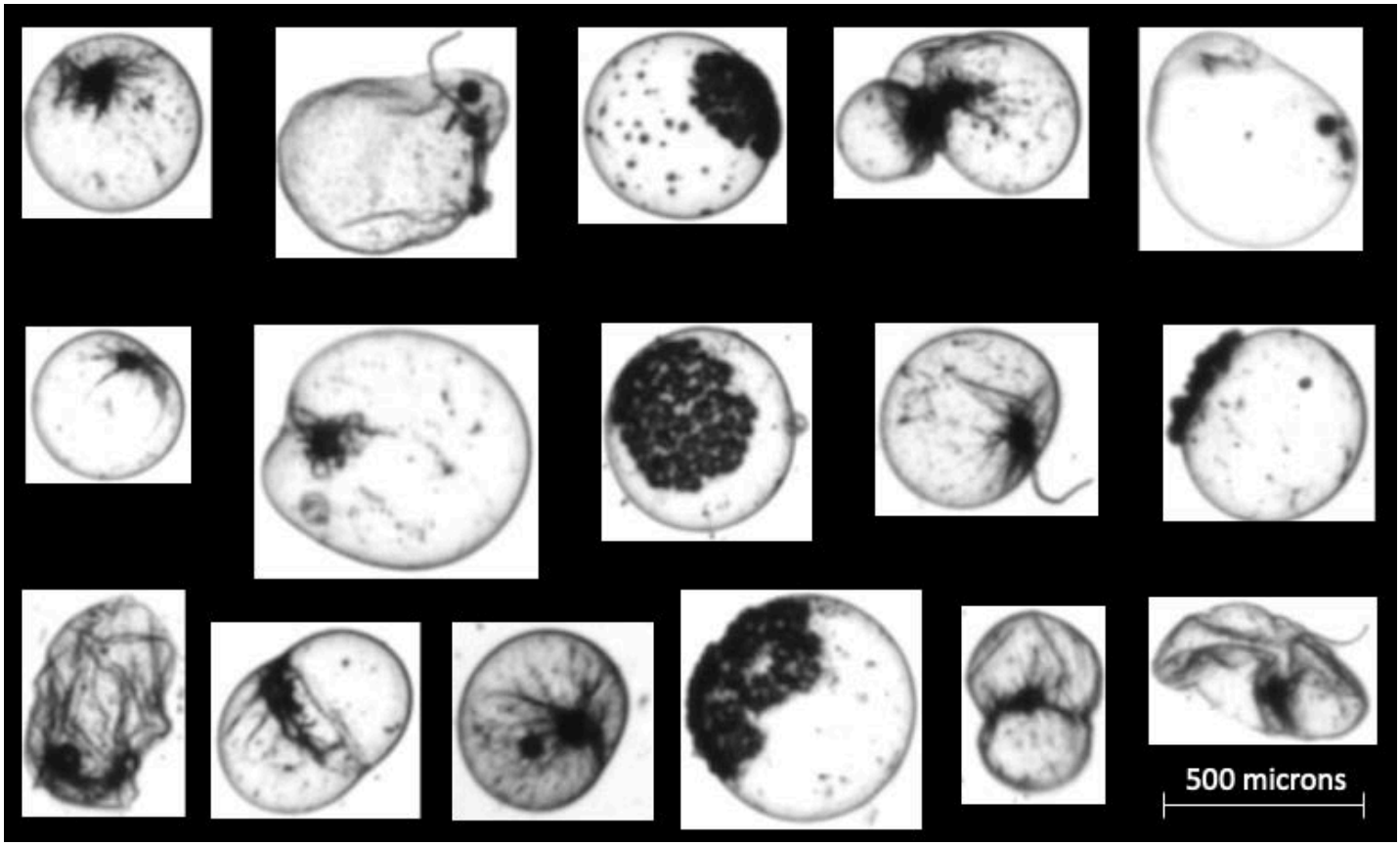

Fig. 1. Examples of morphological and textural variety of Noctiluca scintillans

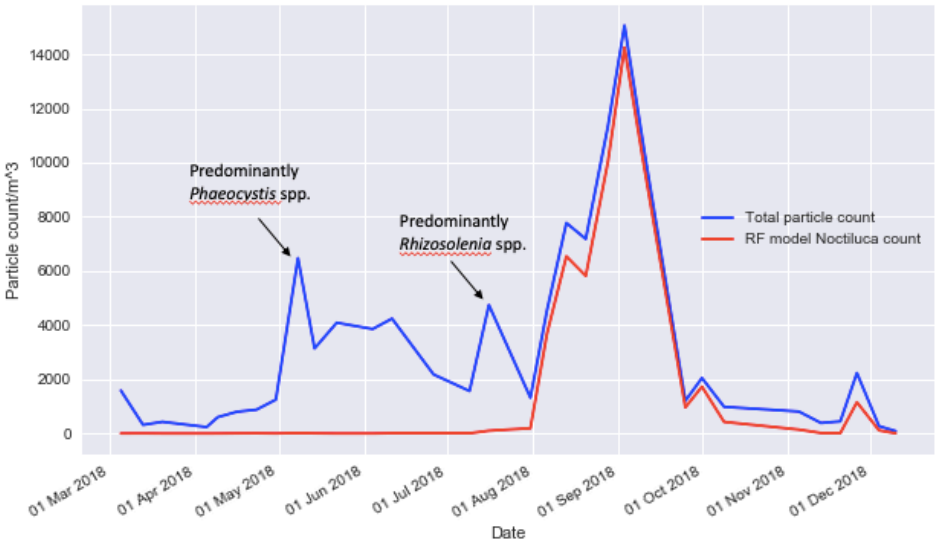

Fig. 2. Temporal abundance pattern of Noctiluca compared with total particle counts for 2018

The advantage of the RF model is its rapid sample processing time, minimal computing power requirement and excellent relative accuracy in classifying *Noctiluca* within mixed plankton communities. This lends itself as a useful tool in monitoring *Noctiluca* abundance and alerting the observer to particle numbers approaching bloom levels. In future work, we will use the model to explore spatio-temporal relationships of *Noctiluca* over longer time periods and with other physico-chemical parameters in an effort to predict bloom occurrence.

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Postponed HAB events



Due to the coronavirus (COVID-19)outbreak, the **19th International Conference on Harmful Algae (ICHA 2020)**,planned to be held in La Paz, Mexico in October 2020 will be delayed until **fall 2021**.Keep posted for the new dates.

Information will be disseminated through Harmful Algae News, and to the list of ISSHA members in good standing.

HAB experts wishing to receive the ISSHA member rate for the conference registration or to participate in the nomination/voting activities of the ISSHA Society must join ISSHA or renew their memberships. More information at: *www.issha.org* and *icha2020.com/Secciones/inicio*



Due to the coronavirus (COVID-19) crisis, the **12th International Conference on Modern and Fossil Dinoflagellates (DINO12)**, hosted by The Canarian HABs Observatory (OCH), was postponed. The new dates are from 5th to 9th July 2021. The conference will be held at the Alfredo Kraus Auditorium in Las Canteras beach, Las Palmas de Gran Canaria, Spain. For further information visit: *www.dino12conference.com*

Given the International Health situation and the confinement enforced in many country around the world, the organizers will be working from home on a temporary basis. If you needed to contact the technical secretariat, please phone between 9am and 2pm (BST or GMT+1) to **+34 676 887 596**, or write your enquiry to *info@dino12conference.com*

International validation and recognition of method for paralytic shellfish toxins in bivalve molluscs

Food safety scientists from Cefas (UK) and Cawthron Institute (New Zealand) have led an international study over the past four years to gain international recognition for a new method to quantify paralytic shellfish toxins in shellfish. It was a truly global study, incorporating 21 participating laboratories from five continents. An extensive process took place involving three pre-trials, and an enormous amount of work sourcing, testing, preparing, stabilising, and characterising study materials and shellfish samples from all around the world.

The collaboration between the two science organisations began in 2014 when Cawthron scientist Mike Boundy spent 3 months in Dr Turner's laboratory at the Weymouth Cefas facility on a Queen Elizabeth II technician's award. It had been known for some time that it was possible to chromatographically separate paralytic shellfish toxin analogues found in contaminated shellfish using HILIC chromatography. However, enhancement/suppression effects observed when monitoring the toxins in shellfish extracts were substantial and needed to be mitigated to allow development of a robust method. This technical challenge was overcome using graphitised carbon solid phase extraction (SPE) cartridges during sample preparation prior to analysis. This procedure could be performed manually or using a liquid handling robot.

Following the development and single laboratory validation of the method [1,2] it was included within the routine regulatory monitoring programme within New Zealand, with ISO17025 accreditation since 2016. During this time, it has had strong support from both regulators and industry. The method is well suited for the high throughput routine testing demands with the workflow allowing more samples to be analysed each day with faster, easier and more accurate results.

Results from the collaborative study demonstrated not just excellent performance across a variety of shellfish species, but also the ability of laboratories that use a wide range of instrument models to successfully run the method for both regulatory testing and research. The method provides accurate quantitation with higher throughput and greater specificity than existing methods of analysis with reduced complexity for the analysts. In addition, the method includes additional paralytic shellfish toxin analogues as well as tetrodotoxin (TTX), which to date have not been incorporated into any other hydrophilic marine toxin official method of analysis. The full results of the interlaboratory validation study have recently been published [3]. It is recommended that the method is used as an official alternative method for the quantitative determination of PSTs and TTX in mussels, oysters, clams, scallops, and cockles. It has been approved for regulatory testing in New Zealand and Australia, with other countries now considering its use.

Acknowledgments

We wish to sincerely thank all study participants for their time and considerable efforts in setting up the method, running the samples, and reporting the results. Thanks also to all those who supplied shellfish tissue materials for use in the study, including those who shipped materials but did not enter into the final study samples.

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Cawthron marine toxin analysts Emilie Burger, Michael Boundy, Dr Tim Harwood (L to R).



CEFAS Aquatic Toxins Team. Study co-ordinator Dr Andy Turner is located front middle.

Aotearoa/New Zealand's nationally significant Cawthron Institute Culture Collection of Microalgae (CICCM)

The CICCM is critical to international research projects and the >500 isolates of microalgae and cyanobacteria in the collection have been sourced from oceans, lakes and rivers in New Zealand, the tropics and even Antarctica. A current research focus is the likely impact of climate change on the dinoflagellate microflora of temperate regions, particularly as the temperatures of the coastal waters around New Zealand have been increasing in recent years (Rhodes et al. 2020).

The CICCM continues to expand, with the freshwater cyanobacteria collection mainly cryopreserved. The marine collection is mainly kept live and regularly sub-cultured. Curator (Fig. 1) hours have increased this year to allow for maintenance of new isolates (an 8% increase in isolates since 2018). In order to reduce the amount of labour involved, a Cawthron Institute PhD Scholarship has been offered to Joseph Kanyi, from Kenya (Fig. 2). Joseph will undertake his PhD through Victoria University of Wellington but will be based in Nelson at the Cawthron Institute. He will endeavor to cryopreserve the more recalcitrant toxic dinoflagellates in the collection and will also investigate any molecular changes associated with the cryopreservation process.

Of concern for New Zealand's marine farmers are the recurrent blooms of *Alexandrium pacificum*, which produces paralytic shellfish toxins (PSTs). Strains of the PST-producer are held in the CICCM to underpin vital research. Recently *A. pacificum* spread from Queen Charlotte Sound to Pelorus Sound in the South Island of New Zealand with a major bloom leading to large-scale closures of Greenshell™ mussel harvesting. Rapid and accurate molecular assays have been developed and tested on CICCM *Alexandrium* cultures and these assays are speeding up critical risk assessments for New Zealand's shellfish industry. The characterisation of benthic cyst beds using the molecular assays enables predications of future blooms and their risk to seafood consumers.

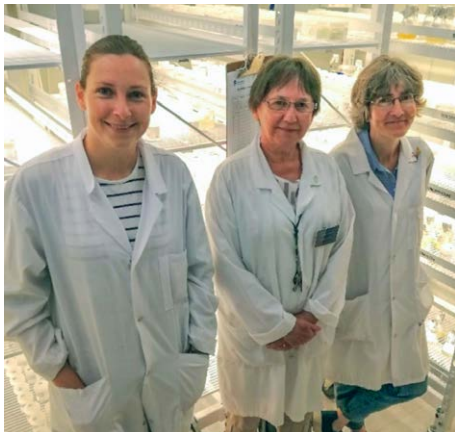

Fig. 1. Cawthron Institute Culture Collection of Microalgae curators. From left, Juliette Butler, Krystyna Ponikla, and Sarah Challenger.

As well as underpinning research into issues of immediate importance to the seafood industry, CICCM isolates are being assessed for compounds of therapeutic benefit. A Cawthron Capability Investment Fund project is underway to screen extracts from the collection in bioassays for therapeutic potential. The project, led by Dr Jonathan Puddick, (Fig. 2) will establish a library of extracts that will be assessed in-house and in partnership with collaborating organisations. This will be an exciting step-change for the collection as the isolation of new microalgal and cyanobacterial strains that show beneficial bioactivities will follow, further growing this national taonga ('treasure' in te reo Māori).

The CICCM's on-line website can be accessed at: *http://cultures.cawthron.org.nz/*

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Fig. 2. Left, Cawthron Institute PhD scholarship recipient, Joseph Kanyi Kihika. Right, Jonathan Puddick, Cawthron algal biochemist investigating the therapeutic potential to be unlocked from the CICCM

The 11th EASTHAB Symposium and 4th Philippine HAB Conference

The 11th EASTHAB Symposium and 4th Philippine HAB Conference were held back-to-back from December 11 – 13, 2019 at the Microtel Hotel, Puerto Princesa, Palawan, the Philippines. The theme was "Advances in Harmful Algal Bloom Research, Monitoring and Management". This served as a transdisciplinary platform for researchers, practitioners, educators, managers, policy makers and fisherfolk to present and discuss the recent trends, innovations and challenges in relation to HABs. This event brought together not just scientists from East and Southeast Asia, but also resource managers, policy-makers and communities in the Philippines to exchange and share their experiences on HAB occurrences, monitoring and management. There was a total number of 134 participants with 10 coming from Japan, 18 from Korea, 11 from China, 3 from Malaysia, 3 from Indonesia, 2 from Vietnam, 1 each from Thailand and Singapore, and 85 from the host country, the Philippines (Fig. 1). The conference was opened by the local government and the head of the Philippine science agency, acknowledging the significant impacts of HABs and the importance of research, monitoring and management efforts. This was followed by Dr. Rhodora Azanza setting the context for the conference and Dr. Yasuwo Fukuyo giving a historical perspective on HAB research in the country and the region as well as the outcomes of strong collaborations across countries and disciplines. Throughout the three days, there were 58 oral presentations and 24 poster presentations spread through 8 sessions: i) Recent HAB Research and Development Initiatives in the Philippines; ii) Managing HABs; iii) HAB Dynamics; iv) HAB Monitoring Tools and Approaches; v) New HAB Species from East Asia; vi) Country Reports; vii) Current HAB Monitoring and Research; and viii) the Community for Alliance for the Sustainability of our Threatened Seas (COASTS) special session.

On the first day, the Director for the Marine Resources Research Division of the Department of Science and Technology- Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD), Dr. Mari-Ann Acedera shared the Philippine accomplishments and roadmap for HAB research in her plenary talk. Dr. Ma. Lourdes San Diego-McGlone highlighted the link between HABs and eutrophication in relation to potential management measures particularly for Philippine mariculture areas. This session on "Managing Harmful Algal Blooms" also showcased a diversity of topics from use of advanced technologies such as drones to community participation in monitoring and management. The COASTS special session uniquely allowed fisherfolk from three HAB-affected sites to present their own perspective on the impact of HABs and their capacity to participate in studies and monitoring.

On the second day, the HAB Dynamics session was opened by Dr. Ichiro Imai where he shared potential preventive strategies against fish-killing raphidophytes, while Dr. Aletta Yñiguez provided a meta-analysis of what is known in relation to knowledge gaps in *Pyrodinium bahamense* bloom dynamics. There were two parallel sessions on HAB dynamics with one focused on molecular studies and the other on larger ecosystem aspects. The last session for the day was on HAB Monitoring Tools and Approaches. Dr. Rhodora Azanza reviewed the monitoring tools and approaches from the common HAB-causative organisms in East Asia. Within this session, studies using traditional microscopy, metagenomics, biochemical profiling, remote sensing and modeling were presented.

On the last day, Dr. Douding Lu discussed different HABs from China and the seas nearby – from pelagic and benthic sources, as well as observations on range expansions by some species such as *Margalefidinium polykrikoides*. He was followed by Dr. Mitsunori Iwataki illustrating the morphology and phylogeny of *Azadinium* and *Amphidoma* from Japan in the session on New HAB Species from East Asia. Studies on benthic and unarmored dinoflagellates were highlighted in this session. Dr. Goh Onitsuka started off the Country Reports on HABs, presenting the case of *Karenia mikimotoi*, the species with the highest number of blooms in the past 3 years in Japan. Together with *Chattonella* spp., this species has caused severe damage to farmed fish. Japan has also experienced an increase in shellfish bans due to Paralytic Shellfish Poisoning (PSP). For South Korea, Dr. Weol-ae Lim explained the new HAB monitoring system and forecast model which included voluntary participation by aquaculture farmers. She also reported results about the *Margalefidinium* blooms in 2019, and the monitoring activities for *Alexandrium* including the use of qPCR.

Dr. Ren-Cheng Yu (China) showed HAB distribution patterns in mainland China, ranging from brown tides in the Bohai Sea, green tides in the Yellow Sea, red tides of dinoflagellates in the East China Sea, red tides of *Phaeocystis* in the South China Sea, and various toxic algal blooms along the coast. Ms. Elsa Furio reported a decline in PSP cases in the Philippines, although a few cases were recorded, from newly affected areas. She also discussed HABs due to *Alexandrium* spp., *Prorocentrum minimum, Noctiluca scintillans* and *Chattonella subsalsa*. The last session was on Current HAB Monitoring and Research. Plenary speaker Dr. Songhui Lu talked about mechanisms of brown tides occurrence in China and their effects. This session was split into two: one dealing with molecular studies and the other with ecosystem scale processes.

The IOC WESTPAC HAB group also had the opportunity to meet on the 12th of December, while the EASTHAB Scientific Steering committee met on the 13th of December. The next EASTHAB Symposium will be held in the Republic of South Korea tentatively in late November 2021.

The Book of Abstracts for this event can be downloaded from: *https://www.philhabs.net/*

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Fig. 1. Participants in the 11th EASTHAB Symposium/4th Philippine HAB Conference



Fig. 2. Department of Science and Technology secretary Fortunato dela Peña with the keynote, plenary speakers and local organizing committee leads. Left to right: Dr. Ichiro Imai, representative of the Vice Mayor of Puerto Princesa City, Dr. Mari-ann Acedera, Dr. Rhodora Azanza, Science Secretary Fortunato dela Peña, Dr. Yasuwo Fukuyo, Dr. Douding Lu, Dr. Aletta Yñiguez, Dr. Lilibeth Salvador-Reyes, Dr. Ma. Lourdes San Diego-McGlone, Dr. Deo Onda, Dr. Mitsunori Iwataki



Fig. 3. A wooden Pyrodinium bahamense pendant (left), crafted by Dr. Takayama, was given to Rhodora Azanza. Dr. Ren-Cheng Yu presenting the country report for China (right).

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics 2020 Meeting

The International Council for the Exploration of the Sea (ICES) and the Intergovernmental Oceanographic Commission of UNESCO (IOC) have collaborated closely for 26 years, stimulating research on HAB population dynamics and monitoring through the joint Working Group on Harmful Algal Bloom Dynamics (WGHABD). This year the WGHABD meeting was hosted by Hanna Mazur-Marzec and Justyna Kobos from the Institute of Oceanography, University of Gdansk in Sopot, Poland from the 3rd – 6th March 2020.

Every year working group members update the IOC-ICES-PICES Harmful Algal Event Database (HAEDAT) with HAB event data from the previous year. These data provide the basis for an ICES Harmful Algal Event Status Report which will be completed during 2020. This ICES report as well as a number of peer reviewed papers from WGHABD members will form the ICES contribution to the forthcoming IOC Global HAB Status Report.

National reports showed that HABs continued to cause a variety of problems in the ICES area during 2019. Paralytic Shellfish Toxins (PSTs) and Amnesic Shellfish Toxins (ASTs) continued to impact the east coast and west coasts of the USA, with Diarrhetic Shellfish Toxins (DSTs) also causing problems in Washington State. *Karenia brevis* continued to cause issues in the Gulf of Mexico. A bloom of *Levanderina fissa* resulted in fish and crab mortalities in the Potomac river in Virginia. Fish and marine mammal mortalities recorded on Mississippi beaches were caused by a cyanobacteria bloom resulting from river discharge from the Mississippi. PSTs, DSTs and ASTs continued to cause problems in Europe. Unusual events included blooms of *Lingulodinium polyedra* in Portugal which resulted in preventative beach closures. Fish kills in Murcia, Spain due to anoxia/mucilage formation after heavy rainfall events. In France, the majority of shellfish closures were recorded in Southern Brittany and associated with DSTs. PSTs were recorded above the regulatory limit in a new region in Ireland for the first time. Human illness was recorded in the UK after consumption of shellfish containing DSTs. An unusual bloom of *Pseudochattonella* resulted in mortalities of farmed Rainbow Trout in Denmark in November 2019. In Sweden, an extensive cyanobacteria bloom was recorded in the Baltic Sea. Beach closures were again enforced in Poland but these were not as extensive as in 2018. No impacts associated with harmful algae were recorded in the Netherlands, Belgium and Germany.



Fig. 1. ICES-IOC WGHABD 2020 meeting

New findings reported on the distribution of *Ostreopsis* in the Bay of Biscay, the extensive fish mortality event in Norway in 2019 caused by *Chrysochromulina leadbeateri*, the importance of understanding monitoring frequency in interpreting shellfish toxin monitoring data in Scotland and a study of the impacts of viruses on cyanobacteria. Investigations into the causative organism of tetrodotoxin (TTX) in the ICES area are still underway. A summary of the successful Co-Clime qPCR workshop held in Germany during October 2019 was presented.

Highlights from the EuroCigua project "Risk Characterisation of Ciguatera Fish Poisoning (CFP) in Europe" were reported. EuroCigua started in 2012 and has had a number of successful deliverables which has improved management and increased awareness of CFP issues in Europe. Epidemiological surveillance protocols were developed and 23 ciguatera outbreaks from Spain, Portugal, Germany and France between 2012 and 2018 have been identified. These involved both endemic and imported fish. *Gambierdiscus* and/or *Fukuyoa* species were identified from the Canary Islands, Madeira, Selvagem, Crete and Cyprus and *Gambierdiscus* was identified in the Balearic Islands for the first time. The majority of strains from the Canary and Balearic Islands were found to produce CTX like toxins. The N2a Assay to identify CTX toxins was standardized and validated by LC-MS-MS and successfully used to test fish in the Canary and Madeira Islands. Currently methods are being optimised to produce reference materials from fish contaminated with CTX.

For the first time WGHABD shared a day with ICES-IOC WG Ballast and Other Ship Vectors (WGBOSV) and ICES WG Introduction and Transfer of Marine Organisms (WGITMO). The Arctic is a region of interest for both ICES and the scientific community. Climate change is envisaged to alter this region in terms of biological impacts as well as increasing potential for shipping. Members from the different WGs exchanged experi-



Fig. 2. Shared day between ICES-IOC WGHABD, ICES-IOC WGBOSV and ICES WGITMO

ences using molecular methods as well as results from studies in Arctic areas. These studies presented data on HAB and invasive non-native species present in Arctic waters.

A USA study focused on the distribution, community structure, and dynamics of *Alexandrium catenella* and *Pseudo-nitzschia* in the Northern Bering, Chukchi and Beaufort Seas. Two extensive *Alexandrium catenella* cyst seedbeds were documented in the region during cruises in 2018 and 2019. This Alaskan Arctic cyst seedbed is approximately five times larger than the equivalent feature in the Gulf of Maine. Bottom temperatures recorded during the cruises were in the range to support relatively rapid germination of cysts. High concentrations of *A. catenella* vegetative cells were also detected during cruises in these regions. The data suggests that blooms in the region may originate locally and are likely self-seeding and recurrent. Established populations transported from the south through the Bering Strait may be a second source of blooms north of the Strait. Multiple species of *Pseudo-nitzschia* were also detected from Bering Strait to Chukchi Sea including *Pseudo-nitzschia australis/seriata, P. pungens, P. delicatissima*, and *P. obtusa*. A review of Canadian data revealed the widespread presence of potential toxin producing species throughout Canadian Arctic waters; Beaufort Sea, Baffin Bay, North West Passages as well as Hudson Bay and the Labrador Sea. Records include species from the genus *Pseudo-nitzschia* (*P. pseudodelicatissima, P. seriata, P. calliantha, P. granii, P. delicatissima, P. obtusa*), *Alexandrium* (*A. catenella, A. ostenfeldii*), *Dinophysis* (*D. norvegica, D. acuminata, D. acuta*), *Phalachroma rotundatum* and *Prorocentrum lima*. Reports from cruises to the west coast of Iceland and Greenland recorded *Phaeocystis* and *Pseudo-nitzschia* species. Measurement of algal toxins from the water was also a useful tool to identify the presence of HAB species. Presentations by all three WGs highlighted similar issues with the use of molecular methods. These included the standardisation of methods between laboratories and over time to allow data to be compared, primer bias and the requirement for bioinformatics expertise and computing and data power.

This was the final year in the three year ICES reporting cycle and the meeting opened with a discussion about the UN Sustainable Development Goals and the UN Decade of Ocean Science for Sustainable Development (2021 – 2030) https://www.oceandecade.org/. The UN decade is an opportunity to make HAB science more relevant to society. Discussions within WGHABD when setting terms of reference (ToRs) for the coming three year reporting cycle were framed within the context of how they would contribute to progress within the UN decade. For the next three year cycle current WGHABD ToRs focused on national reports, new findings and HAEDAT will continue. New ToRs will address increased visibility of the WG, advances in automated imaging and molecular methods for detection of HAB species, emerging toxins, sub lethal impacts of HABs on farmed fish, and the impacts of climate change on HABs. Dave Clarke from the Marine Institute, Ireland was elected as the new chair of ICES-IOC WGHABD.

The next meeting is planned for the 20 -23 April 2021 in Lowestoft, England. WGHABD welcomes new members at any time and is open to experts from all IOC Member States. HABs are a global problem and experts from outside the ICES region, interested in joining the WG (at their own expense) are particularly welcome. Now is your chance to get involved. For further information please contact WGHABD or the IOC.

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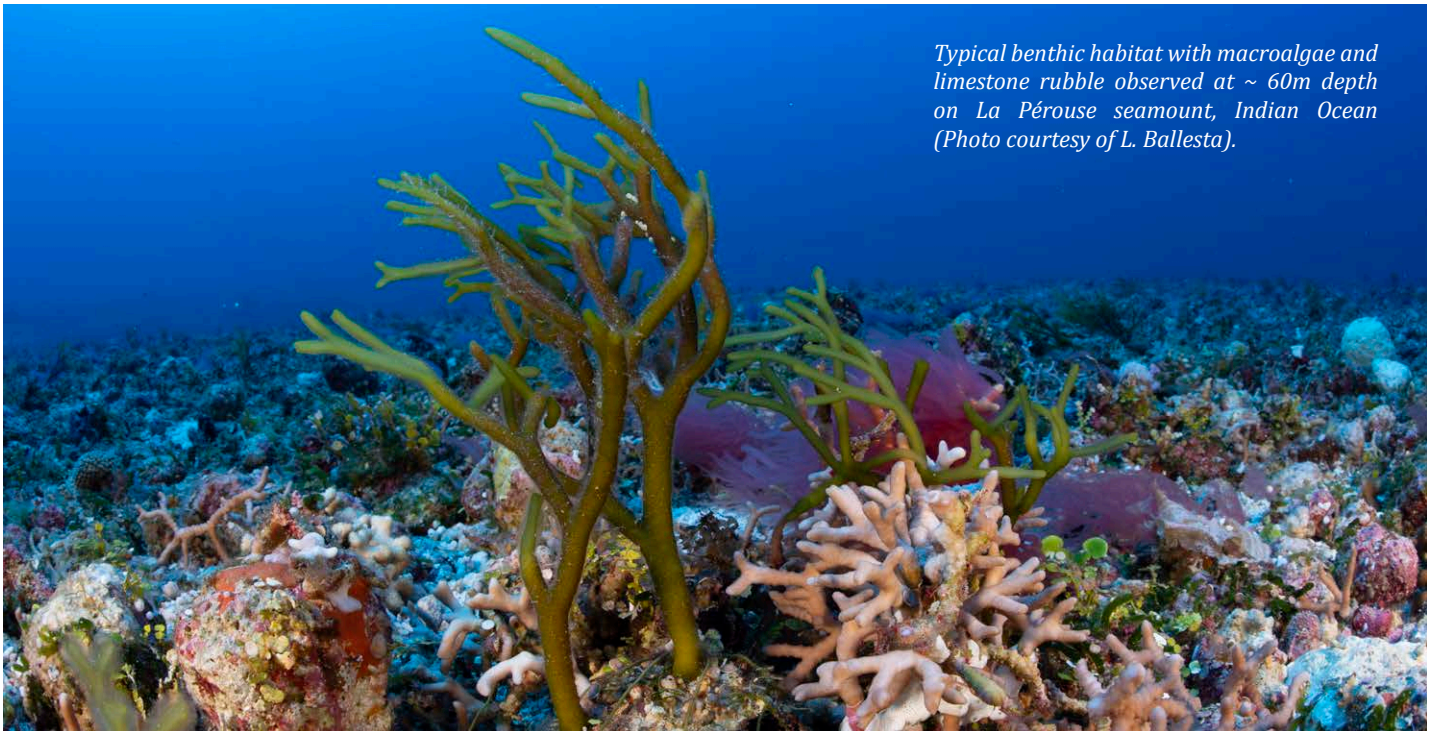

Typical benthic habitat with macroalgae and limestone rubble observed at ~ 60m depth on La Pérouse seamount, Indian Ocean (Photo courtesy of L. Ballesta).

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 65:
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Genomic resources for the domoic acid-producing diatom *Pseudo-nitzschia multistriata*

Species responsible for Harmful Algal Blooms (HABs) are among the best studied unicellular microalgae. HABs pose a serious risk to human health and are responsible for considerable economic losses in the aquaculture industry which has resulted in the funding of monitoring programs to investigate their temporal and spatial distribution, as well as research projects aimed at understanding various aspects of their biology. In recent decades, understanding about HAB diversity, physiology and trophic habits, the mechanisms that regulate toxin production, and the role of environmental variables and interactions with other members of the plankton community on their growth dynamics has increased. The rapid development of genomic- and transcriptomic-based approaches, now available for an increasing number of unicellular eukaryotes including HAB species, is allowing scientists to gain a mechanistic understanding of a broad range of biological features.

The genus *Pseudo-nitzschia* includes the majority of species that produce the neurotoxin domoic acid, the causative agent of Amnesic Shellfish Poisoning. About 50 species have been described to date and half of these are known to produce domoic acid. The importance of this genus motivated the selection of one of the most toxic species, *Pseudo-nitzschia multiseries*, to be one of the few

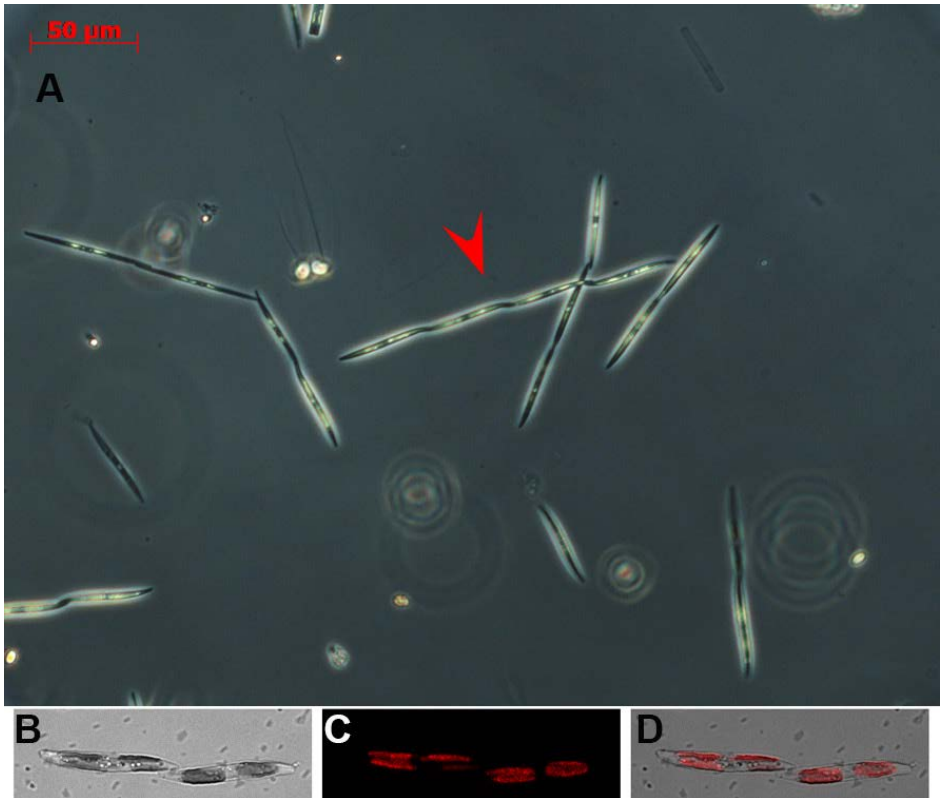

Fig. 1. A. Light micrograph of a natural phytoplankton sample collected at the Long Term Ecological Research station MareChiara (Gulf of Naples, Italy) on October 3rd 2013. Pseudo-nitzschia multistriata cells (a chain is indicated with an arrow) can be easily identified by their slightly sigmoid shape. Photo: Marina Montresor. Two Pseudo-nitzschia multistriata cells approaching cell division, B. bright field, C. chlorophyll autofluorescence in the chloroplasts, and D. merged images.

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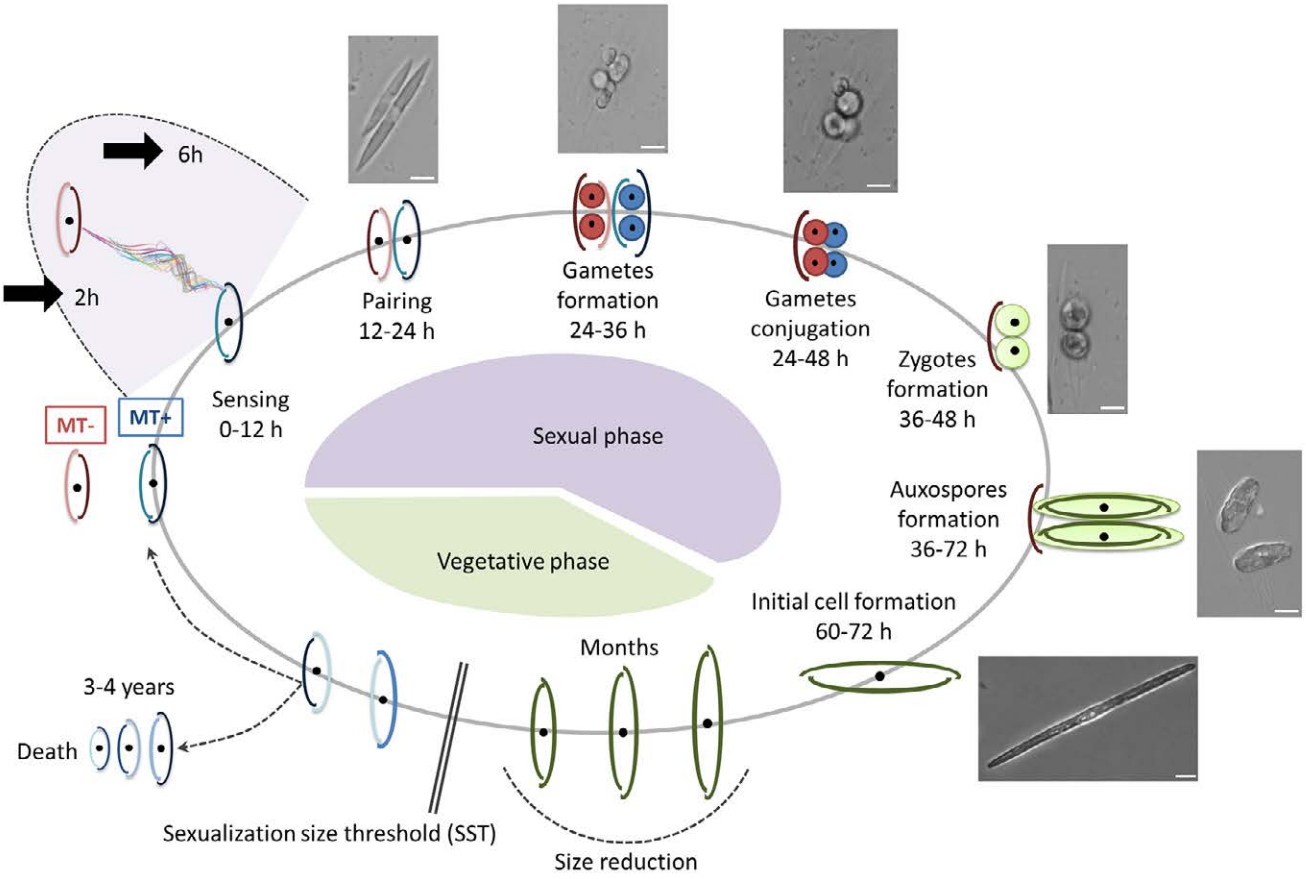

Fig. 2. Schematic drawing of the life cycle of the pennate diatom Pseudo-nitzschia multistriata. The vegetative phase is characterized by progressive cell size reduction of the population. When cells reach the sexual size threshold (SST), they can either keep decreasing in size until they die, or undergo sexual reproduction and escape the size-decreasing process producing large initial cells. The perception of chemical cues deriving from the mating partner brings cells of opposite mating type to pair and haploid gametes are produced following meiosis. Conjugation of gametes produces two expandable zygotes that develop into auxospores, within which an initial cell of maximum size is synthesized, restoring the vegetative phase of the cycle. Representative microscopic images of the different stages are shown outside the circle; bar, 10 µm. From [7].

diatoms selected for genome sequencing when this was still a demanding and time consuming effort. The *P. multiseries* genome has been publicly available since 2011 (*https://mycocosm.jgi.doe.gov/Psemu1/Psemu1.home.html*) and despite being quite fragmented, possibly due to the high content of repetitive sequences it has represented a valuable resource used to explore diatom biology in general and in many comparative genomic analyses. Subsequently, *Pseudo-nitzschia* transcriptomes became available through the Marine Microbial Eukaryotic Transcriptome Sequencing Project (MMETSP), enlarging the repertoire of sequence resources for the genus (*https://www.imicrobe.us/#/search/mmetsp*). Currently, thanks to this initiative, transcriptomics data are available for *P. arenysensis*, *P. delicatissima*, *P. fraudulenta*, *P. australis*, *P. heimii* and *P. pungens*. Transcriptomics data have also been generated in dedicated studies for other species, namely *P. multiseries, P. granii, P. seriata* and *P. obtusa* [1-3].

Here we illustrate genomic resources that are available to the research community for another toxic *Pseudo-nitzschia* species, *P. multistriata.* We also provide a few examples of questions that can be addressed by genomic approaches.

Pseudo-nitzschia multistriata was described in 1993 by Hideaki Takano from coastal waters in southern Japan. The species produces domoic acid [4] and has been reported from various regions world-wide (Mediterranean Sea, Gulf of Mexico, Malaysia, Singapore, New Zealand, the Pacific coast of Mexico, see [5]). Cells of *P. multistriata* can easily be identified in light microscopy due to their distinct 'sigmoid' shape (Figure 1). This species began to be recorded at the Long Term Ecological Research station Mare Chiara in the Gulf of Naples in 1993 and since then is regularly recorded in summer-early autumn. The relative ease of identification made it a model for a long term study of population structure using microsatellites [6]. This study required the isolation of a few hundred strains to obtain their DNA. Availability of all of these strains represented a great resource facilitating the use of *P. multistriata* as a genetic model, allowing genetic crosses and production of new generations in the laboratory.

We would like to avail of this opportunity to publicize our request for *P. multistriata* strains to all of our colleagues working with phytoplankton samples who may find the species, easily distinguishable from other *Pseudo-nitzschia* due to the curved tips and sigmoid shape. Strains from different geographic locations will greatly enrich ongoing population genomics analyses and could reveal more about core and dispensable regions of the genome, selection, and ultimately about the evolution of this toxic species.



Fig. 3. The genome browser available on the SZN BioInforma platform.

The genome could be sequenced exploiting inbred strains, obtained from the cross of a first generation of sibling strains [7]. Because of the lower polymorphism of inbreds, it was possible to reconstruct long fragments of DNA from reads obtained with the Illumina technology, overcoming the limits of short reads assembly. Indeed, the assembly of high polymorphic sequence reads make it harder for common assembly software to bind small sequences together to produce the long fragments called scaffolds.

The 59 Mb genome obtained enhanced the range of possible approaches to explore the genetic basis of many of the species features, including the capability to undergo sexual reproduction. *P. multistriata*, like the majority of pennate diatoms, has a heterothallic mating system. This implies that sexual reproduction, the process in diatoms that counteracts progressive cell size reduction to produce large-sized F1 cells, only occurs when cells of opposite mating type (MT+ and MT-) make contact (Figure 2) [7]. Gene expression studies based on RNA-seq have focused on the life cycle and on the sexual reproduction phase, revealing differences in the transcriptomic behavior between the two mating types, even if they are morphologically identical. One of the most exciting findings enabled by the genome sequence availability was the discovery of the first mating type determining gene for diatoms. How a cell becomes MT+ or MT- was unknown; differential expression of a specific gene between groups of MT+ and MT- strains was linked to structural differences in a specific genomic region, providing the first clue on how the MT is specified [8].

Diatom genomes, when compared to estimates for other toxic phytoplankton such as dinoflagellate species, are generally smaller and less "intimidating", and genome sequencing is approachable for even small teams, without the requirement to establish large consortia. The *P. multistriata* genome sequencing project was a small project funded by a Marie Curie Career integration grant, it mainly involved two teams, the SZN in Naples (Italy) and the TGAC in Norwich (UK) (now Earlham Institute), cost around 10,000 euros and was initially released on a TGAC browser which was password protected in line with institute policies. To make it more easily accessible, the genome browser is now being hosted by the bioinformatics service at the SZN, (*http://bioinfo.szn.it/pmultistriata/*), embedded in the service platform (*http://bioinfo.szn.it/*; Figure 3) where it is freely accessible together with all the related RNA-seq data produced so far. Beyond gene tags, researchers have the possibility to browse the genome information visualizing repetitive elements, conserved regions in common with other species and other features, with links provided to the data files and to the relevant literature. Finally, within the European project EMBRIC and with the assistance of European Bioinformatics Institute teams, additional work on the sequencing data has been done to have it released in Ensembl (*https://protists.ensembl.org/Pseudonitzschia_multistriata/Info/Index*), one of the major genome browsers, originally created for vertebrate genomes but with an expanding section for protists. Availability in Ensembl makes the data readily discoverable by the HAB community, by scientists working on very distantly related organisms, and facilitates its use in large scale comparative studies. Unfortunately due to specific requirements of the Ensembl browser which did not support the original files generated for *P. multistriata*, the SZN and the Ensembl versions of the genome are not uniform; the scaffold names are different and, in Ensembl, all *P. multistriata* proteins are reported as "unknown proteins" while a detailed functional annotation is provided by the platform available from SZN.

While these discrepancies highlight the existence of difficulties in crosslinking –omics data from different resources, especially for non-model systems as well as the need for constant and supported interaction between wet lab scientists and bioinformaticians, the availability of *P. multistriata* genome resources was a big opportunity for comparative analyses. It has enabled and strengthened important discoveries, such as the conservation of a cluster of four genes involved in domoic acid synthesis, originally identified in the *P. multiseries* genome [9].

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Fig. 4. Some Pseudo-nitzschia fans at Stazione Zoologica Anton Dohrn: (standing from left to right) Svenja Mager, Maria Immacolata Ferrante , Francesco Manfellotto, Anna Santin, Maria Valeria Ruggiero, Monia Russo, Viviana Di Tuccio, (sitting from right to left) Rossella Annunziata, Antonella Ruggiero, Pina Marotta, Marina Montresor

When tides collide: Harmful cyanobacterial and microalgal blooms in Florida and implications for risk assessment

Cyanobacterial blooms are a regular occurrence in southern Florida. Water releases from Lake Okeechobee to maintain the water level in this large lake regularly occur along the St. Lucie Canal to the eastern seaboard or along the Caloosahatchee to Fort Myers and the Gulf of Mexico (Fig. 1). Due to increased nutrient loading in Lake Okeechobee, cyanobacterial blooms, frequently composed of *Microcystis aeruginosa* are released from the lake and contaminate both these waterways. This results in large, microcystin-containing cyanobacterial blooms that can affect tourism and fisheries (Fig. 2). Press reports cataloguing these economic and health impacts in Florida are common.

In summer 2018, the situation on the west coast of Florida was exacerbated by the appearance of a bloom of *Karenia brevis* in the Gulf of Mexico. This brevetoxin-producing algal bloom resulted in the deaths of marine mammals and fish with numbers in the thousands reported on the beaches around southwest Florida. Due to the large discharge of water from Lake Okeechobee, pulses of freshwater ended up in the estuarine and coastal environments.

Sampling of the Caloosahatchee in 2018 showed high concentrations of *Microcystis aeruginosa* and microcystin-LR according to microscopy, UPLC-PDA and UPLC-MS [1]. BMAA was also detected by triple quadrupole mass spectrometry at much lower concentration. Although such blooms dominated the freshwater environment of the river, visually intact *Microcystis* colonies were observed in brackish water in the sound and estuary past the tide line. Conversely, although diatoms and dinoflagellates could not be observed in freshwater samples, ELISA analysis for brevetoxins showed low positive concentrations in the Caloosahatchee and large concentrations in the brackish and marine environment.

The findings of the "crossing-over" of blooms raise questions concerning the risk assessment of cyanobacterial and dinoflagellate toxins in estuarine environments. First, how far can toxins produced by freshwater cyanobacterial blooms be found in saline environments? Conversely, can marine diatom and dinoflagellate toxins be routinely found in freshwater environments, most likely through navigation and tidal flows? Our data indicate that both are possible, although a greater understanding of the effect of salinity on the growth of *Microcystis* is required, building upon previous work that has shown that e.g. microcystin concentrations can be increased after salt shock [2]. Furthermore, in other estuarine environments a wide range of marine and freshwater toxins have been identified [3].

If the particular toxin(s) are known within freshwater or marine blooms, then adequate risk assessment is possible to protect human and animal health. However, when multiple blooms occur at the same location, then toxicity assessment and risk assessment are necessary to determine whether there are interactions between the toxicants that may change the toxicological outcome. Communication is also essential in order to alert the public to potential issues that may arise from exposure to one or more classes of aquatic toxin(s), whether from harmful cyanobacterial and/or algal blooms. Further communication may be necessary as, depending on the country, marine and freshwaters may be under the jurisdiction of different agencies with different accountabilities and responsibilities. The presence of such multiple blooms may also have effects upstream as, in the case of Florida, changes to discharges from Lake Okeechobee may have to occur when marine blooms are present. When tides collide, procedures need to be in place to determine the potential risk, communicate with relevant parties and perform toxicity assessments when necessary. In addition, collection of autopsy materials can also determine what toxicants may be causing adverse health effects, whether to wild or domestic animals or humans. Ultimately, legislation and good stewardship of waters and relevant contingencies can minimise the potential risk of adverse health effects from occurring.

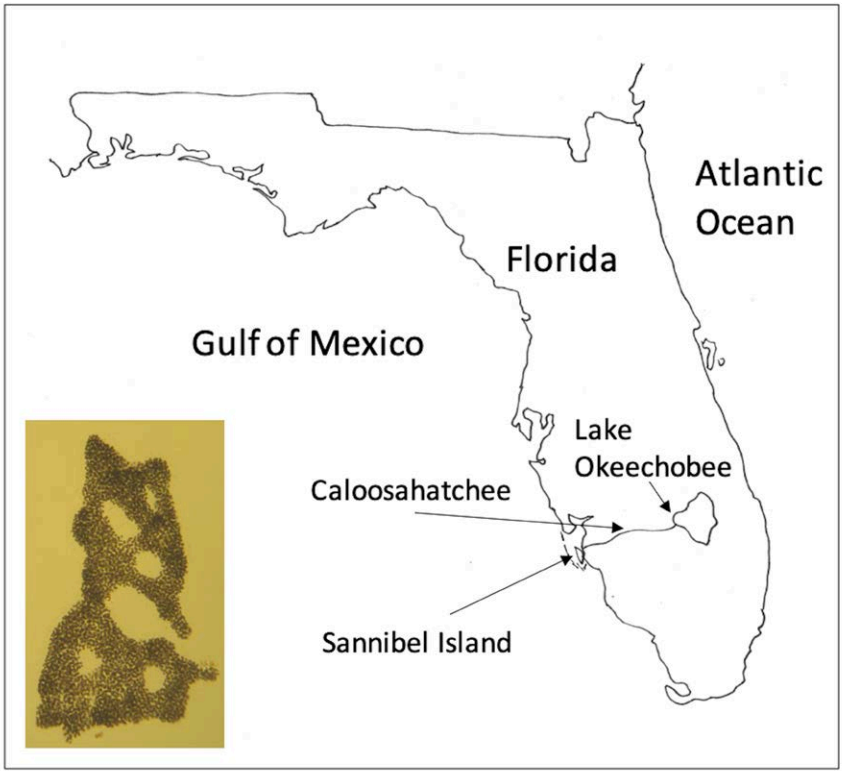

Fig. 1. Location of Lake Okeechobee and Caloosahatchee River in Southern Florida, USA where a bloom of Microcystis aeruginosa released from the lake collided with a red tide of brevetoxin-producing Karenia brevis near Sannibel Island. Bottom left Insert, a colony of Microcystis aeruginosa (approx. 500 m width) from Caloosahatchee River

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We are grateful to Dr. John Cassini, Jason



Fig. 2. Bloom of Microcystis aeruginosa at Lake Okeechobee, Florida, summer 2018

Pim and Calusa Waterkeepers for assistance in sampling.

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Continued from page 3

The raw and processed sequencing data generated can be beneficial for many applications. Some are already routine methods, such as the use of the genome as a reference to map RNA-seq reads and interpret transcriptomics studies, comparative analyses with other genomes to define structural and/or phylogenetic relationships, estimation of the extent of genomic variations and rearrangements, and the identification of regions under selection, contributing to evolutionary insights in populations and species. Other analyses that can benefit from the existence of a reference genome include support in taxonomic assignation and in the interpretation of results from metagenomics and metatranscriptomics projects. Combining information from such approaches can lead to innovative ways to monitor phytoplankton; for example, genes found highly induced under specific experimental conditions in the laboratory can become markers to follow specific processes in the natural environment by qPCR or metatranscriptomics. Examples include genes involved in the synthesis of toxins, genes expressed during sexual reproduction, genes activated during specific stress conditions. We are now familiar with the fact that community composition can be described with barcode sequences. In the future we will be able to identify organisms by retrieving their genomes, as is now the case for bacteria. Moreover, species-specific genes allow specific functions from individual species to be understood, e.g. to obtain a picture of their metabolic state, or the phase of their life history. Finally, for many traditional or more innovative methods for the study of gene function, the presence of a reference genome is mandatory. Sophisticated manipulations and genome editing methods, including the CRISPR/Cas9 technology, are now within reach for this *Pseudo-nitzschia* species.

In view of the potential for *P. multistriata* to be used as a model for planktonic diatom life cycles, more studies are ongoing, and a further leap forward is expected thanks to the support of the Marine Microbial Initiative of the Gordon and Betty Moore Foundation, which is funding a project (*http://www.szn.it/index.php/en/research/integrative-marine-ecology/research-projects-emi/disco*) to dissect the mechanisms underlying sex determination and controlling transitions between life cycle phases, to discover the genes and genomic regions that are under selection in natural populations, and to assess the effects of sex on genome evolution. More *P. multistriata* genomes are being re-sequenced thanks to this project, and technological advances and new technologies gradually becoming available will be exploited to improve the current genome assembly, to explore epigenetic control and to bring our knowledge of *Pseudo-nitzschia* to a higher functional level.

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Record levels of Dinophysistoxin-2 in clams from Douarnenez Bay, France, after an unusual bloom of *Dinophysis acuta*

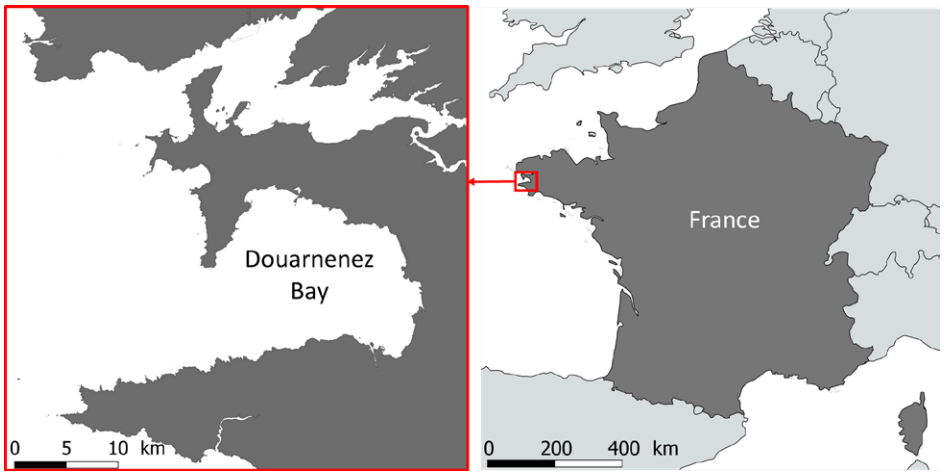

Fig. 1. Location of Douarnenez Bay (48° 5' 29" North; 4° 19' 51" West), Western French Atlantic coast.

The official monitoring network for phytoplankton and algal toxins in French shellfish production areas (REPHY) was established by Ifremer in 1984 after several thousand cases of diarrhetic shellfish poisoning occurred in Western France [1]. The monitoring program has evolved over time. From January 1, 2010 chemical analysis by liquid chromatography coupled with mass spectrometery in tandem (LC-MS/MS) became the official method for monitoring diarrhetic shellfish poisoning (DSP) toxins as a result of uncertainties about the reliability of the mouse bioassay for detection of these toxins. As a result an almost ten year time series of toxin profiles in shellfish exists for some sites along the French coast, including Douarnenez Bay in Brittany (Fig. 1).

This shallow semi-enclosed bay, with an area of 230 km^2 (15 km wide by 20 km long) has a weak circulation which favours the accumulation of nutrients from different watersheds [2]. Water temperatures range between 7 and 21°C and salinity between 32 and 36 (minimum and maximum over 10 years). These features, combined with its particularly clear waters, favour phytoplankton growth in this area. This is particularly true from April to September when days are longer and sea surface temperatures increase. The Douarnenez coastline includes steep rocky seashore areas in the north and south and large sandy beaches to the east, with optimal conditions for clam (*Donax* spp.) cultivation (Fig.2 A-B).

Phytoplankton communities in Douarnenez Bay (Fig.1) have been monitored twice a month since 1987, as part of seafood safety and environmental quality control programmes. In parallel with phytoplankton monitoring, clams (*Donax* spp.) has been regularly analysed for lipophilic toxins before being marketed. These toxins include two groups of polyether compounds: i) diarrhetic shellfish poisoning toxins (DSP): okadaic acid (OA) and dinophysistoxins (DTXs: DTX1 and DTX2) and ii) pectenotoxins (PTXs: PTX1 and PTX2). These toxins are mainly produced by dinoflagellate species belonging to the genus *Dinophysis*.

During a typical year, the main phytoplankton species responsible for the occurrence of lipophilic toxins in Douarnenez Bay, from spring until late autumn or early winter, was *Dinophysis acuminata* (Figs.2C, 3A) [3]. In the last decade, densities of *D. acuminata* varied from 1 x 10^2 to 12 x 10^3 cells L^{-1}. The predominant toxin associated with this taxon was okadaic acid (OA) (Fig. 3 B). The maximum OA concentration

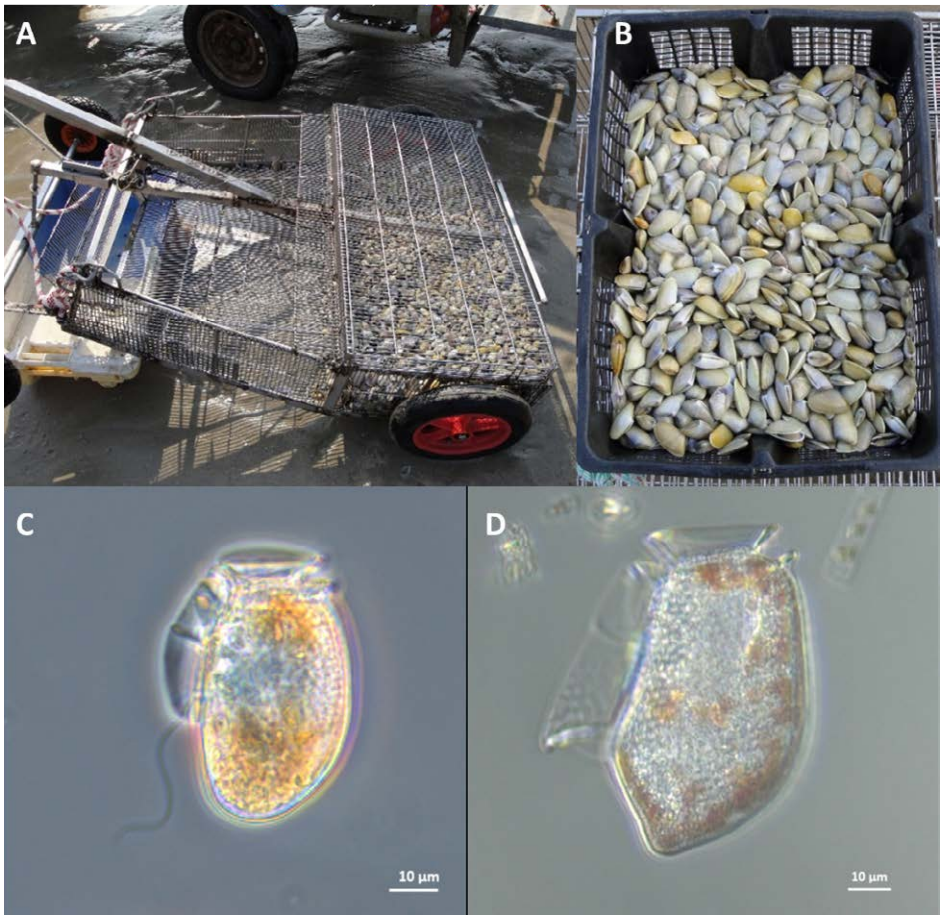

Fig. 2. (A) Sampling device to collect (B) Donax clams. Light micrographs of (C) Dinophysis acuminata and (D) Dinophysis acuta. (Photos A, B by Dominique Le Gal; C, D by Audrey Duval).

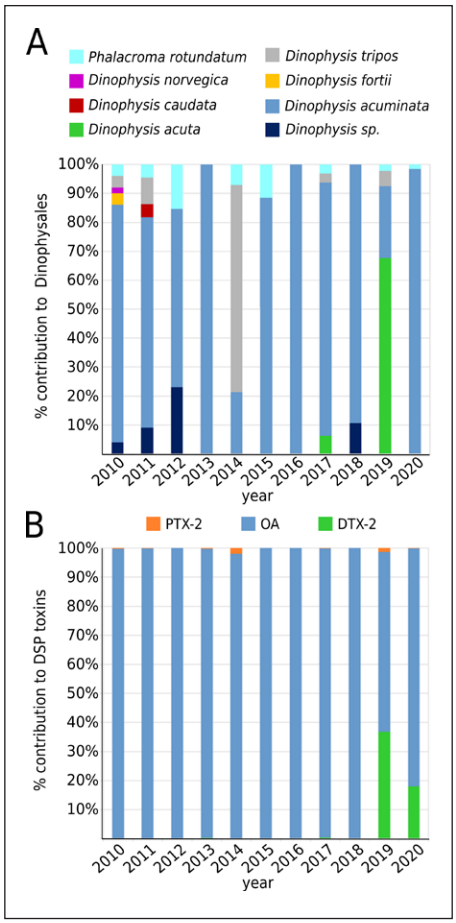

Fig. 3. (A) Percentage of Dinophysis species in Douarnenez Bay water samples between 2010 and August 2020. (B) Mean percentage of DSP toxins in Douarnenez Bay in Donax spp. between 2010 and August 2020.

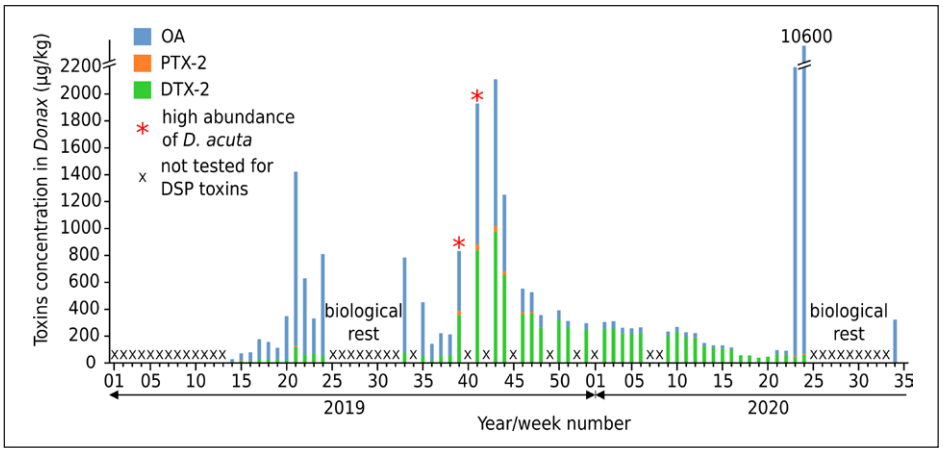

Fig. 4. Weekly lipophilic toxin concentrations in Donax spp. in 2019 and 2020.

recorded in *Donax* spp. was 9,853 µg kg^{-1} in June 2020 (Fig. 4) [4]. The maximum toxin concentration as well as the duration of the episodes (concentration above the regulatory threshold) varied considerably from year to year. Usually, the other regulated DSP and PTX toxins were not detected (DTX1, PTX1) or were present at very low concentrations (DTX2, PTX2).

However, 2019 was a very exceptional year in terms of phytoplankton and toxin composition in Douarnenez Bay. Indeed, for the first time in over 30 years, *D. acuta* (Fig. 2D), known to produce OA and DTX2, was present in high cell densities in late summer and early autumn. Since 1987, *D. acuta* had never been observed in densities above 10^2 cells L^{-1} in Douarnenez Bay, but in September 2019, densities of 5.5 x 10^3 cells L^{-1} (week 39) and 3.3x 10^3 cells L^{-1} (week 41) were detected. After this bloom of *D. acuta*, *Donax* spp. were found for the first time to contain almost the same concentration of DTX2 as OA (Fig. 4). During week 39, when *D. acuta* was first detected (5.5 x 10^3 cells L^{-1}), DTX2 and OA concentrations in *Donax* spp. were 356 µg kg^{-1} and 443 µg kg^{-1}, respectively and in week 41, after the second detection of *D. acuta* (3.3x 10^3 cells L^{-1}), 840 µg kg^{-1} and 1,045 µg kg^{-1} respectively. Finally, in week 43, maximum concentrations of DTX2 (974 µg kg^{-1}) and OA (1089 µg kg^{-1}) and low concentrations of PTX2 were recorded. Analysis of the full dataset from the REPHY monitoring network reveals that this is the first time such high DTX2 concentrations have been found in Douarnenez Bay. These are also the highest concentrations of DTX2 recorded in France to date.

The difference in depuration time for DTX2 and OA in *Donax* spp. is striking. While OA concentrations decreased very quickly, DTX2 was hardly eliminated at all (Fig. 4). During week 46, three weeks after the toxicity peak, DTX2 concentration was double that of OA, and still five times higher than that of OA eight weeks later (week 51). The presence of DTX2 at such concentrations and the difficulty of eliminating this toxin resulted in the regulatory threshold of 160 µg kg^{-1} (OA+DTXs+PTXs) TEF being exceeded until early March 2020. Hence, the toxic episode of 2019 and first quarter of 2020 lasted a total of 49 weeks, including 27 weeks caused by the presence of DTX2 produced by *D. acuta*. Considering that in previous years *Donax* spp. harvesting bans due to DSP toxins in Douarnenez Bay lasted 10-29 weeks per year, the 2019 outbreak was an exceptionally long event. If blooms of *D. acuta* become recurrent, causing lengthy contamination by DTX2 in shellfish, a strong economic impact on *Donax* spp. harvesting activity is to be expected. Further investigations should aim at better understanding the reasons of this shift in the phytoplankton community.

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New insights on the diversity of the dinoflagellate genus *Ostreopsis* in lagoons of French Polynesia, South Pacific Ocean

French Polynesia is a vast territory in the South Pacific Ocean, stretching over an expanse of more than 1,200 miles with a surface area as large as Europe. It is composed of 118 geographically dispersed islands and atolls regrouped into five distinct archipelagoes: Society Archipelago, Tuamotu Archipelago, Gambier Archipelago, Marquesas Archipelago, and Australes Archipelago (Fig. 1).

Historically, French Polynesia has long been concerned by harmful algae events, especially ciguatera poisoning (CP) which is, by far, the most prevalent seafood poisoning in the region [1]. Some areas like Gambier Islands undergo recurrent high toxicity CP outbreaks which became the focus of major research conducted in the late 70s by R. Bagnis, T. Yasumoto and Y. Fukuyo. Their pioneering work led to the formal identification of *Gambierdiscus* as the dinoflagellate responsible for CP [2-3]. Since this milestone discovery, several decades of research on ciguatera have been conducted in French Polynesia, which is the unique Pacific island territory with a permanent ciguatera research unit (Louis Malardé Institute, ILM) [1].

In addition to *Gambierdiscus* species, other benthic and potentially toxic dinoflagellates have been identified in benthic assemblages of French Polynesian ciguateric biotopes [4-5], including *Ostreopsis* and *Prorocentrum* species, but their potential harm has remained unstudied. In the past decades, *Ostreopsis* has become highly problematic in several temperate and subtropical areas, due to the formation of intense blooms associated with the production of toxic compounds analogous to palytoxin that have negative impacts on human health [6]. As the risk posed by *Ostreopsis* spp. proliferations in French Polynesia has never been assessed, investigations were undertaken as part of the research project TATOO to study the diversity and toxicity of *Ostreopsis* species in various French Polynesian lagoons. The present study was based on both field material collected between 2016-2019 from eight distinct islands (Fig. 1) and several clonal strains that are part of the Laboratory of Marine Biotoxins culture collection at the *Institut Louis Malardé* (Tahiti, French Polynesia), where cultures are deposited. Samples from islands of the five archipelagoes were obtained. Taxonomic identifications were carried out using microsocopy (LM, SEM) coupled with molecular characterization of DNA extracts prepared from cultures or single cells isolated from field samples. Toxicity screening analyses were initially conducted using the neuroblastoma cell-based assay (CBA-N2a), and toxin profiles were further characterized in toxic strains by liquid chromatography tandem mass spectrometry (LC-MS/MS) [7,9].

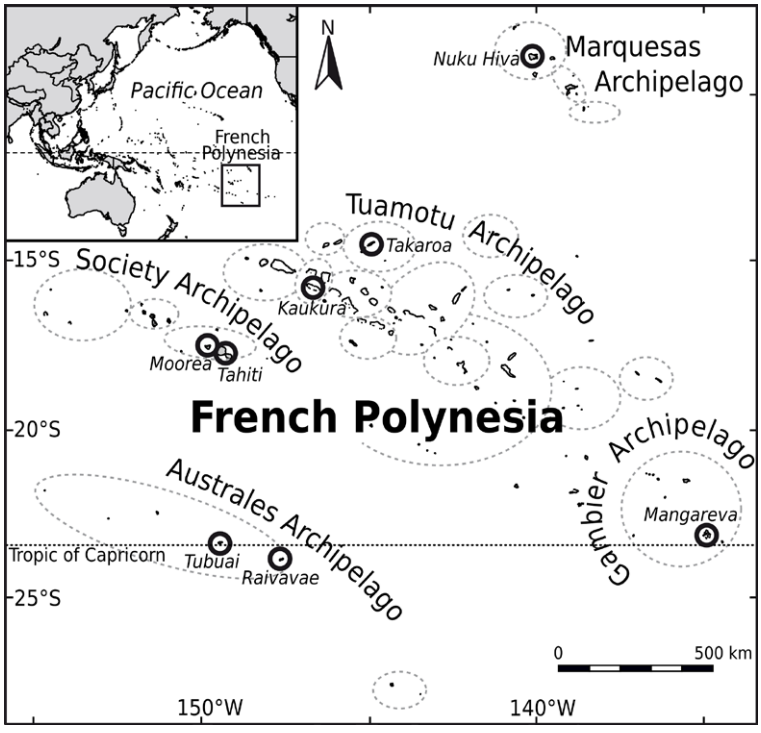

Fig. 1. Map of French Polynesia showing the five archipelagoes. Islands where samples have been collected for this study are circled and their names are in italics.

Our analyses revealed that two species, namely *Ostreopsis lenticularis* and *Ostreopsis* cf *ovata,* were present in all five archipelagoes of French Polynesia and that they constituted the most commonly observed *Ostreopsis* species in the area. This result is in agreement with previous observations by Bagnis and Fukuyo [4-5] and it was suggested that *Ostreopsis lenticularis* filled the ecological niche following *Gambierdiscus* outbreaks [5]. Thanks to our study, it was possible to re-investigate the type locality (Tahiti island) and unambiguously identify *Ostreopsis lenticularis*, confirming its morphological features and resolving its genetic identity (= *Ostreopsis* sp. 5) [7].

In addition to these two species, two other previously unreported species were found in several locations (Fig. 2).

In Kaukura and Takaora Islands (Tuamotu) as well as in Nuku Hiva Island (Marquesas) (Fig. 1), a small species (ca. 40–50 µm diameter) was present, and its thecal plate pattern was not significantly distinctive from other *Ostreopsis* species. Interestingly, it possessed a long second apical plate 2', reaching the fourth precingulary plate 4'' dorsally (Fig. 2B). Genetically, these specimens were identified as *Ostreopsis rhodesiae*, a species described rather recently from Heron Reef Lagoon, in the southern Great Barrier Reef (Coral Sea, Australia) [8] and, to our knowledge, not reported elsewhere to date.

An additional species was also observed in 2019 in Tahiti Island forming a large benthic bloom [9]. Morphologi-

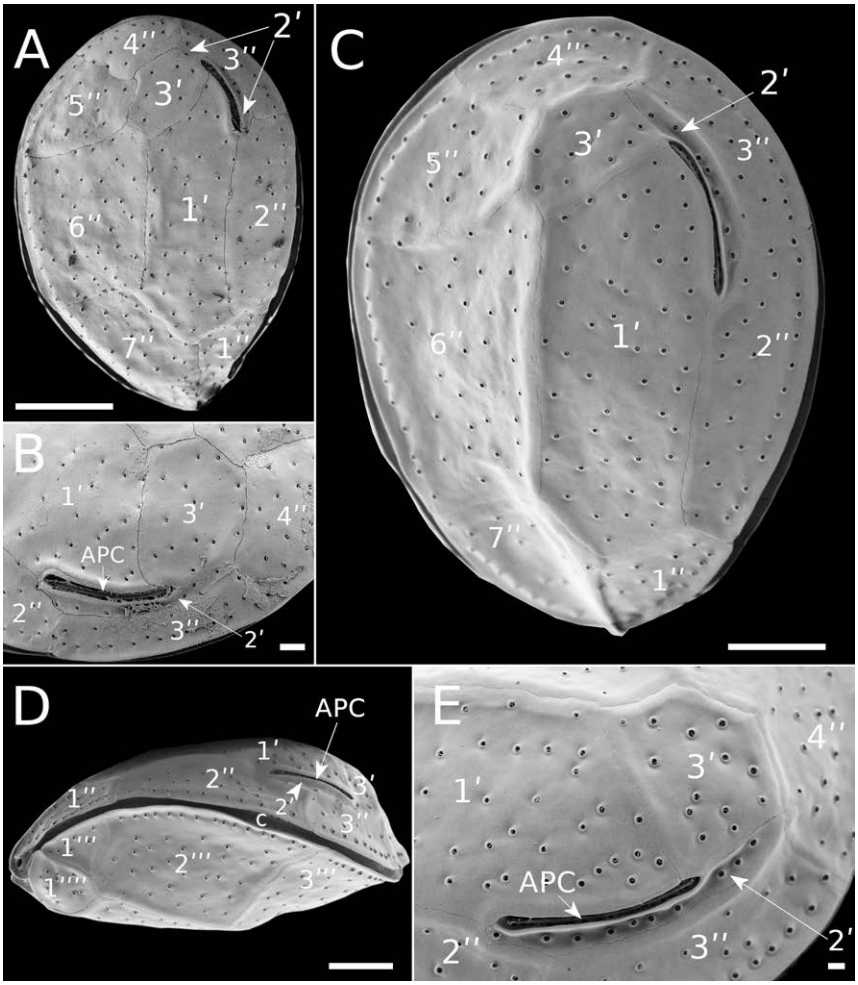

Fig. 2. Scanning electron micrographs of two previously unreported Ostreopsis species in French Polynesia. (A) Apical view of O. rhodesiae. (B) Detail of the apical pore complex (APC) of O. rhodesiae. (C) Apical view of Ostreopsis sp. 6. (D) Left lateral view of Ostreopsis sp. 6 showing the undulated cingulum (c). (E) Detail of the APC of Ostreopsis sp. 6. Scale bars = 10 µm (A and C, D), 2 µm (B and E).

cally, it was easily distinguished from other species by a typical undulation of the cingulum (Fig. 2D) and a rather large size (58-82 µm) [7]. Interestingly, this species is closely related morphologically to *O. siamensis*, as described by Schmidt [10] and interpreted by Fukuyo [4]. However, our detailed study revealed that it also has a long plate 2' (Fig. 2E), and this feature now appears to be common to several *Ostreopsis* species and probably not a relevant taxonomic character [9]. In this species, thecal pores were surrounded with a small collar rim, which was typical and absent in *O. rhodesiae* (Fig. 2). Genetic analysis revealed that this species corresponds to *Ostreopsis* sp. 6, which is also found in several tropical areas such as Gulf of Thailand, Malaysia, Viet Nam, Japan and more recently Korea [9]. Following this outbreak in Tahiti, this species was also observed in Moorea Island in November 2019, indicating that it is widespread in the Society archipelago. Both the genetic proximity of *Ostreopsis* sp. 6 sequences from Tahiti from those of the Gulf of Thailand, and the type locality of *O. siamensis* [10] suggest that it can be the same species. Fukuyo [4] emphasized the absence of *O. siamensis* in the samples from French Polynesia in the early 1980s, and it was not observed before 2019. Hence our observations constitute a new record of this species in the Pacific Ocean.

Preliminary toxicity analyses revealed that none of the *O. lenticularis* (× 47), *O.* cf. *ovata* (×13) and *O. rhodesiae* (× 1) clonal strains screened by CBA-N2a showed toxic activity, whereas strains of *Ostreopsis* sp. 6 (× 8) proved toxic, with a toxin profile dominated by ostreocin-D as confirmed by LC-MS/MS [9]. Due to the lack of analytical standards, its toxin profile is not completely resolved as yet, however, as *Ostreopsis* sp. 6 is capable of forming large blooms, these findings warrant further investigation on the potential environmental and/or health hazards posed by the proliferation of this species in French Polynesian lagoons. Future studies should aim at developing a better understanding the biogeographic distribution of this species, as well as assessing the impacts of its associated toxins on coral reef ecosystems and/or putative accumulation in marine organisms.

Acknowledgements

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Toxin profiles of *Gambierdiscus lapillus* from the Cook Islands

Species of the dinoflagellate genus *Gambierdiscus* produce the toxins responsible for ciguatera fish poisoning (CFP), an illness that has been prevalent throughout the Pacific and particularly in the Cook Islands [1]. The illness is caused by ciguatoxins (CTXs) and possibly maitotoxins (MTXs). Although CTX production ability is genetically associated with the *Gambierdiscus / Fukuyoa* complex, the presence of *Gambierdiscus / Fukuyoa* in a particular area does not mean a risk of CFP, as the toxicity of the species and even strains of species can vary [2]. The key species known to be responsible for CFP in the Pacific is *G. polynesiensis*, but other species need to be considered and their toxin profiles determined to enable better risk assessments. This is particularly important for regions where illnesses have been reported but *G. polynesiensis* has not been detected.

The epiphytic species *Gambierdiscus lapillus* was first isolated from Heron Island, Queensland, Australia, where CFP is endemic [3]. Culture extracts of these Queensland isolates were tested by mouse biaoassay (MBA) and were toxic to mice, however liquid chromatography with tandem mass spectrometry (LC-MS/MS) analyses of these isolates were negative for the known microalgal Pacific ciguatoxins (P-CTXs; P-CTX-3B and C, P-CTX-4A and B) and maitotoxin-1 (MTX-1). They did, however, produce 44-methylgambierone (44-MG) [3-5] although this compound is not considered a cause of CFP [6]. Trace-level toxin activity was also detected using the Ca^{2+} influx SH-SY5Y cell Fluorescent Imaging Plate Reader bioassay. It was hypothesised that unknown compounds were responsible for the MBA results and that *G. lapillus* could still be a contributor to CFP intoxications in Australia [3].



Fig. 1. Sampling area in Rarotonga, Cook Islands.



Fig. 2. Typical macroalgal substrate, Halimeda species.

Sampling of macroalgae was carried out in Rarotonga, Cook Islands, at Muri Lagoon in March 2017 and at Titikaveka Beach and Muri Beach (Fig. 1) by Prof Muharrem Balci in August 2019. Samples were returned to the Cawthron Institute, New Zealand, under quarantine regulations and living cells were immediately isolated and on-grown for molecular and toxin analyses. Species determination was by DNA sequencing (large subunit ribosomal DNA, D8-D10 region) as described previously [7]. Production of P-CTXs [8], MTX-1 [9] and 44-MG [4] by isolates was determined by LC-MS/MS. Isolates were deposited in the Cawthron Institute Culture Collection of Micro-algae (CICCM).

Two isolates from samples collected in 2017 (CAWD263 and 264) and six isolates from the 2019 sampling effort were confirmed as *G. lapillus* (CAWD330-333, 335, 338) (Fig. 2). All were positive for 44-MG production and negative for microalgal P-CTXs and MTX-1 production.

Other *Gambierdiscus* species isolated from the 2017 event were *G. polynesiensis* (CAWD267) and *G. pacificus* and in 2019 *G. pacificus* (CAWD337) (Fig. 3). Only the *G. polynesiensis* isolate produced the known microalgal P-CTXs [unpublished data], while for both *G. polynesiensis* and *G. pacificus* 44-MG was detected. Other *Gambierdiscus* species isolated from the Cook Islands from earlier sampling efforts were *G. australes, G. cheloniae* and *G. honu.* Only *G. australes* produced MTX-1 but all produced 44-MG [10].

The use of molecular assays to determining the presence of *Gambierdiscus* species in field samples is proving useful for guiding isolations [e.g., 11-13]. As *G. lapillus* has been considered a potential candidate for CFP [3,14], a species-specific quantitative PCR assay for *G. lapil-*

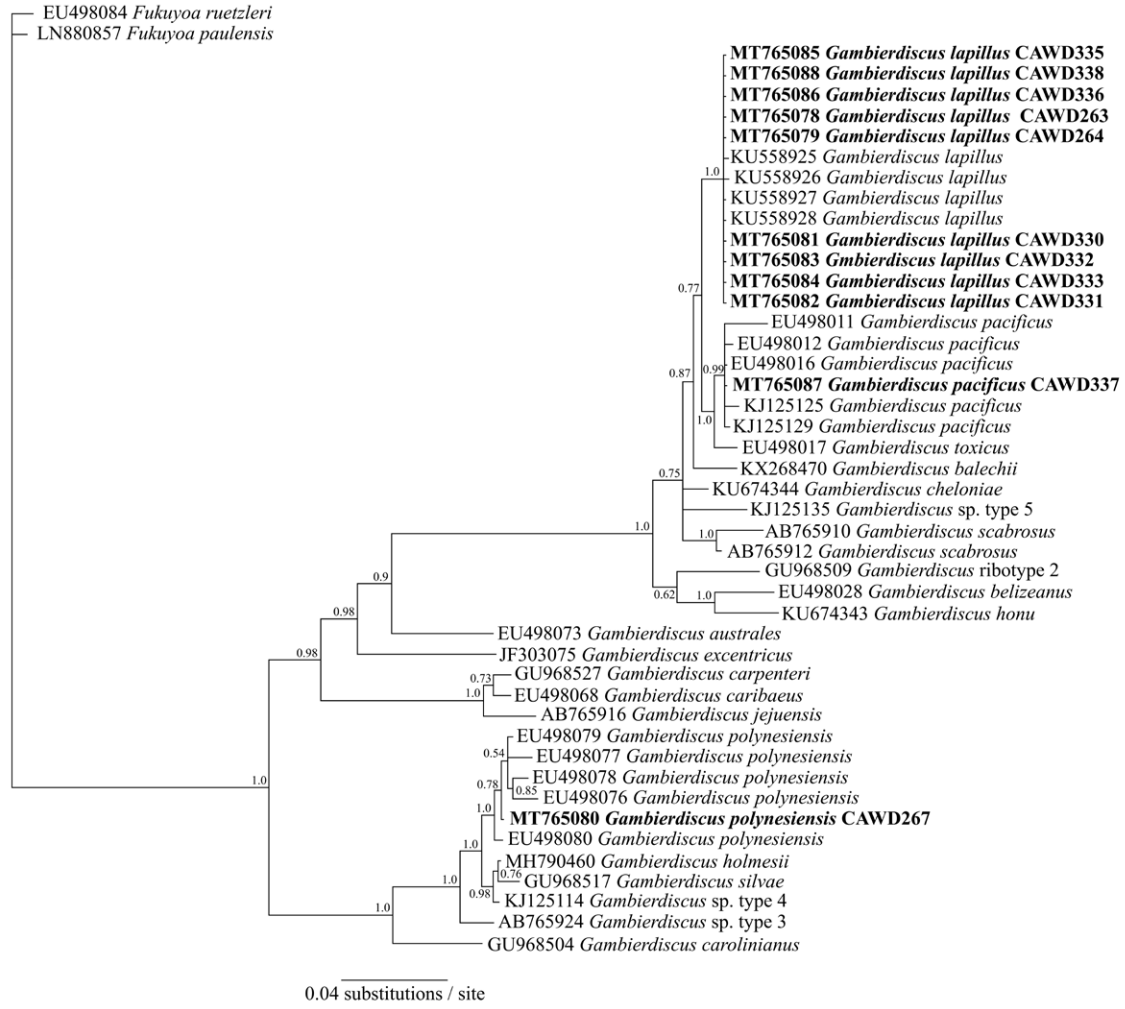

Fig. 3. Phylogenetic analysis of partial large subunit ribosomal DNA sequences (D8–D10 region) from the Gambierdiscus strains isolated in this study (in bold font) using Bayesian analyses. Values at nodes represent Bayesian posterior probability support. Scale bar is substitutions per site.

lus was developed and has proved sensitive and accurate [14]. Molecular tools will be used to analyse future samples collected not only from the Cook Islands but from throughout the Pacific region including Aotearoa/New Zealand and its territories (Rangitāhua/Kermadec Islands) where, due to climate change, CFP is considered an emerging risk.

Based on the current results, it is unlikely that *G. lapillus* is a primary cause of CFP, but further toxicity work is planned to determine whether it does produce currently undescribed compounds which could contribute to CFP cases.

Acknowledgements

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Assoc Prof Muharrem Balci on sabbatical at Cawthron Institute, Nelson, New Zealand, 2019

Unusual bloom of the red alga *Ceramium* sp. (Ceramiales, Rhodophyta) in Cartagena, Colombia, SW Caribbean Sea

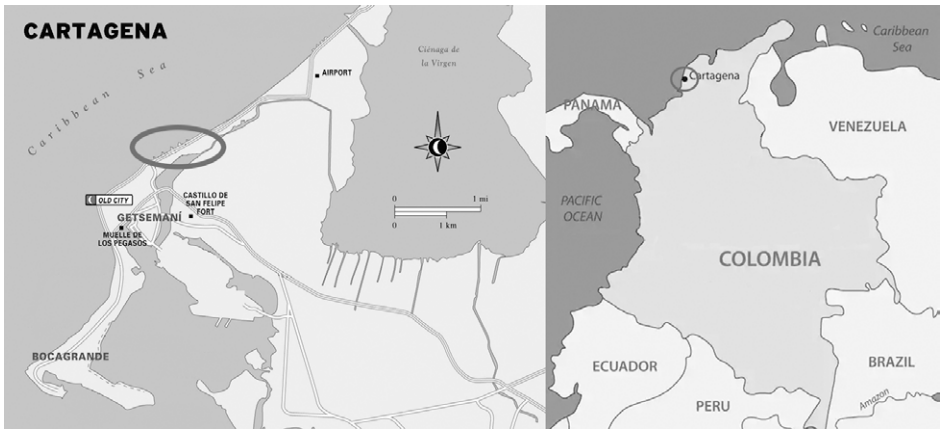

Fig. 1. Map of the study site.

Macroalgal blooms are frequently associated with eutrophication of coastal waters [1]. These blooms are mainly composed of ephemeral and opportunistic green algae belonging to the genera *Ulva*, *Chaetomorpha* and *Cladophora*. Less frequently, red algal species are involved, and when this occurs, the genera *Hypnea* and *Gracilaria* are usually involved [2-4]. Macroalgal blooms have been increasing in frequency, geographic distribution and duration in the last four decades [4]. Unlike toxic phytoplankton blooms, macroalgal blooms lack chemical toxicity but have other ecological impacts. They may displace indigenous species, alter biogeochemical cycles, increase grazing and cause die-off of seagrass and coral reefs [5-6]. Furthermore, when drift algae reach the coastline they foul beaches and shorelines important to local tourist economies and require expensive biomass removal programs [6-7].

Cartagena de Indias is one of the main tourist attractions in Colombia. In addition to the historical town center and its architecture, tourists are attracted by its beaches. Since the 1980s there are records of macroalgal blooms in Marbella beach, at the north end of town [8]. The authors reported a mixed bloom occurring in 1988, dominated by the red algae *Gracilaria cylindrica*, *Hypnea musciformis* and *Grateloupia filicina*. During September-October of the last two years (2018 and 2019), a monospecific red algal bloom has been observed in the same locality in Marbella beach (Figs 1-2). The taxon involved is an unidentified species of *Ceramium* (Fig. 3). Its morphological features do not correspond to any of the listed taxa for the Caribbean Sea, and it was not possible to identify it to species level. During 2019, several species of dead animals were observed, entangled by the wrack biomass of *Ceramium* (Figs 4-5). Species of *Ceramium* are not re-



Fig. 2. Aerial view of the bloom. Photo courtesy of Luz Marina Mejia.

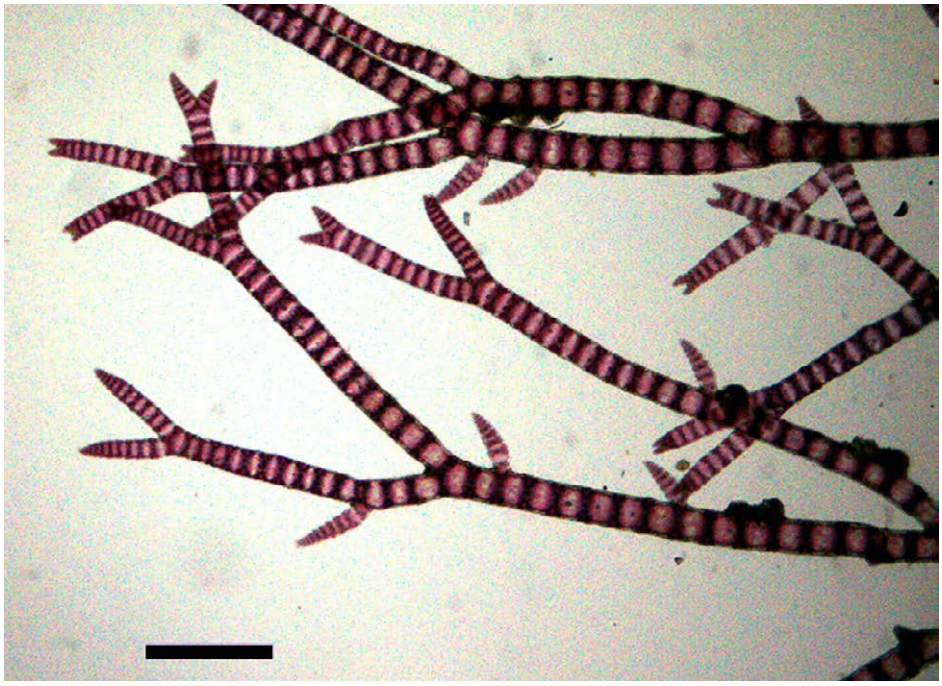

Fig. 3. Ceramium sp. Scale bar: 200µm.



Fig. 4. Dead jellyfish entangled by Ceramium stranded on the beach.



Fig. 5. Dead fish recovered from wrack Ceramium.

ported as bloom forming species, and this finding adds another genus to the group of harmful bloom-forming macroalgae. Furthermore, this report highlights the potential introduction of a new species which has passed undetected until now. This would not be the first case of a potentially introduced bloom species: an undescribed species of the genus *Anadyomene* was reported forming a persistent bloom in nutrient enriched waters in Florida [9].

The invasive species *Caulerpa brachypus* f. *parvifolia* (Harvey) A.B. Cribb has bloomed in Florida as well [3]. Molecular analyses needs to be carried out in order to assess the identity of this bloom-forming species of *Ceramium*.

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Distribution of the fish-killing dinoflagellate *Karlodinium* (Dinophyceae) in the Johor Strait, Malaysia

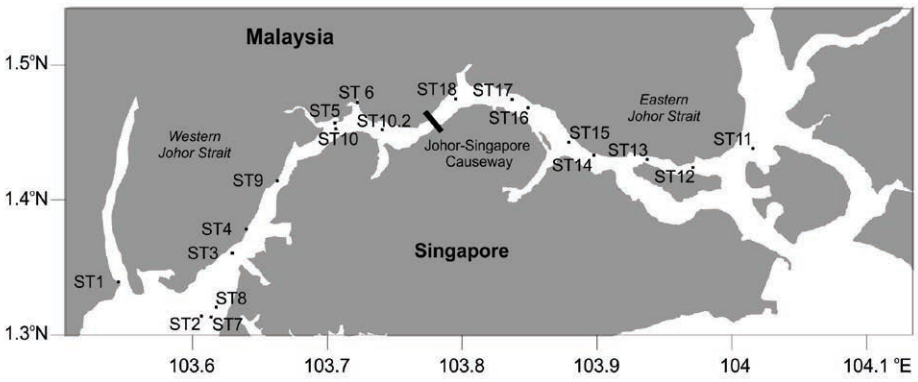

Fig. 1. Sampling sites in the Johor Strait

Species of *Karlodinium* are naked dinoflagellates. More than one third of the named species have been known to cause fish mortality. Toxigenic *Karlodinium* species have gained notoriety due to their capability of producing ichthyotoxins (karlotoxins) that damage the fish gill epithelial tissues causing mortalities by suffocation [1]. In this study, the spatial and temporal distribution of the dinoflagellate *Karlodinium* in the Johor Strait was investigated. The strait is a narrow waterway that separates Peninsular Malaysia and Singapore (Fig. 1) and is an important aquaculture area for both countries. Blooms of *Karlodinium australe* previously reported in the western Johor Straits have led to massive caged-fish mortality and serious economic losses in 2014 and 2015 [2-4].

A total of 19 stations along the Johor Strait were sampled during plankton monitoring surveys between January 2018 and September 2019 (Fig. 1). *Karlodinium* species were present in the Strait but at low cell densities ranging from 2×10^2 to 5.5×10^3 cells L^{-1} (Fig. 2). The average cell density of *K. australe* recorded during the 2014 bloom was 1.25×10^6 cells L^{-1}, with a maximum (2.34×10^6 cells L^{-1}) recorded at the innermost part of the Strait [2]. Densities of *Karlodinium* exceeding 5,000 cells L^{-1} were observed in October 2017 (S18), February–April 2019 (S4, S17, and S18), and July 2019 (S14). Results from a Spearman's rank correlation revealed that phosphate (PO_4-P) availability in the Strait was significantly correlated with high abundances of *Karlodinium* ($p < 0.05$). It has been suggested that the 2014 bloom was probably triggered by high nutrients input from the nearby river discharges and anthropogenic inputs from mariculture, coastal reclamation and dredging activities in the Strait [2]. The stagnant water conditions during neap tides also contributed to sustain the bloom [2]. This view is supported by Leong et al [5] who showed that dinoflagellates blooms including *K.* cf. *veneficum* and *K. australe* in the Eastern Johor Strait occurred during neap tides with high nitrogen but low silicate concentrations. Diatoms were likely suppressed under silicate depletion, allowing the dinoflagellates to thrive in that environment, and subsequently prolong their bloom development in the Strait [5-6].

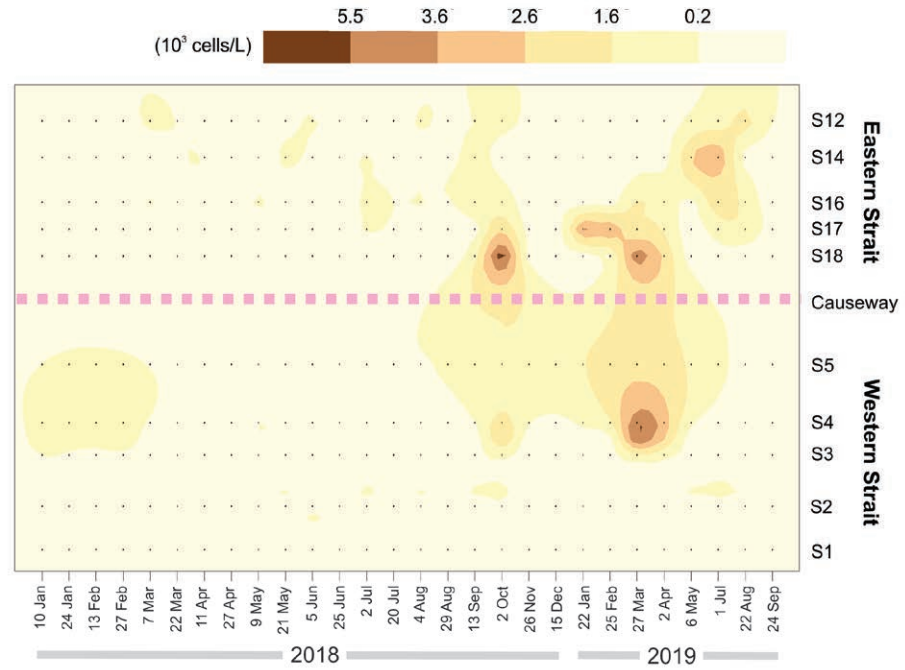

Fig. 2. Spatial and temporal distribution of Karlodinium along the Johor Strait during the study period between January 2018 and September 2019

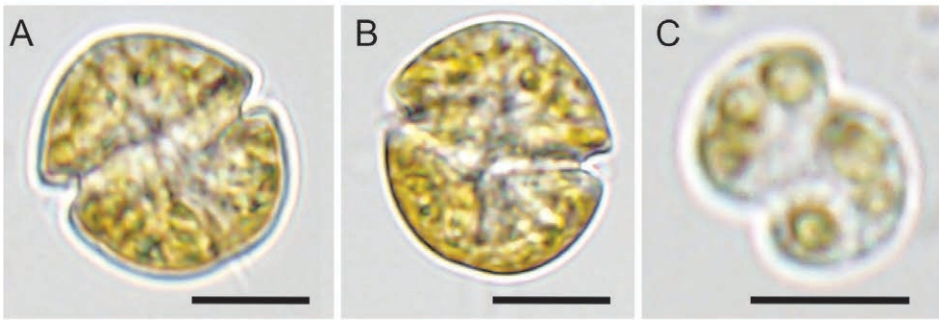

Fig. 3. Light micrographs of Karlodinium found in the Johor Strait. Scale bar = 20 µm.

Under the light microscope (LM), cells of *Karlodinium* appear oval with epicone and hypocone equal in size. The shape of the epicone is conical or rounded while the hypocone is hemispherically rounded (Fig. 3A) and the

Continued on page 16

Bloom of a red tide species *Akashiwo sanguinea* in Semerak Lagoon, Kelantan, Malaysia

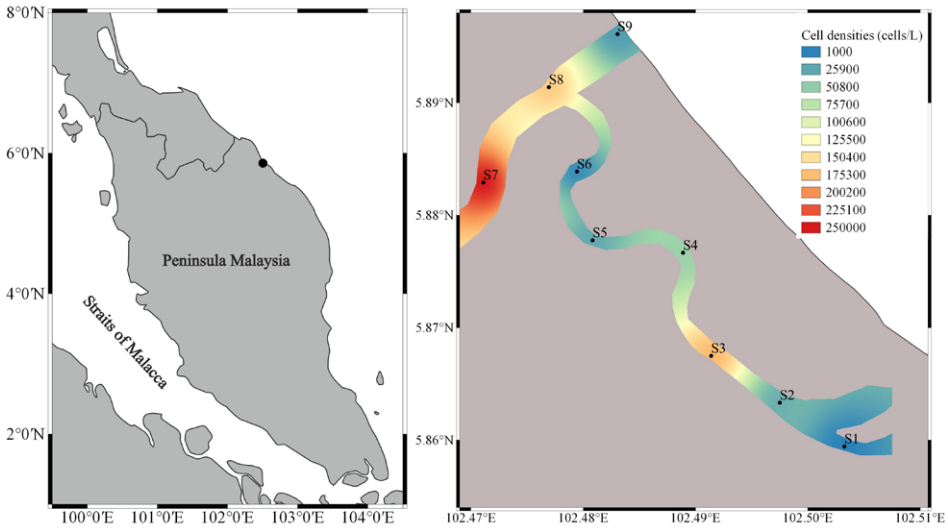

Fig. 1. Chart of sampling sites at the Semerak Lagoon and cell densities of Akashiwo sanguinea

The species *Akashiwo sanguinea* is a cosmopolitan naked dinoflagellate known to form harmful algal blooms (HABs). This bloom-forming species has been reported to cause seabird mortality due to saponification in Monterey Bay, California, USA [1]. In Malaysia, there are no reports of fish kill incidents caused by *A. sanguinea* to date, but the species is commonly found in Malaysian waters, especially in aquaculture areas [2-3].

The Semerak Lagoon, located in the north east of Peninsular Malaysia, is an important aquaculture area for finfish and shrimp. On May 23th, 2019, water discoloration was observed in the lagoon (Fig. 1). Following the incident, morphological and molecular analyses were performed to identify the bloom species. Water samples from nine selected sampling sites (S1–S9; Fig. 1) were collected at 1 m depth using an 8-L Van Dorn water sampler. The sampling stations S1 to S3 were located inside, S4 to S8 outside the aquaculture area, and S9 at the river mouth. Clonal cultures were established through single-cell isolation from field samples. Field and cultured cells were observed using light microscopy and confirmed the species as *Akashiwo sanguinea*. Cells were 60.6 to 81.0 µm long and 46.6 to 60.6 µm wide with numerous ribbon-like chloroplasts in the periphery (Fig. 2).

Gene amplification of the large subunit (LSU) region and the internal transcribed spacer (ITS) region was performed by polymerase chain reaction. The species was previously reported to comprise four ribotypes (A, B, C, and D) [4]. Our results based on both LSU rDNA and ITS sequence analysis clearly indicated that the *A. sanguinea* population in Semerak Lagoon belonged to ribotype B; sharing the same ribotype with the strains found in Tumpat, Kelantan [4]. The LSU phylogenetic inference revealed that the strains from Semerak Lagoon shared identical sequences with those from China, Mexico, Singapore, and South Korea (Fig. 3A; MP/ML/BI: 99/99/1.0); while using ITS phylogeny, all strains from Semerak Lagoon shared identical sequences with one strain (GSXM02) from Xiamen Harbour, China (Fig. 3B; MP/ML/BI: 100/100/0.99).

During the bloom event, the highest density of *A. sanguinea* was found at S7 (248,658 cells L^{-1}; outside the aquaculture area), followed by S3 (177,773 cells L^{-1}; inside the aquaculture area), and S8 (164,269 cells L^{-1}; outside aquaculture area) (Fig. 1); the densities were higher as compared to the previous occurrences in 2015 and 2016 (highest density of 3,460 cells L^{-1}; [3]). The phytoplankton community assemblage in the lagoon was diverse, with a dynamic shifting of phytoplankton community composition over time related to the nutrient dynamics in the lagoon. Aside from blooms of *A. sanguinea*, several different algal blooms were encountered between the study period of September 2015 and March 2016: i.e. *Pseudo-nitzschia* spp. (potentially toxic species), *Chaetoceros*, *Skeletonema*, and *Blixaea quinquecornis* (red tides, fish kills) [3].

In this survey, we confirmed the blooming species as the dinoflagellate *A. sanguinea* belonging to ribotype B. The potential occurrence and distribution of other ribotypes require further investigation, and the drivers of the recurrence of this species remain unknown. Therefore, routine monitoring along the lagoon is needed for early warning to minimize losses due to fish kill events.

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Fig. 2. Light micrograph of Akashiwo sanguinea from Semerak Lagoon. Scale, 20 µm

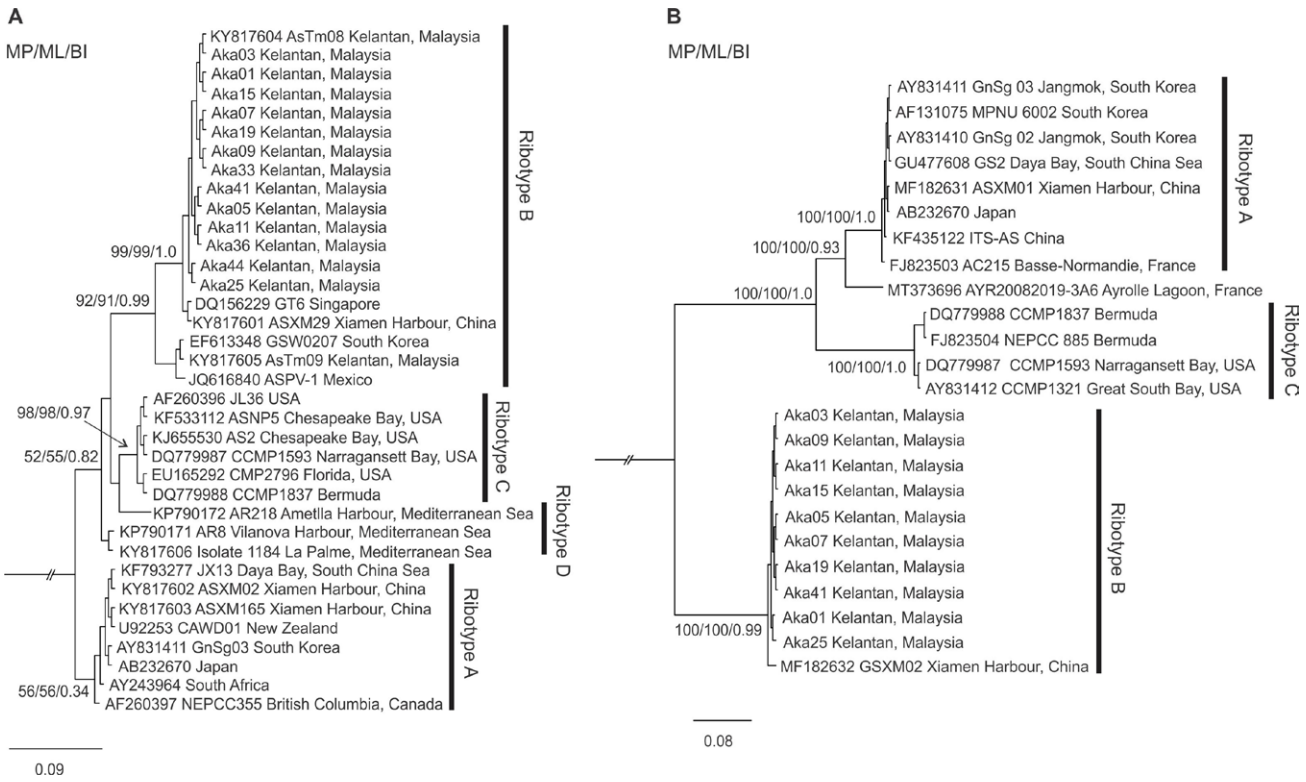

Fig. 3 Bayesian trees of Akashiwo sanguinea inferred from (A) LSU rDNA and (B) ITS datasets. Values on nodes represent bootstrap supports of MP, ML, and posterior probabilities of BI

Continued from page 14

sulcus extension invading the epicone is visible (Fig. 3B). Cells are slightly pigmented, with chloroplasts observed along the epicone and hypocone, each with a lenticular pyrenoid (Fig. 3C). Observations of *Karlodinium* cells collected during the sampling period were 10.2 – 17.4 µm long (11.8 ± 1.2 µm, n = 10) and 8.4 – 13.6 µm wide (8.9 ± 1.1 µm, n = 10), with a length: width ratio of 1.60 ± 0.2 µm. Thus, their size was slightly smaller than those described in [2] and [7]. However, with the data obtained here, it was not possible to identify the specimens at species level.

To gain a better insight into the species distribution and diversity, the assemblages of *Karlodinium* in Johor Strait were characterized with a genetic approach using ribosomal DNA (rDNA) metabarcoding and real-time quantitative PCR (qPCR). Plankton samples collected between May 2018 and September 2019 were further analysed with the 18S-V9 rDNA metabarcoding approach using a high throughput sequencing under the Illumina Miseq platform. Our results revealed a total of 27 operational taxonomic units (OTUs) associated with harmful microalgal species. Among the harmful algal OTUs, *K. veneficum* was detected at the Eastern Johor Strait (S14 and S16) during the Northeast- (January 2019) and Southwest (July-August 2019) monsoons but in relatively low (number of OTU) abundance. Results from a Pearson correlation showed that nitrate concentration in the strait were significantly associated with OTU abundances of *K. veneficum* ($p < 0.05$). Nevertheless, *K. australe*, the previously reported fish-killing species, was not detected in our metabarcoding datasets during the study period. Likewise, the qPCR species-specific detection of *K. australe* [8] confirmed no detection of this species during the study period.

Clearly, further monitoring efforts are warranted to alleviate the adverse impacts of HABs on the aquaculture industry in the Strait of Johor.

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CLEFSA project identifies Harmful Algal Blooms as a threat to food safety resulting from climate change



Fig. 1. Organizations involved in the CLEFSA project

Climate change is one of the key drivers of emerging risks for food and feed safety, plant and animal health (including terrestrial and aquatic species) and food nutritional quality. In order to ensure food and feed safety in the future, analysis of environmental drivers may contribute to supporting long-term anticipation of food safety challenges and risk assessment needs, as well as the design of prevention measures.

The CLEFSA project (Climate change and Emerging risks for Food Safety) has developed and tested new methodologies for the identification, characterisation and analysis of a large number and variety of emerging risks linked with climate change, including those associated with marine toxins. European Union and United Nations (e.g. UNESCO-IOC) agencies, international organisations and coordinators of relevant international projects and programmes including some directly related to harmful algal blooms (EuroCigua project, GlobalHAB programme) were engaged to build a knowledge network of experts (Fig. 1). The overall CLEFSA procedure is illustrated in Fig. 2.

Horizon scanning and crowdsourcing were used to collect a broad range of signals or emerging issues from a variety of information sources. CLEFSA has characterised them through a Multi-Criteria Decision Analysis (MCDA) tool (Fig. 3).

Characterisation has been conducted in a participatory manner through the involvement of a multidisciplinary group of 60 experts, who have assessed how climate change affects the impact and likelihood of emergence of issues. Climate change scenarios, using the climate data store provided by the Copernicus C3S platform implemented by the European Centre for Medium-Range Weather Forecasts (ECMWF), were used.

The project has developed methodologies and indicators for the analysis and visualisation of the information collected during the characterisation, as well as for addressing the uncertainty associated with these assessments.

Rather than comprehensively describing all issues individually, the CLEFSA project has focused on the multifaceted effects of climate change, the diversity of hazards to be considered, the large uncertainties involved and the interconnections between the different issues.

Over 100 emerging issues were identified and characterised, among

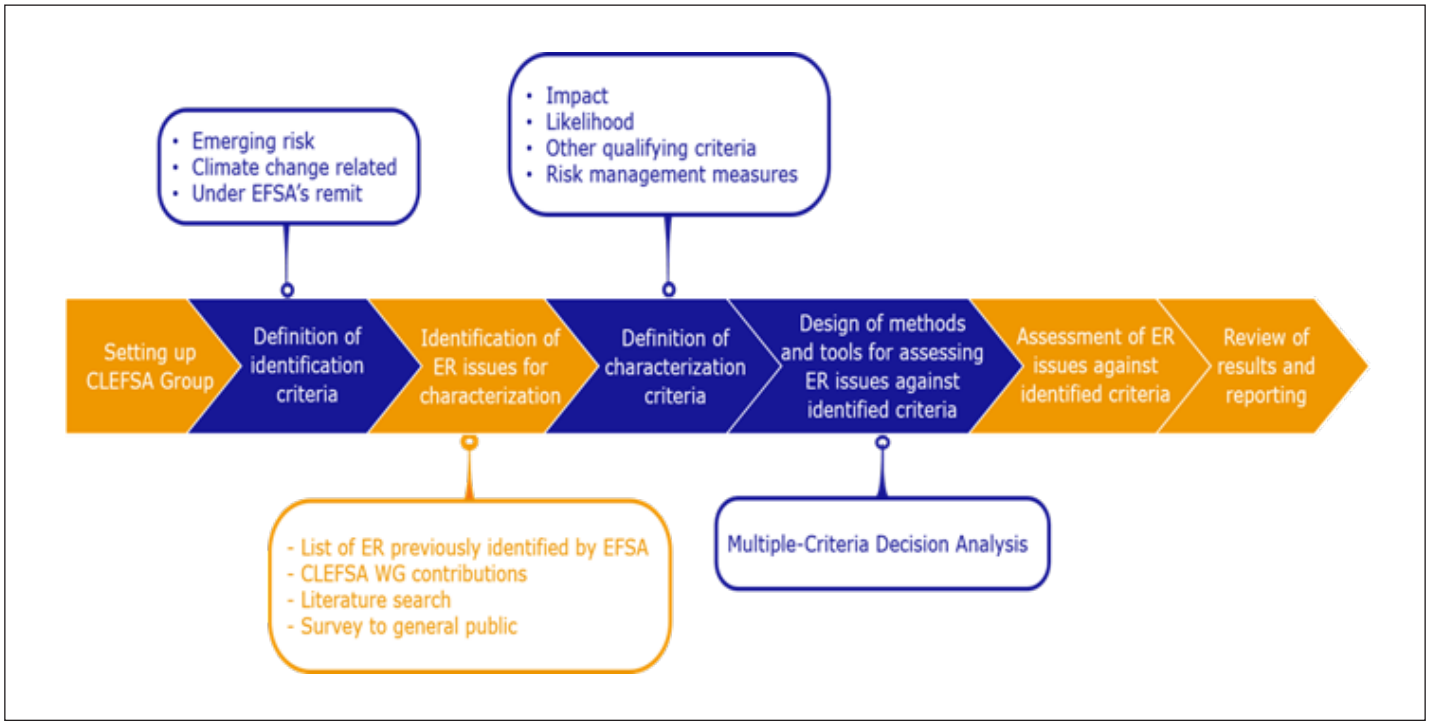

Fig. 2. Chart diagram of CLEFSA protocols

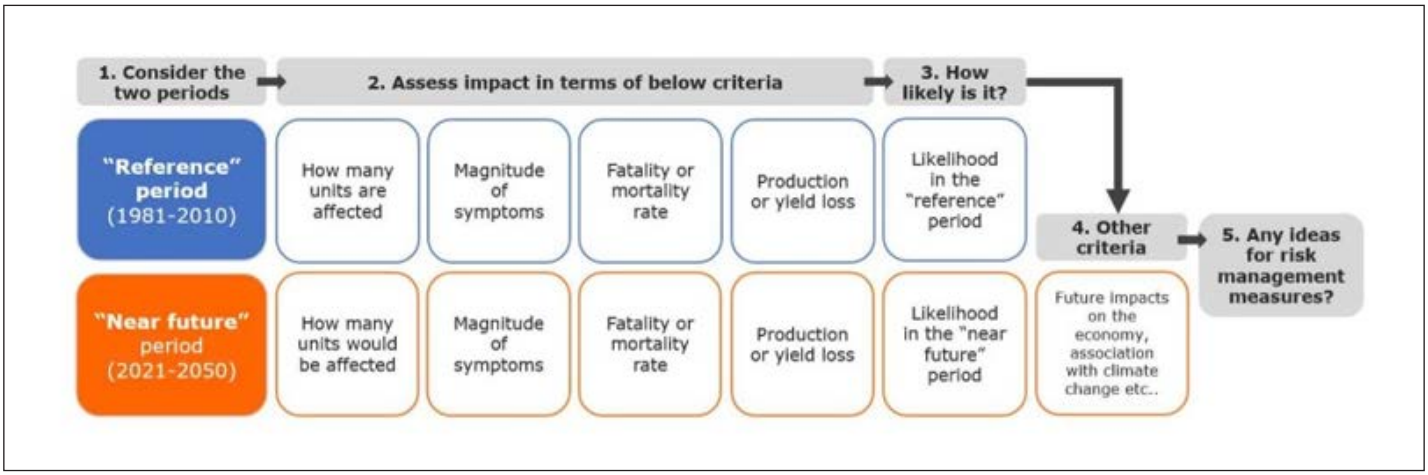

Fig. 3. CLEFSA Multi-Criteria strategy

which several directly related to toxins produced by harmful algal blooms (HABs). These include: ciguatoxin, domoic acid, okadaic acid, saxitoxin, pinnatoxin, tetrodotoxin, beta-methyl-amino-L-alanine (BMAA) and palytoxin analogues. The analysis indicates that climate change may increase severity, duration and/or frequency of the potential effects of these hazards. However, a more pronounced effect is expected on the likelihood of emergence. The analysis showed that issues related to HABs were also among the ones with the highest likelihood of emergence in the climate change scenario. Scientific literature and previous EFSA work (e.g. EuroCigua project) [1] indicate that climate change may be influencing the occurrence and intensity of blooms of potentially toxic marine algae and bacteria resulting in risk for food and feed safety.

The CLEFSA project has stressed the need for policymakers and other relevant actors in the food system to consider adjusting surveillance and monitoring to prepare for emerging risks to food and feed safety linked to climate change, and for risk assessors to consistently include climate change scenarios in risk assessments. Considering the increasing demand for new sources of food and feed from the ocean, the CLEFSA project contributes to raising awareness on the need of integrated policies for ocean protection and food safety by building synergies across the relevant institutional actors involved. The complete CLEFSA report can be found at *https://www.efsa.europa.eu/it/supporting/pub/en-1881*.

Acknowledgements

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Blooming Buddies: MSc Research Projects Extend our Knowledge on Bloom-Forming Freshwater Cyanobacteria

Freshwater cyanobacteria blooms are an increasing problem globally and much work is focussing on understanding bloom dynamics and toxin production in order to better manage the inherent health risks they pose. In New Zealand, the major toxin-producing cyanobacteria are microcystin-producing *Microcystis* and anatoxin-producing *Microcoleus* (previously classified as *Phormidium*) which frequently form blooms in New Zealand lakes and rivers, respectively. These cyanobacterial blooms have led to numerous animal deaths (dogs, sheep and cattle) and the closure of recreational swimming sites due to the human health risk at certain times of the year. In New Zealand, there has been a good deal of research conducted on microcystins which was recently reviewed in the *New Zealand Journal of Botany* [1]. This review revealed interesting observations about microcystin-production in New Zealand *Microcystis*; that there are predominantly two microcystin congener profiles produced by New Zealand *Microcystis*, and that there are geographical differences in microcystin quotas between international data and New Zealand *Microcystis* strains. It also identified that New Zealand research on microcystin production has contributed significantly to international knowledge on the topic.

In 2017, funding from the Marsden Fund of the Royal Society of New Zealand was awarded to investigate cyanobacterial bloom ecology and whether cooperation occurs between toxic and non-toxic strains of cyanobacteria. The premise for the project was that microcystins were sequestered by the non-toxic cyanobacterium *Synechocystis* and modulated its photosynthetic performance [2]. The project also included funding for two MSc scholarships that were awarded to Jenna Mumford and Rossella Nicolai. Jenna's research project centred around assessing the impacts of environmental contaminants on cyanobacteria (microcystins themselves and herbicides) and how this might affect the community composition. Rossella's work was on the potential active export of microcystins and the optimisation of a cell lysis staining technique for use with cyanobacteria. Both are enrolled through Victoria University of Wellington, but undertook their research projects at the Cawthron Institute in Nelson (New Zealand).

Jenna, from Wellington, has a passion for environmental sustainability. As such, she wished to investigate how global change is affecting aquatic environments and chose to investigate the effects of herbicides (glyphosate and 4-chloro-2-methylphenoxy acetic acid; MCPA) on New Zealand cyanobacteria. For her experiments, she used a selection of planktonic cyanobacteria from the Cawthron Institute Culture Collection of Microalgae [3] (Fig. 1) spanning seven species from four genera; including *Cuspidothrix issatschenkoi*, *Dolichospermum circinale*, *Microcystis aeruginosa* and *Nodularia spumigena*. During screening experiments, Jenna found that the cyanobacteria species assessed were not affected by MCPA (up to 5×10^3 µg L^{-1}) but they did show varied susceptibility to glyphosate. In a real-world situation, the differential effects observed with glyphosate might disturb the balance of cyanobacterial communities in lakes and reservoirs, and could potentially modify proliferation of toxin-producing species. Follow-up experiments are now being conducted to investigate the effect of glyphosate photosynthetic performance and toxin production in a selection of the susceptible cyanobacteria species.

Rossella hails from Rome, Italy, but has undertaken her tertiary education at Victoria University in Wellington (New Zealand). Rossella's passion for art has led her to develop an interest in algal microscopy during her university education, finding beauty in their cellular structure. Rossella's work on the potential active export of microcystins employed the fluorescent nucleic acid stain, SYTOX™ green [4-5], to measure the amount of cell lysis observed during culturing experiments using cyanobacteria. The SYTOX green stain is particularly useful for analysing cyanobacteria (and other microalgae) because its fluorescence characteristics (excitation 504 nm; emission 523 nm) avoid interference from chlorophyll autofluorescence (excitation 440/660 nm; emission 700 nm). During her project Rossella was able to optimise staining procedures for *Microcystis aeruginosa*, *Nodularia spumigena* and *Planktothrix* sp. (Fig. 2). However, the presence of heterotrophic bacteria in the non-axenic cyanobacteria cultures did prove troublesome in *Nodularia spumigena* samples, as heterotrophic bacteria were too abundant in the mucilage produced by the cyano-



Fig. 1. Microcystis aeruginosa CAWBG11, one of the strains used by Jenna to assess the effects of herbicides on cyanobacteria.

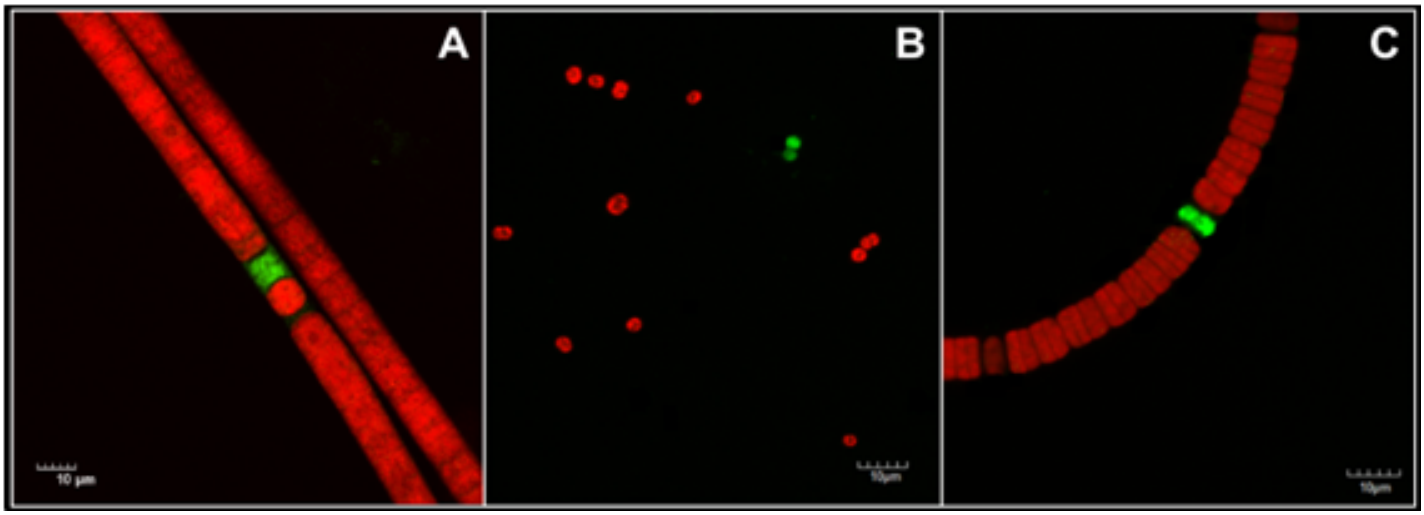

Fig. 2. Confocal microscopy images of Planktothrix sp. CAWBG59 (A), Microcystis aeruginosa CAWBG617 (B) and Nodularia spumigena CAWBG21 (C) stained with SYTOX™ green so that lysed cells fluoresce green, whilst intact cells are detected by red chlorophyll autofluorescence.

bacterium interfering with the cyanobacterial signal. Rossella is now using this fluorescence microscopy technique to assess whether cell lysis or active transport is the main contributor to the extracellular microcystins observed in cyanobacteria cultures. Because data on cell lysis has been lacking in previous experiments performed on microcystin export, the SYTOX green staining technique has proven valuable for our work in this area.

As Cawthron operates outside of the New Zealand University system and is largely staffed by scientists, technicians and consultants conducting government-funded and contract research, it's always refreshing to have the added vibrancy and enthusiasm of research students being based onsite. This latest collaboration with Victoria University of Wellington builds on previous collaborations working on toxic benthic cyanobacteria in rivers and will further cement the relationship between the two organisations.



Rossella Nicolai (left) and Jenna Mumford (right), MSc research scholars from Victoria University of Wellington working on bloom-froming freshwater cyanobacteria.

Acknowledgements

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25 years of service enhancing the capacity to monitor and manage HABs



Fig. 1. Participants from the first course held at the IOC Centre in Copenhagen in 1995

The IOC Science and Communication Centre on Harmful Algae opened in May 1995 at the University of Copenhagen, Denmark. It was a new concept for the IOC to collaborate so closely with a national research institution to deliver international training, capacity development, coordination and support research initiatives as well as to function as a decentralized programme office of the IOC itself.

For the first decade, the Centre had a strong focus on assisting the least developed countries to build capacity for monitoring and managing HABs as the bulk of the funding was provided to the IOC by Danida, the Danish foreign aid programme. After then, activities diversified to more broadly underpin the activities of the Intergovernmental Panel on Harmful Algal Blooms (IPHAB) including the GEOHAB/GlobalHAB programmes, while training activities became targeted to the HAB community in general including an exam at the end of the course with an '*IOC Certificate of Proficiency in Identification of Harmful Algae*'. The course activity was later expanded to include close cooperation with the Marine Institute (Ireland) implementing the IPI, the International Phytoplankton Intercalibration scheme.



Fig. 2. Jacob Larsen, driver behind the training courses offered by the Copenhagen Centre since the beginning (1995). In the photo, in action during a course preparing samples for the microscope.



Fig. 3. Santiago Fraga, who became one of the permanent teachers at the courses offered by the Centre in Copenhagen and Vigo, here during a taxonomy class.

UNIVERSITY OF COPENHAGEN

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Some +700 people have attended training courses on HABs either at the Centre in Copenhagen or at courses held around the world. These courses have been driven by the scientists at University of Copenhagen including Jacob Larsen, Øjvind Moestrup, and Nina Lundholm and assisted by many dedicated colleagues from all over the world, nobody mentioned, nobody forgotten. It should be fair to say that the course activity has functioned as a major networking mechanism between the less and the more experience colleagues in the HAB community over the 25 years. Many research partners and new friends have been found among trainees and trainers.

What about the future? Both Jacob Larsen and Øjvind Moestrup seem to run on very strong batteries and the course activity is not planned to change, although in the short term amendments for the impact of COVID-19 need to be made. The Centre is working on a new version of the on-line / OceanTeacher part of the '*IOC Identification Qualification in Harmful Marine Microalgae*' to facilitate the consideration of course activity while international travel is restricted in the immediate future and perhaps beyond. Using a longer term perspective the shortage of in depth, detailed knowledge of species and identification based on morphology continues to diminish and there may be a day when there is no 'one stop shop' to get training on HAB species identification.

Continued on next page

International Phytoplankton Intercomparison (IPI) exercise in abundance and composition of marine microalgae

Dear participants of the annual IPI (International Phytoplankton Intercomparison) exercise in abundance and composition of marine microalgae:

This note is to confirm that due to the ongoing pandemic across the globe caused by the SARS-COVID-19 virus, the IPI organising committee after discussion with our Advisory Group and partners have decided to cancel the IPI exercise for 2020. The proficiency testing scheme will continue from 2021 onwards. It is unfortunate that due to the current situation and the uncertainty surrounding it, the organisation of the exercise which includes the generation and posting of biological materials to many different laboratories around the world, development of educational tools like the *Oceanteacher* online test and organisation of the IPI workshop, which requires the presence of participants in a teaching room, has become too challenging and difficult to coordinate as a result of the ever changing situations in different countries. We are aware that many laboratories use the results of this exercise as part of their annual quality assurance requirements for their test method accreditation by their national accreditation bodies and also at the laboratory level for the ongoing competency of their analysts. This decision is not taken lightly and it is obviously going to affect everyone but I hope that given the circumstances, allowances should be made by the competent authorities to permit laboratories to maintain their accredited methods credentials until 2021 when we will resume the exercise.

The IPI exercise is organised by Rafael Salas (Marine Institute) and Jacob Larsen (IOC Science and Communication Centre on Harmful Algae) in collaboration with Claudia Delgado (IODE office). If you are interested in participating in 2021, you can register through our website *www.iphyi.org* next year and find all the information about the exercise. You can also e-mail me directly if you want to be included in future mailing about this test.

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The Centre is taking part in delivering on IOC's obligation to prepare the UN Decade of Ocean Science for Sustainable Development (2021-2030). The Decade may bring new opportunities for HAB science to be relevant to society, new demands for HAB expertise in order to reach the ambitious objectives of the Decade, and the Centre will strive to adapt to facilitate this.

We hope to work with all you for many years to come!

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Henrik Enevoldsen, Head of the IOC Science and Communication Centre since its establishment

Canadian review: Marine harmful algal blooms and phycotoxins of concern to Canada

As has been reinforced all too well in recent months with the COVID-19 pandemic, the world is indeed interconnected. The international harmful algal bloom community recognized this early on, with a series of international conferences on HABs, starting in 1975 [1]. The International Society for the Study of Harmful Algae (ISSHA) was initiated in 1997. ISSHA made a commitment at the Florida international conference in 2002 to help develop a worldwide map of HABs and, beginning with the XII HAB conference (2006) in Copenhagen, ISSHA (along with the local organizing committee), also committed to help in the production of the proceedings of future international HAB meetings [2]. Also in 2002, the international HAB community developed the program Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) now called GlobalHAB [3,4].

Each country impacted by HABs has also developed national HAB programs. In Canada, the Phycotoxins Working Group (PWG) was established in 1988, by Fisheries and Oceans Canada (DFO), as a result of human poisonings by a novel phycotoxin (domoic acid) in late 1987, in eastern Prince Edward Island. One goal of the PWG was to "apprise senior management within DFO on harmful algae, including planning, coordinating and prioritizing research activities" [5]. This resulted in an unpublished review, written by the PWG in 1994, with the goal of informing management at DFO headquarters in Ottawa of the status and ongoing risks of marine HABs and phycotoxins in Canada.

Over the years since then, information was added to that review regarding new HAB events and novel phycotoxins discovered in Canada as discussed in a Canadian HAB Working Group meeting (Fig.1). The authorship was expanded to include 16 other scientists from DFO, the National Research Council of Canada, the Canadian Food Inspection Agency, the Canadian Museum of Nature, and Microthalassia Consultants Inc. (see Acknowledgements). This allowed the co-authors to contribute their individual expertise in the diverse fields of phytoplankton taxonomy, physiology, ecology, and the analytical chemistry of phycotoxins. The result is a comprehensive review of marine HABs and phycotoxins of concern to Canada [6]. This 322 page review contains sections on the history and distribution of HABs in Canada, the toxic and otherwise harmful phytoplankton that compose the HABs, and the phycotoxins that they produce. Links to Canadian reports in the ICES-IOC Harmful Algal Event Database (HAEDAT) since 1988 [7] are included. It also reviews HAB issues in Canada relating to climate change, ocean acidification, ships' ballast water, the Canadian Arctic, as well as monitoring programs and how they have changed over the years. Finally, it points out knowledge gaps and provides recommendations for further research. The text is supplemented by 13 tables, 13 figures, nine chemical structures of phycotoxins, and four appendices. A 126-page reference list contains links to PDF files or abstracts at journal websites. Below is an extended abstract of the Canadian HAB review:



Fig. 1. Participants at the Third Harmful Algal Bloom Working Group Meeting (25-27 September 2018, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada), which included a discussion of the Canadian HAB review

In Canada, as in many parts of the world, reports of marine harmful algal blooms (HABs) have increased over the past few decades. HABs are caused by the growth of certain phytoplankton that produce phycotoxins or otherwise cause harm. Phycotoxins are problematic not only to human health, but their cumulative effects are also stressors to marine ecosystems; some cause the mortality of marine fish, birds and mammals, including species designated at risk. Paralytic Shellfish Poisoning (PSP), caused by saxitoxin group toxins produced by *Alexandrium* spp. has been problematic for years along virtually the entire coast of British Columbia (BC), and on the east coast in the Bay of Fundy, the lower St. Lawrence Estuary, and the Gulf of St. Lawrence (Figs. 2-3). Since 1982, saxitoxin group toxins have been present sporadically in Newfoundland and Labrador (NL) and the Atlantic coast of Nova Scotia (NS). Amnesic Shellfish Poisoning (ASP, caused by domoic acid produced by *Pseudo-nitzschia* spp.) was identified for the first time worldwide following consumption of blue mussels (*Mytilus edulis*) from Prince Edward Island (PE) in 1987. Domoic acid has since been found in molluscan shellfish in the Bay of Fundy, New Brunswick (NB), NS, Quebec (QC), NL, offshore banks (Georges and Browns Banks), and in BC (Fig. 2). Diarrhetic Shellfish Poisoning (DSP, caused by okadaic acid group toxins produced by *Dinophysis* spp. feeding on ciliates and by benthic and epiphytic *Prorocentrum* spp.) was first recognized as a hazard in Canadian waters in 1990,

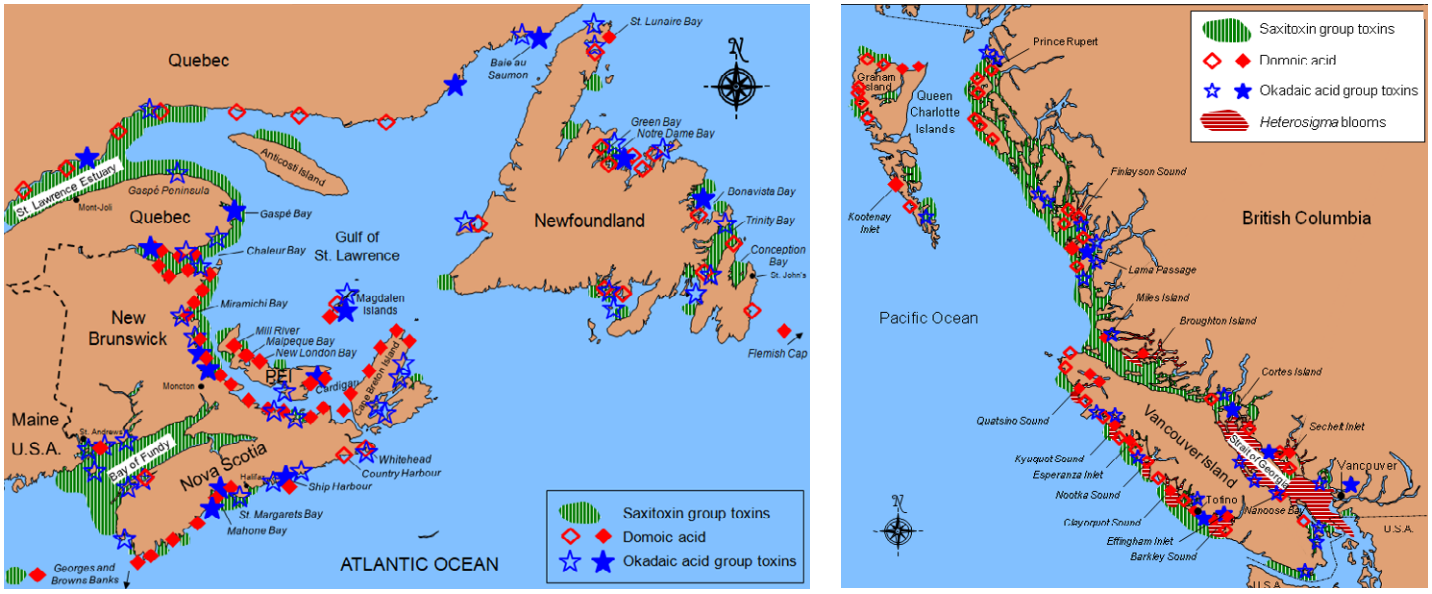

Fig. 2. Maps showing the location of selected phycotoxins on the Canadian east (left) and west (right) coast. Symbols represent domoic acid and okadaic acid group toxins above (closed symbols) and below (open symbols) the regulatory action level. The green shaded areas show the distribution of saxitoxin group toxins. The red shaded areas in the map on the right show the distribution of fish-killing Heterosigma blooms.

in NS. These toxins have since been found in the Bay of Fundy, the Gulf of St. Lawrence, QC, NL, and BC (Fig. 2).

Low levels of other phycotoxins that may cause harm to human health have recently been discovered in Canadian waters. These include pectenotoxins, yessotoxins and azaspiracids, as well as the cyclic imine group toxins (spirolide toxins, pinnatoxins, and gymnodimines). Pectenotoxins (produced by *Dinophysis acuminata*), spirolides (produced by *Alexandrium ostenfeldii*), and pinnatoxins (produced by *Vulcanodinium rugosum* in other parts of the world, but the Canadian producer has not yet been identified) have been detected in waters of both the Atlantic and Pacific coasts. Yessotoxins (produced by *Gonyaulax spinifera*, although not proven for Canadian waters, and *Protoceratium reticulatum*) are reported from NL, PE, NB, NS, and BC. Azaspiracids (produced by *Azadinium spinosum* and *A. poporum* in other parts of the world, but the Canadian producer has not yet been identified) have so far been found only in NL, NS, and QC. Gymnodimines (produced by *Karenia selliformis* and *A. ostenfeldii*, although both not proven for Canadian waters) are present in waters of NL, NS, QC, and BC. With the exception of pectenotoxins, no regulatory limits have yet been established for the above newer phycotoxins.

Multiple harmful algal species have been associated with fish-killing blooms in BC, including the diatoms *Chaetoceros convolutus* and *C. concavicornis*; the raphidophyte *Heterosigma akashiwo* (Fig. 4); the dinoflagellates *Margalefidinium polykrikoides* (formerly *Cochlodinium polykrikoides*), *M. fulvescens* and *Alexandrium catenella*; and the silicoflagellates (dictyochophytes) *Dictyocha fibula*, *Octactis speculum*, and *Pseudochattonella verruculosa*. Harmful species that have been associated with fish kills on the Atlantic coast include the diatoms *Eucampia zodiacus* and *Leptocylindrus minimus*, the dinoflagellate *A. catenella*, and the ciliate *Mesodinium rubrum* (Fig. 5). Several other algae *Chrysochromulina polylepis* (syn. *Prymnesium parvum*), *Gyrodinium aureolum* (now called *Karenia mikimotoi* in many regions), known to be problematic elsewhere in the world, are reported at low concentrations on both coasts.

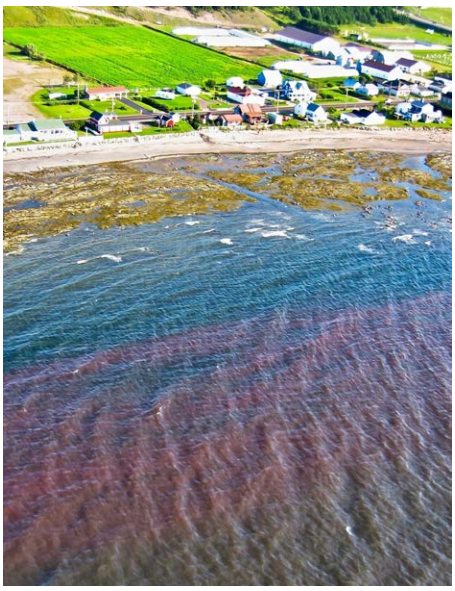

Fig 3. Red tide of Alexandrium catenella (PSP toxin producer) previously called A. tamarense in the St. Lawrence Estuary near Ste-Flavie, Quebec, 2008. Photo courtesy of Michel Starr, Maurice Lamontagne Institute – DFO)

The range of exotic toxic/harmful algae is expanding in Canadian waters due to introductions by ships' ballast water and spreading related to climate change and other natural and anthropogenic vectors. Canada's experience in dealing with toxic events has resulted in research and monitoring programs designed to understand HABs and to assist the fishing and aquaculture industries. In spite of decreases in research and phytoplankton monitoring efforts, consumers of molluscan shellfish are still protected by phycotoxin monitoring, which is conducted by the Canadian Food Inspection Agency. Nevertheless, novel phycotoxins and toxic algae will continue to be discovered. Questions remain about the mechanisms associated with the initiation and decline of some HABs, the source organisms of several of the phycotoxins, the role of toxins and other mechanisms of fish-killing HABs, and about the causes of inter-annual variability in HABs. Further study of novel toxigenic species and phycotoxins will be required to understand the degree of harm being done. HAB-related problems will be exacerbated with continued ship ballast water exchange and climate change.



Fig. 4. Heterosigma akashiwo bloom in Kyuquot, British Columbia, 1996 (Photo courtesy of Nicky Haigh, Microthalassia Consultants Inc., Nanaimo, BC)



Fig 5. Red tide of the ciliate Mesodinium rubrum, potential prey of Dinophysis, in Passamaquoddy Bay, southwest NB, 1999 (Photo courtesy of Jennifer L, Martin, Saint Andrews Biological Station – DFO)

The detection of domoic acid and the discovery of several toxigenic diatoms and dinoflagellates in the Canadian Arctic is of increasing concern because of the limited knowledge of HABs in this region. Continued vigilance and the maintenance of an effective capacity to manage developing problems via strategic research programs is essential. The goal of this document is to review Canadian marine harmful algal events and phycotoxins up to late 2018 (including relevant literature from Canada and internationally up to 2020) and to provide a foundation for any future research in this area.

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We thank the contributors to the comprehensive review of marine HABs and phycotoxins of concern to Canada: Stephen S. Bates & Luc A. Comeau (DFO-GFC, Moncton, NB); Andrea Locke (DFO-IOS, Sydney, BC); Jennifer L. Martin (DFO-SABS, Saint Andrews, NB); Christine Michel (DFO-FI, Winnipeg, MB); Michael G. Scarratt & Michel Starr (DFO-MLI, Mont-Joli, Quebec); Daniel G. Beach, Nancy I. Lewis, Pearse McCarron, Christopher O. Miles & Michael A. Quilliam (NRC, Halifax, NS); Nicola Haigh (Microthalassia Consultants Inc., Nanaimo, BC); Cynthia H. McKenzie & Terri Wells (DFO-NAFC, St. John's, NL); Michel Poulin (Canadian Museum of Nature, Ottawa, ON); Wade A. Rourke (CFIA, Dartmouth, NS).

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ISSHA's Corner

CHA 2021 Hybrid Conference, NEW DATE!!

Dear ISSHA members and colleagues:

Due to the COVID-19 pandemic and following recommendations of the World Health Organization and National Health Authorities, the 19th International Conference on Harmful Algae has been postponed to October 10-15th, 2021.

We are currently working on changes for having a live and virtual hybrid meeting. The virtual meeting will be held in the same venue, the International Conference Center in La Paz, Baja California del Sur, Mexico. Our goal is to provide a high-quality conference to motivate future harmful algae research. We will keep you informed of any conference updates through our web page (www.icha2021.com), email and social media.

All ISSHA members who renewed their membership after October 1st, 2019 will maintain their membership until the date of the conference. Please take the time to renew your ISSHA membership to allow for future student travel, awards and exciting speakers.

We appreciate your understanding and look forward to your participation.

Christine Band-Schmidt, Chair of the Local Organizing Committee on behalf of the ISSHA Conference Organizing Committee



18th ICHA Proceedings. Harmful Algae 2018

Dear ISSHA members and colleagues,

I am pleased to announce that the Proceedings of the International Conference on Harmful Algae, held in 2018 in Nantes, France, are now available on *the ISSHA website*.

Ph. Hess [Ed]. Harmful Algae 2018 – from ecosystems to socioecosystems. Proceedings of the 18th International Conference on Harmful Algae. International Society for the Study of Harmful Algae, 2020. 214 pages.

Thanks to Dr. Philipp Hess, IFREMER, and all his colleagues for a successful conference. Thanks to all the authors, reviewers and editors for their dedication to HAB science.

Vera Trainer, ISSHA President

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

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The Recurring Great Atlantic Sargassum Belt Impacts the Caribbean and South Florida

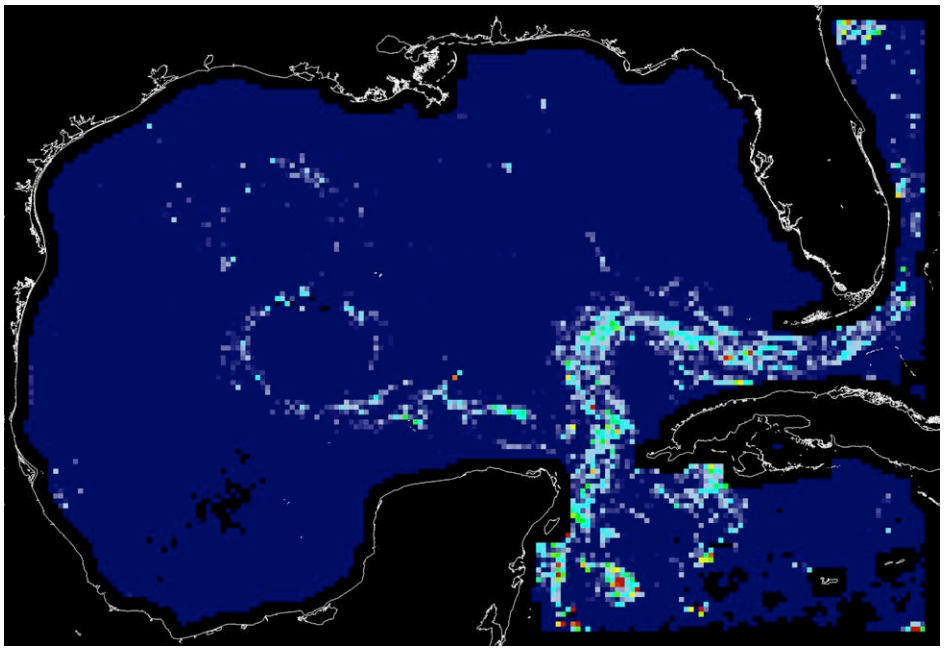

Fig. 1. MODIS satellite image of Sargassum transport from the Caribbean through the Yucatan Channel and into the Loop Current and Florida Current on July 16, 2020

Since 2011, the floating brown seaweeds known as pelagic *Sargassum* have formed a *Great Atlantic Sargassum Belt* (GASB) in the tropical Atlantic Ocean extending between Africa and South America [1]. The GASB represents an expansion in the distribution of this unique floating vegetation, which was historically restricted to the Gulf of Mexico (GOM), Loop Current, Gulf Stream, Sargasso Sea, and Caribbean Sea (Fig. 1). This vegetation was first reported by Christopher Columbus in the Sargasso Sea in 1492 and includes two pelagic species – *Sargassum natans* and *Sargassum fluitans* - that reproduce by vegetative fragmentation (Fig. 2). Early oceanographers were impressed with the vigorous appearance of *Sargassum* in the Sargasso Sea and estimated that ~ 90% of this vegetation was comprised of *S. natans* with the remainder being *S. fluitans* and several benthic species recruited from shallow coastal waters of the West Indies [2].

The precept that pelagic *Sargassum* flourishes in surface waters of the Sargasso Sea became a paradox to modern oceanographers who referred to this oligotrophic gyre as a "biological desert" [3]. Studies of the productivity and nutrition of *Sargassum* showed that photosynthesis and growth of these oceanic populations were nutrient-limited in the Sargasso Sea, compared to more productive nutrient-enriched plants in neritic waters of the GOM, Loop Current, and Gulf Stream [4]. These studies documented the importance of nutrient cycling by associated fish and invertebrates within the pelagic *Sargassum* community that has been designated "Essential Fish Habitat" by the National Oceanic and Atmospheric Administration (NOAA). A variety of nutrient sources in neritic waters of the western North Atlantic Ocean and GOM explained the higher nutrient contents and productivity of *Sargassum*, which included shelf-break upwelling, continental runoff via rivers (Mississippi River, Everglades), submarine groundwater discharge, benthic sediment recycling,

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and atmospheric inputs [5]. Human influences on several of these nutrient sources greatly increased in the 1980s and 1990s as a result of increased application of agricultural fertilizers, urbanization, atmospheric pollution, and extreme rainfall and flooding associated with climate change. These increasing nutrient inputs correlated with increasing accumulations of *Sargassum* on beaches and shorelines around the GOM [5].

A variety of nutrient sources appear to also be supporting the development of the GASB in the tropical Atlantic Ocean since 2011. Studies using MERIS (Medium Resolution Imaging Spectrometer) and MODIS (Moderate Resolution Imaging Spectroradiometer) satellite remote sensing described a new *Sargassum* bloom event north of the Amazon River in April 2011, which by July had spread eastward to Africa and westward to the Lesser Antilles and Caribbean Sea [6]. With the exception of 2013, the GASB has since formed annually, developing during spring months and peaking in abundance in summer; in July 2018, the GASB extended for 8,850 km from Africa to the GOM and ranked as the largest algae bloom on earth [1]. Over its broad distribution, the GASB can be supported by nutrients from the Amazon, Orinoco, and Congo rivers, upwelling in the eastern tropical Atlantic off the African coast [1,7,8], and atmospheric deposition of Saharan dust and biomass burning in South Africa [9]. Annual flooding events in the Amazon basin since 2009 combined with increased nutrient supply from deforestation and fertilizer use are factors potentially supporting bloom initiation in the central western Atlantic Ocean in 2011 [1,6,8,10].



Fig. 2. Collection of Sargassum natans (left side of photo) and Sargassum fluitans (right side of photo) off Ft. Pierce, Florida, for nutrient analysis



Fig. 3. Sargassum influx to the south side of Big Pine Key in the lower Florida Keys, June, 2014. Note absence of abundant fishes associated with Sargassum in the bottom photo

The excessive *Sargassum* biomass transported from the GASB to coastal waters in the Caribbean region have been causing a myriad of environmental impacts. Offshore, the extreme abundance of *Sargassum* has fouled fishing gear and caused challenges for safe navigation in and around the extensive mats. Decreased catches of the dolphinfish or mahi mahi (*Coryphaena hippurus*; -37%) and flying fish (family Exocoetidae; -52%) have been reported in Barbados. After stranding on beaches and coastlines, decomposition of *Sargassum* releases large amounts of brown leachate and results in *hypoxia* and *anoxia,* releasing toxic hydrogen sulphide and ammonia. The resulting water quality degradation from *Sargassum* exacerbates coastal eutrophication, resulting in fish kills, sea turtle mortality, and increased die-off of seagrasses and corals [11]. The massive influx of *Sargassum* to the Mesoamerican reef system was identified as a contributing cause of decreasing water quality and the outbreak in stony coral tissue loss disease (SCTLD) in 2018 [12].

As in the Caribbean, *Sargassum* influx to South Florida has increased



Fig 4. Sargassum stranding event on the south side of the Ft. Pierce inlet, Ft. Pierce, Florida, July, 2020

since 2011, especially following the larger size of the GASB after 2014 [1]. Extensive mats of *Sargassum* washed ashore in the Florida Keys between May and August 2014 (Fig. 3), a trend that has continued through summer of 2020 (Fig. 4). The massive strandings and decomposition have impaired water quality in nearshore waters of South Florida, especially in coastal bays and canals where *Sargassum* can become concentrated (Fig 4). Since 2014, SCTLD has spread from Miami through the Florida Keys during the same time frame when *Sargassum* influx has increased. This correlation parallels research in the Caribbean that has linked increasing *Sargassum* influx to worsening water quality and coral reef health [12].

Public health issues related to the increasing *Sargassum* influx have also increased in recent years. During large-scale strandings in the summer of 2018, more than 11,400 residents in Martinique and Guadeloupe were diagnosed with acute exposure to toxic H_2S gas produced by decaying *Sargassum* [13]. Additionally, bacterial (fecal coliform, enterococcus) and heavy metal (arsenic, cadmium) pollution associated with excessive *Sargassum* strandings are causing concern for recreational beach use, as well as attempts to safely dispose of *Sargassum* or recycle its biomass into fertilizers and soil conditioners for use on food crops.

The serious environmental and public health issues resulting from the harmful *Sargassum* influx pose major economic challenges to the Caribbean and South Florida region. *Sargassum* removal from Texas beaches during earlier, less severe inundations was estimated at $2.9 million per year. In 2018, the Caribbean-wide clean-up cost $210 million [8] and Florida's Miami-Dade County alone estimated removal expenses of $45 million in 2019. In addition, the direct economic impacts to tourism and commercial fishing are substantial, underscoring the urgent need for research focused on the various physical, chemical, and biological mechanisms underlying the development of the GASB.

The United Nations Environment Program (UNEP - GPNM) in collaboration with IOC UNESCO and the IOC-SCOR GlobalHAB programme hosted four webinars on *Sargassum* in 2020, and the Secretariat has shared the current realities of the impacts and approaches being taken to address the issue. Various stakeholder groups participating in the webinars have expressed their concern for the recurring *Sargassum* events in West Africa and the Caribbean region, highlighting the necessity for mainstreaming the issue in the policy arena, possibly a resolution at the United Nations Environment Assembly.

The scale of the GASB, which extends from Africa to the GOM and South Florida, is unprecedented for a HAB, as is the complexity of physical, chemical, and biological factors supporting initiation, seasonal development, and transport of the GASB.

Recently, the Scientific Steering Committee of the IOC-SCOR GlobalHAB Programme, through its Science and Implementation Plan, has recognized the *Sargassum* expansion as an emerging HAB issue. A new subcommittee dedicated to this topic has been established (see GlobalHAB this issue)

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The authors are grateful to Drs. Chuanmin Hu and Mengqiu Wang (University of South Florida) for providing remote sensing imagery of Sargassum, and to Ileana Lopez (UN Environment, Cartagena Convention Secretariat) and Dr. Elisa Berdalet (Institute of Marine Sciences, Barcelona; GlobalHAB SSC Chair) for their review and comments.

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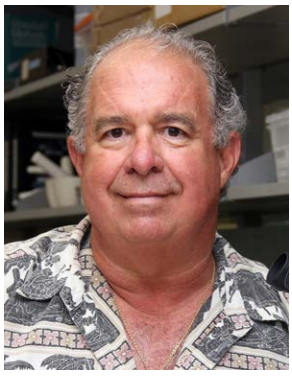

Ciguatera fish poisoning event in New Zealand from imported tropical reef fish and confirmation of Pacific ciguatoxins by LC-MS/MS

Ciguatera fish poisoning (CFP) is an illness caused by the consumption of fish contaminated with ciguatoxins (CTXs) and possibly maitotoxins (MTXs). The causative organism is a single-celled alga in the genus *Gambierdiscus* (Dinophyceae). *Gambierdiscus* is epiphytic on macroalgae, eel grasses and coral and is consumed with the host substrate by herbivorous and coral grazing fish. These fish are predated upon by omnivorous and carnivorous reef fish species and the ciguatoxins are bioaccumulated and biotransformed up the marine food web [1].

The only known Pacific-CTX (P-CTX) producer in New Zealand waters is *G. polynesiensis*, which has been isolated from Rangitāhua/Kermadec Islands (a New Zealand territory). This toxic dinoflagellate species is common throughout the tropical Pacific Islands. A related genus, *Fukuyoa* (previously named *Gambierdiscus*), has been isolated from the Bay of Islands, but does not produce P-CTXs.

Both genera were considered sub-tropical to tropical, but in recent years *G. carpenteri* has been isolated from the temperate waters south of Sydney, NSW, Australia. Cases of CFP in Australia have historically been reported from Queensland waters but, with warming ocean temperatures causing changes in the prevailing currents, and expansion of the sub-tropical and temperate latitudes, fish caught in NSW waters have caused CFP in that state. The risk of locally contracted cases of CFP occurring in New Zealand is therefore greater as the coastal waters around New Zealand continue to warm and as some species become adapted to cooler waters [1].

Cases of CFP reported in New Zealand to date have resulted from the consumption of contaminated reef fish (including eels) by tourists who have returned to New Zealand after consuming reef fish in the Pacific Islands and then become ill, or from New Zealanders consuming imported reef fish they have brought back into New Zealand themselves or have purchased from a commercial vendor. Several cases of CFP, linked to the consumption of Moray eel brought back from Samoa, were reported in Wellington, New Zealand, in 2016 [2]. Another series of cases were clinically diagnosed, based on symptomology, in 2019 following the consumption of Moray eel brought back from the Kingdom of Tonga and home-smoked [3]. No cases of CFP due to consumption of locally caught and consumed fish have been reported.

In May 2020 there was another intoxication event when five people from two families became ill after the consumption of reef fish, reported to be Fiji Kawakawa (*Epinephelus* sp.). There were three males aged between 19 and 58 and two females, one in her 40s and the other in her 50s. The symptoms were typical of CFP (inversion of hot and cold, paraesthesia and dysaesthesia) and the patients were clinically diagnosed as suffering from CFP. Immediately following the report, a product recall was put in place to try and prevent any additional intoxications occurring (Fig. 1).

Two samples of meal remnants, fried fish and fish curry (imported as *Epinephelus* sp.; Fig. 2), were received at Cawthron Institute, 2 June 2020. The results of analyses, as reported to the Canterbury District Health Board, indicated quantifiable levels of P-CTX-1B and two additional fish metabolite P-CTXs (Table 1). The samples had the skin and bones removed prior to being homogenised in their entirety. Analysis was carried out in duplicate using an in-house quantitative LC-MS/MS method for P-CTXs and MTXs [4]. The LoQ for P-CTX-1B was 0.1 µg/kg. Three other P-CTX fish metabolites, 52-epi-54-deoxyCTX-1B (P-CTX-2), 54-deoxyCTX-1B (P-CTX-3) and 51-hydroxy-CTX-3C were reported by presence/absence. Results for P-CTX-1B were adjusted based on the spike recovery (45% and 48% respectively).

In the Pacific region P-CTX-1B has been well documented in many fish species [5] and is the typically dominant P-CTX analogue detected from

Krazy Price Mart Ltd frozen camouflage grouper (kawakawa)

27 May 2020: Krazy Price Mart Ltd is recalling a specific batch of its frozen camouflage grouper (kawakawa) due to the presence of ciguatoxin, which may cause ciguatera poisoning if eaten.

KRAZY PRICE MART
Indian | Island | Asian Foods

Product identification	
Product type	Frozen whole fish (gutted)
Name of product	Camouflage grouper (kawakawa)
Batch marking	The fish is not labelled.
Date marking	No date marks appear on this product. The product was available for sale between 10 March and 21 May 2020
Package size and description	The product is sold as an individual whole fish (gutted) wrapped in clear plastic.
Distribution	The product is imported from Fiji. The product is sold only at Krazy Price Mart Ltd in Christchurch.
Notes	This recall does not affect any other Krazy Price Mart products.

Fig. 1. Notification of recall of product due to ciguatera fish poisoning cases in New Zealand

intoxication events. No regulatory limits have been officially set for CTXs, although the FDA has an established action level of 0.01 µg/kg P-CTX-1B equivalents for Pacific CTXs and 0.1 µg/kg C-CTX-1 equivalents for Caribbean CTXs [6]. Two additional fish imported in the same batch as the recalled product were also tested but were negative for P-CTXs (Fig. 3). Upon receipt these additional fish were identified as two different species – Camouflage Grouper (*Epinephelus polyphekadion*; Fig. 3) and Squaretail Grouper (*Plectropomus areolatus*; Fig. 4). The fact these fish were imported as a single species demonstrates the complications associated with species identification and linking intoxication events to a particular species. In addition, as these fish were not toxic and caught in the same area, it illustrates the complexities associated with ciguatera fish poisoning and the determination of risk fish species. It is always desirable therefore to get meal remnants so that analytical testing can confirm the clinical diagnosis.

Acknowledgements

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Table 1. Concentrations of P-CTX-1B recorded in reef fish samples (imported as Epinephelus sp.) linked to a ciguatera fish poisoning event in Christchurch, New Zealand.

Sample type	P-CTX-1B (µg/kg)	P-CTX-2	P-CTX-3	51-hydroxy-CTX-3C
Fried fish	0.29	Present	Present	Absent
Curried fish	0.21	Present	Present	Absent



Fig. 2. Remnants of fish meals linked to ciguatera fish poisoning in Christchurch, New Zealand. Remnants contained ciguatoxins



Fig. 3. Camouflage Grouper (Epinephelus polyphekadion) imported in the same batch of fish that caused the ciguatera fish poisoning event. This fish was negative for Pacific ciguatoxins



Fig. 4. Squaretail Grouper (Plectropomus areolatus) imported in the same batch of fish that caused the ciguatera fish poisoning event. This fish was negative for Pacific ciguatoxins

First report of *Ostreopsis* in a tropical mangrove lagoon in São Tomé Island (Gulf of Guinea)

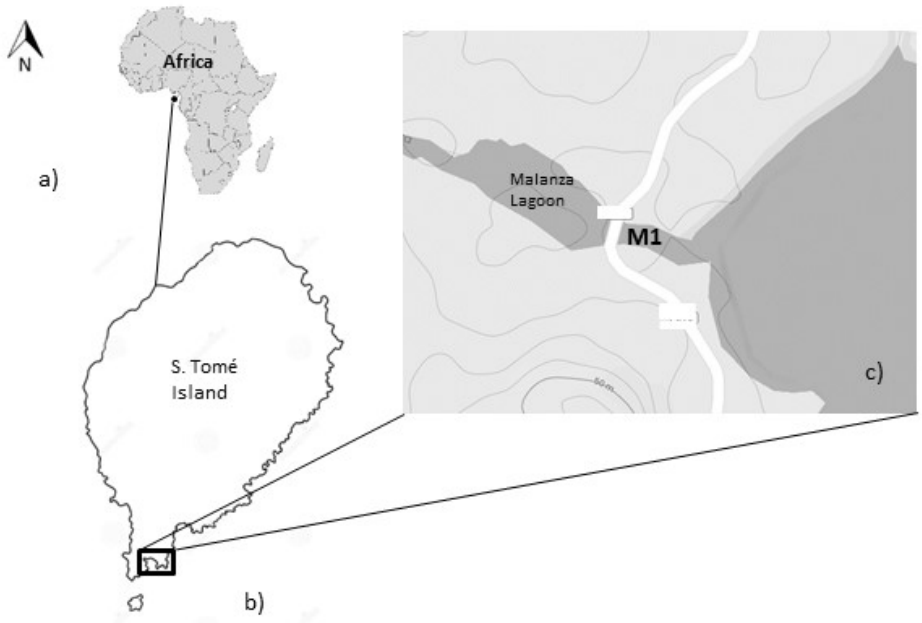

Fig. 1. Location of a) S. Tomé Island in the Gulf of Guinea (African Continent), b) the Malanza lagoon (0°02'46"N, 6°31'48"E) at the south of S. Tomé Island, and c) the sampling station (M1) in Malanza lagoon.

We report for the first time the presence of *Ostreopsis* in a tropical mangrove lagoon of the São Tomé and Príncipe Archipelago (Gulf of Guinea).

In-situ sampling was performed during the rainy season in November 2014 at the Malanza mangrove system. This is located in São Tomé Obô Natural Park (PNOST) in the southernmost part of the São Tomé Island (0°02'46"N, 6°31'48"E) [1]. The Malanza lagoon is a nutrient-enriched mangrove system with a central basin connected with offshore seawater through a constricted inlet [1] (Fig. 1). Water samples were collected by hauling a plankton net (20 µm mesh size) horizontally at the lagoon inlet (station M1 location in Fig. 1). Samples were collected during a flood when the landward current was relatively strong. Water samples were then stored in 125 ml glass bottles and preserved with neutral Lugol's iodine solution. The sampling site was 2.2m deep and at the time of sampling, the water temperature was 27.3 °C and salinity 29.1.

The morphology of isolated single cells stained with the fluorochrome Calcofluor White [2] were characterized under the light microscope (Olympus BX50), using bright field and epifluorescence microscopy. Tentative identification to species level was made according to Penna et al. [3]. All cells (n=6) were oval shaped and anterioposteriorly compressed (Fig. 2), with dorso-ventral diameters ranging from 39.40 - 51.48 µm and width from 28.75 - 34.91 µm (Table 1). Thecal pores were only of one type (Fig. 3). The apical pore (Po) measured less than ca 8.5 µm (n=2). Given the occurrence of cryptic species in the genus *Ostreopsis*, an identification based only on morphological characteristics is not sufficient and further work using molecular tools, to confirm

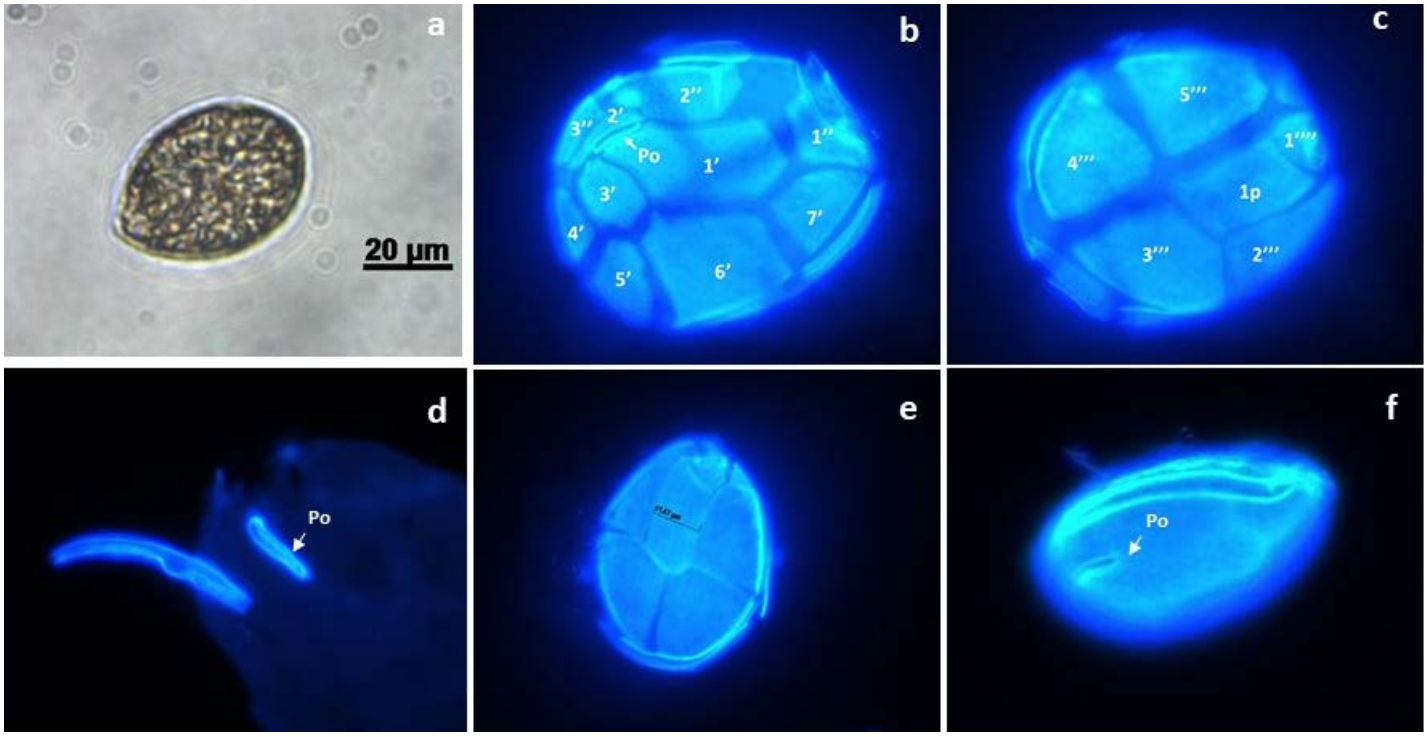

Fig. 2. Ostreopsis sp. bright field (a) and epifluorescence (b–f) micrographs. Epitheca (b) and hypotheca (c and e) showing plate patterns. Apical pore plate (Po) (d). Side view of Ostreopsis sp. (f). Po, apical pore plate. Scale bar = 20 µm. For cell size, see Table 1.

Table 1. Morphometric characteristics of Ostreopsis cells (n=6) analyzed in this study. Dorso-ventral diameter and width (including mean ± standard deviation values).

Dorso-ventral diameter (µm)	mean ± standard deviation	Width (µm)	mean ± standard deviation
48.44		34.91	
39.40		28.75	
45.99	45.44 ± 4.075	31.95	32.47 ± 2.215
41.31		30.83	
51.48		34.83	
46.00		33.53	

the species identification is required.

Ostreopsis cells were detected in the Malanza lagoon in a station located close to the ocean (M1). *Ostreopsis* is an epiphytic-benthic dinoflagellate genus which colonizes a variety of substrates including macroalgae, rocky and soft sediments and coral reefs. Cells belonging to this genus can also occur in the water column usually associated with higher concentrations of benthic cells [4]. In this study, free living *Ostreopsis* cells were collected from the water column, suggesting that their presence may be the result of a high benthic cell concentration inside the lagoon. Another hypothesis is that it may arise from a cell resuspension and transport mechanism, probably associated with the high tide water flow from offshore into the lagoon. The presence of coral reefs that represent potential habitats for benthic dinoflagellates were observed along the coastal zone. Further work is needed to clarify whether the species is present inside the lagoon or if it has been transported from the adjacent coastal zone.

Species within the genus *Ostreopsis* produce potent neurotoxins belonging to the palytoxin group which have been related to serious human poisoning through the consumption of toxin-containing seafood [5] and the aerosolization of toxins [6]. *Ostreopsis* is often associated with other nuisance dinoflagellate genera such as *Gambierdiscus*, *Coolia*, *Prorocentrum* and *Amphidinium*, which can develop benthic harmful algal blooms (BHABs), causing severe problems in economically relevant activities such as fishing and tourism. BHABs have become a major concern because of their biogeographical expansion from endemic tropical and subtropical areas to temperate areas. To date, there are no previous reports on the occurrence of BHAB species in São Tomé Island. The presence of *Ostreopsis* sp. cells in the water column of the Malanza mangrove system highlights the need for further studies on the distribution and ecology of this species.

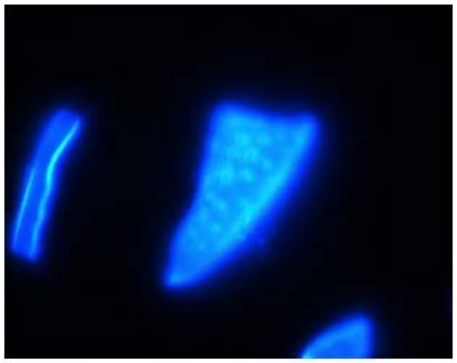

Fig. 3. Ostreopsis sp. from the Malanza lagoon. Epifluorescence microscopy, showing the plate pores.

Acknowledgements

The authors would like to thank all people who assisted during sampling in São Tomé and Príncipe and with laboratory work in Portugal. Special thanks to Tiziano Pisoni and Mariangela Reina from NGO Alisei, Grabriel Santos from Monte Pico, Márcio Gonçalves, from MARAPA. The authors are also thankful to Arlindo Carvalho, General Director for the Environment of Sao Tomé and Príncipe, for permission to conduct the work and export the specimens. This study was conducted in the framework of the Global Environment Facility (GEF) Project Contract nº 4494 'Integrated Ecosystem Approach to Biodiversity Mainstreaming and Conservation in the Buffer Zones of the Obô and Principe Natural Parks'. It received further support from Fundação para a Ciência e a Tecnologia, through the strategic project (UID/MAR/04292/2020).

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The impact of toxic *Dinophysis* spp. on the productivity of Scottish shellfish farms

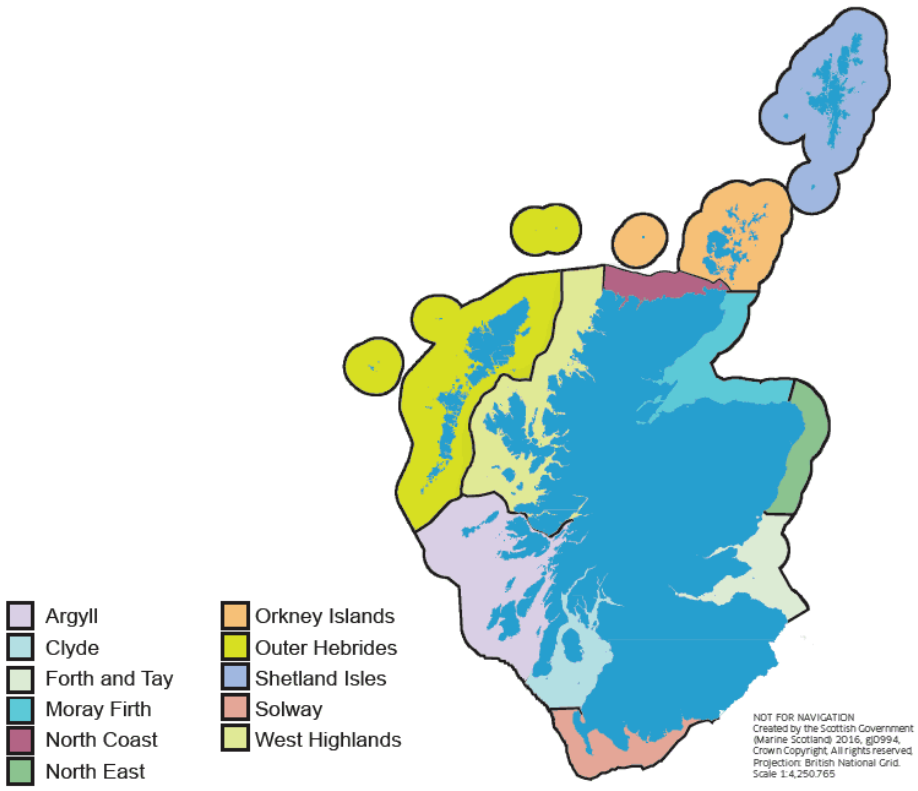

Fig. 1: Map of the Scottish marine regions. Shellfish farms considered in this study are located in the Western Scotland (Clyde, Clyde, Outer Hebrides and Shetland Islands) (Credit Scottish Gov).

There is limited evidence in the peer-reviewed literature on the monetary impacts of Harmful Algal Blooms (HAB) on European aquaculture. Direct estimates of damage, sensitive information for businesses, are not easily disclosed, and independent studies from public sector are limited, if not missing. The Scottish shellfish sector is not an exception. However, revealing and possibly mitigating likely damages on production caused by HABs is an important goal because of the social and economic importance played by aquaculture for rural communities characterized by limited job opportunities. For instance, in the Scottish Highlands, shellfish farming is carried out predominantly by small family enterprises generating a gross value (turnover) of £12.4m [1]. This sector is composed of 205 separate enterprises that support, in rural coastal areas, more than 300 employees mainly involved in the production of Blue mussels (*Mytilus edulis*) (~ 96% by weight of the total shellfish production) [2] with a value (turnover) of £ 10.1 m in 2017 [1;3]. While this turnover might be considered of low relevance in comparison with other industries, it is very important for the sustainable development of the local economy and the protection of jobs. Realising this importance, the Scottish Government is supportive of doubling the industry's economic value and the number of jobs by 2030. However, to achieve this goal, a series of technical and environmental issues should be resolved: among these an accurate valuation of factors that are limiting the current shellfish production.

In Scottish waters, Diarrheic Shellfish Poisoning (DSP), Paralytic Shellfish Poisoning (PSP) and Amnesic Shellfish Poisoning (ASP) are the shellfish toxicity syndromes of greatest concern [4;5]. PSP toxins produced by the genus *Alexandrium* are regularly detected in mussels during the summer months [6], while DSP toxins are more frequent still, often as a result of the advective transport of the causative *Dinophysis* to coastal aquaculture [7;8] (Fig. 2), with *Prorocentrum lima* being another source of these toxins. *Pseudo-nitzschi*a mediated ASP is less frequent [9] and may also require advective cell transport [10].

To ensure shellfish safety for public consumption, EU regulation EC No 853/2004 requires the monitoring of the concentration of biotoxins in shellfish flesh and their causative harmful phytoplankton. In Scottish waters, sampling is undertaken weekly during spring and summer, and fortnightly in winter and autumn at a set of Representative Monitoring Points (RMP) with the aim of minimising the risk of not detecting biotoxin concentrations above regulatory threshold shellfish [11]. Monitoring is overseen by the competent authority Food Standards Scotland (FSS), and carried out by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) for biotoxins and the Scottish Association for Marine Science (SAMS) for phytoplankton. If biotoxin concentrations at the relevant RMP exceed regulatory threshold, the farms associated with this RMP are closed to harvesting and risk management procedures based on phytoplankton and biotoxin data from the current and previous four weeks are used to determine the appropriate harvesting action. Mussels can be left in place to depurate toxins naturally and reach health and safety conditions required by the market. However, because much of Scottish shellfish production fulfils commercial contracts to supply a particular quantity of product at a certain time, it is likely that this product will go out of phase with market demand and remain unsold.

While considerable effort is expended to ensure shellfish safety, the impact of HABs on the economic sustainability of this regionally important industry remains unquantified. Statistics shows that mussel production in Western Scotland regions (Fig. 1) has been quite stable during the period 2009-2018 at an average of nearly 7,200 tonnes per year. The lowest production occurred in 2013 (6,935 tonnes), and the highest in 2016 (10,586) carried out in nearly 160 active sites employing globally 330 workers. The region that exhibits the highest production is the Shetland Islands with 4,825 tonnes, followed by West Highlands (850 tonnes), Clyde

(843 tonnes) and Outer Hebrides (678 tonnes). The Shetland Islands is the region characterised by the highest capital-intensive production with 75 sites employing in total 112 staff.

Using a production function approach applied to a panel data of mussel farms in the four most productive Scottish shellfish harvesting regions (Shetland Islands, West Highlands, Outer Hebrides and Clyde) (Fig. 1) over the period 2009-2018, we have estimated the impact of DSP on shellfish industry production [12]. We found that at the average DSP biotoxin concentration of 24% above the safe threshold, recorded in the period of investigation, these toxins are expected to cause a yearly average reduction of nearly 15% in production (95% confidence interval -20% to -10%). This is equivalent to a loss of 1,080 ton of shellfish per year (95% confidence interval -1,490 ton to -670 ton). At the average price of £ 1,272 per ton (in 2015 constant GBP), the average annual economic damage caused by DSP is equivalent to £ 1.37m (95% confidence interval £-1.9m to £-0.85m) over a turnover of approximately £ 10.1m. Our results offer insight into likely regional differences in operation and environmental characteristics of the sites. In the West Highlands, characterized by a lower productivity than Shetland Islands, the impact on production is marginally more damaging, suggesting that managers consider strategies to minimize the impacts of harmful algae, from shifting production sites to rearranging contractual agreements with wholesalers and retailers. The full study has recently been published in Harmful Algae [12].

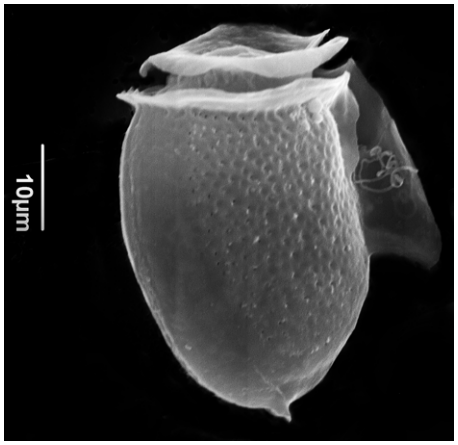

Fig. 2. Electron micrograph of Dinophysis sp. (Credit Callum Whyte, SAMS)





Fig. 3. Mussel farming in Scotland. Top: Shellfish longlines in a Scottish sea loch, Bottom: a long line being raised

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Bloom of *Cylindrotheca closterium* originating from shrimp farming discharges in the SE Gulf of Mexico

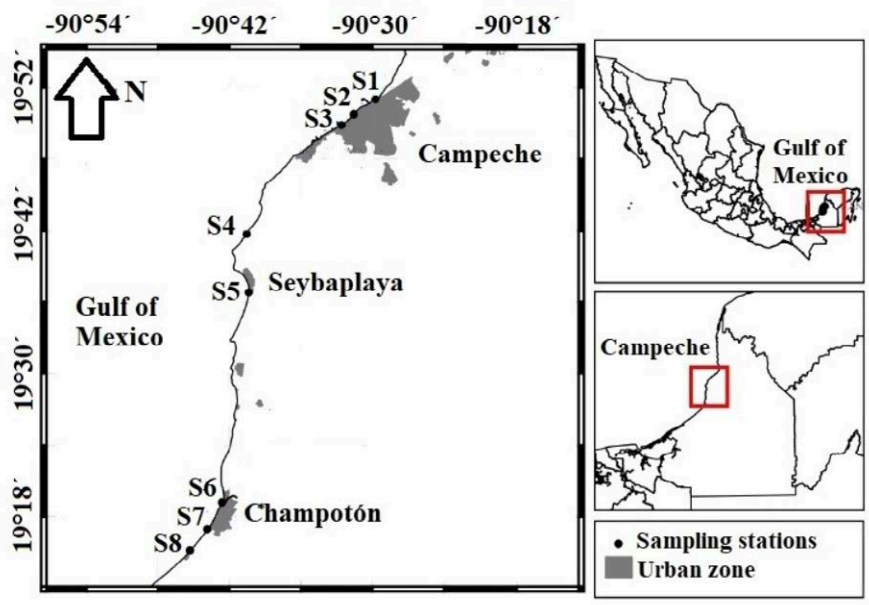

Fig. 1. Location of sampling sites (S1-S8) in coastal waters of Campeche (red frame), Peninsula de Yucatán, SE Gulf of Mexico.

Discharges from shrimp farming into the sea are common in the coastal zone of Campeche, located on the western side of the Yucatán Peninsula, SE Gulf of Mexico (Fig. 1). These discharges, with heavy loads of nutrients added to stimulate phytoplankton production, are a major source of eutrophication [1]. Despite the environmental impact of these activities in shrimp cultivation areas in the Caribbean, very little information is available on the phytoplankton species composition within the discharge waters. A project was carried out to identify anthropogenic impacts along the central coast of Campeche. The project included a study of phytoplankton species composition, with an emphasis on potentially harmful species. Eight sampling stations (depths ca. 50 cm) from three coastal localities (City of Campeche, Seybaplaya and Champotón) corresponding to sites affected by anthropogenic inputs were chosen (Fig. 1). These stations were sampled in September and November 2016. A bloom of the pennate diatom *Cylindrotheca closterium* (Ehrenb.) Reimann & J.C. Lewin, with a maximum of 1.4×10^5 cells L^{-1}, was observed in September. The maximum cell density was found at station S4, a drainage area for shrimp farming discharges (Fig. 1). Environmental conditions in this station at the time of sample collection were: salinity 41.5, temperature 31.5°C, pH=9.2 and dissolved oxygen 7.1 mg L^{-1}. Water discoloration was observed (Fig. 2).



Fig. 2. Water discoloration from a bloom of Cylindrotheca closterium observed in September 2016 in shrimp farming discharge waters in Campeche, SE Gulf of Mexico.

Cylindrotheca closterium, a well known bloom-forming species, has been reported in high cell densities in the SE Gulf of Mexico [2]. In coastal waters of northern Yucatan, the species is present in high numbers throughout the year. Blooms have been observed mainly during the warmer rainy season from mid-May to mid-October [3]. Our observations are in agreement with these results. In Yucatan waters, especially in the marinas, *C. closterium* blooms appear to be linked to high concentrations of nitrite, phosphate, nitrate and urea. These phytoplankton blooms are indicators of eutrophication due to fluctuations in environmental conditions because of the intensity and type of human activities occurring in the study area. The species is ubiquitous, has a high potential to dominate both planktonic and benthic microalgal assemblages, and is characterized by its high growth, reproduction rates and its wide geographical distribution.

Acknowledgements

The financial support to project "Fitoplancton marino potencialmente nocivo de la costa central de Campeche, México", 022.16-P04 (2016-2017) from COSDAC-SEMS-SEP (Coordinación Sectorial de Desarrollo Académico de la Subsecretaría de Educación Media Superior de la Secretaría de Educación Pública, México) is acknowledged.

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New GlobalHAB Theme: *Sargassum* Blooms

The GlobalHAB Science and Implementation Plan (*www.globalhab.info*) identifies that new, emerging HAB related issues can be incorporated into the program. This has been the case with the Sargassum mass occurrences in the Caribbean and the west coast of Africa (Fig. 1). In 2020 GlobalHAB established a subcommittee to develop the new GlobalHAB topic. The subcommittee is comprised of four experts on *Sargassum* and macroalgae ecology, Brigitta van Tussenbroek (Universidad Nacional Autónoma de México, Mexico), Brian Lapointe (Harbor Branch Oceanographic Institute, Florida, US), Ester Serrao (University of Algarve, Portugal) and José E. Martinelli Filho (Federal University of Parà, Brazil) (Fig. 2) and, from the GlobalHAB Scientific Steering Committee itself, Raffaele Siano (Ifremer, France) and Elisa Berdalet (ICM, Spain). The subcommittee may expand with new members to cover additional scientific aspects and geographic regions as required.



Fig. 1. Sargassum accumulation on sea turtle nesting beaches in Guinea-Bissau, Bijagós Islands. Photo from the Instituto da Biodiversidade e das Áreas Protegidas (IBAP, Guinea-Bissau)

The overall task of the subcommittee is to contribute to the identification of the key research questions to understand *Sargassum* growth dynamics, and to develop long term preventive measures, adequate forecasting and warning systems for *Sargassum* beaching. To achieve this objective, the subcommittee is organizing an Open Science Meeting (OSM) where the international scientific community working on *Sargassum* related issues is invited to participate. The OSM will facilitate a thorough review of current knowledge on *Sargassum* dynamics and identify research and technology development priorities. This will be achieved through expert workshops as part of the OSM addressing key questions. The results will be published as a white paper and possible peer reviewed papers. The OSM will also help identify the need and focus for further activities to enhance the general knowledge of the biology of *Sargassum*, to contribute to capacity building in the area and to support management and mitigation possibilities. A summary of this research plan will be included in the GlobalHAB Science and Implementation Plan.

The *Sargassum* topic is complementary to other ongoing Sargassum initiatives in the world. Until now, GlobalHAB has been linked to UNEP and GESAMP activities, collaborating in the organization of four webinars in 2020. These webinars increased the visibility of the active and multidisciplinary community working on *Sargassum* related issues. Several of the GESAMP sponsoring agencies and the European Funded project EuroSea (*https://eurosea.eu*) have shown an interest in the topic and the OSM. The OSM on *Sargassum* will be hosted by the Universidad Nacional Autónoma de México, Mexico. Due to COVID-19 the OSM is planned for 2022.

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Fig. 2. Members of the new GlobalHAB Sargassum subcommitte. From left to right: Brigitta van Tussenbroek, Brian Lapointe, Ester Serrao and José E. Martinelli Filho

Insights from the first US HAB Symposium Science Communication Workshop

The 10th US Symposium on Harmful Algae was held November 3-8, 2019, in beautiful Orange Beach, Alabama – hosted jointly by researchers from the University of South Alabama, Dauphin Island Sea Lab, Auburn University and the University of Oklahoma. This was the first US HAB meeting to host a Science Communication workshop, and it was such a success the hope is to continue with these at future meetings. A broad panel of experts was assembled to discuss their unique vantage points for communicating HAB science, including approaches to research, translation of research findings, testifying before Congress about the importance of HABs, and integrating information about HABs into weather forecasts. While these are all unique opportunities for communication, they share the same challenges in providing concise, clear, but non-alarmist, messaging regarding the human health, ecological, and socioeconomic impacts of HAB events. The goal of the workshop was to identify the tools available for participants to communicate HAB science in their respective regions.

Legislative landscapes are inherently complex and perplexing environments, as they require navigating both scientific and political factors. However, common ground can be found with the proper preparation, engagement, and approach. When conveying your message to political representatives, it is important to understand the legislative process and assemble your HAB colleagues (locally, regionally, nationally as appropriate) into a unified voice (involving your institutional liaison if available) and remain active throughout the entire process with letter writing, public commenting, and in-person meetings with representatives and staff (**Peter Hill**). Be prepared for dialogue in various forms, including invited testimony, briefings with staffers, and the quick "elevator speech" for unexpected encounters with key people. Messages should be simple, clear, and have succinct "asks" – all supported with pertinent materials, reinforced by post-meeting communications (**Don Anderson**). Whether oral or written, remember to provide the "big picture" compelling stories related to human and economic impacts, and avoid painstaking details and data sets (**Dan Ayres**). Engaging with representatives can be done in a variety of ways, including in-office visits, targeted events like "lobby days," invitations to visit the lab and/or field site, and offering to be a point person for HAB expertise (**Margaret Lansing**).

Communicating HAB science with ***industry*** entities can be challenging due to the potential for direct financial damages (particularly long term market impacts) through misunderstandings and alarmist language translated into public perceptions of product safety. Keeping information concise, consistent, and jargon-free supports constructive exchanges between researchers and industry, which, in turn, leads to progress in developing relevant early warning detection systems (**Dan Ayres**). To further these efforts, attend in-



Fig. 1. View of a session during the first US HAB Symposium-Science Communication Workshop

dustry-focused meetings such as those related to the local fishing community or aquaculture.

An educated ***media*** is vital for effective community response to threats against human health and local economies that arise from HAB activity. Too often, the discussion coincides with an active bloom event when tensions can be high and uncertainties abound. HAB scientists are quickly thrust into a situation where digestible information is being sought by the media and laypeople with diverse backgrounds and interest levels. When called upon, use the communication tips outlined above to share what is and is not known (simple graphics can be highly engaging), provide timely updates as more information becomes available, and describe recommended actions and approaches needed to further knowledge (**Kate Hubbard**). Agencies that are frequently called upon should designate a contact person and develop a written response plan for formulating consistent messaging that provides simple information, void of speculation, for a variety of target audiences (**Heather Raymond**). Inquiries will come from many sectors, therefore extensive documentation and re-visiting communication successes and challenges will aid in refining this plan for future use. When the media do call, be prepared by having written (and practiced!) your talking points ahead of time so you can stay focused and provide concise information during the interview (**Ken Kostel**). Do not feel you have to answer every question, but do offer to help fact-check, ask for a deadline, and follow-up with additional contacts and resources as appropriate.

We are presented with daily opportunities to discuss the impacts of HABs with the ***general public*** on an individual scale and can do so by identifying connections to the health and well being of communities and families within. The key to success is to empower people of all ages and backgrounds with achievable solutions and ways to become involved. For example, citizen science programs have mutual benefits for the general populace and research programs. By giving citizens a voice, validating their point of view, and having empathy for their situation, we can build strong relationships within and outside times of crises (**Tracy Fanara**). Building trust and rapport comes through introducing yourself first as a person, then as a scientist. Develop connections through shared interests, and family and community attributes (**Barbara Kirkpatrick**). Working with those in the public sector can further the dissemination of important information, particularly in times of uncertainty. Reach out to these people directly or through social media, as they can help dismiss rumors, translate science jargon, and serve as the voice of reason and provide the 'bottom line' for the lay public (**Ryan Wichman**).

Given that many adults (and even more young people) glean their information from ***social media***, it is imperative to develop and refine strategies that are both engaging and constructive. These can be highly useful platforms, but they also lend themselves to providing multiple messaging from various angles (from agencies to citizen journalists). Successful tips for engaging and empowering a broad base of readers include being timely with information, using images and humor where appropriate, providing context while keeping posts concise, remaining personable, asking a question or employing a poll, and including relevant hashtags of collaborators and influencers so the information reaches larger audiences (**Margaret Lansing, Ken Kostel**). These platforms can be used effectively to steer interested parties to translational products, such as newsletters, blog posts, and well developed agency websites with tiered information that allows the user to choose where they want/need to navigate.

Public communication has not been a traditional offering in science disciplines, however the world is now more connected than ever and the delivery of timely, valid and understandable scientific information is of increased importance. The tools highlighted above afford us the unique opportunity to step out from behind the bench and participate in the expanding information network available to help ensure informed citizenry.

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Advances in Colombia to address the Ciguatera problem

In the last two decades, an average of 17 cases of ciguatera poisoning (CP) have been registered each year in the Colombian Caribbean. Given that epidemiological surveillance is not very comprehensive, the number of cases is likely to be much higher. This situation prompted the interest in developing an inter-institutional and inter-sectorial plan in Colombia aimed at monitoring, forecasting and evaluating the risks of CP. The IV International HAB- National HAB Workshop in Colombia was planned to advance the development of the structure and operation of a risk management system.

The organization of the workshop was in charge of several institutions: Colombian Ocean Commission (CCO), Universidad Nacional de Colombia, Institute of Marine and Coastal Research (INVEMAR), General Maritime Direction, Ministry of Health and Social Protection, National Institute of Health and had the support and input from IOCARIBE and the ANCA group.

The IV HAB workshop, held virtually on October 7 and 8, 2020 focused on three thematic areas: health, environment and economy. Invited experts from Spain (**Beatriz Reguera**), Mexico (**Christine Band Schmidt**, **Erick Núñez Vázquez**) and Cuba (**Gustavo Arencibia**) shared their views and experience. The sessions were broadcast via YouTube, with more than 120 participants from Colombia and other countries in the region [1-2]

During the workshop a review of HAB seminars held in Colombia was presented (**Juliana Mancera**, **CCO**); as well as the main problems related to CP showing a historical analysis between 2010 and 2019 (**Milena Borbón Ramos, National Institute of Health**). The potential fish vector species in the Seaflower Biosphere Reserve where most Colombian cases of CP have occurred, were presented (**Adriana Santos-Martínez, Universidad Nacional de Colombia**), as well as the HABs impacts on the tourism sector (**Mónica Puyana Hegedus, Jorge Tadeo Lozano University**). The current capacities of the environmental sector (**Luisa Fernanda Espinosa, INVEMAR**) and the development of risk indices of HABs (**José Ernesto Mancera Pineda, ANCA**) were detailed. Scientific and socioeconomic criteria were discussed for the design of early warning systems for HABs (**Beatriz Reguera, Institute of Oceanography, Vigo Oceanographic Center**) as well as guidelines for the generation of the early warning protocol in Colombia (**Joana Pérez, National Unit for Disaster Risk Management**).





The workshop was an opportunity to widely disseminate information about CP amongst a diverse audience, as well as to identify Colombian strengths and weaknesses to address the impacts from HABs. Among the strengths, the existence of a regulatory framework (Law 1523 of 2012) was highlighted. By implementing this law, Colombia adopted a disaster risk management policy and established the National Risk Management System. Risk management (RM) is understood as the process of planning, execution, monitoring and evaluation of actions to be aware of, avoid, reduce or control risks to avoid or mitigate disasters. The RM has the explicit purpose of contributing to the safety, well-being and quality of life of people and to sustainable development. Although in Colombia there are no management protocols or an early warning system regarding HABs, Law 1523 represents the legal framework to develop a national plan for HAB management.

Another of the strengths identified in the country is the support of different organizations and public and private institutions interested in HABs, as well as the technical advice provided by different Colombian experts and international networks. Likewise, the experience gained with HAB research projects carried out so far, and the current activities such as the monitoring and the development of HAB risk indices, were recognized as an important advance.

Currently, INVEMAR is carrying out a monitoring project co-financed by the IAEA, in which the CIOH and the National Universities of Colombia and Cartagena participate. It is expected that the monitoring data will be useful to validate the IRAFAN index proposed by the IOCARIBE-ANCA group.

Among the weaknesses identified, the limited recognition of the potential negative impacts generated by HABs was highlighted. This is a matter of concern considering that the Caribbean development model is based mainly on tourism, an industry that each year reports a significant economic income. Fishing also provides an important source of protein for local communities. In this sense, HAB events and particularly CP represent a socio-economic development problem.

It was concluded that the National Technical Committee on Marine Pollution should continue to lead this process. For this purpose, more support for financial resources for scientific research is required as well as more information for decision making; better fishing controls; training for the health sector and greater dissemination through different media.

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Day 1. https://www.youtube.com/watch?v=L61cpwUv8sA&t=8s
Day 2. https://www.youtube.com/watch?v=oZE6o4lz5o4&t=51s)

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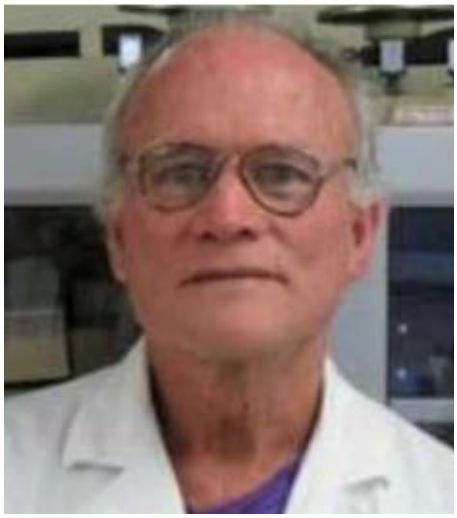
Luis Alfonso Vidal Velásquez (1950-2020) – In Memoriam

On November 8, 2020, Professor Luis Alfonso Vidal Velásquez, the academic authority on marine phytoplankton in Colombia, sadly passed away. Alfonso graduated as a Marine Biologist from Jorge Tadeo Lozano University and since then he dedicated his life to the study of marine phytoplankton. He completed his master's degree at Universidad Nacional de Colombia. His master's thesis was meritorious and became a guide book on estuarine phytoplankton of common use in many universities of the country.

Luis Alfonso taught at various universities in Colombia, participated in numerous courses on phytoplankton taxonomy, collaborated with activities of the ANCA-IOCARIBE Group, and was a mentor to many biologists. His charisma, discipline and ability to manipulate microalgae under the microscope and his profound acuity to infer biological information, made him a permanent source of reference. He made his own capillaries to be able to manipulate microalgae a few microns long, under the microscope. His human qualities and sensitivity to social problems made him highly appreciated by his students and colleagues.



Alfonso was a free spirit; he never accepted a permanent position because he wanted to be independent, even at the cost of his financial stability. He was a true gentleman, his kindness was his signature, and all the people who had the opportunity and the luck to meet him, remember this as his most characteristic trait.

Insensitive to the lure of material life, his universe was in a drop of seawater, where he could immerse himself and admire the true beauty of this world: the infinite forms of microscopic life, its elegance and variety. Both inside and outside the laboratory, Alfonso kept his spirit as a child alive, and this is the most remarkable aspect of his legacy: never lose your amazement when you look at nature, because in each being there is a hidden beauty; and this was true when it came to people, he always saw the best in each one and celebrated it.

The death of Luis Alfonso leaves a huge void and saddens the entire Colombian scientific community, especially in marine sciences. Fortunately, Luis Alfonso leaves an indelible mark; his peace will be preserved in the hearts of the people who knew him. His scientific work was the cornerstone of phytoplankton studies in the country, and will continue to be so for a long time.

Our condolences to his wife Ligia, his daughter Anayanci and his son Josué.

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Masaaki Kodama – In Memoriam

Masaaki Kodama was born on September 7th, 1943 in Saitama Prefecture near Tokyo. He passed away on August 30th, 2020 at the age of 76 after more than six months in hospital. This was a big shock for all his colleagues, especially for those he had trained in toxin-chemistry. Dr. Kodama was awarded a doctoral degree from the University of Tokyo in 1972 and moved to Kitasato University as an associate professor. He started HAB research in Ofunato Bay, Sanriku, Japan in the late 1970s where he monitored PSP toxins in field populations of *Alexandrium tamarense* and shellfish. Kodama's detailed research opened new avenues to the physiology and chemistry of toxin production processes in dinoflagellates and diatoms, and to toxin accumulation and excretion in bivalves. Based on these studies, he found that PSP toxins are combined with nucleotides and are released by enzymes (ribonuclease RNase). He further developed modifications in toxin monitoring methods by introducing an ELISA assay method.

Dr. Kodama played a pioneering role in the development of HAB science in Western Pacific countries. His first international cooperation on HABs research took place in Thailand in 1984, a country where PSP cases had occurred the previous year. He organized a mission comprised of four Japanese researchers, including ourselves, and collected samples in the innermost areas of the Gulf of Thailand (Figure 1). He confirmed the presence of PSP toxins in cultures of *Alexandrium tamiyavanichii* isolated by Ms. Suchana Wisessang (a pioneer on toxic dinoflagellate physiology in Thailand). The species was named by Dr. Enrique Balech after the name of another Thai scientist, Dr. Stichai Tamiyavanich who died from an accident during a toxic dinoflagellate sampling trip.

Dr. Kodama participated in various international HAB meetings, and especially in the 7th International Conference on Toxic Phytoplankton held in Sendai in 1995, where he or his co-workers presented seven papers on PSP, DSP, and ASP toxins in shellfish, and also a joint contribution, with local and international colleagues, about symbiosis of bacteria with toxic plankton. One evening, during that conference, he invited over 10 foreign participants to a bar for drinks and music. Figure 2 recalls Kodama's jolly attitude to music.



Fig. 2. Playing guitar during a break in the VII International Conference on Harmful Algae in Sendai, 1995. (Photo DM Anderson)

He had more than 30 different musical instruments, and often played them, even during conferences and sampling trips abroad.

After the Sendai Conference, he hosted a HAB training course in Kitasato University, with more than 30 invited scientists. His friendliness and easily



Fig. 1. Drs Masaaki Kodama, Yasuwo Fukuyo and Takehiko Ogata on a sampling trip at Pran Buri, Thailand. The man with a helmet on the left is Dr Tamiyavanich



Fig. 3. Having a discussion with Dr Rhodora Azanza during a training course in Kitasato University, July 1995



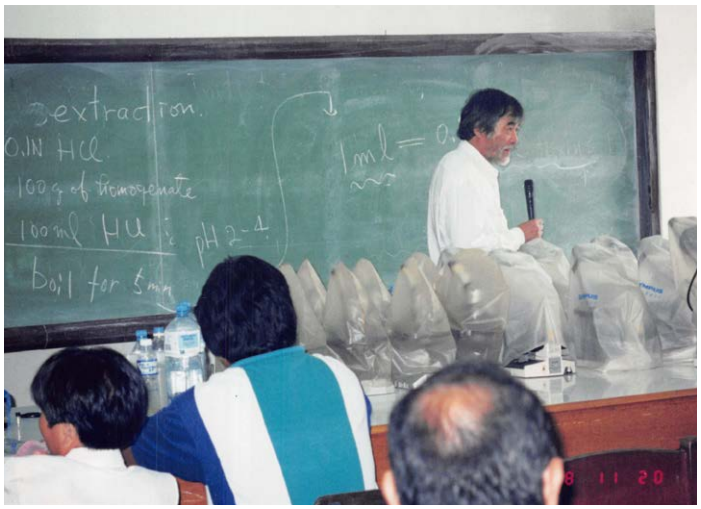

Fig. 4. International cooperation in the Philippines. Top, Banka boat during sampling break; bottom, Training the monitoring staff at a local school

understandable lectures attracted all participants. Figure 3 shows him during a discussion about toxins with Dr. Rhodora Azanza, a participant from the Philippines. Dr. Kodama visited the Philippines quite often, because of the widely endemic PSP problems of that country. He joined a JICA (Japanese International Cooperation Agency) project to establish PSP toxin monitoring centers and joined seminars and training courses at various places organized by Mr. Cielito Gonzales of the Bureau of Fisheries and Aquatic Resources. Figure 4 shows him during a break and giving a seminar for local staff working on toxin monitoring at an elementary school in the Philippines.

Domoic acid in bivalves was another of his research interests and a major issue at the time of his cooperative work in Vietnam. His collaborator there was Dr. Dao Viet Ha, currently the director of the Institute of Oceanography (Nha Trang, Vietnam). Dao Viet Ha thesis research was about identification of the phytoplankton species producers of domoic acid that contaminated shelfish at Phu Quoc Island. She was the last of Kodama's PhD students (Figure 5)

Outside Eastern Asia, he traveled to Central American countries (Guatemala and Puerto Rico) for PSP-related research. Figure 6 shows him bathing in a hot spring on the Atlantic coast of Guatemala during a sampling break, together with Guatemalan Coast Guard officers.

During the last years he had trouble in walking and stayed at home. Sometimes we visited him and enjoyed talking on various HAB topics, especially on toxin producing bacteria and their contamination in organs of protists and animals. Figure 7 was taken together with Dr. Yasukatsu Oshima at his home. We pray that he rests in peace smiling as in the photo.

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Fig. 5. International cooperation on ASP toxicity in Vietnam. Graduation of Dr. Dao Viet Ha, currently the director of the Institute of Oceanography of Nha Trang, last PhD student of Kodama.



Fig. 6. Bathing in a hot spring, with the Coast Guard officers, Atlantic coast of Guatemala, during a sampling break.



Fig. 7. Dr Masaaki Kodama with Dr. Yasukatsu Oshima at Kodama's home.

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ISSHA's Corner

NEW DATE: October 10-15 2021

Hybrid Conference - Call for abstracts - ICHA 2021



Dear ISSHA members and colleagues, The organizing committee is pleased to announce the call for abstracts for the *19th International Conference on Harmful Algal Blooms* to be held from the 10th to the 15th of October 2021 in the Conference Center of La Paz, B.C.S., Mexico. We will have a hybrid meeting, providing you the choice to participate either in person or remotely.

We invite the submission of abstracts within a broad range of topics related to the study of harmful algae, ranging from basic research to future applications. For the full list of topics, please visit the *conference website*. The deadline for submitting an abstract for a poster, speed-talk or an oral presentation is **9 April 2021**. All abstracts must be submitted at the *conference website*.

All ISSHA members who have already renewed their membership (after 1 October 2019) will remain active members until after the conference. If your membership has expired, please *renew your ISSHA membership*. Your membership fees ensure that the conference is successful, and are used to help support student travel, awards, and plenary speakers. **Participants wishing to receive the ISSHA member rate for conference registration must join ISSHA or renew their memberships prior to 11 June 2021. Discounted membership is provided for students.**

A visa may be required for participants from some countries. Please check your country's visa requirements well in advance with your local Mexican Consulate for official instructions on the specific visa, travel regulations, and application procedures.

As the major host of the conference, ISSHA will support the following activities: student travel awards, achievement awards, and the popular ISSHA auction!

On behalf of the conference organizing committees, we look forward to receiving your submission.

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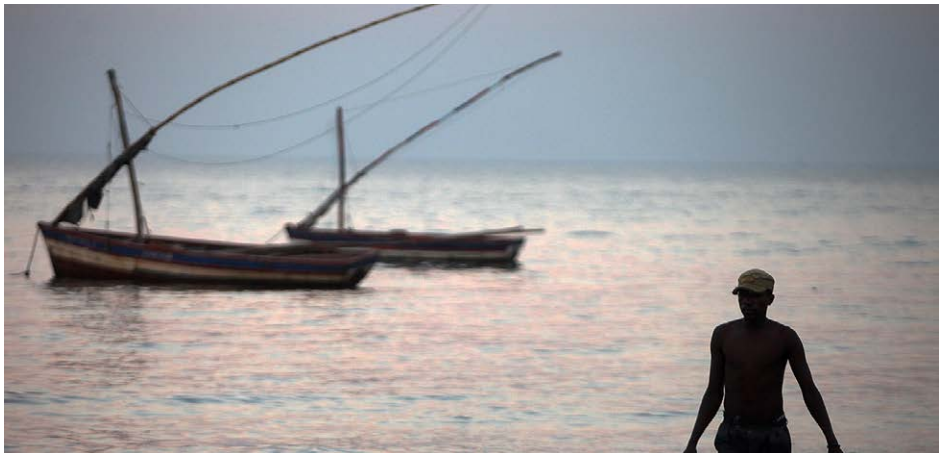

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Deadline
Deadline to submit material for HAN 67:
March 15th, 2021

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SHIOHIGARI and PSP toxins in Japan: Initiatives to save traditional recreational clam picking

Shiohigari has been enjoyed by people in Japan for centuries, as depicted in the Japanese art style Ukiyo-e by the artist Hiroshige in 1852 (Fig. 1). Although much time has passed since Hiroshige's day, today *Shiohigari* remains a popular marine recreational activity. It is a traditional as well as recreational springtime event, when crowds of people flock to tidelands to gather shells during low tide. Nowadays, *Shiohigari* is a rare opportunity for not only families but also schools to teach children the importance of conservation of marine resources and the environment.

In spite of the cultural importance of *Shiohigari*, it is at risk due to the occurrence of toxic algal blooms. Paralytic shellfish poisoning (PSP) toxins in bivalves (PST) exceeding regulatory limits have been detected in the seas surrounding Japan [1] and almost every year in shellfish collected from the seas along Osaka prefecture since 2002 [2]. The main causative species has been identified as *Alexandrium pacificum*, (formerly *A. tamarense*). Operators of recreational clamming parks in Osaka prefecture (Fig. 2) have taken the initiative with innovative measures to keep recreational clamming parks open for *Shiohigari* even when paralytic shellfish toxins levels exceed the regulatory limit.

In Japan, people can enjoy clamming in two different locations: coastal areas where anyone can take clams from their natural habitat free of charge and clamming parks, where an entrance fee is charged and the clams are under the control of the operators of the clamming park. In most cases, the operators of these clamming parks are fisheries cooperatives. These fisheries cooperatives monitor growth of wild juvenile clams and ensure enough are available in the clamming areas so that people can dig them up and take them home. They even purchase clams to ensure that stocks are sufficient.

In general, operators of clamming parks voluntarily refrain from opening parks when PST exceeding regulatory limit is detected to prevent shellfish poisoning cases (Fig. 3). At clamming parks in Osaka, however, they keep parks open by purchasing non-toxic clams and exchanging the non-toxic ones for those collected by clammers. This is extraordinary.

The exchange of the non-toxic clams for those collected by clammers started at Tannowa clamming park run by Tannowa Fisheries Cooperative. Surpris-



Fig. 1 Ukiyo-e drawing of "Shiohigari" at Shinagawa seashore by Hiroshige Utagawa (from Digital Collection of National Diet Library, Japan)

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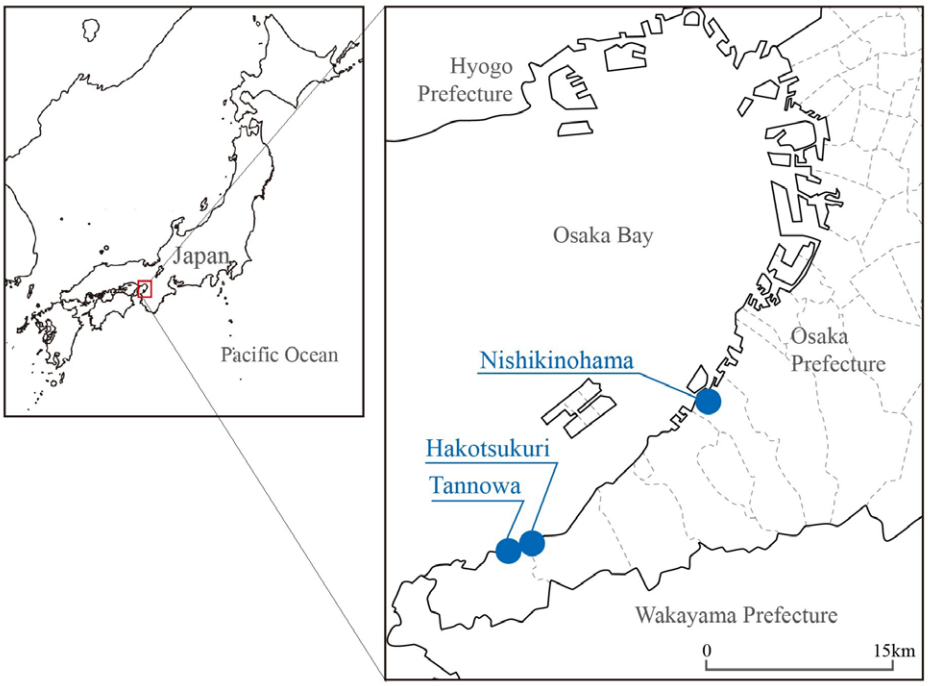

Fig. 2 Clamming parks in Osaka Prefecture (Modified from a digital map of The Geospatial Information Authority of Japan)

ingly, they had developed the exchange system to secure viability of their operations before 2002 when PST exceeding the regulatory limit were first detected in clams from seas along Osaka prefecture. .

One of the species most targeted at clamming parks is *Ruditapes philippinarum*. Commercial catch of *R. philippinarum* in Osaka prefecture had its peak at 556 tons in 1953, then decreased sharply in late 1950's, 30 years before its decrease throughout Japan [3]. The major reasons for the rapid decrease and lack of recovery of commercial catch of *R. philippinarum* are considered to be the extensive reclamation of tidal flats and severe deterioration of the environment of Osaka Bay. Because of this, by the 1960s at the latest, operators of clamming parks in Osaka prefecture had to purchase clams to meet the requirements of people visiting for recreational clamming.

Tannowa clamming park is not a natural but an artificial beach. Tannowa Fisheries Cooperative originally operated recreational clamming in natural tidal flats in different coastal areas. However, these tidal flats were reclaimed to build a harbour for yachts which led to a subsequent shift of focus to the present clamming park. Operations were restarted where clams cannot grow naturally due to the nature of bottom sediments. Due to these circumstances, the Cooperative can only operate the clamming park by purchasing clams from outside areas. Through operation of clamming parks with purchased clams, fishermen of Tannowa Fisheries Cooperative developed the system of exchanging a fixed quantity of clams for those collected by visitors to prevent visitors from taking away too many.

Fortunately, this exchange system worked well addressing the new challenge, i.e., how to keep the clamming parks open despite the unexpected detection of shellfish toxins exceeding the regulatory limit. The fishermen of Tannowa Fisheries Cooperative kept the park open by ensuring the safety of the externally purchased clams by independently getting toxin tests (Fig. 4). The clam exchange system then spread to other clamming parks, such as Nishikinohama and Hakotsukuri (Fig. 2). When Nishikinohama Tourism Association, operator of Nishikinohama clamming park self-closed the park when shellfish toxins exceeded the regulatory limit in 2002, they received numerous phone calls from citizens requesting the opening of the park and enquiring the reason for its closing. In order to meet the needs of people for clamming, they introduced the system of exchanging clams in 2007 after the third incidence of PSP exceeding the regulatory limit detected in Osaka prefecture.

At first, the Fisheries Division of Osaka prefectural government requested operators of clamming parks not to open the parks until a safety declaration was issued. In the last few years however, self-closure of the parks on detection of shellfish toxins surpassing the regulatory limit is no longer requested. Thorough management of both clams and visitors by the operators resulted in no record of poisoning cases from the clamming parks, which led to no requests for self-closure of the parks by the Osaka prefectural government [4]. The once decreasing number

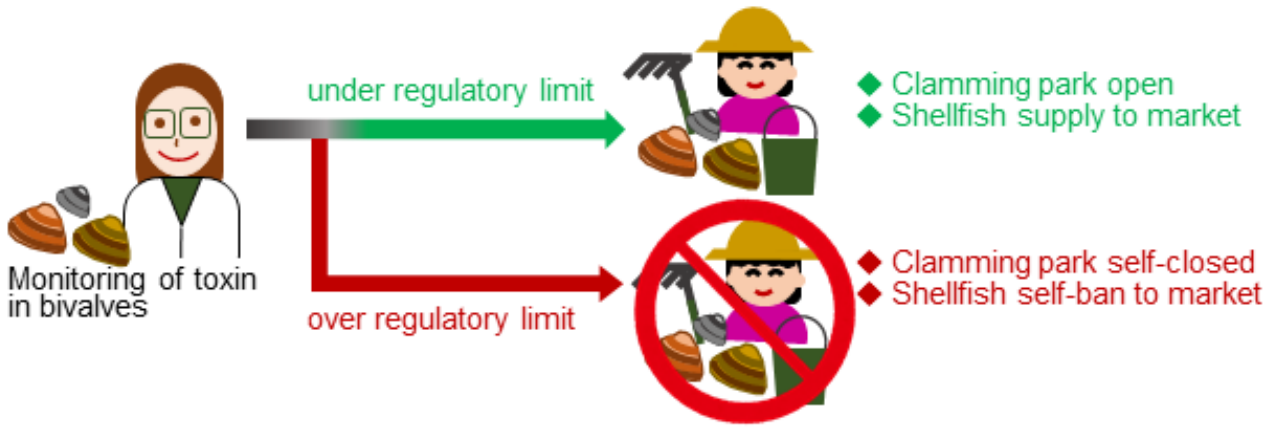

Fig. 3 General framework of risk management of shellfish poisoning in Japan

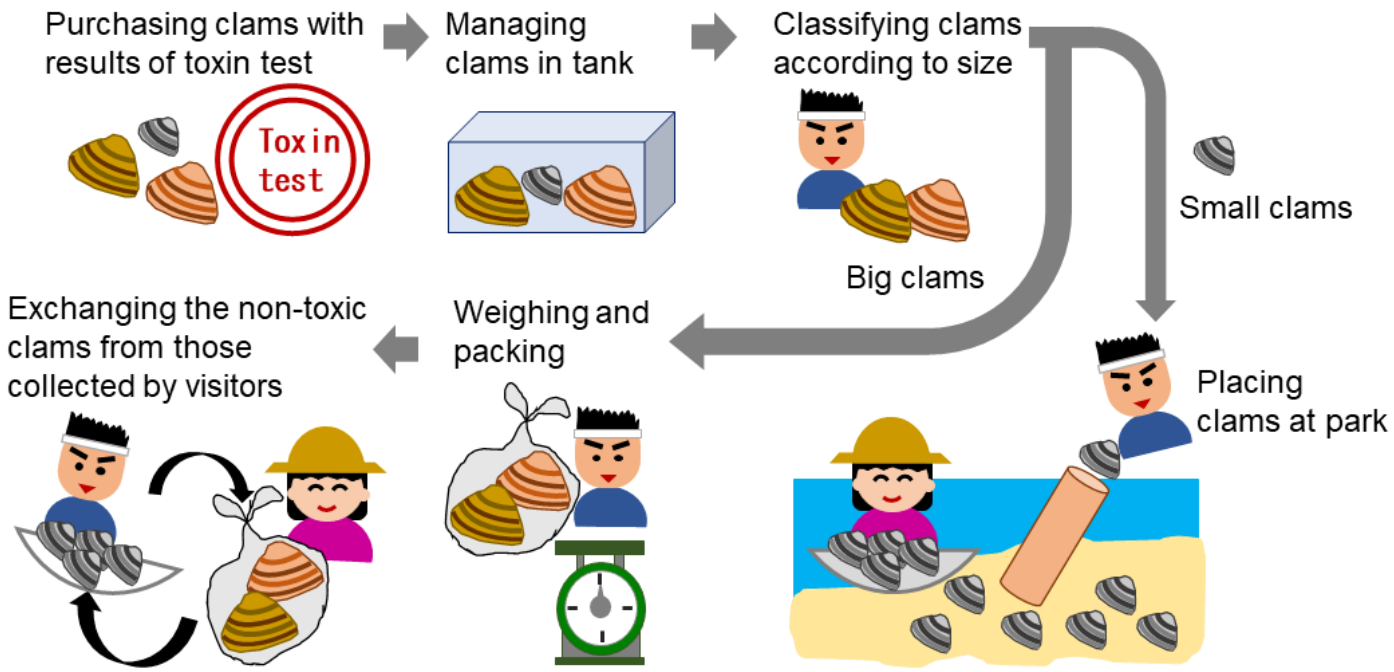

Fig. 4 System of exchanging clams which secure food safety at Tannowa clamming park (Modified from [3]

of visitors to clamming parks has now recovered.

This is a success story of how to mitigate socio-economic impact on recreational clamming in Osaka prefecture due to PST. Thanks to the initiatives and careful measures to ensure safety by the operators and the understanding of the Osaka prefectural government, opportunities for recreational clamming have continued to be provided even when shellfish toxin exceeding the regulatory limit is detected. The complete absence of any poisoning cases from clamming parks is what has made this mechanism possible. *Shiohigari* has recreational, cultural, and educational values for citizens in Japan. How can we pass on the traditional culture of *Shiohigari* to the next generation given the spread of shellfish toxins as well as the critical status of clam resources? This is one of the most urgent issues facing Japan if we are to prevent a further decrease in the number of people enjoying and being interested in the sea.

Marine recreation such as *Shiohigari* has a strong influence on enhancing people's behaviours around marine conservation [5]. Thus, the exchange system of clams at clamming parks will have an effect not only on mitigating the socio-economic impact PST but also on promoting people's willingness to conserve the marine environment by continuously providing opportunities for *Shiohigari*. Although introduction of the exchange system at clamming parks is still limited, park operators in other prefectures might become interested if PST over the regulatory limit becomes more widely spread and more frequently detected. For *Shiohigari* to succeed, further study on social acceptance of the clam-exchange measures is necessary, as is a better understanding of the challenges for securing food safety and the continued operation of clamming parks.

Acknowledgements

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HABs and the Mixoplankton Paradigm

Mixotrophs are defined as organisms that are "*able to use photo-autotrophy and phagotrophy or osmotrophy to obtain organic nutrients*" [1]. It is notable that all phototrophic protists are potentially mixotrophic if only through expression of osmotrophy enabled by the uptake of amino acids and sugars, processes that were much studied back in the 1970's and 1980's. Over the last decade, however, there has been an increasing awareness of the importance of the activity of plankton combining phototrophy and phagotrophy [2]. And that includes many HABs, which are not just "algae", but also predators.

That awareness of the importance of phagotrophy in so-called algae prompted a reclassification of protist plankton functional groups [3] according to their different modes of photo+phago 'trophic nutrition. The most basic division was between those species with a constitutive (innate) ability to photosynthesise, and the non-constitutive species that acquired phototrophy from their prey. Subsequently, to remove the ambiguity between mixotrophy enabled by {photo' + osmo' trophy} versus {photo' + osmo' + phago' trophy}, the use of the term "mixoplankton" was proposed [4] for those capable of phagotrophy. "Phytoplankton" was then proposed to describe protists capable of phototrophy but not phagotrophy. Planktonic cyanobacteria, including HAB species, would by default also be phytoplankton.

The role of mixotrophic plankton in HABs has long been recognised [5]. With the newly proposed mixoplankton term and its allied paradigm [4] that sees the potential for bacteria-mixoplankton and mixoplanktonic predator-prey interactions, the question arises as to how HAB species align with this new take on marine science?

In order to identify and hence differentiate between the mixotrophic and mixoplanktonic HABs, we have adapted the new protist functional group classification [3] specifically for Harmful Algal Bloom species (Fig. 1). In Fig. 1 example species names have been obtained from the IOC-UNESCO HABs list [6] and assigned to functional groups according to the mixoplankton database [7]. The primary difference between the HAB functional group classification presented here and the protist functional group classification [3] is the inclusion prokaryotic plankton as there are various known HAB species within this group.

As before [3], this HAB functional group classification chart starts with the question of whether the HAB species is capable of carbon fixation. A negative response to this leads us to one end of the spectrum – the pure heterotroph describing the traditional protozooplankton (or, microzooplankton) functional group. Within the current IOC-UNESCO HABs list [5] there is one only species, *Phalacroma rotundatum* which falls within this group. However, if the HAB species has the ability to photosynthesize, the next question is whether the species can also engage in osmo-heterotrophy. A null response results in what would be a purely photo-autotrophic organism; there are no known HAB species which would satisfy this description – indeed there is no known microbe that aligns with this state. A *yes* response gives us a mixotroph capable of photo-autotrophy and osmo-heterotrophy. Once we have a mixotroph, the next criteria to test is whether the mixotroph is also capable of phagotrophy. A negative leads to the traditional "phytoplankton" which include the HAB species within the Bacillariophyceae (diatom) and cyanobacteria groups. A positive response results

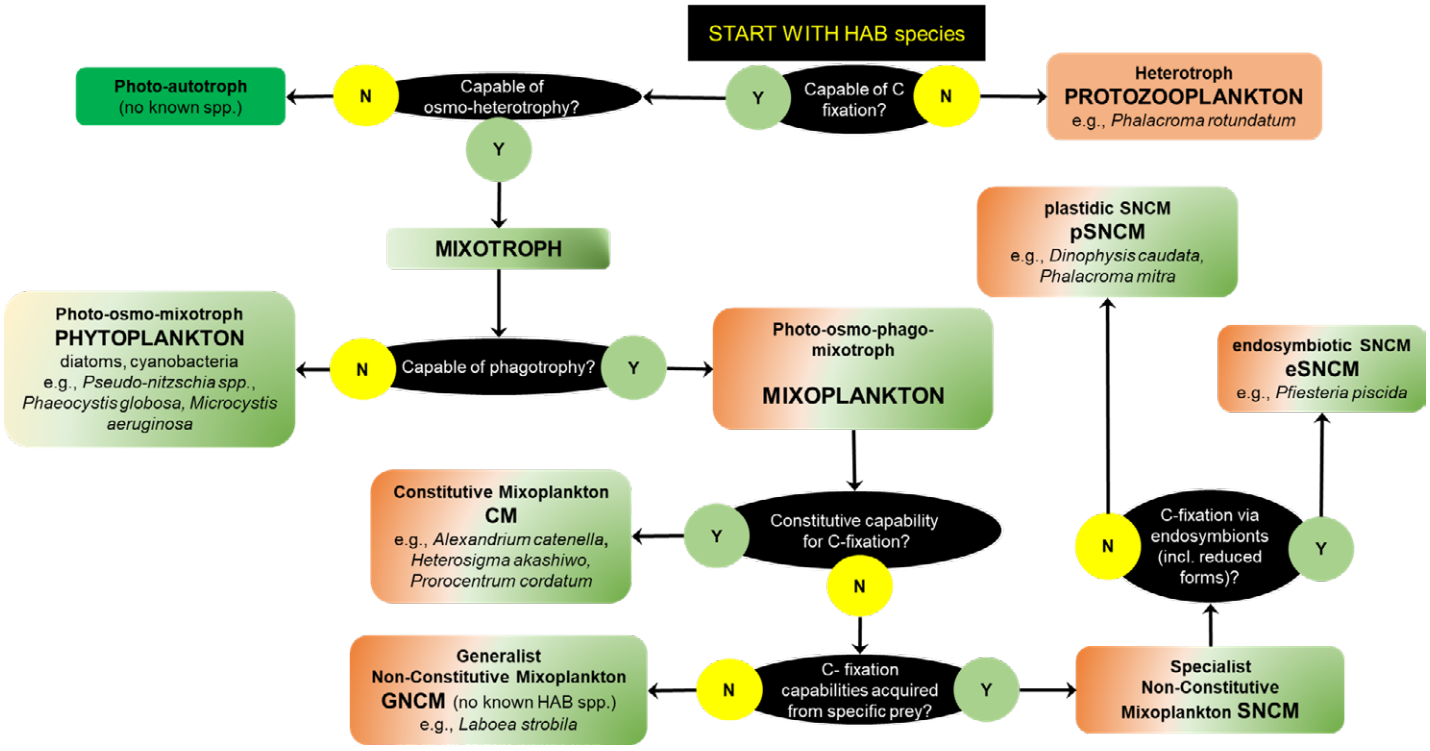

Fig. 1. Functional group classification key for Harmful Algal Bloom Species. N, no; Y, yes. Key developed from the protist functional group key [3] with example species from the IOC-UNESCO HABs list [6] aligned to functional groups according to the Mixoplankton Database [7]

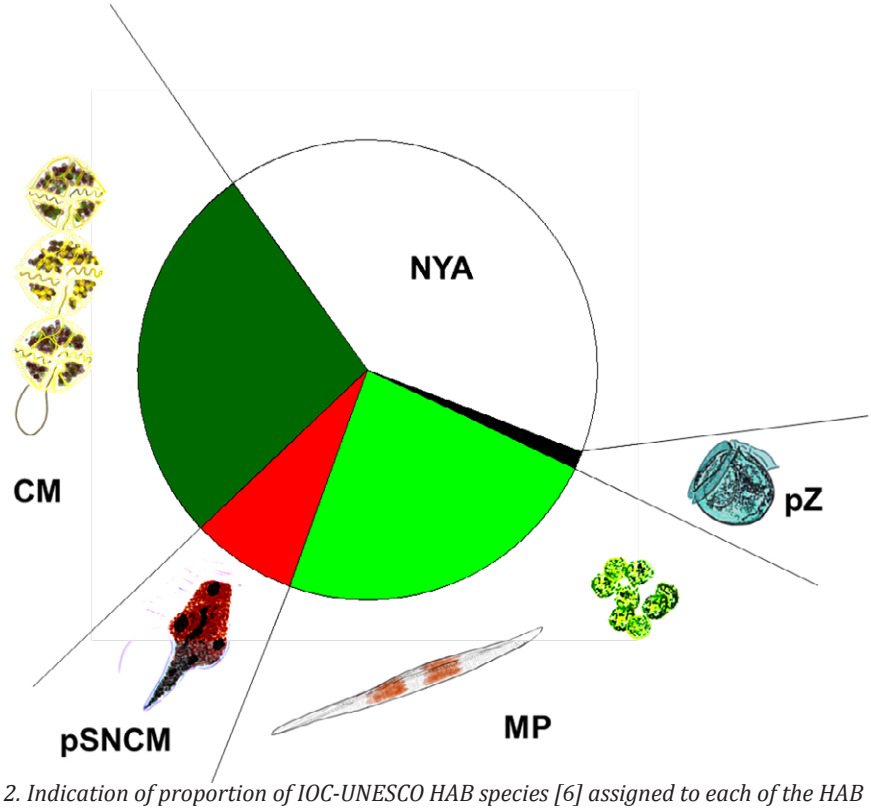

Fig. 2. Indication of proportion of IOC-UNESCO HAB species [6] assigned to each of the HAB plankton functional groups according to key in Fig.1 compiled by cross-reference to a database on mixoplankton species. CM, constitutive mixoplankton; pSNCM, plastidic specialist non-constitutive mixoplankton; MP, mixotrophic phytoplankton including eukaryotic diatoms and prokaryotic cyanobacteria; pZ, protozooplankton; NYA, not yet assigned. See text and Fig. 1 for further explanations.

in a photo-osmo-phago-mixotroph – the mixoplankton - functional group; these are primarily planktonic protists.

The mixoplankton HABs are then broadly classified into constitutive and non-constitutive mixoplankton. Here, the constitutive mixoplankton (CM) are HAB species which have innate photosynthetic capabilities; these include various species from the Dinophyceae (e.g., *Alexandrium minutum*, *Karenia brevis*, *Karlodinium veneficum*), Raphidophyceae (e.g., *Chattonella marina*, *Heterosigma akashiwo*) and Haptophyta (e.g., *Chrysochromulina leadbeateri*, *Prymnesium parvum*) groups.

The non-constitutive mixoplankton (NCM) are those that need to acquire their obligate phototrophic capabilities from either generic species (generalist non-constitutive mixoplankton, GNCM) or specific species (specialist non-constitutive mixoplankton, SNCM). There are no known HAB GNCMs; GNCMs comprise various ciliates, such as *Strombidium* and *Laboea* species [8]. The SNCMs are further divided into those that retain plastids and parts of the prey (plastidic SNCM, pSNCM) and those that maintain endosymbionts (endosymbiotic mixoplankton). The well-known HAB *Dinophysis* fall within the pSNCM group while the HAB *Pfiesteria piscida* with their reduced endosymbiont fall within the r-eSNCM group. *Dinophysis* acquires its plastids from the SNCM ciliate, *Mesodinium*, which in turn acquires its plastids from CM cryptophytes such as *Teleaulax*. Thus, SNCM species always depend on the availability of other species for acquired phototrophy while CMs have no such restriction on their growth. Furthermore, the conditions of growth effects the longevity of acquired plastids in NCMs and thus the components of the food chain are closely coupled.

Fig. 2 presents preliminary results from assigning this functional group classification to the 190 HAB species currently recorded in the IOC-UNESCO list. The "not yet assigned" (NYA) group represents HABs within the Dinophyceae group which are known to be mixotrophic by virtue of being photo-osmo-heterotrophic but we have not found any published records evidencing their capability (or, otherwise) to engage in phagotrophy. The primary reason behind allocating "NYA" to these groups rather than "mixotrophs" is the presence of various well-known mixoplanktonic species within the same genus (e.g., the CM *Alexandrium catenella* vs the NYA *A. hiranoi*; the CM *Karenia brevis* vs the NYA *K. cristata*).

Phagotrophy has a direct consequence for trophic dynamics, with the removal of competitors and potentially also predators by the collective action of high abundance blooms of HAB species. Phagotrophy also provides nutrients which, according to traditional inorganic nutrient analysis, may otherwise be considered as limiting. The consumption of bacteria, for example, appears common. The traditional food web considers bacteria as a competitor for nutrients with phytoplankton; indeed, nutrient stressed phytoplankton release increased amounts of organics that promote bacteria growth in a positive feedback loop [10]. Protozooplankton then control the dynamics of the interaction (Fig. 3). According to the mixoplankton paradigm [3-4], the protists are not in competition with the bacteria, but *de facto* farm them to acquire nutrients (Fig. 3). The consequential dynamics of the growth of the phototrophic protist ('phytoplankton' in the traditional scenario; 'mixoplankton' under the mixoplankton paradigm) differs greatly (Fig. 3),

Labelling HAB species as mixoplankton, or otherwise, has obvious important consequences for how we consider factors affecting the growth and demise of these organisms. It also then affects how those responsible for monitoring water quality and ecosystem services may view the ecosystem. In short, assuming the activity of HAB species aligns with 'phytoplankton' needing light and inorganic nutrients, can be considered for many if not most species as inappropriate. That *Chrysochromulina* is a mixoplankton, and grows to such perfusion around salmon farms [11] is perhaps no coincidence.

For more about mixoplankton, please see the article about the recent Mixoplankton International Conference in this edition of HAN, and also visit *www.mixotroph.org*

Acknowledgements

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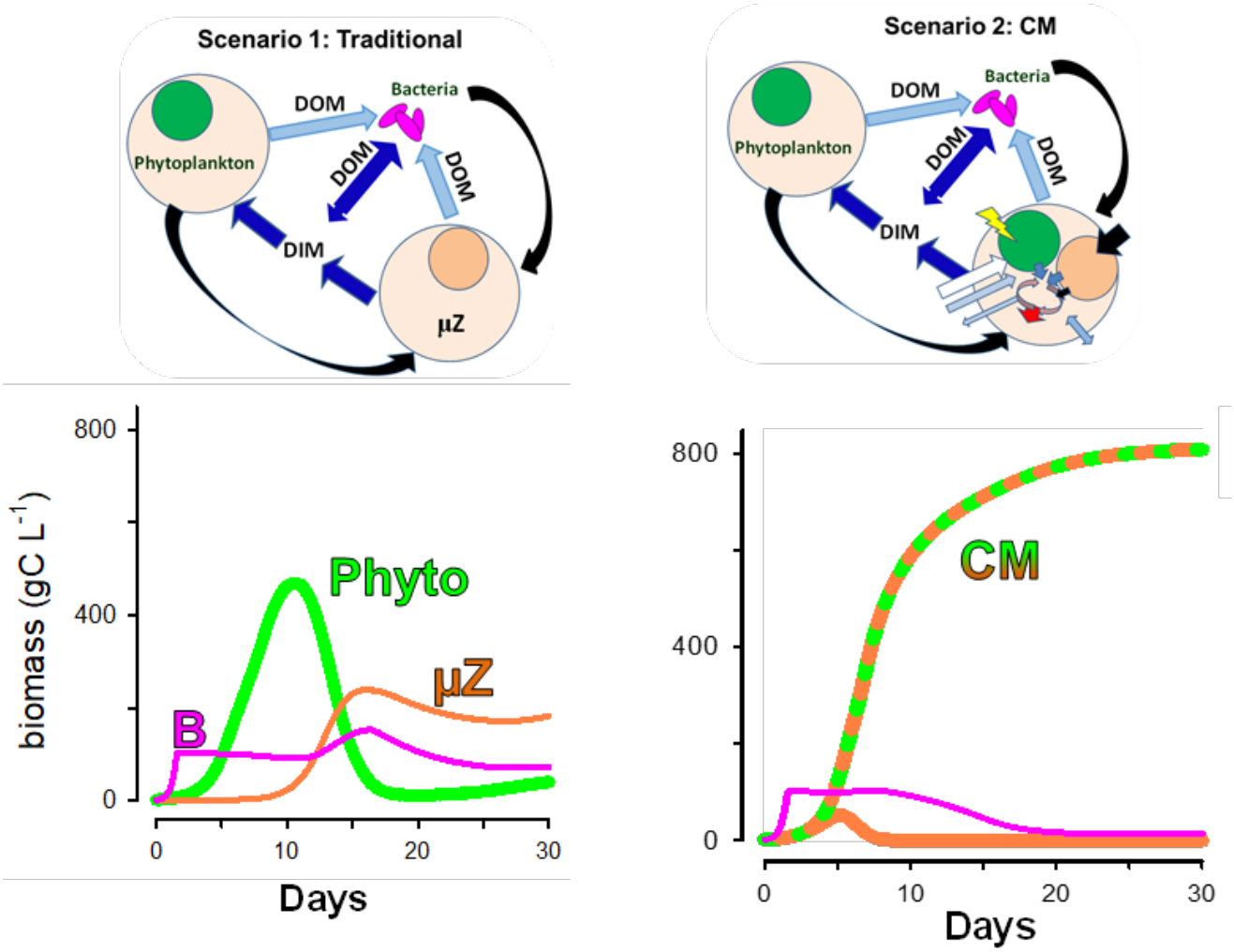

Fig. 3. Schematics and model simulation outputs run under the traditional paradigm (left) versus the mixoplankton paradigm (right). See text for explanation. B – bacteria; Phyto – phytoplankton (non-phagotrophic phototroph); µZ – protozooplankton; CM – constitutive mixoplankton (photo-phago-trophic); DIM – dissolved inorganic matter (nutrients); DOM – dissolved organic matter. Adapted from [3]

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Tiny cells with a big impact: An unexpected bloom in the mid-Atlantic

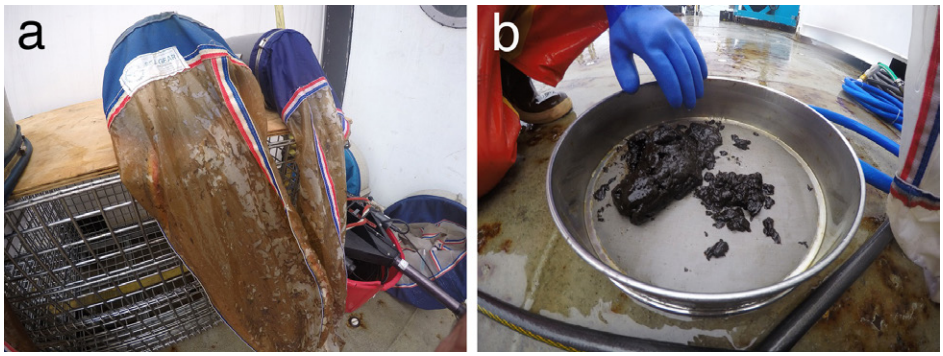

Fig. 1. a) Bongo nets fouled with the brown mucilaginous plankton. b) Dark and gelatinous content of the plankton nets scraped into a sample tray.

Since 1992, the US NOAA Ecosystem Monitoring (EcoMon) cruises survey the Northeast U.S. Continental Shelf between 36° N and 41° N three to four times a year. Plankton and larval fish are sampled along with physical and chemical oceanographic parameters. During November 2018, the cruise encountered an unusual massive bloom of mucilaginous plankton that completely clogged 165-µm and 335-µm zooplankton nets with a puzzling dark brown slimy substance (Fig. 1). The plankton nets were extensively fouled at 16 of the 60 stations, predominantly north of 40° N (Fig. 2). The fouled double-oblique Bongo plankton tows ranged in depth from the surface to ~ 5 m from the bottom, i.e. between 26 and 57 m, depending on the station (CTD depths). With no microscope on board to observe fresh material, some of the brown slime was scraped from the nets into 95% ethanol, which changed it to a dark green color.

Upon return to onshore laboratories, identification of the material by light microscopy remained somewhat elusive, although the super abundant ~7.5-µm cells hinted at the presence of either a diatom or the prymnesiophyte *Phaeocystis*. Aliquots of the preserved material from station 60 were digested in H_2O_2 and prepared for scanning electron microscopy (SEM).

We identified the tiny cells enmeshed in chitan threads as *Thalassiosira mala* [1]: a single strutted process (fultoportula) offset from the center of the valve; a ring of marginal strutted processes 1 to 1.5 µm apart from each other; a single labiate process (rimoportula) located within the ring of marginal strutted processes; and 25 to 30 areolae in 10 µm (Fig. 3). Copious chitan threads emanate from the ring of marginal strutted processes and are responsible for creating the mucilaginous consistency of the colonies; the threads were markedly obvious on micrographs (Fig. 4). Additional morphological features of this taxon have been previously described [2-3]. Subsequent review of data at 3 m from the Imaging FlowCytobot (IFCB) onboard the EcoMon vessel indeed revealed the presence of numerous colonies as well as single cells of what we can now identify as *T. mala* (Fig. 5) [4]

Because of its small size, this diatom is often misidentified or goes unnoticed by light microscopy; it is however easily detected when intense blooms form massive gelatinous colonies, such as during the widespread event of the EcoMon 2018 cruise. The species is considered cosmopolitan within temperate and tropical waters [3,5] its presence in the mid-Atlantic region is not unusual [5]. It is observed in waters closer to the coast, such as in Narragansett Bay (41° 34' N, 71° 23' W), so far at non-bloom densities [6] (Figure 6).

Given that the EcoMon crew did not visually notice any discoloration of the surface water at the time, one can surmise either that a bloom occurred at depth or that the colonies were abundant and somewhat dispersed through the water column. Vertical profiles of temperatures and salinities indicated generally well-mixed water columns, thus may suggest colony dispersal at most stations rather than thin layers of concentrated materials at depths. For example, at station 56, chlorophyll concentrations were nearly constant at 2.4, 2.4 and 2.1 µg L^{-1} at 2, 20 and 30 m, respectively (in situ fluorometry) over a very slight sigma-t difference of 0.001 unit. However, at station 60 where the heaviest fouling occurred, the chlorophyll concentrations were

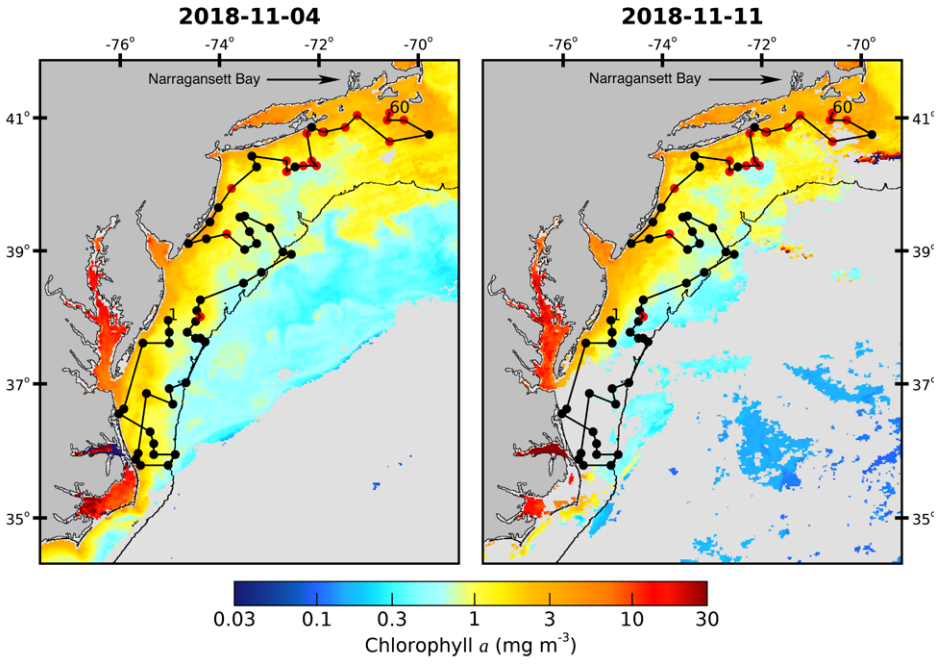

Fig 2. Composite maps of chlorophyll a concentration over a range of 0.03 to 30 µg L^{-1} from the MODIS-Aqua, Suomi-NPP-VIIRS and NOAA20-VIIRS satellite sensors: Nov 3-5 (left panel) and Nov 10-12 (right panel) 2018 during the time of the EcoMon cruise. Sampling on the R/V Sharp took place November 2-12, 2018. Dots on top of the cruise track indicate sampling stations; red dots specify stations with extensive fouling of the nets. The cruise ended at station 60. The thin topographic grey line represents the 100-m depth contour.

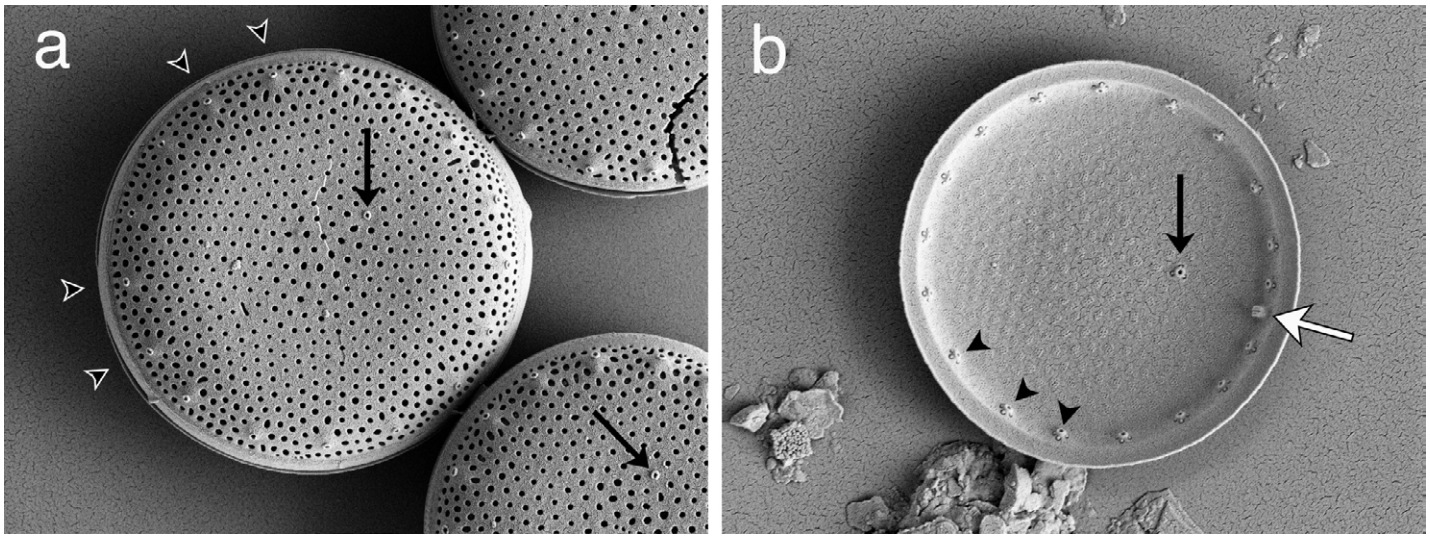

Fig. 3. Scanning electron micrographs of frustules in valve view of Thalassiosira mala. Note the single eccentric strutted process (black arrow), the ring of marginal strutted processes (arrowheads) and the single labiate process (white arrow) located within the ring of marginal strutted processes seen on the outside (a) and inside (b) of the frustule. 3a EcoMon Station 60, November 12, 2018; 3b Narragansett Bay December 10, 2018.

5.2, 4.9, and 9.3 µg L^{-1} at 2, 20 and 30 m (over a 0.0175 sigma-t unit), respectively, possibly suggesting a deep somewhat denser bloom. These November observations may thus document a settling or settled later stage of a bloom. We compared ship-based observations to remote sensing data for this time (Fig. 2). Satellite imagery shows coastal chlorophyll-*a* concentrations of up to 3 mg m^{-3} in the area of the survey where the heaviest fouling of nets and instruments occurred.

This *T. mala* bloom may have been more widespread, and of longer duration than the EcoMon data can document. Independently, and a few weeks earlier, an observer on a pelagic monitoring vessel noted the daily presence of conspicuous 'brown clouds' on the surface of the sea off the coast of Delaware (~39° N), sometimes in large patches (Fig. 7, September 29, 2018) (S. McConnell, pers. comm.). Unfortunately, no samples were taken, thus we must stress that although it seems likely, we can only speculate that this may have been an earlier surface expression of the widespread regional *T. mala* bloom, which by the time of the EcoMon cruise had settled to depth or been dispersed.

Returning to Narragansett Bay, observations on local plankton help to document the extent of the presence of *T. mala* in Northeast U.S. coastal waters. The taxon was occasionally reported from various locations from September to December 2018, but always at low densities: water samples and plankton tows (our own samples and Dr. D. Borkman, RI Department of Environmental Management, pers. comm.), and the local IFCB ([7]). The morphology of living cells and colonies from Narragansett Bay was documented with light microscopy (Figure 6) and identification was confirmed with SEM (Fig. 3b).

Intense blooms of *T. mala* mucilaginous colonies can impair the growth of filter-feeding shellfish, primarily documented in bivalves and suspected of affecting efficient gill function [8]. The deleterious mode of action on shellfish is thus likely physical in nature rather than chemical, this tiny harmful alga is not believed to produce a toxin. We know of no reports of harmful consequences outside of coastal events; specific intense offshore blooms are not likely to be investigated, unless by accident, such as during the EcoMon cruise. No adverse effects on cultured or wild shellfish were reported for Narragansett Bay.

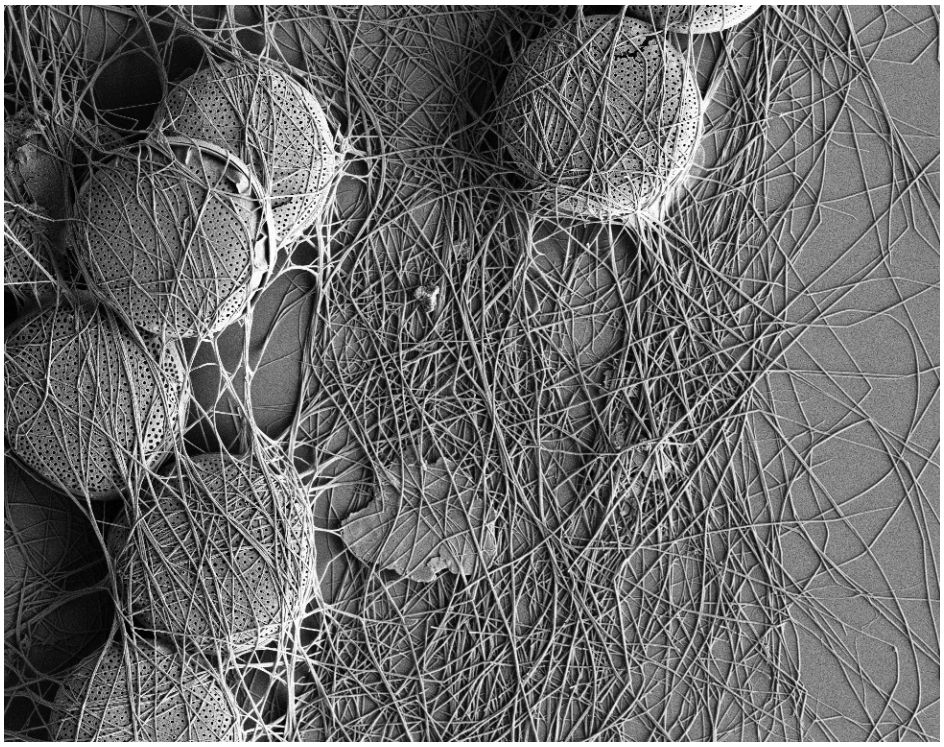

Fig. 4. Thalassiosira mala frustules enmeshed in chitan threads. EcoMon Station 60, November 12, 2018.

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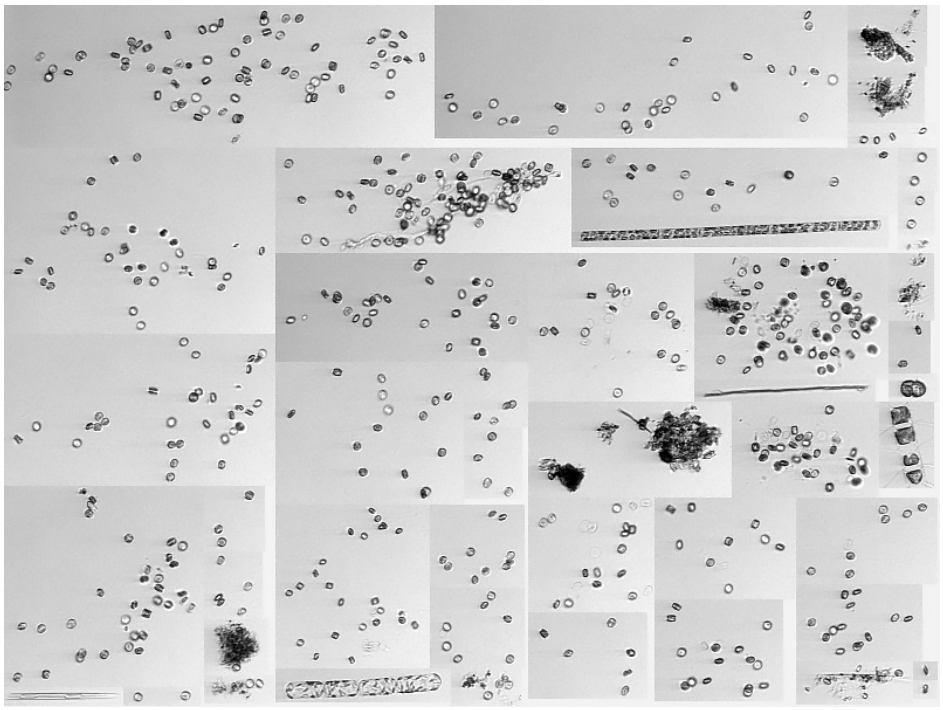

Fig. 5. Live Thalassiosira mala as viewed with the continuous Imaging Flow Cytobot aboard the R/V Sharp during the EcoMon 2018 cruise, near station 45 at 3 m, November 11, 2018 (https://ifcb-data.whoi.edu/timeline?dataset=NESLTER_broadscale&bin=D20181111T094425_IFCB127)

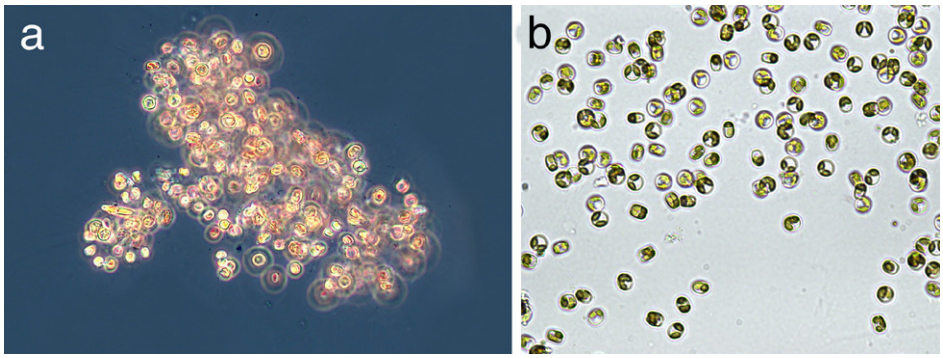

Fig. 6. Live Thalassiosira mala a) in dense mucilaginous colonies (phase contrast), and b) as loosely associated cells where two chloroplasts per cell are clearly seen (bright field). Photographs from Narragansett Bay; identification confirmed by SEM (Fig. 3b). Note that as a diatom, T. mala appears round in valve view and rectangular in girdle view, which is clearly seen here, indicating that these images could not depict Phaeocystis, whose cells appear irregularly spherical, with no rectangular profiles.



Fig. 7. Surface patches of a dense phytoplankton bloom several miles off the coast of Delaware on September 29, 2018. Photo credit S. McConnell.

First report of an *Ansanella granifera* bloom in Cuban waters, Caribbean region

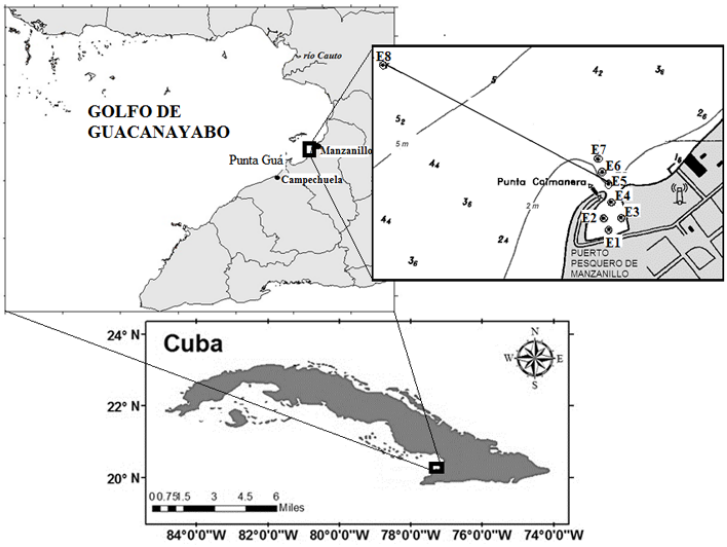

Fig. 1. Map of the study area showing the location where the dinoflagellate bloom occurred in southeastern Cuba.

Harmful Algal Blooms (HABs) have been associated with fish and shellfish kills, ecosystem damage, human health impacts, and significant economic losses to the aquaculture and tourist industries throughout the world. The increase of HABs in recent years is generally related to direct and indirect anthropogenic activities (e.g. eutrophication, climate change)[1].

In August 2018, an intense red water discoloration was observed in the Fishing Port of Manzanillo city, southeastern Cuba (20°19.891'N and 077°09.350'W) (Fig. 1). The discoloration was caused by a small dinoflagellate later identified by genetic sequencing as *Ansanella granifera*. The bloom, located near the coast, occupied an area of about 1.85 km^2 inside the Port and its adjacent waters, and was large enough to be detected by satellite imagery (Fig. 2).

Surface water samples (0.30 m depth) were collected with a Van Dorn bottle for phytoplankton and nutrient analysis ($N\text{-}NO_3^-$, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$). Water temperature, salinity and dissolved oxygen were measured in-situ with a multi-parameter sonde (HI 9828, Hanna Instruments, Inc., USA) at eight sampling stations (Fig. 2). Phytoplankton samples were preserved with acidic Lugol's solution and analyzed with a Zeiss Axiovert 40 inverted microscope (Zeiss, Oberkochen, Germany) using a Sedgwick–Rafter counting chamber. Micrographs were taken with a digital camera (Canon ELH 135) and cell length and width measurements collected from 20 individuals. For Scanning Electron Microscopy (SEM) examination of the bloom sample, cells were filtered on a 3 µm polycarbonate filter, desalted with a 10% step gradient of seawater to freshwater and dehydrated using a 10% step gradient of freshwater to ethanol followed by 100% hexamethyldisilazane (HMDS). The resulting dehydrated sample was placed on an aluminum stub using double stick tape and sputter coated with gold-platinum using a Denton Vacuum Desk II Sputter Unit prior to examination with a JEOL 5600LV SEM.

Individual cells were isolated with microcapillary pipettes, and placed into 25 µL of Chelex solution (InstaGene™ Matrix; Bio-Rad, Hercules, California, USA). Single cell DNA extractions were performed following protocols adapted from Richlen & Barber (2005) [2], as outlined in Gómez et al (2017) [3]. The D1-D3 domains of the LSU rRNA gene were amplified using primers D1R and D2C [4], and PCR products were visualized, cloned, and sequenced as described in Gómez et al (2017) [3]. DNA sequences were analyzed using Basic Local Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi) against databases in GenBank. The most closely related sequences (99.08-99.85% identity), as determined using BLAST searches, were those of *Ansanella granifera* (GenBank Accession no. HG792066.1). The newly generated consensus sequences were deposited in DDBJ/EMBL/GenBank under accession numbers MW698929-MW698931.

Ansanella granifera occurred in a nearly monospecific bloom at a maximum concentration of 2.16×10^8 cells L^{-1}. Other dinoflagellates (*Alexandrium* sp., *Blixaea quinquecornis*, *Scrippsiella trochoidea*) and diatoms such as *Cylindrotheca closterium*, *Hemiaulus hauckii, Nitzschia longissima*, *Skeletonema* sp. were found at very low cell densities.

Ansanella granifera cells were pentagonal to oval in shape; the episome was conical with a round apex, and larger than the trapezoidal hyposome. The nucleus was oval and located in the anterior to central part of the cell. A bright red eyespot was located near the sulcus. Cell length 9.70–13.60 µm (average 10.75 ± 1.11, n=20); cell width 8.0–12.5 µm (average 9.11±1.22, n=20) (Figs. 3,



Fig. 2. A. Satellite image of the dinoflagellate bloom along the coastline of the Fishing Port, Manzanillo city, Cuba. B. Red water discoloration due to the bloom.

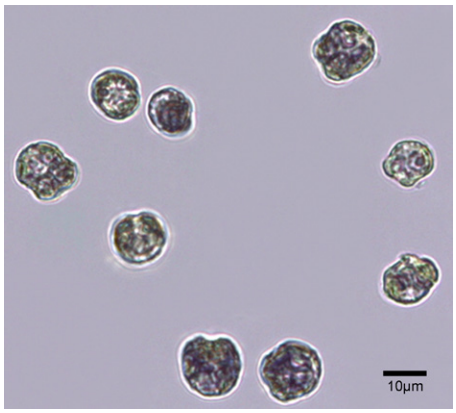

Fig. 3. Light microscopy images of fixed cells of Ansanella granifera.

4). *Ansanella granifera* is a dinoflagellate belonging to the family Suessiaceae (order Suessiales) that was recently described from Korea [5]. To our knowledge, the occurrence in waters from southeastern Cuba represents the first record outside its type-locality, including the first report of the taxon for the Caribbean region, for the entire Western Atlantic, as well as the first record as bloom forming species worldwide.

The application of molecular methods is particularly valuable for small dinoflagellates (e.g. "gymnodinioid forms") of ambiguous taxonomy like *Ansanella granifera* (very similar to *Gymnodinium* species). They are nanoplankton sized forms that cannot be properly identified in optical microscopy examinations. Recently, a *Gymnodinium* species (*G. natalense*) has been transferred to the genus *Ansanella* (*A. natalensis*) by combining morphological and genetic studies [6].

When the event occurred, weather conditions were favorable for bloom formation in southeastern Cuba, with high water temperature (30.66 ^{o}C) and salinity (37.44 psu); and ammonium concentration ($NH4^{+}$) was also moderately high (0.20 mg/L). This level of ammonium could be due to organic nutrient input linked to discharges in the coastal zone from the wastewater treatment plant of the nearby local fishery industry. In spite of its location in the open sea, symptoms of eutrophication such as high values of chemical oxygen demand (COD) (average of 5.31 mg/L) and concentrations of oxygen below 5 mg/L (average of 4.76 mg/L) were recorded along the coastline of the study area (Fishing Port and adjacent waters), an area that is characterized by organic rich sediments. Further work is needed to determine the composition and distribution of dinoflagellate cysts from sediments of this coastal area to determine the historical occurrence of bloom-forming species.

Many dinoflagellates are better adapted to use ammonium and other organic nitrogen forms like urea, in comparison with diatoms which are nitrate specialists [7, 8]. *Ansanella granifera*, similar to other red tide dinoflagellates, is also a mixotrophic species that is capable of photosynthesis and acquiring nutrients in pre-packaged or particulate form (including heterotrophic bacteria and other small photosynthetic microalgae) together [9]. Mixotrophy is a competitive advantage for many dinoflagellate species that allows them to dominate in the ocean. Mixotrophic species are responsible for ~ 40% of the species forming red tides globally [10, 11]. *Ansanella granifera* is one of the fastest growing mixotrophic dinoflagellates reported to date. Mixotrophic dinoflagellates can increase their populations by migrating between well-lit surface and eutrophic bottom waters [6, 12].

Eutrophication processes associated with nitrogen loading in the coastal zone have resulted in algal blooms, fish kills, altered trophic interactions, and oxygen depletion, and have caused other environmental problems in different coastal regions around the world [1, 13]. Dinoflagellate blooms including toxic species have also been reported near areas of sewage in semi-enclosed bays from Cuba [14, 15].

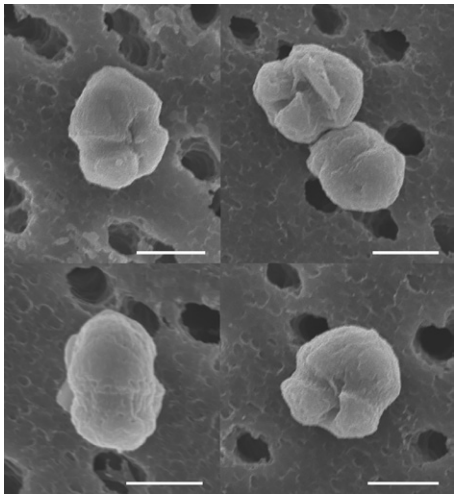

Fig. 4. Scanning electron micrographs of Ansanella granifera cells from field samples. Scale bar = 5 µm.

Acknowledgements

This work was supported by CiguaPire (NSF OISE Award # 1743802), which facilitated collaboration among researchers from USA and Cuba, leading to studies and training on HABs. Additional support was provided by the Greater Caribbean Center for Ciguatera Research (NIH 1P01ES028949- 01 and NSF 1841811). We thank Evie Fachon for her valuable technical and laboratory assistance.

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Dolichospermum spiroides blooms in a man-made lake in Sarawak, Borneo

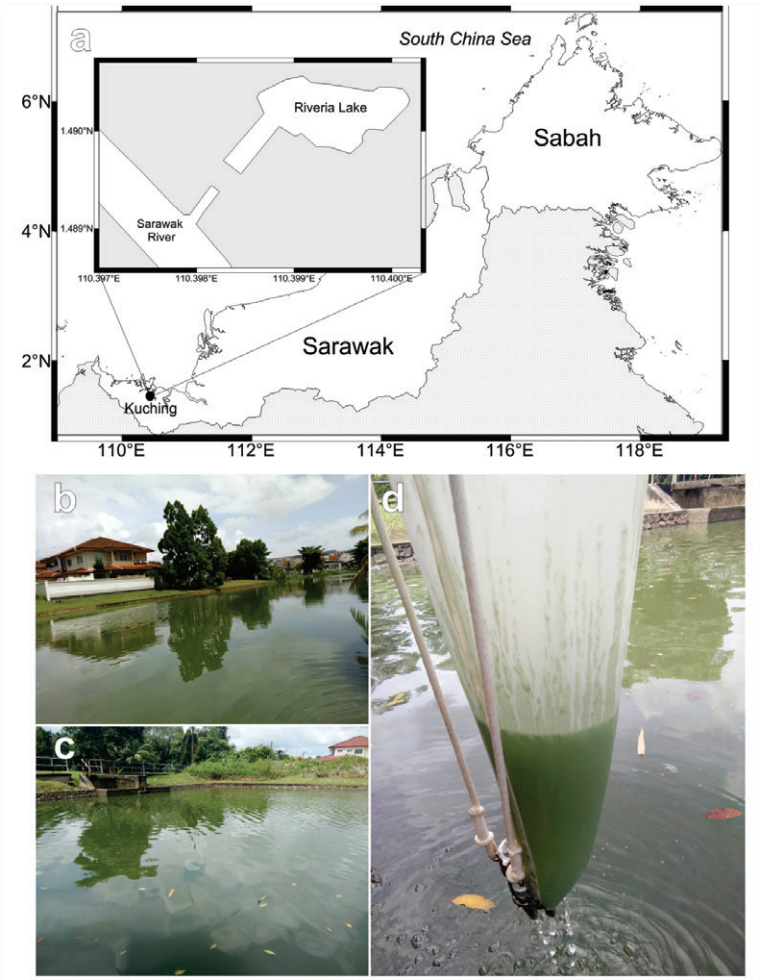

Fig. 1. (a) Map of Malaysia Borneo showing the bloom location at the man-made lake in Kota Samarahan, Sarawak. (b, c) Green water discoloration detected at the study site. (d) Concentrated plankton net haul samples.

Dolichospermum (formerly *Anabaena*) is a cyanobacterial genus that includes toxic bloom forming species [1]. Species of *Dolichospermum* are free-living cyanobacteria found in lentic water bodies such as freshwater lakes, ponds, reservoirs as well as brackish waters [2].

In this study, we report a water discoloration caused by a massive bloom of a filamentous heterocyst-forming cyanobacteria, between February and March 2020. The discoloration, with an extension of approximately 2.22 acres, was formed in a lake at the center of a residential area and connected to the Sarawak River in Borneo (Fig. 1). The cyanobacteria was identified as *Dolichopermum spiroides* using light microscopy (Fig. 2) and reached densities of 1.21 to 1.25×10^6 cells L^{-1}. Cells were filamentous and often curly. Trichomes were surrounded by a mucilaginous envelope.

To date there are a very limited number of studies of cyanobacteria [3,4,5] in Sarawak. In Malaysia, a bloom of *Dolichospermum* (reported as *Anabaena*) was recorded in a freshwater fish pond in Serian, Sarawak, and co-existed with a *Microcystis* bloom. However, the species and cell density for both genera were not recorded [3].

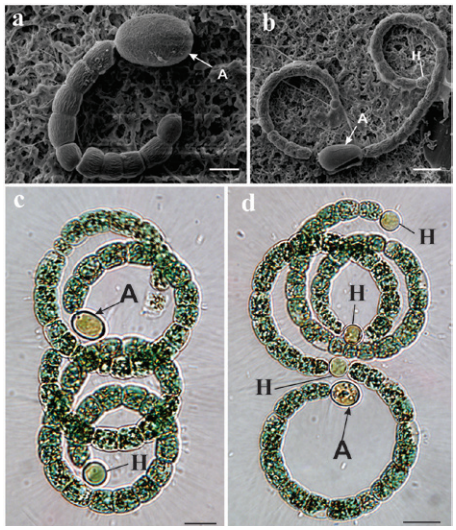

Fig. 2. SEM (a, b) and LM (c, d). A: Akinete, H: Heterocyst of Dolichospermum spiroides

This is the first documented report of *D. spiroides* in Sarawak waters. The occurrence of *Dolichospermum* blooms encouraged the implementation of monitoring the freshwater system to avoid any intoxications by cyanotoxins. Fortunately, there were no human health problems as the lake water is not used for drinking, however there are some recreational activities, such as fishing and kayaking which may be affected in the future.

Acknowledgements

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An online platform (GEE App) for Trophic State Index monitoring of inland waters in Latin America

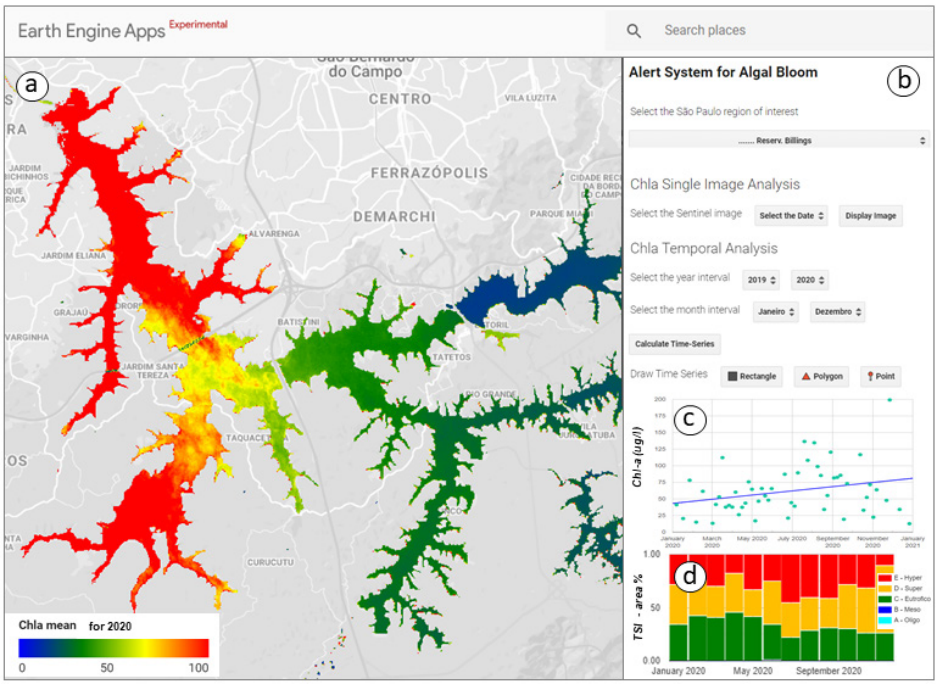

Fig. 1. a) The dark gray region shows the Paraná River Basin in Brazil; b) Water masses within Paraná River Basin palette according to the Chl-a concentration average for 2020. The red rectangle indicates the Billings Reservoir taken as an example for the GEE App (see Fig. 2).

Human activities on a global scale have significantly contributed to the change in quality of water bodies caused by increasing nutrients levels. In these circumstances, algae have proliferated rapidly and their concentrations are above normal values. The lack of *in situ* data in Latin American countries limits the capacity of water quality management due to the paucity of information about the current status of surface waters. In this scenario, remote sensing and cloud computing techniques are fundamental tools to deliver precise and quick indicators of algal bloom (AB) occurrence and Trophic Levels over large areas, supporting decision-makers and management actions.

Last year, in collaboration with several researchers, a project to develop a tool that maps ABs over the main water bodies and reservoirs in Latin America was approved by Google Earth Engine (GEE) [1] and Earth Observation Data Science (EO) [2]. So far, the proposed methodology uses Sentinel-2 images corrected for atmospheric and sun-glint effects to generate an image collection of the Normalized Difference Chlorophyll-a Index (NDCI) [3] for the entire time-series (August 2015 to present) of a given area. The NDCI retrieved from the imagery are compared with chl-a measured *in situ* with a time window of ± 2 days for match-ups. NDCI is used to estimate chl-a concentration by applying a non-linear fitting model, and is also used to classify every pixel into 5 classes of Trophic State Index (Oligo, Meso, Eutrophic, Super and Hypereutrophic) [4] based on a tree-decision model. Once the approach is developed and validated using pilot sites, the method will be transferred to stakeholders at several levels to provide AB information for the main water bodies/reservoirs in Latin America.

In this article, we report the first results for the Tiete River Basin, one of the Paraná River Basin's main tributaries, located in the State of São Paulo, Brazil (Fig. 1). Water in large urban areas in São Paulo is supplied by a variety of reservoirs. The rapid urbanization and industrialization process led to high nitrogen and phosphorus concentrations in surface waters and consequently degradation of water quality and eutrophication. For example, the Billings reservoir, which is located in the upstream Tietê basin, has faced serious water pollution problems due to the expansion of urban slums with no sewage or solid waste collection system. As a result, high chl-a and TSI levels were often observed throughout the past year (Fig. 2).

The use of cloud computing (GEE) to process all Sentinel-2 imagery and generate the NDCI collection, allows for quick access to the chl-a and TSI (Trophic State Index) levels for any water body within the study area (Paraná River Basin, Fig. 1), which includes several reservoirs and dams, such as Itaipú, Três Marias, Cantareira System and others.

More importantly, all of this information will be freely accessible for the general public via an Earth Engine APP (Fig. 2) that allows personalized evaluation of a given water mass that can either be chosen from a list or drawn by the user on the map canvas. The APP is currently in development and soon will be released in the GEE APP gallery. The user will be able to define date range, ROI (Region of Interest), time-series charts (either NDCI or Chl-a) and TSI area charts (% class area), and save these plots as texts or image formats.

For the project's following activities, we plan to improve the APP and release it on-line within the following weeks. We will also extend the NDCI collection to other important South America regions, such as Uruguay, Argentina, and North-eastern Brazil. However, this extention depends on the availability

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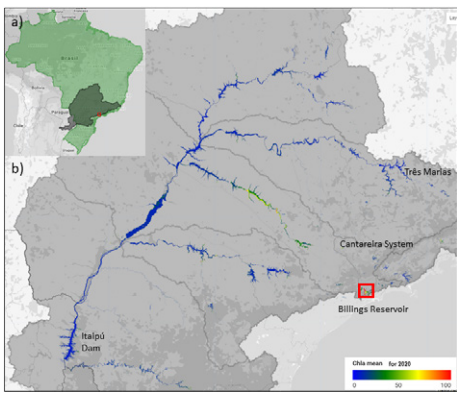

Fig. 2. Screen grab of the current version of the experimental GEE App. a) Display of maps and classifications; b) User interface, where the user can pick date range, ROI and select the products (NDCI, Chl-a and/or TSI) to be displayed as charts (c and d).

Remote sensing of recurrent cyano-HABs in Patos Lagoon, Brazil

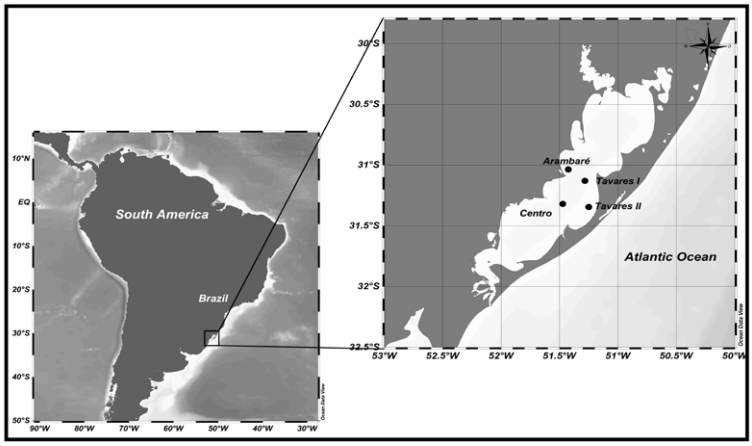

Fig. 1. Map of Patos Lagoon (southernmost part of Brazil) taken from [7]. Black circles indicate the four sites chosen forNDCI values retrieval [4].

Every austral summer, dense surface growth and accumulations of cyanobacteria threaten public health and local economic and leisure activities in the Patos Lagoon system (PL) in the southernmost part of Brazil (Fig. 1) [1]. *Microcystis* and *Dolichospermum* species can be the main components of these cyanobacterial harmful blooms (cyanoHABs). *Microcystis* species produce microcystins and *Dolichospermum* species, saxitoxins [2,3]. Due to the great spatial coverage of PL by these blooms, *in situ* studies and monitoring programs for cyanoHABs are major tasks that require resources that are not always available.

We are conducting studies using relatively new tools, remote sensing and modeling, that can jointly address two important questions: 1) To what extent can the spatial and temporal distribution pattern of cyanoHABs be better understood? 2) Are distribution patterns being modulated by climate change? We used an index of phytoplankton biomass derived from Sentinel-2 images, i.e., the Normalized Difference Chl-*a* Index (NDCI) [4], for the central part of PL spanning July 2019 to June 2020 (Fig. 1; see all four sites chosen for retrieving NDCI and water temperature values). NDCI, where values greater than 0 imply bloom occurrence, is based on the use of the 708 nm and 655 nm bands combined to evaluate bloom status in the water. We observed two cyanoHAB events during the summer of 2019–2020: one on the east (Tavares town) on December 30th 2019 (Fig. 2) and the other on the west margin (Arambaré town) on January 10th 2020 (Fig. 3). To correlate meteorological data with the NDCI index, two meteorological stations were considered: Mostarda's for the Tavares and Camaquãfor the Arambaré bloom events, both for the same time period. In addition, a 30-year meteorological time series of data assembled from the literature was retrieved with a closer grid point for the two locations [5]. The available information included rainfall (1980–2015), air temperature (1980–2013) as well as wind speed and direction for the summer 2019–2020. This approach helped us to classify the years 2019 and 2020 within a changing climate scenario. Lastly, water temperature values for 2019-2020 were obtained using MODIS (Moderate Resolution Imaging Spectroradiometer)-Terra imagery. Preliminary findings showed strong winds (>6 ms^{-1}) were recorded before the observed blooms, followed by relatively weak winds (<<6 ms^{-1}) during the bloom days. Thus, calm winds would have allowed the growth and surface accumulation of phytoplankton cells (Fig. 4). NDCI peaks coincided with the highest water temperature values, confirming an expected correlation between high temperatures and exponential cyanobacterial growth, especially near the margin of Tavares town (Fig. 5) [3,6]. We concluded that in a climate change context, summer 2019–2020 was drier in comparison to the historical mean, in particular during November-December 2019 and February 2020. Meanwhile, Tavares' region showed a negative (-100-mm) anomaly in cumulative rainfall during the summer. Currently we can state that relatively weak winds and low rainfall, especially in Tavares (highest NDCI values) contribute to bloom formation. This is possibly due to the increase of water residence time within PL, a situ-

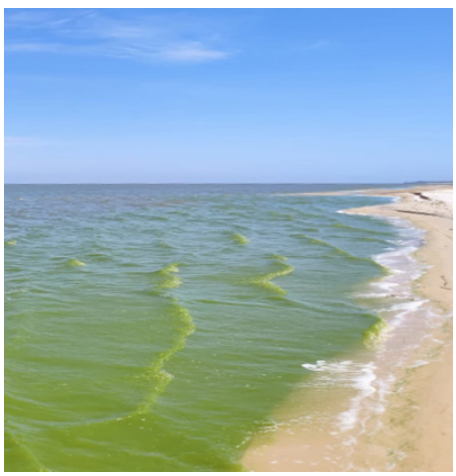

Fig. 2. Bloom on the Tavares' margin, on December 30th 2019 (Source: a local, anonymous inhabitant).

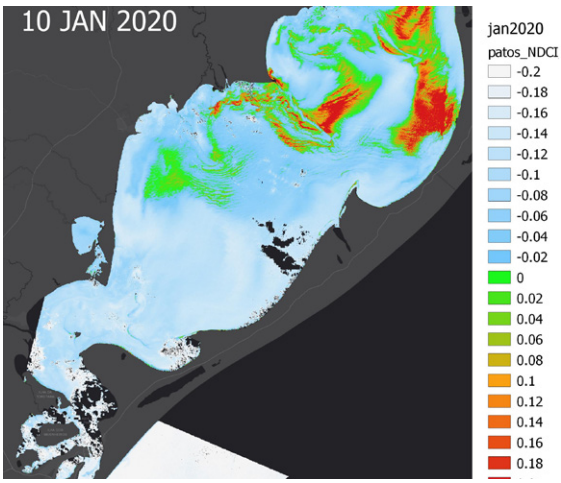

Fig. 3. Sentinel-2 image on January 10th 2020 depicting NDCI features across the central part of the PL system, particularly near the west margin, including Arambaré town (see Fig. 1). Unfortunately, there was no image available for December 30th 2019.

ation promoting the prevalence and duration of cyanoHABs.

More detailed information will be published soon adding modeling tools to locate dominant cyanoHAB accumulation sites within the PL, and their potential exportation to the ocean. Future studies are needed to discriminate between local effects from agricultural inputs near the PL's margins over the recurring appearance of cyanobacterial blooms, transport and advection processes within the PL and the modulation associated with climate change effects.

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We are grateful to the Programa de Pós-graduação em Oceanologia for the MSc. fellowship to B. Canever. M. de Souza is granted a PNPD-CAPES fellowship at the Programa de Pós-graduação em Oceanografia Biológica (FURG). This short communication is endorsed by the GlobalHAB/IOC-SCOR and the Pesquisador Gaúcho Research Grant from FAPERGS-RS-Brazil. Last but not least, we thank the teacher William Marques (*in memoriam*) for his comments and suggestions.

Table 1. 30-year average mean and standard deviation (1980–2015; retrieved from [5]) and monthly rainfall. The monthly values were retrieved from the Camaquã's and Mostardas' meteorological stations for 2019 and up to June 2020. Note the monthly values highlighted in italics and bold denoting that almost all months since November 2019 were drier than their respective historical average.

	30-year average rainfall		Camaquã's station		Mostardas' station	
Month	Mean	Standard deviation	2019	2020	2019	2020
January	76.5	52.2	139.2	155.6	83.6	85.4
February	88.1	49.0	***70.8***	***35.4***	***50.4***	***19.8***
March	90.9	59.0	***81.2***	***44.8***	-	***27.4***
April	98.9	62.3	***78.6***	***48.2***	-	***28.6***
May	115.5	69.1	278.4	117.6	-	0.00
June	123.1	65.4	41.2	165.2	47.6	25.2
July	137.8	80.4	179.8		137	
August	114.5	70.1	155		131	
September	133.8	65.1	108.8		103.8	
October	120.5	79.0	127.8		238.2	
November	80.6	56.4	***44.6***		***59.2***	
December	85.8	64.5	***76.6***		***19.2***	

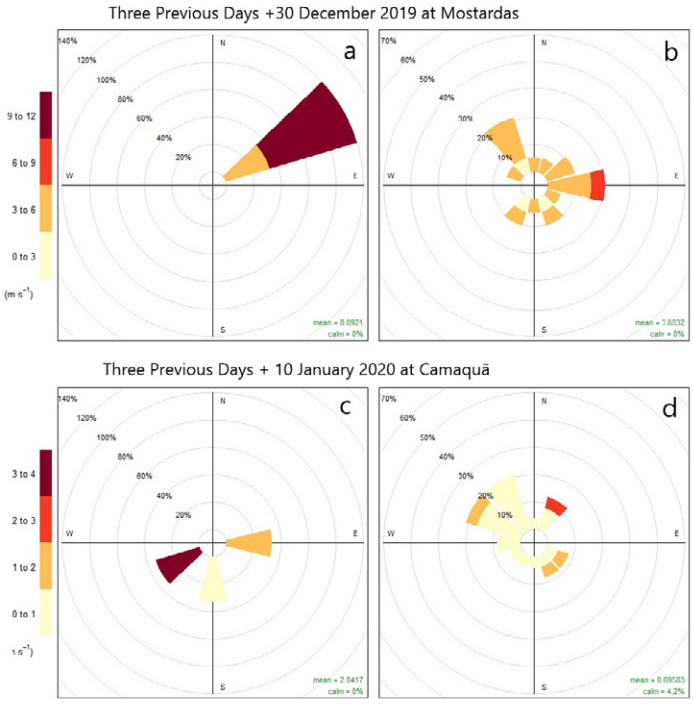

Fig. 4. Frequency of occurrence of wind direction and intensity by wind roses for the three previous days (a,c) and highest NDCI value days (b,d) collected from the Mostardas and Camaquã meteorological stations, respectively. Note the different scales adopted for each corresponding meteorological data, with higher values obtained at Mostardas (>6 ms^{-1}) whereas Camaquã gave values lower than 6 ms^{-1}.

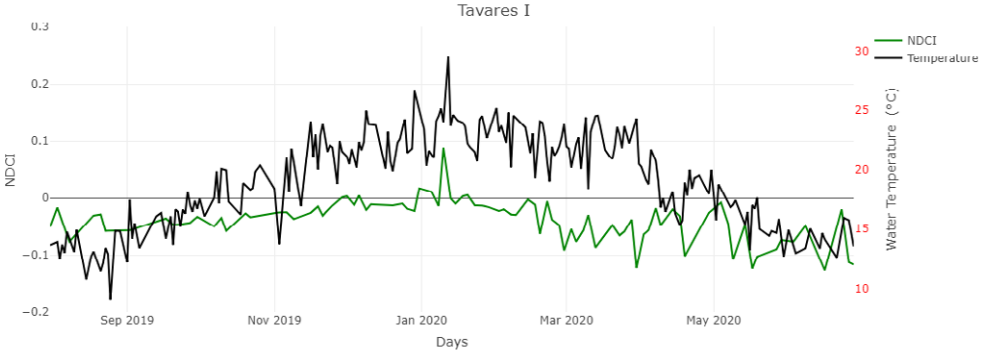

Fig. 5. Water temperature (black line) and NDCI values (green line) for one of four sites, Tavares I (see also Fig. 1), spanning July 2019 to June 2020.

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Blooms of *Akashiwo sanguinea* (Dinophyceae) in a tropical estuary in northeastern Brazil

We report an inter-annual bloom of the unarmored dinoflagellate *Akashiwo sanguinea* in a pristine estuary (Figure 1F) in Brazil. The estuarine section of the Serinhaém River, Camamu Bay is a species-rich ecosystem located in the state of Bahia (Northeastern Brazil, Figure 1G), comprising preserved mangrove vegetation and remaining fragments of Atlantic Forest. It is an important regional center of economic activities based on coastal tourism and fishing. We sampled ten areas along a 30 km transect starting downstream in March 2013 and April 2014 during the rainy period (260 mm), and October 2013 and November 2014, during the dry period (235 mm). Water and surface sediment samples were collected along a salinity gradient, stored in plastic bottles and preserved in Lugol's solution and formaldehyde. Cell counts were made following the Utermöhl method with 5 ml of material observed using an inverted microscope Olympus CKX 41. Linear dimensions, such as length, width, and height were measured under 400x magnification prior to calculating biovolume by geometric shape.

Based on discontinuities in spatial variability, the estuary was divided into three sections (Figure 1E) according to Ward agglomerative clustering of log transformed biovolume: (I) the four downstream sites (SE1–SE4) with high values of salinity, mainly in the dry seasons, high pH, and where *A. sanguinea* did not occur; (II) the three intermediate sites (SE5–SE7) where salinity varied from ~17 to ~28 and *A. sanguinea* biovolume varied from intermediate values in 2013 to high values in the rainy season of 2014, along with local extinction during the dry season in this same year; and (III) the three upstream sites (SE8–SE10), which formed a group due to remarkable enhanced abundances of *A. sanguinea*, reaching up to 2 million cells L^{-1}, as well as enhanced environmental heterogeneity (variability in nutrient and hydrological conditions). The highest biovolumes observed in the recent study were during the first dry season, specifically at the warm, stratified upstream conditions occurred during October/November (Figure 1C).

Bloom events occur in response to specific environmental conditions, such as high nutrient concentrations and low turbulence [1]. These constraints partly explain the high accumulation of *A. sanguinea* during the first dry season, when stratification from reduced turbulence created vertical gradients of nutrients and light [2]. Under these conditions, *A. sanguinea* is able to vertically migrate in spiral movements and overcome the vertical displacement of nutrients, optimizing its resource exploitation and generating advantages over non-motile taxa. The local source of nutrients or disturbance also explains high accumulations upstream, since SE10 is the site nearest to the town of Ituberá (68 meters), where the river receives an average effluent discharge of 18.08 m^3 s^{-1}. It has been demonstrated that potentially harmful algae blooms (among them *A. sanguinea*) can be spatially correlated with effluent discharge gradients [3]. On another level, a disturbance source

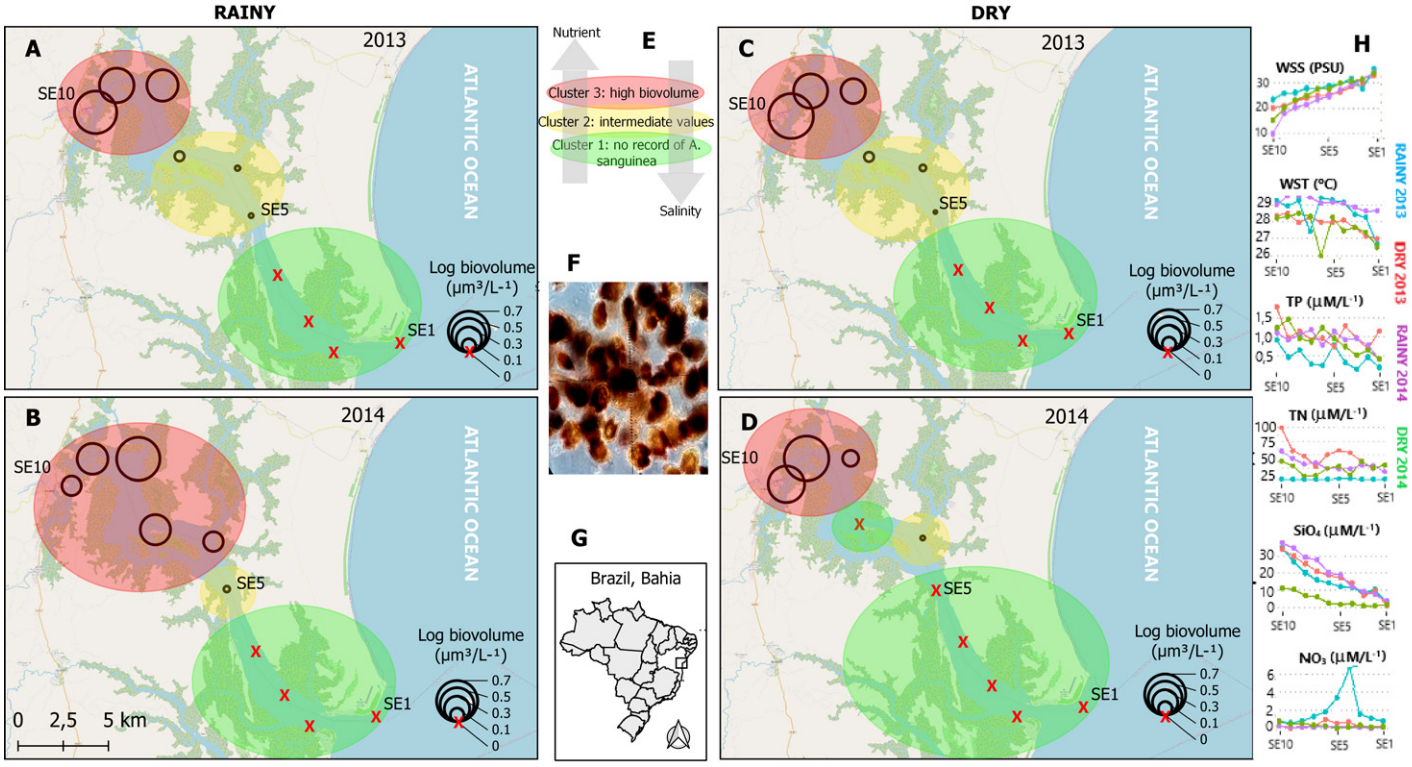

Figure 1. Map showing (G) the geographic location of the study area in Brazil, (A-D) the sampled sites along the Serinhaém River, (E) biovolume (log transformed) of A. sanguinea along the Serinhaém River (point density) and clusters based on spatial discontinuities of biovolume values along sites, (F) light micrograph of sample from site SE10 during the dry season of 2013 (highest accumulation) showing several cells at different side views (200x magnification) and (H) temporal and spatial variability of the main nutrient and environmental descriptors of the Serinhaém River. The colors represent each season (blue and purple lines for rainy seasons, red and green lines for dry seasons), the figure is plotted downward in order to match the map visualization.

can affect the entire structure of a community due to changes in composition due to outcomes of biotic interactions with one species being benefited while another one is harmed. The spatial location of SE10 within an area of potential disturbance driven by urban tributaries alters ecological stoichiometry locally and regionally, since the sites are connected by dispersal [4].

In Camamu Bay the main significant variables explaining the biovolume site-based typologies according to discriminant analysis were salinity and silicate. However, Si:DIN ratio and dissolved oxygen were significant only in 2013, while pH, nitrate and Si:TP were significant only in 2014. While total nitrogen peaked in the first rainy season (6.7μM L^{-1}), it reached higher values in the dry seasons, coincidentally when the highest biovolume of *A. sanguinea* was detected, contradicting most of the research elsewhere. There was a remarkable decrease in biovolume during the rainy seasons, especially in 2014. Although there was no phosphorus limitation at the sites where high biovolumes predominated (Figure 1, density plots), the decrease in phosphate from 0.7 μM to 0.3 μM L^{-1} represents an important stoichiometric consequence that could lead to changes in maximum abundance. Nitrogen, on the other hand, was a limiting resource at several sites throughout the second rainy season indicating a strong bottom-up effect, given that DIN:TP and Si:DIN ratios, used to determine nitrogen limitation, were significant variables in explaining biovolume variability in both rainy seasons.

The environmental variables (Figure 1H) clearly demonstrated the estuarine characteristics of the downstream section of the Serinhaém River, wherein well marked gradients were observed with salinity increasing seaward and nutrient concentrations increasing towards the warm, nutrient-rich upstream sites. The annual mean temperature ranged from 21ºC to 25ºC and significantly explained biovolume during the second rainy season (discriminant analysis, p = 0.02). Conversely, temperature differed remarkably between the two rainy seasons at SE7 (where biovolume increased inter-annually) with ~27ºC in 2013 and ~29ºC in 2014. These gradients are important ecological features for understanding both the distribution and development of *A. sanguinea* blooms in tropical waters, especially due to the lack of causal explanations for them in regions with rainy and dry periods and temperatures above 20ºC the entire year, instead of four well-marked seasons as in temperate regions, where most studies have been concentrated. The role of temperature is then more important when considering the current scenario of global climate change, which leads to the hypothesis, through supporting experimental studies, that *A. sanguinea* may benefit from ongoing climate change or global warming due to increased acidification, temperature and irradiance [5].

The formation of harmful algae blooms by the dinoflagellate *Akashiwo sanguinea* in estuarine waters is considered to be ecosystem disruptive [6,7], demanding conservative attention and management efforts.

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of *in-situ* data for algorithm validation, which could be accomplished with more *in-situ* chlorophyll data provided by the users and collaborators. Finally, this project represents the state-of-art application of Remote Sensing and Cloud Computing to provide near-real-time algal bloom alerts in the Latin America region, helping governments, institutions, and decision-makers protect and mitigate environmental impacts derived from algae bloom events.

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Can artisan fishermen help to prevent HABs intoxication? A science communication project in Rio de Janeiro, Brazil

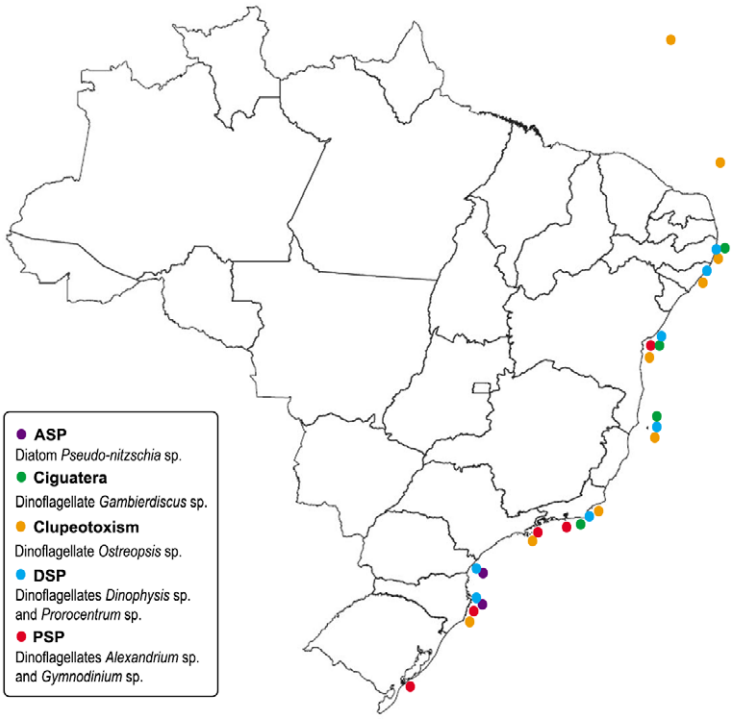

Fig. 1. Geographic distribution of the main harmful microalgae genera in Brazilian coastal waters. These genera may induce different poisoning syndromes: amnesic shellfish poisoning (ASP, purple circle), ciguatera (green circle), clupeotoxism (also called as Ostreopsis poisoning, yellow circle), diarrhetic shellfish poisoning (DSP, blue circle), and paralytic shellfish poisoning (PSP, red circle).

The occurrence and expansion in the distribution of toxic microalgae is a global concern, considering their toxicity to marine ecosystems and human health [1]. Thirty nine species from the main toxic genera of marine microalgae (e.g., *Alexandrium*, *Dinophysis*, *Gambierdiscus*, *Gymnodinium*, *Ostreopsis*, *Prorocentrum*, *Pseudo-nitzschia*) have been reported along the Brazilian coast (Fig. 1). Given the geographic distribution of these microalgae species, five different poisoning syndromes could be expected during bloom events depending on the region of the country (Fig. 1) [2-4]. Brazilian society, shellfish farmers and fishing communities are barely aware of the existence of toxic microalgae and poisoning syndromes induced by contaminated seafood and/or direct contact with toxic cells during HAB events. In the United States and European countries, commercial and recreational fisheries closures are put into place when toxin levels in seafood exceed regulatory limits for human consumption [5,6]. In contrast, Santa Catarina is the single state over the Brazilian territory to periodically monitor microalgal densities in coastal waters to control shellfish production in marine farms. Despite all the related effects to vulnerable fishing communities [5,7], fisheries closures are not only an effective management response for preventing poisonings due to the consumption of contaminated seafood, but also a way to elucidate the occurrence of HABs on coastal areas and their toxicity to human health.

In light of the ecological, economical and human health impacts of HABs, in 2019 we have started a science communication project in Rio de Janeiro coast (Brazil), entitled "Knowing the HABs" (in Portuguese, Conhecendo as HABs). This project has been developed in collaboration with other colleagues and laboratories from the Federal University of the State of Rio de Janeiro (UNIRIO). Artisan fishermen are daily in contact with seafood and effectively working in aquatic systems; thus, we believe that local fishing communities can serve as a starting point for the recognition of HABs in coastal areas and, consequently, for the prevention of human poisonings induced by the consumption of contaminated seafood. We have started creating a process for frequent contact with artisanal fishermen. Within the "Knowing the HABs" project, science communication activities have been planned and developed for the artisan fishermen organized in the main fishing association of Rio de Janeiro city (Colônia Z-13). Colônia Z-13 includes more than 950 artisan fishermen whose daily activities are undertaken from Urca Beach (22°57'07"S; 43°09'49"W) to the Pontal do Recreio (23°02'10"S; 43°29'31"W). This area includes the Rodrigo de Freitas coastal lagoon (22°58'05"S; 43°11'57"W), which comprises more than 50 km along the coast of Rio de Janeiro city. Science communication activities consist of small meetings, photo and video presentations to the fishermen to "introduce" them to the most common HABs clues in marine ecosystems (e.g., water color changes, changes in fish behavior), as well as ichthyotoxic mechanisms of noxious microalgae and poisoning syndrome symptoms.

Until the beginning of the Covid-19 pandemic and social restrictions, science communication activities started in two units of the fishing association – Z-13 Copacabana (Fig. 2) and Z-13 Rodrigo de Freitas Lagoon (Fig. 3). After their training in the recognition of HABs in marine systems, most of the artisan fishermen related that they had never heard about HABs or their impacts on fishing, ecology and human health. However, they have already noted changes in water coloration, mainly a brown color, when fishing in the coastal areas of Rio de Janeiro. Some of them have also related symptoms that could be indicative of phycotoxin poisoning. This project, based on personal contact to promote collaboration between the research conducted in the university and the reality of fishermen, had to stop during the Covid-19 pandemic. After social restrictions, we expect to restart the science communication activities in areas we have previously visited, as well as to visit the other units (e.g., Z-13 Ipanema, Z-13 Urca, Z-13 Barra) in order to extend the training for more artisan fishermen. Our insights from the



Fig. 2. Photos of the meeting, held in the fishing association Z-13 Copacabana on 9th May 2019, with the team of the science communication project "Knowing the HABs" and the artisanal fishermen from the Rio de Janeiro, Brazil.



Fig. 3. Photos of the meeting, held in the fishing association Z-13 Lagoa Rodrigo de Freitas on 2nd October 2019, with the team of the science communication project "Knowing the HABs" and the artisan fishermen from the Rio de Janeiro, Brazil.

first contacts with artisan fishermen led us to believe that the fishing communities of Rio de Janeiro city are extremely vulnerable to HABs impacts, particularly economic and human health effects, and that efforts to inform the artisan fishermen may provide future records of HABs on Rio de Janeiro coast and prevent the capture of contaminated shellfish and fish and, consequently, human poisonings.

Acknowledgements

We are grateful to the fishermen from the fishing association (Colônia Z-13) that received us and dedicated their time during our meetings and presentations, and Mariana G. Tavares from the Project "Ilhas do Rio" that introduced us to the fishing association.

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8 Meeting HAB-ANCA IOCARIBE Group

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission



The VIII Workshop of the Group Harmful Algal Blooms of the Caribbean (ANCA) of IOCARIBE (Intergovernmental Oceanographic Commission for the Caribbean and adjacent waters of UNESCO) was held virtually from March 3rd – 5th 2021. Experts from different countries of the Caribbean region such as Colombia, Costa Rica, Cuba, El Salvador, Guatemala, Honduras, Jamaica, Mexico, Panama and Venezuela participated in the workshop. The event was broadcast on social networks and was attended by 82 people from 16 countries in America, Europe and Asia. *https://unesco-org.zoom.us/webinar/register/WN_Ourz98shSdqNp993LnuMEQ*

After the opening, Elisa Berdalet, presented the GlobalHAB program and discussed possible mechanisms for the inclusion and promotion of HAB-ANCA-IOCARIBE in this initiative. Cesar Toro, Executive Secretary of IOCARIBE, spoke about the United Nations Decade of Ocean Sciences for Sustainable Development (2021-2030). Ernesto Mancera presented the contribution of ANCA-IOCARIBE to the HAB status report for Latin America and the Caribbean based on OBIS and HAEDAT.

Carlos Seixas and Adriana Santos-Martínez, paid a posthumous tribute to María Esther Meave (Mexico) and Luis Alfonso Vidal (Colombia). Their dedicated work contributed significantly to improve knowledge on phytoplankton taxonomy and training of scientists.

On the second day of the meeting, Henrik Enevoldsen presented new features of the IOC HAIS/HEADAT tool, widely used for the documentation of HAB events around the world.

Each country presented a complete report on the HAB events in recent years, as well as the actions, achievements and results related to HAB issues. In some of the Caribbean countries there are specific government entities to deal with HAB matters, but only a few have a national HAB monitoring program. Universities and research institutions are important allies, but it is necessary to improve cooperation mechanisms between government entities and those allies. Country delegates recognized the importance of including HAB events in HAEDAT but expressed the difficulty of doing so in many cases. There is an important group of experts in the region but it is necessary to strengthen capacity development, mainly on issues such as cyanotoxins and mass accumulations of *Sargassum* on beaches. Although local institutions and international cooperation projects provide financial support in some cases, in general the delegates consider it urgent to finalize a regional proposal to integrate HAB research in the Caribbean region.

On the third day, the participation of the HAB-ANCA-IOCARIBE group in the XIX International Conference on Harmful Algae (ICHA) was discussed. The XIX ICHA will be held in October 2021 and organized by Mexico (Dr. Christine Band Smith). Erick Nuñez also provided a complete report on Mexico's progress with issues associated with ciguatera.

During the development of the workshop, commitments were also made for a management of the HAIS / HEADAT webinar; ANCA-IOCARIBE participation in ICHA; design of a virtual HAB course; elaboration of a macro-regional project and design of a web page- ANCA-IOCARIBE. Finally, Gustavo Arencibia Carballo, representative of Cuba, was elected as president of HAB-ANCA-IOCARIBE.

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Aotearoa/New Zealand – Japan collaboration strengthened through HAB research at Cawthron Institute

The collaboration between New Zealand and Japan has been continued and strengthened since Dr Tomohiro Nishimura (Fig. 1) has been working at Cawthron Institute, Nelson, New Zealand. He arrived in 2018 on a two year *Japan Society for the Promotion of Science (JSPS)* Overseas Research Fellowship. During that time, Tomohiro's research focused on the diversity of, and diarrhetic shellfish toxin production by, the benthic dinoflagellate genus *Prorocentrum* in some regions of Oceania [1–3]. He also reported the first instance of DTX1 production for multiple New Zealand *P. lima* complex strains [T. Nishimura, unpublished data]. Several new records of benthic *Prorocentrum* species and other benthic/planktonic dinoflagellate genera/species were also recorded during that research period [T. Nishimura, unpublished data] and will be added to the New Zealand checklists of benthic and planktonic dinoflagellates [4,5]. He was also involved in other HAB research, relating to New Zealand or Japan, published between 2018 and 2020 [6–14].

Cawthron Institute then offered a scholarship for a further two years, beginning in April 2020. Despite the difficulties encountered during COVID-19 lockdowns, Tomohiro focused on the amnesic shellfish poisoning toxin producing planktonic diatom genus *Pseudo-nitzschia* and cultured more than 100 isolates from New Zealand's coastal waters for investigating species diversity and toxin production. The presence of fourteen species, several of which were new records for New Zealand's coastal waters were confirmed. Preliminary toxin analyses suggested that multiple strains of these newly reported species did not produce domoic acid and epidomoic acid [15]. The results from this research were presented to the New Zealand seafood industry and regulatory representatives at the Seafood Advisory Group meeting, held by the Cawthron Institute in Nelson in December 2020. The upside of the research was that the new species reported did not alter the current monitoring regime and may even allow for some relaxing of the risk alerts for some *Pseudo-nitzschia* species.



Fig. 1. Tomohiro Nishimura, sampling at Te Uenga Bay, Northland, New Zealand, 2019

A further project has recently been funded through the Catalyst: Seeding New Zealand - Japan Joint Research Project Programme, collaborating with Kochi University, Japan. This will lead to the mapping of the predicted distribution of toxic benthic microalgae given the forecasted global warming of Japan's and New Zealand's coastal waters.

In 2020 Tomohiro was awarded the '19th Young Investigators Award' from the Plankton Society of Japan and the '16th Young Researchers Award' from the Japanese Society of Phycology for his research on harmful algae in Japan.

Acknowledgements

The technical help of Lucy Thompson and Jacqui Stuart was appreciated. Diarrhetic shellfish toxin and amnesic shellfish poisoning toxin analyses were carried out by J. Sam Murray, Joshua Fitzgerald and Michael J. Boundy. The research was partly supported by the NZ government-funded Seafood Safety programme (Contract No. CAWX1080).

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ANNOUNCEMENT:
The International Phytoplankton Intercalibration

The International Phytoplankton Intercalibration (IPI) Proficiency Testing scheme in abundance and composition of marine microalgae programme 2021 is now open for registration for 2021 through www.iphyi.org. The schedule for 2021 and all other information relating to the programme can be found at the website. The IPI is open for participation to all relevant laboratories globally. The purpose of IPI is to compare and evaluate the performance of testing laboratories and to monitor the laboratories continuing performance over time on the composition and abundance of marine microalgae in preserved marine samples. We work mainly with laboratories engaged in national official/non-official phytoplankton monitoring programmes, water framework directive, marine strategy framework directive and others (environmental agencies, consultancies, private companies) working in the area of analysis of water samples for marine phytoplankton abundance and composition.

From 2021 to 2025, the IPI moves from being hosted by the Marine Institute Ireland to the Canary Islands Harmful Algal Observatory (OCHAB) at the University of Las Palmas de Gran Canaria (ULPGC), Spain. The programme continues under the auspices of IOC UNESCO (http://hab.ioc-unesco.org) through its Science and Communication Centre on Harmful Algae in Denmark.

The IOC collaboration involves the provision of algal cultures, the elaboration of a marine phytoplankton taxonomy assessment (online HAB quiz) using the IOC online platform 'Ocean Teacher Global Academy' (OTGA), and the organisation of a training workshop which is held annually to discuss the results of the intercomparison exercise and to provide training on phytoplankton taxonomy. This workshop has become an important forum for phytoplankton taxonomists working on phytoplankton monitoring programmes from around the world to convene and be able to discuss taxonomical matters related to monitoring, new advances and finds, taxonomical nomenclature changes, as well as looking at samples from different geographical areas and listening to relevant stories from other laboratories about harmful algal events in their regions of relevant ecological importance. This workshop has taken the format of a full 3 days training workshop with at least 2 days dedicated to lectures on algal groups in rooms equipped with microscopes and using live cultures and preserved samples from participants and locations across the globe.

OCHABS recognises that regular quality control assessments are crucial to ensure a high quality output of phytoplankton data. OCHAB is planning to apply for the accreditation of this proficiency testing scheme under ISO 17043 in due course. All OCHAB work is carried out following the technical and managerial requirements for PT schemes (ISO17043) and the data is statistically analysed using the statistical methods as laid out in ISO13528. OCHAB use the statistical database software ProLab Plus from QuoData to do the statistical evaluation of the participant's data.

The IPI is divided into two main parts: The first part includes the analysis of water samples sent to the participating laboratories comprising a number of unknown Phytoplankton species which analysts must identify and enumerate. The second part is an online taxonomic assessment where participants must show theoretical proficiency. At the end of the exercise participants are invited to attend a full 3 day annual workshop.

The calendar for 2021 is as follows:
Registration: Open from **Monday, 8th March 2021**
Samples sent to analysts + Oceanteacher quiz from July 30th to September 30th 2021
Deadline for submission of samples and online test results to lead laboratory: Tuesday, 30th November 2021
Intercalibration Report (January 2022) and Workshop: From 5th January 2022 to 1st March 2022

If you have any queries about registration or the programme, please contact

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https://classroom.oceanteacher.org/

The 19th International Conference on Harmful Algae 2021 (ICHA2021) is going virtual! We appreciate the responses that many of you provided in the recent survey which indicated that >85% of respondents will participate in a virtual meeting. The abstract submission deadline is 9 April 2021. Details on *abstract submission can be found here*. The International Society for the Study of Harmful Algae (ISSHA) Council is busy preparing the meeting with the local steering committee and is excited to announce that we will provide options for live participation in several time zones. We ask for your patience as we continue to update the *conference website*. The meeting will feature:

1. Pre-recorded presentations
2. Live Q&A sessions with oral presenters
3. Interactive poster sessions
4. Exciting side sessions on Early Warning Systems, Fish Farming, New HAB Technologies, and more!
5. Opportunities for informal discussion and "coffee breaks"
6. Online fun (inter)activities!

I am sure you will be pleased to see the greatly reduced registration rates for the virtual conference found *here*. Please register by 11 June 2021 to enjoy the early bird rates of 100 USD (student, retired, Yasumoto awardee, Lifetime achievement awardee), 200 USD (regular participant). You also have an option to register as a "full participant" to help support a student or a colleague from an under-represented nation.

Please also provide your nominations for the 2021 Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award *(both nominator and nominee need to be current ISSHA members)*

ISSHA members are invited to submit nominations for the Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award. The nominator and the nominee both need to be current ISSHA members. Further information on the appropriate profile of the nominees can be found *here*.

Any ISSHA member in good standing may submit nominations for either achievement award, **using the nomination forms that can be found on the website *here*.** The nominator and the nominee both need to be current ISSHA members. Nominations should include a description of the nominee's contribution (not more than one page). Please, make sure that you and your nominee have renewed your membership for the period 2020-2021.

Nominations for the 2021 Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award should be sent by e-mail to Dr. Marta Estrada (*marta@icm.csic.es*), Chair of the Committee on Achievement Awards. **Deadline: May 15, 2021.**

Please write "ISSHA Achievement Awards 2021" in the message subject. Nominations will be considered by the ISSHA Council, and eventual awards will be presented at the virtual 19th ICHA Conference, October 11-15, 2021. La Paz, B.C.S. Mexico.

Student Registration Fee Award for ICHA 2021 Mexico

In order to encourage student participants and young investigators to participate in our biennial conference, ISSHA is glad to announce student registration fee awards to cover registration fee for ICHA2021 Mexico.

Selection criteria

Membership of ISSHA (If you are not a member, please *join the society* prior to the submitting of your application;

1. application of non-member will not be considered).
2. Quality or nature of science of the paper to be presented in an oral or a poster presentation. Students who are first authors will be given higher priority for funding.
3. Priority will be given to first time applicants and from least developed or developing nations over award recipients of the past ICHA conferences.

Please indicate your wish to apply for the Student Registration Fee Award during the registration, and submit your application letter as an e-mail attachment to Dr Po Teen LIM, Email: *poteenlim@gmail.com*, Chair of Student Travel Award Committee.

The letter should contain the following information:

1. Name, address, telephone, FAX, e-mail
2. Institutional affiliation and educational status
3. Name(s) of advisor(s)
4. Research interest (please use keywords)
5. Activity at HAB 2021: poster or oral presentation
6. Copy of the abstract

Please state "**ISSHA Student Registration Fee Awards 2021**" in the subject of your e-mail. Applications not submitted correctly may be rejected. The deadline for receipt of applications will be **15th May 2021**. Applications will be reviewed by the ISSHA Travel Award Committee and classified according to merits presented. Decisions will be announced by 1 June 2021.

More details will be provided soon on conference side sessions, the schedule to accommodate different time zones, and the popular auction. Please check the *ICHA2021 website* frequently for updates. Please take some time to submit your abstract and nominate your colleague for an award.

Vera Trainer (ISSHA President)

In memoriam

Maria Esther Angélica Meave del Castillo (1960-2020)

María Esther Meave (who also received the nickname "Teté" or "Tey" by many of her friends) was born in Mexico city, Mexico (September 5th, 1960) and passed away on December 6th, 2020, after contracting COVID-19. She earned her Master and Doctorate degrees in Biology from the Universidad Nacional Autónoma de México (UNAM). In her early professional career she worked on the biology and taxonomy of freshwater Chlorophyta, in particular with *Cladophora* from waterfalls and lotic environments. A topic that was reflected in her graduate and postgraduate theses

Teté began her study of marine phytoplankton in 1989 when she was appointed by the Universidad Autónoma Metropolitana-Unidad Iztapalapa (Mexico). There she increased her scientific knowledge about marine phytoplankton through participation on a number of national and international courses. These courses were an opportunity to meet and interact with academics and researchers from different parts of the world and through these she established not only academic links, but also a number of friendships.

Teté developed her original research on marine phytoplankton with a focus on the taxonomy and ecology of phytoplankton. She successfully led a number of external funded and institutional scientific projects, and was an author and coauthor of 24 scientific papers in national and international journals, some of which focused on the study of toxic and non-native species in Mexican coasts (Mexican Pacific and Gulf of Mexico). She was also an author/coauthor on 23 book chapters and coeditor of two books.



Teté was very enthusiastic about educational issues and participated in many courses at graduate and postgraduate levels. She was an advisor of several students in graduate and postgraduate levels, and was director of 7 Master and 4 Doctorate theses.

Teté also contributed to numerous national and international scientific meetings (including various HA meetings), and organized three academic congresses. She was part of the Sistema Nacional de Investigadores (National Researchers System) (Level I), where she had an important coordinating role (e.g. Chief of Area, Coordinator, part of Committees). She was the Editor-in-chief of the scientific journal Hidrobiológica, from UAM-I.

Teté made a number of important contributions to the taxonomy and description of new marine phytoplankton genera/species including;

(1) *Calyptrella* Hernández-Becerril *et* Meave
(2) *Calyptrella robusta* (Norman) Hernández-Becerril *et* Meave
(3) *Neocalyptrella* Hernández-Becerril *et* Meave
(4) *Neocalyptrella robusta* (Norman) Hernández-Becerril *et* Meave
(5) *Triposolenia fallax* Hernández-Becerril *et* Meave
(6) *Ceratium balechii* Meave del Castillo, Okolodkov *et* Zamudio (present basyonym of *Tripos balechii* (Meave del Castillo, Okolodkov *et* Zamudio) Gómez)
(7) *Ceratium balechii* f. *longum* Meave del Castillo, Okolodkov *et* Zamudio (present basyonym of *Tripos balechii* f. *longus* (Meave del Castillo, Okolodkov *et* Zamudio) Gómez)
(8) *Pleurosigma gracilitatis* Sterrenburg, Meave *et* Tiffany
(9) *Fryxelliella sepulvedana* Meave, Zamudio *et* Fernandes (present synonym of *Fryxelliella pacifica* Hernández-Becerril *et* Barón-Campis)

We regret her passing away and will certainly miss Teté very much. We extend our deep condolences to her family (her father, two sisters and two brothers, and her son) and friends.

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Leif Bolding, graphic designer and webmaster at the Department of Biology, University of Copenhagen, Denmark, has been an unsung hero of Harmful Algae News. Working behind the scenes since 2000 as responsible for the layout of each issue. Leif retires at the end of April 2021. He will have plenty of time to follow his passion as a nature photographer (www.boldings.dk). We thank him for all of his efforts over the years and wish him all the best for his retirement.



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The GLOBAL HAB Status Report (GHSR): 4 years later

Why a GHSR was planned

In issue 58 of HAN, we made a call to participate in the initiative to develop the first Global Harmful Algal Bloom Status Report (GHSR) to illustrate trends of HAB events by collecting all available data and published information at a global scale [1]. This exercise was motivated by a number of different reasons. Firstly, it was realised that after several decades of intensive research, the HAB field was dominated by the common perception of an increase of these phenomena around the world's seas. Yet so far no sound assessment of HAB patterns and trends had ever been attempted. That HABs were expanding and increasing has been commonly stated in the introductions of many scientific publications and research projects. Furthermore IPCC and GOOS reports used HAB increases as a flag to point to global change. Fluctuations and trends in the aquatic environment under the impact of human activities and hydrographic/climatic change can reasonably cause changes in abundance and distribution of algal species, either toxic or non-toxic. The underlying question is whether environmental and human-driven changes unequivocally determine a global increase of algal blooms or their impacts. On the other hand, human activities can act as a multiplier for HAB detection because of intensification of observational activities driven by increased exploitation of the coastal zone, as well as progress in science, but the extent of these effects had never been quantified. Therefore, a data-based assessment was needed to disentangle the different issues leading to the current perception of HAB trends, and discern a possible spreading and intensification of bloom events driven by environmental and/or anthropogenic changes from the effects of a growing awareness resulting from improved knowledge and monitoring systems.

A second motivation for GHSR was the need to demonstrate the relevance of HAB data collection and systematization for scientific research and management activities. Data related to harmful algae events are regularly produced in relation to human health protection and seafood marketing issues, while detailed studies on toxic algae and algal bloom occurrence are published in a myriad of scientific papers not freely available. To collect these data in well organised and shared datasets allows us to perform robust statistical analy-



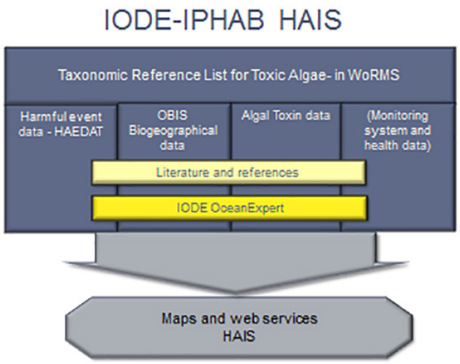

Fig. 1. The Harmful Algae information system (HAIS) provides one user entry point for access to data and information on harmful algal species, their effects, their occurrences, the toxins, expertise on HABs, and key references.

unesco
Intergovernmental Oceanographic Commission

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Table 1. The three main databases concerning harmful microalgal species and their impacts. Toxic species cover all those known to include strains that produce toxins and are hence only potentially toxic. Asterisks indicate mandatory data and information (from [2]).

	Taxonomic Reference List of Harmful Micro Algae	HABMAP-OBIS	HAEDAT
Field	Taxonomy and toxins	Biogeography	Socio-economics, health, ecology
Includes	List of toxic species	Toxic species records with or without impact	Non-toxic or toxic harmful events with an impact
Excludes	Non-toxic HAB species	Records of non-toxic HAB species	Records of blooms or toxic species or toxin concentrations in shellfish without impacts
Literature	References on species taxonomy* and toxicity*	References of species records*	References of the event or reports by national representatives entered when appropriate
Geographic coordinates	No	Yes*	Yes*
Time	No	Yes	Yes
Environmental data	No	At times in the literature cited	At times in the reports
Impacts	No	Not needed	Yes*
Source	Editors of the list	Regional groups of Editors	National representatives of IOC-ICES-PICES groups
Web site	www.marinespecies.org/hab/	ipt.iobis.org/hab	haedat.iode.org/

ses and meta-analyses aimed at tracing trends and variations of HABs at multiple spatial and temporal scales. Such analyses contribute to understanding the ecological and biological mechanisms underlying these phenomena, providing effective knowledge and forecasting tools in support of management and coastal space planning activities, to benefit the whole of society[2].

Thirdly, the GHSR was planned to give more visibility to a field of investigation that is important for both scientific and societal reasons in order to draw public attention and attract continued funding in support of HAB research. The case of the success of IPCC provides a remarkable example of how scientific knowledge can be disseminated outside academia, fuelling and supporting scientific research, influencing public opinion and decision-makers, and provoking societal change. In this respect HABs can be seen as the tip of the iceberg of complexity of the marine world, showing that invisible creatures that dominate it provide humankind with multiple benefits, but at times can pose health and economic risks to society. Capitalising on the knowledge gained through over 50 years of studies and monitoring of HABs to assess their actual distribution and impacts is one of the ways to attract public attention.

How GHSR was produced

To address the GHSR aims a winning move was to involve the scientific community in a region by region collection, review and analysis of the relevant information made available by data input from national institutions, as well as the large HAB science community who had produced and published it in the literature. A main element of the operation was the support from the International Oceanographic Data Exchange programme IODE of IOC, which is in charge of the maintenance and development of the Harmful Algae Information System (HAIS, Fig. 1). The process was placed under the responsibility of an IOC Task Team under the Intergovernmental Panel on Harmful Algal Blooms (IPHAB). The first GHSR was produced with dedicated funding from the Government of Flanders (Belgium), which was used for meetings, training workshops and information technology development, and in-kind contribution from the International Atomic Energy Agency (IAEA) in data compilation workshops, meetings and training.

GHSR planning and goals

GHSR consists of a number of intertwined initiatives and goals:

Improve and expand the databases on HABs. Among the HAB related databases, OBIS/HABMAP includes information on the distribution of potentially toxic species based on published literature while HAEDAT, collects data on harmful events produced by toxic or non toxic species that have an actual impact

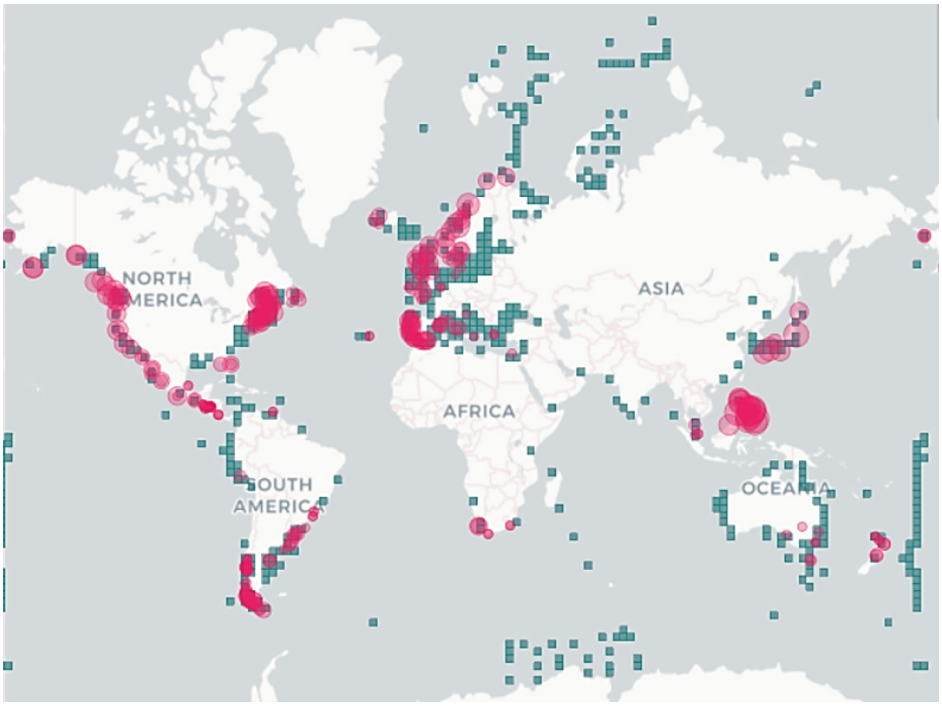

Fig. 2. World map of the distribution of events associated with Paralytic Shellfish Toxins (red) combined with that of the occurrence of Alexandrium (blue). The HAIS mapping tool allows for any combination of species occurrence data (OBIS/HABMAP) and event data (HAEDAT).

on human health or activities, or on the environment (Table 1). Both databases rely on the volunteer contribution and inputs by regional editor groups and national representatives within ICES and IOC regional groups. Following our calls at the HAB conference in Brasil [3] and in HAN [1] as well as further contacts with HAB scientists from different areas of the world, the existing databases on HAB species distribution and HAB events were enriched with a considerable amount of new information. The whole activity led to an increase of more than double the number of HAEDAT records between March 2017 and December 2019 (from 4517 to 9503) and to increment the OBIS/HABMAP with data on HAB species distribution from several regions of the world. To coordinate this effort, workshops were organised during which databases and data input details were illustrated, to make data collection as easy and consistent as possible. Connected to this activity, a new user-interface was developed for HAIS (Harmful Algae information System, *https://data.hais.ioc-unesco.org*) with different options of data visualization combining OBIS records on the occurrence of toxic species with HAEDAT records on harmful algae events with impacts (Fig. 2).

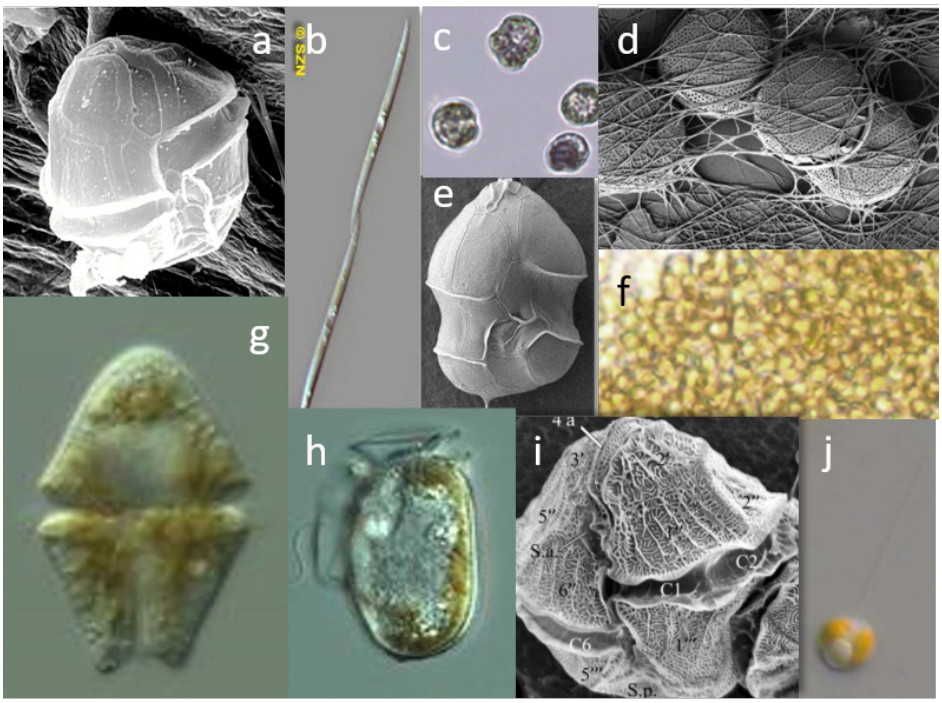

Fig. 3. Microalgae of different taxonomic groups causing distinct HAB types. a) Paralytic Shellfish Toxin producing dinoflagellate Alexandrium minutum; b) Amnesic Shellfish Toxin producing diatom Pseudo-nitzschia multistriata; c) dinoflagellate Ansanella granifera causing discolorations off Cuba (from [5]); d) mucilage-producing diatom Thalassiosira mala associated with fish mortality (from [6]); e) Azaspiracid producing dinoflagellate Azadinium dexteroporum; f) picoplanktonic pelagophyte Aureococcus anophagefferens causing ecosystem disruptive blooms (from[7]); g) dinoflagellate Akashiwo sanguinea causing seawater discolorations; h) Diarrhetic Shellfish Toxin producing dinoflagellate Dinophysis sacculus; i) dinoflagellate Gonyaulax fragilis associated with mucilages damaging tourism and fisheries; j) prymnesiophyte Chrysochromulina leadbeateri causing salmon mortalities in Norway [8].

Produce a collection of regional HAB overviews. Lead HAB scientists from different countries were invited to constitute groups of co-authors and conduct regional overviews based on the HAB databases described above as well as on literature overviews and expert judgement. An effort was made to cover all regions of the world, although some areas had to be left out because of lack of information. An agreement was made with the Elsevier Journal *Harmful Algae* to host a special issue collecting the regional overviews.

From the work of 109 scientists from 35 countries, a variegated picture of HAB events and trends across the globe emerged, which reflects the diversity of the ca. 188 (toxic to humans and animals) to about 250 (including non-toxic) marine microorganisms that cause those events. Because of their multifaceted nature, harmful algal species may produce different kinds of adverse effects (Fig. 3) and respond in different ways to environmental drivers and their changes over time. Nonetheless the evidence was striking that HABs are posing a risk to human health and economics and affect human activities in all regions of the world[4].

Perform a meta-analysis of distribution trends of harmful event records. In this effort, the expanded HAEDAT database was analysed, summing up the results in the first global scale analysis based on sound statistical analyses,

TEXTBOX 1. The main GHSR outcomes

- The GHSR has documented that **HABs are widespread** all over coastal waters of the world, wherever human activities and observations are in place, with impacts on human health, aquaculture, fisheries and tourism and overall environmental quality.
- An **extremely variegated picture of bloom types** has emerged at the regional and subregional scale, paralleled by that **of the causative species** which show different geographic and temporal ranges and ecological characteristics, as well as highly variable responses to environmental changes.
- Remarkable **differences in HAB socio-economic impacts** have also been revealed, mostly kept under control through ever improving monitoring activities, while the impacts on aquaculture, fisheries, use of natural marine resources and tourism continue to pose an economic risk in many regions.
- The intensity and frequency of HABs vary at a regional and local scale, with increasing or decreasing trends and sudden occasional outbursts but with **no uniform global trend that can be discerned from that of increased observational efforts**.
- The link between HABs and aquaculture has pointed to the relevance of observational activities and awareness to the perception of a global HAB increase, at the same time indicating that the **growing need to exploit marine resources and expand human activities in the coastal zone, driven by a growing human population will exacerbate HAB impacts in future years**.

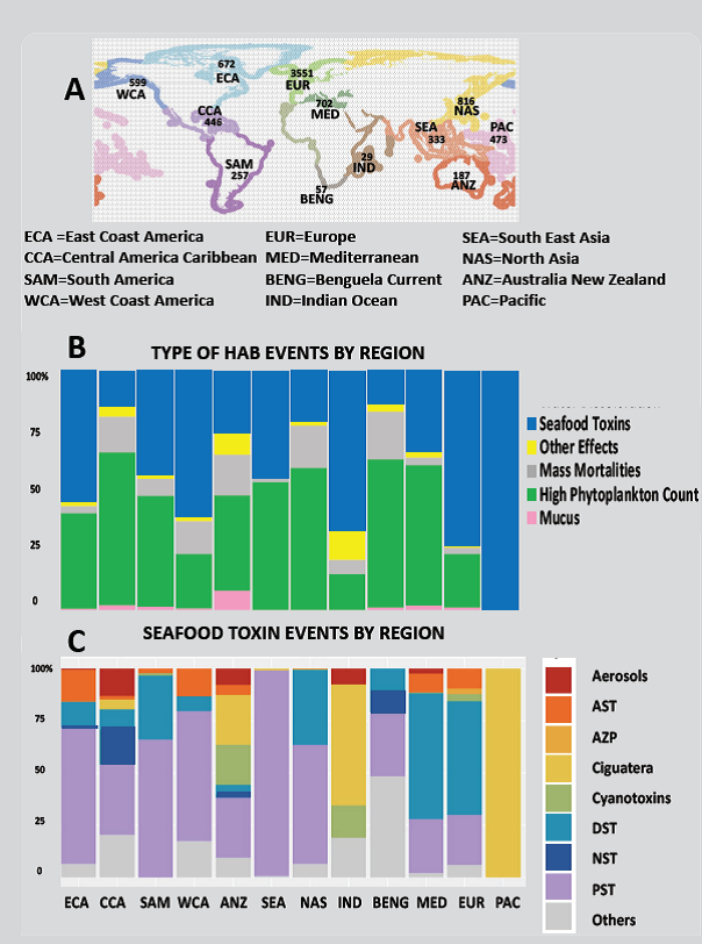

Fig. 4. A. Numbers of HAB events recorded in twelve geographic regions. B. Relative abundance of different HAB types; and C. Seafood toxin syndromes. Paralytic Shellfish Toxins prevailed in East Coast America (ECA), South America (SAM), West Coast America (WCA), South East Asia (SEA) and North East Asia (NAS), Diarrhetic Shellfish Toxins in the Mediterranean (MED) and Europe (EUR), and Ciguatera in the Indian Ocean (IND) and tropical Pacific (PAC). The Australia/New Zealand (ANZ) and Central America/Caribbean (CCA) regions displayed mixtures of events, while Benguela (BENG) had a large proportion of other syndromes (from [9]).

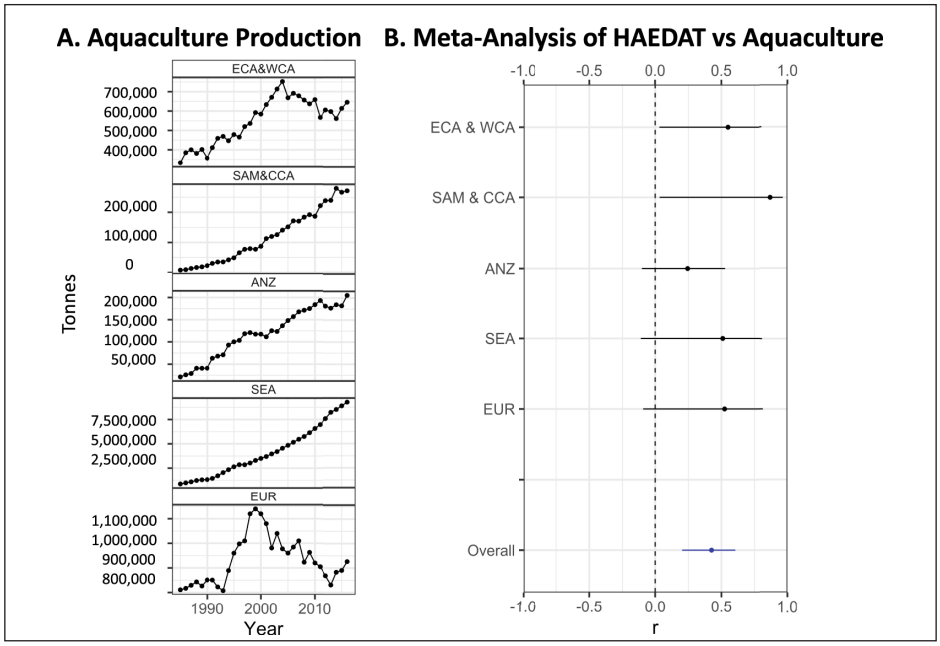

Fig. 5. Relationship between changes in aquaculture production and HAB events recorded in HAEDAT in the period 1985-2018. A. Changes in five regions of tonnage of Aquaculture Production of fish, molluscs, crustaceans and aquatic plants; see the legend of Fig. 4 for abbreviations; and B. Meta-analysis of HAEDAT events over time vs. Aquaculture. Weighted mean correlations (filled circles) are shown with 99% confidence limits (bars). The overall number of HAEDAT events over time was significantly correlated with aquaculture production (bottom), as seen from the confidence bar limit above the 0 value line (from [9]).

using the whole OBIS phytoplankton dataset as a proxy for observational effort changes over the years. In addition, a FAO dataset on aquaculture in different regions was used to take into account the trends in activities that imply an increase of monitoring operations. Trends were calculated at the regional and global scale. The main conclusion was the lack of any uniform global trend in the number of harmful algal events and their distribution over time, once data were adjusted for regional variations in observational activities. Trends were instead variable and contrasting among different regions, where they were characterised by different types of events, harmful species and emerging impacts (Fig. 4). A clear relationship was found however between the trends of harmful algal events and the monitoring effort associated with the development of aquaculture operations in coastal areas (Fig. 5), leading to the conclusion that the latter is at the base of the perceived increase in harmful algae events, while there is no empirical support for broad statements regarding increasing global trends [9].

GHSR outreach to the broader community

It has been imperative not only to communicate the main results of our work (see textbox 1) within our HAB Science discipline (i.e. the *Harmful Algae* special issue) but to reach the climate, environmental, fisheries, health disciplines as well as broader society and politicians. Therefore a **'Scientific summary for policy makers'** was prepared aimed at sharing the GHSR results but also to explain the complexity of the HAB phenomena to a larger community[10]. Basically using the material from the scientific publications, the summary was written in plain language, supplied with colour images, and published on the web at *https://unesdoc.unesco.org/ark:/48223/pf0000378691*.

To further share the GHSR results, at the end of the whole project, with the release of the various publications and the new arrangement of the OBIS/HAIS website, a series of intiatives were taken to disseminate and promote the results obtained to the media and the scientific community. Interviews with the main GHSR Authors were arranged with press agents from all over the wor-

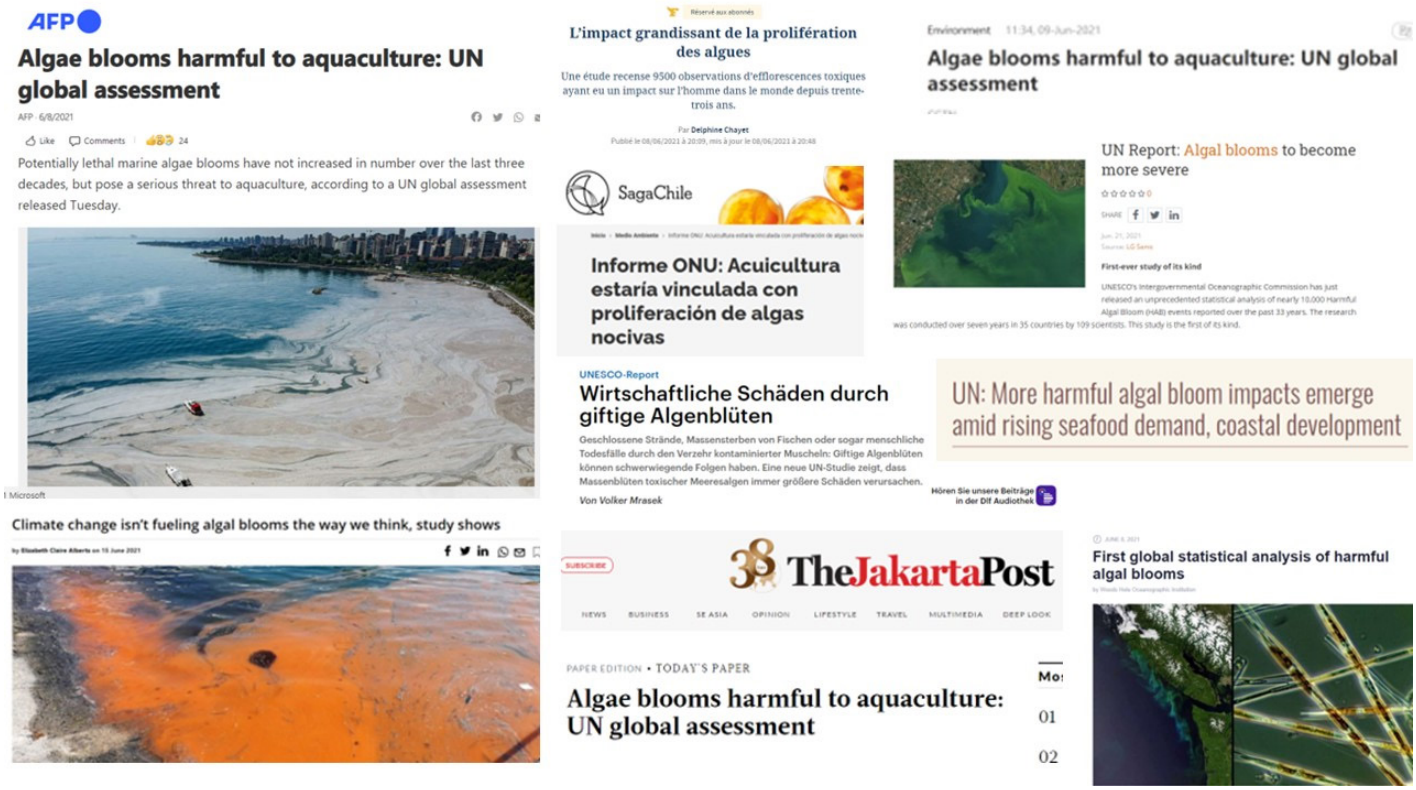

Fig. 6. The launch of the GHSR was well covered in the global press; here some examples from different continents.

ld, resulting in numerous newspaper articles as well as web news (Fig. 6). An open webinar was organised inviting the Editors and the lead Authors of the Special Issue papers, along with the OBIS contributors to HAIS development, to communicate to the rest of the scientific communities the condensed results of the regional overviews and of the GHSR as a whole. In addition, more extended presentations of each of the regional overviews were prepared in the form of videos which are currently available on YouTube (Textbox 2).

The Nature Communications Earth & Environment synthesis publication [9] was downloaded 6400 times in its first 2 months, 110 on-line stories and 6 newspaper stories were published, 5 TV and radio programs delivered, which reached 27 countries, and were communicated in 8 languages. Finally, two GHSR presentations are planned at the 19th International Conference on Harmful Algae, Mexico, 10-15 October 2021.

GHSR problems and limitations

The GHSR identified a number of limitations in the current formats of the databases, HAEDAT and OBIS/HABMAP, which are far from complete. Whole regions such as the Indian Ocean, Africa, eastern south Atlantic coasts and southern-eastern Mediterranean coasts are largely unexplored. Even in well studied parts of the world, no data are available for large coastal strips.

The way the data were historically collected make them sometimes difficult to handle quantitatively [2]. For example, the number of harmful algal events recorded in HAEDAT may not correspond to actual HAB trends in a given region, but vary in relation to changing seafood marketing or HAB monitoring methodologies. Similarly, the higher number of potentially toxic species in selected regions and lack of species in others may simply reflect taxonomic expertise at the local scale.

While the GHSR synthesis attempted to address questions of global increase of frequency and distribution of HABs, data on the **intensity of HABs** as well as the **magnitude of their societal impacts** were not widely available. Using the number of HAB events blurs the actual impacts they may have exerted, because of the lack of quantitative data on the associated economic or well-being losses. An aquaculture ban (toxins in seafood), the closure of a beach (toxic aerosol), a human victim of seafood poisoning, a water discoloration perceived by the public or impairing tourism, currently all count in the same way in the present settings of the HAEDAT databases.

While some regional studies touched upon trends in toxins or in harmful species, neither regional nor global statistical analyses systematically took into account quantitative data of potentially toxic species abundances, and their link to ecological/climate conditions.

TEXTBOX 2.
Regional overview presentations

GHSR Synthesis
https://youtu.be/Xn5kGJxeiHs

Canada
https://youtu.be/_UbTWeV9wtY

Ciguatera
https://youtu.be/kvDxVFVHEf0

Australia and New Zealand
https://youtu.be/QRRTED76E7g

East Asia
https://youtu.be/DCsEXtyX8bs

The Philippines and Malaysia
https://youtu.be/vi-fHGk0Qnc

US
https://youtu.be/uvDMQUtIICk

North Atlantic Margin
https://youtu.be/Hmois4MOI6o

Northern Europe
https://youtu.be/tV7SxVDs43g

Mediterranean
https://youtu.be/VJh--cg-N8Y

Future perspectives

The GHSR was initially planned to be the first of a series of periodic global overviews along the line of IPCC climate reports. However, the results obtained so far demonstrate that, while impacts from HABs are expected to increase driven by the growth of the human population and increasing exploitation of marine resources, the question whether HABs are increasing globally is inappropriate. Considering the heterogeneity of the phenomena and their differing importance from place to place, the issues of HAB status and trends should be addressed at the regional and subregional scale with a focus on individual HAB types and species involved and using a fully multidisciplinary approach. Therefore, having this first phase of GHSR pursuing a global assessment come to an end, a new series of goals and initiatives need to be planned for future years.

At the global scale, the need remains to keep on collecting data and analyse them in a coordinated way, promoting the development of existing and new organised databases and analytical tools and supporting comparative ecological research on individual species and case-studies, thus scaling up the results of local research to a wider scale. The involvement of climate modellers and establishment of nutrient data bases (e.g., in relation to expanding aquaculture) is also needed.

The IPHAB Task Team on HAIS and GHSR will continue its activities, acting as an editorial advisory group for HAIS/GHSR and advise on requirements for HAIS adjustments, quality control and web site edits as well as advise and encourage regional groups and editors on HAB data compilation and their submission to OBIS/HABMAP and HAEDAT. The great spatial heterogeneity highlighted by the GHSR and lack of information for whole areas will be addressed by the IPHAB Task Team by engaging focal points in those regions to build up data for better geographical coverage.

The Task Team is also responsible to engage with working groups, groups of experts and individual scientists to identify time series of phytoplankton data including information on HAB species. There is potential for collaborations with the IOC-SCOR GlobalHAB science programme and the IOC Working Group to Investigate Climate Change and Global Trends of Phytoplankton in the Oceans (TrendsPO, Chair *Peter.A.Thompson@csiro.au*) to assist with a quantitative approach to investigate the relationships between environmental conditions and blooms of individual harmful algal species and their impacts.

Future development work for HAIS as a data system and thus future editions of the GHSR will depend on new sponsorships. While countries or institutions may be willing to support regional trend analysis and data collection it is a central challenge not to lose track of the bigger global aims that play at the level of IPCC (climate) and FAO (aquaculture). A coordination effort is needed to join up regional activities into a global approach.

The ideal would be to attract IPCC type funding that could support a sustained process leading to the regular GHSR delivery. This could include the establishment of a centralised service to coordinate and facilitate data entry for HAEDAT and OBIS, and act as a facility for data analysis workshops and training. One option would be to develop the GHSR as a UN Decade activity as a component of several larger programmes already submitted for endorsement to the Decade Secretariat. There could also be a potential for HAIS/GHSR to be a separate Decade activity, if philanthropic organisations are to be approached for funding. These options will be pursued in more detail by the IPHAB Task Team chaired by *Eileen.Bresnan@gov.scot*.

To put our ultimate GHSR aims in perspective, it emerges that, beyond considerable progress in management and several reasonable hypotheses, no clear explanations are still available for the prevalence of certain HAB types in some areas and their absence in others, nor for the locally decreasing or increasing trends and range expansions of some species, while questions on the outcomes of the ongoing environmental and anthropogenic changes remain mostly unanswered. There is thus a direct link that needs to be reinforced between our scientific understanding of HAB species population dynamic in a changing environment and our ability to analyse and understand trends for species occurrence patterns and HAB events.

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Fish filler *Heterosigma akashiwo* in Comau Fjord, Southern Chile

In early spring of 1988, a massive bloom of the alga *Heterosigma akashiwo* was observed in Comau Fjord (42°22′10″S), Los Lagos, southern Chile, resulting in severe impacts to the local salmon industry [1]. Over three decades later, on March 16th, 2021, approximately 400 cell/mL of *H. akashiwo* were detected, with a particularly noticeable surface distribution at the head of Comau Fjord (Fig. 1). Ten days later, concentrations increased to 35000 cell/mL, forming intense brown patches in the water and a rise in HAB_f INDEX to 333 units, resulting in significant impact to local salmon farms [2]. During Easter celebrations on April 1st 2021 notable brown patches were seen extending approximately two thirds of the length of the fjord and at both margins. Most of the cell aggregation was contained within the Comau Fjord, although some patches were also observed in areas near the mouth of the fjord (Fig. 1).



Fig. 1. Top: Map showing location of Comau Fjord in Los Lagos province, Chile. Middle: Patches of Heterosigma akashiwo in Comau Fjord, where maximal cell concentrations were found at the head of the fjord (red spot in the map). Bottom: A salmon farm in Magallanes province, Chilean Patagonia.

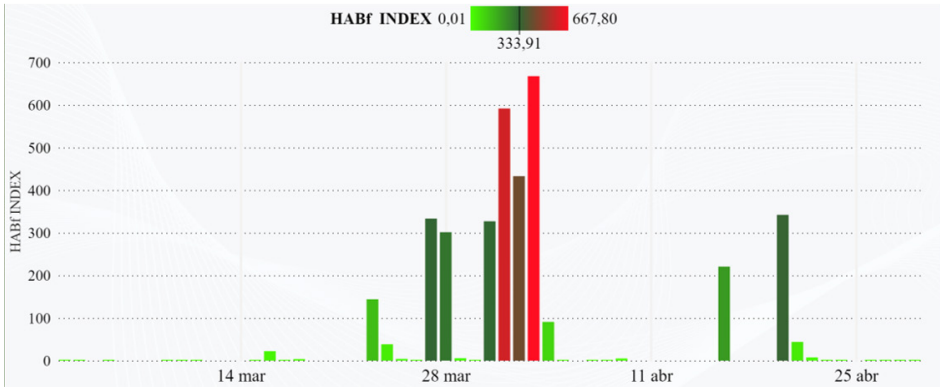

Fig. 2. Temporal distribution of the HABf INDEX in Comau Fjord during the bloom

The Oceanographic and Environmental Program of Salmonids (POAS) made it possible to clearly define the spatio-temporal origin of the bloom at the head of the fjord just prior to March 16th, 2021. Analysis of fish behavior, meteorological conditions, microscopic/FlowCam imaging and fluorescent photosynthesis analysis all indicated the bloom was over by April 4th, 2021. However, on April 16th, 2021 a second peak occurred, further damaging local fish farms (Figs. 2-3). Cells in the second peak of the bloom were healthy, highly mobile and had very high photosynthetic efficiency (Fv/Fm) based on FRRf3 [3]. Induction curves of active fluorescence of PSII show clear differences between the mouth and head of the fjord, and also in the water column due to species composition of phytoplankton and their respective abundances.

The phytoplankton composition and abundance within the Comau Fjord showed a mono-specific assemblage with more than 93 % comprised of *H. akashiwo* cells (Fig. 3). Few diatom species were observed and only outside the fjord, i.e., a typical ecological attribute of this biological event [4, 5].

Final remarks

The *H. akashiwo* bloom originated at the head of Comau fjord on March 16th, 2021 with cells that were poorly shaped, fragile, inactive and with low rates of photosynthesis, albeit at high concentrations (400 cells/mL, with a HABf INDEX = 111). Within two weeks cells recovered, restoring morpho-physiological signals (Fig. 3) and remained in the fjord for 40 days until April 25th, 2021. The very high concentrations of *H. akashiwo* and brown patches set a record of the HABf INDEX = 668, even higher than those

observed in the 2016 *Pseudochattonella* bloom due to the high concentrations of raphidophyte cells.

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We thank Robert Hatfield, from Cefas, UK for the English correction of this note. We also thank the people and customers involved in POAS program.

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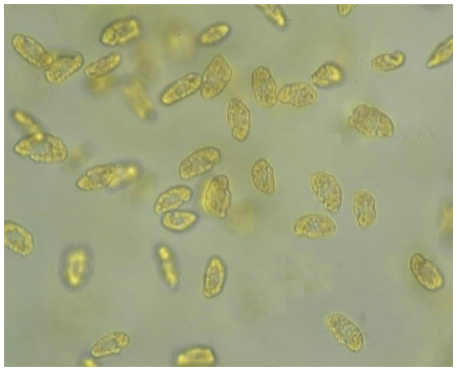

Fig. 3. Microscopy image of a sample from Comau Fjord, March 28th, 2021 showing a mono-specific bloom of H. akashiwo

Harmful Algae News (HAN) DOI Available

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Harmful Algae News 46:
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First report of a *Fibrocapsa japonica* Toriumi & Tanako bloom in the northern Humboldt current ecosystem

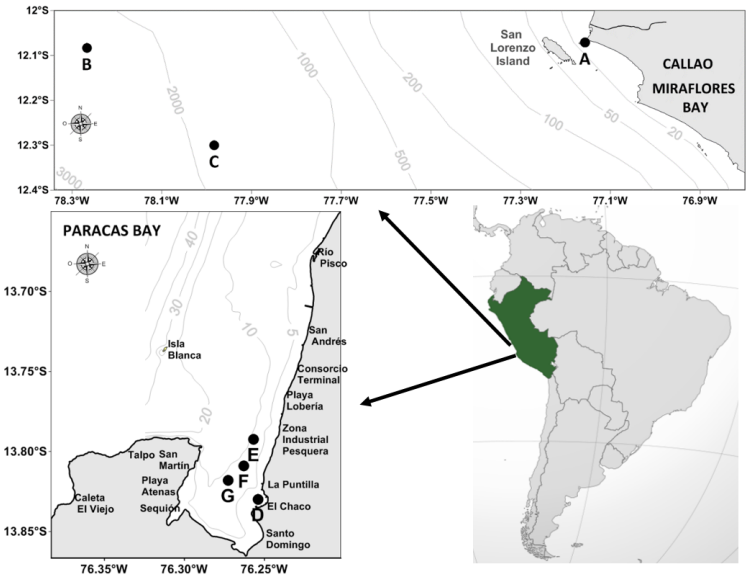

Fig. 1. Sampling sites in Miraflores Bay, Callao (12 °S) and Paracas Bay, Pisco (13.70 °S). February – March 2020

Raphidophycean flagellates associated with massive fish kills have been reported worldwide. High density blooms of species from this class cause physical obstruction to fish gills and mucus excretion, and are associated with production of hemolytic substances, such as polyunsaturated fatty acids (PUFAs) and reactive oxygen species (ROS) [1].

Among the most representative Raphidophycean species, *Olisthodiscus luteus* and *Heterosigma akashiwo* have been recorded in Peru forming extensive Harmful Algal Blooms (HABs) between spring and autumn [2-5], although their potential effects in the ecosystem have not been elucidated. *Fibrocapsa japonica* is widely distributed in temperate and tropical seas. In South America, there are records of this species from Brazil and Uruguay [6-7]. This is the first report of *Fibrocapsa japonica* blooms in two upwelling areas: off Callao (Miraflores Bay 12°S) and Pisco (Paracas Bay 13.70°S in the coastal waters of Central and Southern Perú (Fig. 1). This HAB event occurred during the austral summer, between February 25 and March 11 2020. Yellowish-brown patches, extending 60nm from the coast (off Callao) were observed. In Pisco, patches were observed just inside the bay.

Surface water samples were collected from seven stations for analysis of phytoplankton and measurement of hydrobiological parameters (Fig. 1). Microscopic examination and micrographs were made with a NIKON ECLIPSE TiU inverted microscope using a Nikon Digital Sight DS-U3 camera and Nis element D software. Differential Interference Contrast (DIC-Nomarski) was used to capture details of living cells. Cell counts were carried out using 1 mL Sedgwick Rafter chambers. The specimens from Perú were characterized by slightly dorso-ventrally compressed oval shaped cells with the presence of groups of large trichocysts or mucocysts, located in the posterior end (Fig. 2a). The cell size was approximately 24 µm long and 16.73 µm wide. In general, morphological characteristics observed agreed with those from previous description of the species [8-9].

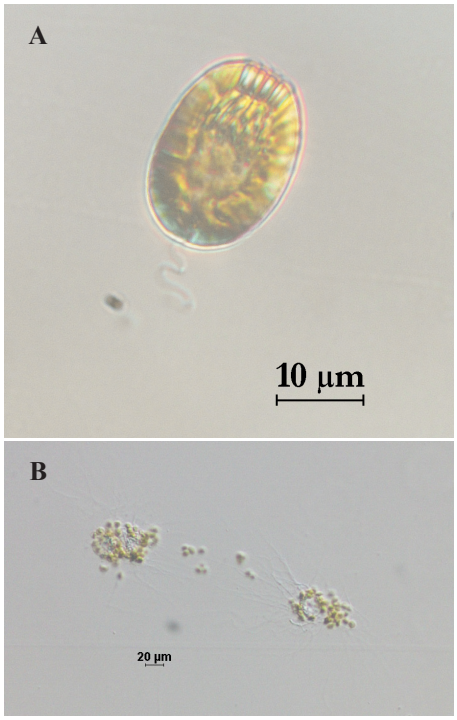

Fig. 2. a) Fibrocapsa japonica living cell, the mucocysts, trichocysts and the flagella are observed in the sub-apical part of the cell. b) F. japonica in cell lysis a few seconds after its death (without preservation) expelling the trichocysts.

Fixed samples were not suitable for a clear observation of the cells, because fixatives commonly used in monitoring projects caused disintegration of the cells, with irregular filaments attached to the raspberry-like specimen produced after the ejection of the trichocysts (Fig. 2b).

This *F. japonica* event was related to unusual environmental conditions that promoted bloom development (Fig. 3). In Callao, the bloom was associated with sea surface temperature (SST) values ranging from 23.5 to 25.6 °C, salinity 35.01 to 35.26 PSU, dissolved oxygen between 5.90 and 12 mL L^{-1}, with an average saturation of 166.45%. Mean cell concentration was 38 x 10^5 cells L^{-1}. And the cell maximum (87 x 10^5 cells L^{-1}) was recorded at station A (oxygen level 12 mL L^{-1}). In Pisco, environmental conditions were influenced by a frequent event in summer, the outflow of continental waters into the coastal zone (Fig. 4) [4]. SST values were 23.4 - 26.1 °C, salinity 33.829 - 34.102 PSU, and oxygen 6.04 - 7.90 mL L^{-1} with an average saturation of 146.90%. Mean cell concentration of *F. japonica* was 8.41 x 10^5 cells. L^{-1}. A cell maximum of 18 x 10^5 cells L^{-1} was found at station D off El Chaco, associated with dissolved oxygen of 6.04 mL L^{-1}.

No mortalities of fish or any other marine organisms were reported associated with the *F. japonica* blooms in the two above mentioned areas but there was abundant production of foam.

According to [10], the wide temperature range tolerated by this species (4 to 32 °C), with an optimal growth rate associated with a salinity of 26 PSU [1] classifies *F. japonica* as a eurythermal and eurohaline species, a fact which would explain its wide distribution.

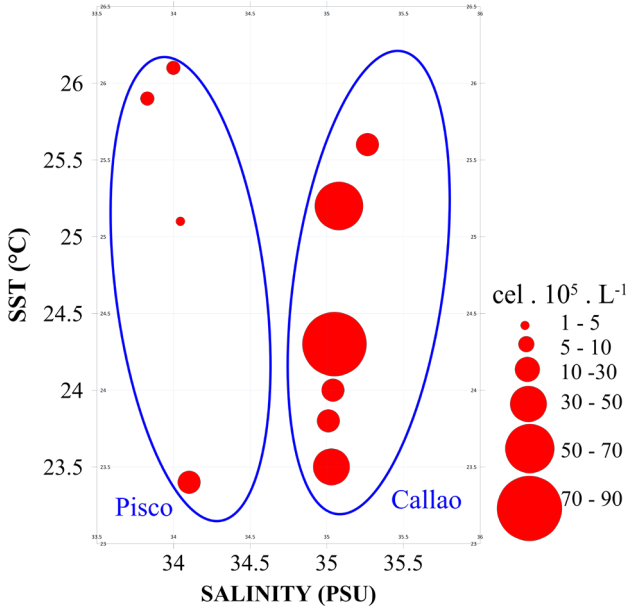

Fig. 3. T-S Diagram for cell concentrations (cell .10⁵. L⁻¹) of F. japonica in Callao (12°S), Pisco (13.70°S)

This species thrives on eutrophic environments and is frequently observed in coastal and brackish waters [11]. These conditions agree with those observed in Miraflores and Paracas bays on the Peruvian coast.

This is the first report of *Fibrocapsa japonica* in these latitudes. Blooms of this species in Perú may pose a potential risk for the fisheries and aquaculture sectors. Further research is needed to unveil the harmful or toxic effects produced by this Raphidophycean species in the ecosystem.

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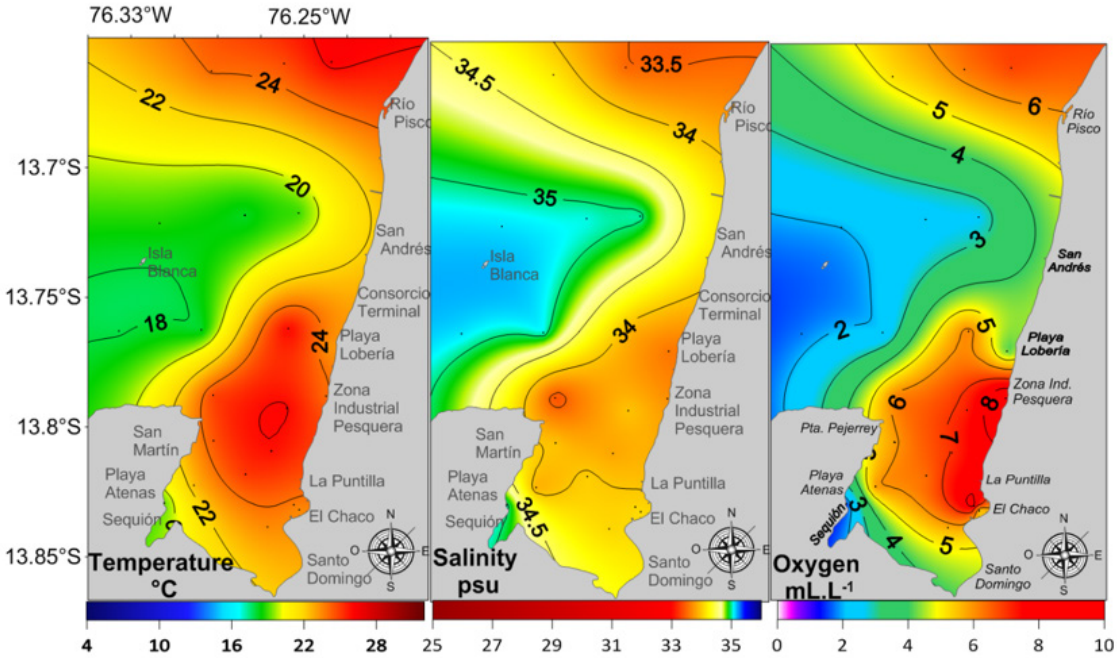

Fig. 4. Surface distribution of temperature (°C), salinity (psu) and oxygen (mL L⁻¹) in Paracas Bay, Pisco (February 2020).

First major bloom of *Trichodesmium erythraeum* (Cyanophyceae) and *Prorocentrum rhathymum* (Dinophyceae) in Yelapa Jalisco, México

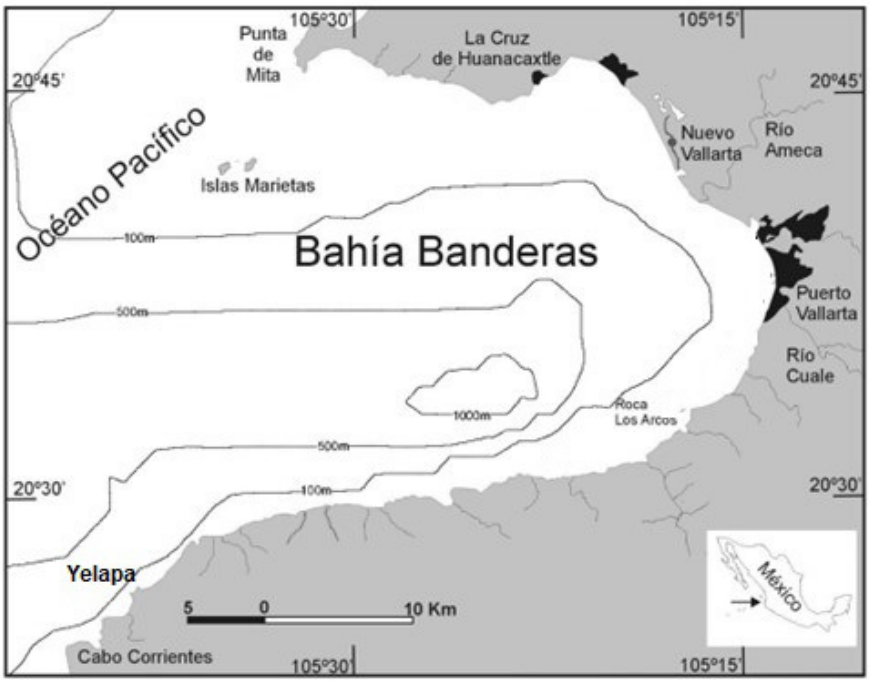

Fig. 1. Sampling station in Yelapa Jalisco, Bahía de Banderas, Mexican Pacific coast, during the September 2019 bloom of Trichodesmium erythraeum and Prorocentrum rhathymum

This report of a harmful algal bloom (HAB) event from Yelapa Jalisco in Bahía de Banderas, México (Fig.1) describes for the first time a bloom of *Trichodesmium erythraeum* tufts co-occurring with high densities of the benthic dinoflagellate *Prorocentrum rhathymum* in phytoplankton samples during September 2019.

The microalgae checklist from a regional monitoring programme 2000 – 2001 consisted of five categories: i) high biomass bloom forming species; ii) toxin producing species harmful to human health; iii) species which can cause skin problems through direct contact; iv) species which are toxic to other marine organisms (especially ichthyotoxic), and v) species that do not cause any problems *in situ* but have been found to produce toxins in culture [1, 4].

Blooms of *T. erythraeum* and *P. rhathymum* have not previously been recorded in the historic phytoplankton data from this region, even though both species are included in the list of microalgae that form HAB events in the coastal waters of the Gulf of California and in the Mexican Pacific [3]. The phytoplankton sample consisted almost entirely of tufts (parallel arrays, filaments) of *T. erythraeum* and oval cells of *P. rhathymum* (Fig. 2) as co-dominant species with densities of 1658 x 10^6 and 78 x 10^3 cells l^{-1} respectively. Some local fishermen reported dead fish, possibly caused by the anoxic conditions that may arise from decaying marine blooms [4], however due to equipment limitations, cyanotoxins and other phycotoxins were not analyzed. In addition, the presence of these microalgae reveals a potential risk of DSP contamination and hemolytic toxins from *P. rhathymun* [4], and a latent threat of neurotoxins, hepatotoxins, ciguatoxins, microcystin analogues, cylindrospermopsin and saxtitoxin from the presence of *T. erythraeum* [2, 3].

The occurrence of these species has increased the algae checklist and current geographical expansion in Banderas Bay, where the factors that could favor these blooms are mostly related to coastal nutrient enrichment, mainly due to seasonal rains and high river discharge. On the other hand, the high abundance of the epibenthic *P. rhathymum* in surface waters could be associated with floating debris due to the seasonal increase of *Sargassum* and other macroalgal drift. This floating debris provides adequate substrate and an important vector for the dispersal of potentially toxic and benthic species of dinoflagellates. Satellite chlorophyll-*a* concentration from NOAA data sets were relatively low (< 3 mg · m^{-3}) (Fig. 3A). Typically temperature values range from 32.58 to 24.3 °C (Fig. 3B) and salinity from 0.43 to 36.59 psu for this period of the year due to the influence of tropical water [5].

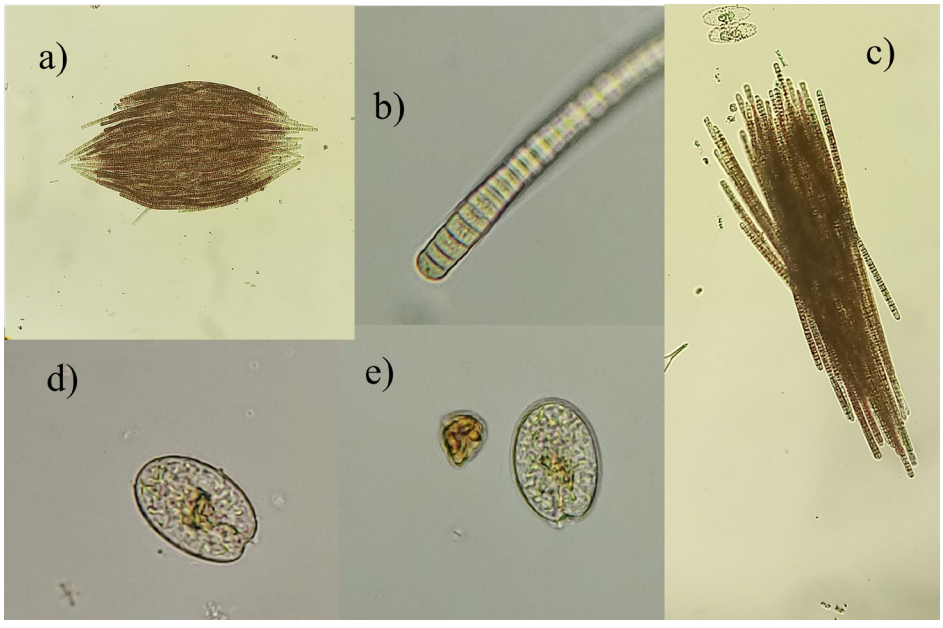

Fig. 2. Light microscope (LM) micrographs of phytoplankton samples: a-c Trichodesmium erythraeum, d-e Prorocentrum rhathymum from Yelapa Jalisco, México

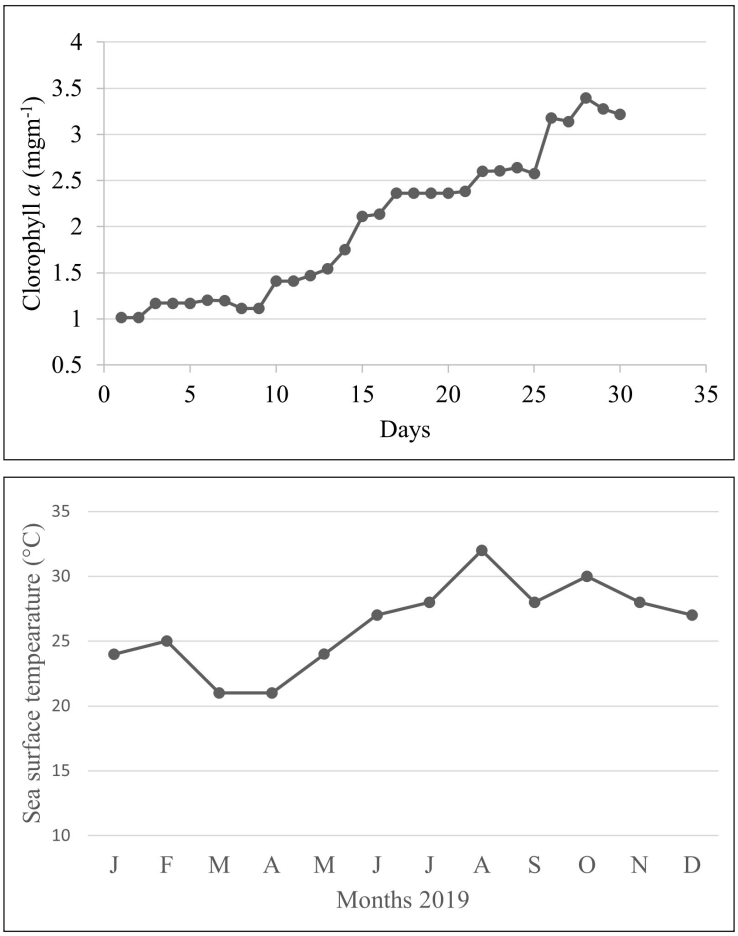

Fig. 3. Top: Temporal distribution of Chlorophyll a concentration (mg m^{-3}) in the study area during September-2019. Bottom: Mean sea surface temperature (°C) in Yelapa Jalisco México

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Training HAIS-HAEDAT to ANCA-IOCARIBE

The regional IOC HAB group ANCA-IOCARIBE is preparing training on the use of and data upload to the Harmful Algal Information System and its Harmful Algal Event Database (HAIS-HAEDAT) at the end of November 2021. This course aims to strengthen the registration of HAB events in the Caribbean region. The information available in HAIS it expected to be used for mechanistic models of biological and ecological processes associated with HAB events.

An example of this type of model is the IRMA, Aerobic Anoxic Mortality Risk Index, developed as a result of massive fish kills in the Ciénaga Grande de Santa Marta - Colombian Caribbean [1-2], which occur recurrently, not only there, but also in many other coastal systems of the Caribbean. Implementing IRMA will serve as an early warning tool, an important input for a risk management system.

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Unexpected *Prorocentrum* bloom and associated gelatinous zooplankton in Alexandria, Egypt

We investigated an unprecedented, massive bloom of the dinoflagellate species *Prorocentrum minimum* and *P. triestinum* which co-occurred with dense swarms of the salp *Thalia democratica*. To our knowledge this bloom, considered an exceptional spring bloom in Alexandria waters, has never been reported anywhere else in the world. Blooms of *P. minimum* and *P. triestinum* during spring and summer have been recorded as a fairly regular feature of the annual phytoplankton succession in Alexandria waters [1-3].

The small salp, *Thalia democratica*, by virtue of its rapid asexual reproduction [4] is able to form extensive swarms [5] yet the physiological and oceanographic factors influencing the magnitude of these swarms are poorly understood [6-8]. The species is a common component of the zooplankton population in Egyptian waters, but not abundant [9]. Information about co-occurring blooms of pelagic tunicate and dinoflagellate species is still poor. We believe these dinoflagellate and gelatinous zooplankton species behave as opportunistic groups.

During routine weekly sampling for the project "Assessment of red tide phenomena in the Egyptian neritic waters: causes and impacts", a visible reddish water discoloration extending approximately 10 Km was suddenly observed near the Alexandria coast between 4-6 May, 2021. The water discolouration at a semi-enclosed station (about 400m long) on 5 May was very unique. The basin was distinctly divided into two separate parts where two water masses of different water discoloration were seen; a reddish area at the Eastern part where dinoflagellates were significantly abundant, and dense white layers of *T. democratica* swarms at the west (Fig. 1). The whole area is part of an ongoing initiative for the protection of Alexandria coastal areas where thousands of cement blocks and tons of rocks are dispersed into the sea, creating semi-closed small basins. These small basins act as traps to extend the duration of the bloom. No fish and/or invertebrate mortality was detected, but the bloom appeared to be harmful for macroalgae as hundreds of individuals of the phaeophyte *Colpomenia sinuosa* were found dead on the beach.

The physicochemical conditions during the peak of the bloom included low temperature (23.8 ^{o}C), relatively high salinity (37.075), intensely stratified water column, high pH (8.65), and very high nitrate levels (8.98 µM), while phosphate levels were severely reduced (0.55 µM). The bloom was composed of *P. minimum* (2.2 x 10^6 cells L^{-1}, contributing to 98.21% of the total standing crop) and *P. triestinum* (0.04 x 10^6 cells L^{-1}). Chlorophyll *a* levels during the bloom were 19.17 µg L^{-1}, DO_2, 10.69 mg L^{-1}, 160.81, and OM 5.6 mg O_2 l^{-1}. The bloom was of a very short duration, with partial dissipation recorded on 6 May with scattered aggregations near the coast driven by changes in wind speed and direction.

Grazing by *T. democratica,* which is known to exhibit efficient feeding strategies [6], capable of depleting phytoplankton production and feeding actively on autotrophic dinoflagellates [10,11], may be the reason behind the rapid dissipation of the dinoflagellate bloom. Blooms of the toxigenic *P. minimum* may constitute a good food source for salps [12].

Why the intense masses of *T. democratica* occurred at a certain stations is unclear. The proliferation of *T. democratica* were associated with the relatively lower temperature, salinity and adequate food supply in agreement with previous findings [13]. The disappearance of the white-coloured swarms of *T. democratica* can be probably attributed to wind dispersal and increased temperature. Similar to other studies [8] our results were unsuccessful in relating abundances of *T. democratica* to physical conditions. Among other suggested causes is the salp clogging due to the high concentration of organic materials and phytoplankton production. Salps themselves serve as prey for numerous fish species [14]. These factors should be further verified. Our results agree with other studies concluding that blooms of *T. democratica* may appear suddenly over different spatial scales, last for a short time and suddenly disappear with no specific environmental drivers defined [15].

These blooms might be blamed on anthropogenic stressors, such as eutrophication, and modification of habitats. It is expected that the paradigm of gelatinous zooplankton blooms will increase in the future with cascading

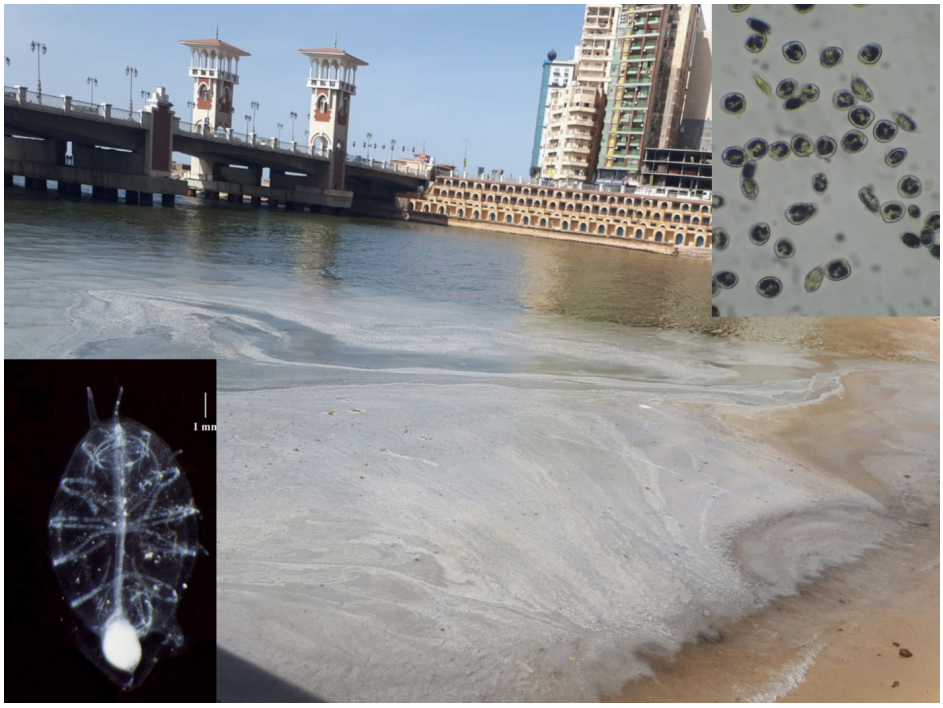

Fig. 1. Photo of the dense white discolouration due to Thalia democratica swarms

Continued on page 15

Elucidating the origin of tetrodotoxin in New Zealand bivalves

Tetrodotoxin (TTX) is a potent neurotoxin that acts by selectively targeting sodium channels, blocking propagation of action potentials and causing paralysis [1]. It has been responsible for countless human intoxications and deaths around the world, particularly in Japan, from consumption of pufferfish. The distribution of TTX and its analogues is remarkably diverse, and the toxin has been detected in organisms from marine, freshwater and terrestrial environments [2]. Increasing detections of TTX in aquaculture species not typically associated with TTX, such as edible bivalves and gastropods, has drawn considerable attention to the toxin, reinvigorating scientific interest and regulatory concerns. We found reports of TTX in 18 species of edible shellfish species from ten countries since the 1980's, with some reports above the safe threshold established by the European Food Safety Authority (EFSA) of 44 µg of TTX per kg of shellfish [3].

Despite decades of research, the exact origin of TTX remains a mystery. Current literature supports three hypotheses: endogenous production, symbiotic bacteria (including cyanobacteria), or direct bioaccumulation through a dietary source. In 2009, the sea slug *Pleurobranchaea maculata* was found to contain high concentrations of TTX in New Zealand [4] and an extensive research programme to explore the origin of TTX in *P. maculata* in New Zealand was launched. During this research, *Paphies australis*, an endemic clam, was found to accumulate high concentrations of TTX (800 µg kg^{-1}) [5]. A Ph.D. study was undertaken to elucidate the source of TTX in these clams that are a common food source and are culturally important in New Zealand. *Paphies australis* are largely sessile and found in subtidal habitats, making them highly amendable organisms to investigate the source of TTX.

In this Ph.D. study, a multiple line of evidence approach was used to investigate potential TTX producers. This included histological and analytical techniques to explore the micro-distribution of TTX within the organs of *P. australis*, aquaria studies to investigate the depuration and uptake of TTX, field studies to explore the variations in TTX concentrations from different *P. australis* populations and other bivalve species in New Zealand, and molecular analyses. Immunohistological analysis of *P. australis* tissues employing a TTX monoclonal antibody demonstrated that TTX was present in the outer cells of the siphons, and also in the digestive system, foot and gill tissues [6]. These results were supported by chemical analysis using LC-MS/MS. Observing TTX in organs involved in feeding provided initial evidence to support the hypotheses of an exogenous source in *P. australis* and presence in siphon tips shows that TTX could be used as a chemical defence against predators.

The TTX depuration rate in *P. australis* was then assessed by maintaining TTX-bearing individuals in captivity and feeding them a non-toxic diet for 150 days. The bivalves significantly depurated the toxin (0.4% of total toxin content per day) and there was a rapid decline of the toxin in their digestive glands with only traces amounts remaining after 21 days [7]. This result provides evidence to support the hypothesis that *P. australis* do not endogenously produce TTX.

During field surveys, *P. australis* from 10 different sites around New Zealand were harvested and tested for TTX. There were significant differences in TTX concentrations from the different sites. All *P. australis* contained TTX but bivalves from the warmer North Island sites contained significantly higher concentrations than those from the South Island. These results provided further evidence to support the proposition that the source of TTX in *P. australis* is likely exogenous, varying in abundance by location [7]. I hypothesised that the source is a warm-water-adapted TTX producer that is mostly present or more prevalent in warmer climates, or that TTX production is triggered by warmer temperatures.

To explore this possibility further, metabarcoding was used to investigate the bacteria, including cyanobacteria, present in the digestive glands and siphons of *P. australis* from the 10 sites with different TTX concentrations and collected monthly over a year from one site with high TTX concentrations. Marine cyanobacteria, in particular picocyanobacteria (e.g., *Cyanobium*, *Synechococcus*, *Pleurocapsa*, and *Prochlorococcus*), were found in all samples collected from sites containing the highest amount of TTX and were present in the core microbiome of TTX-bearing *P.*

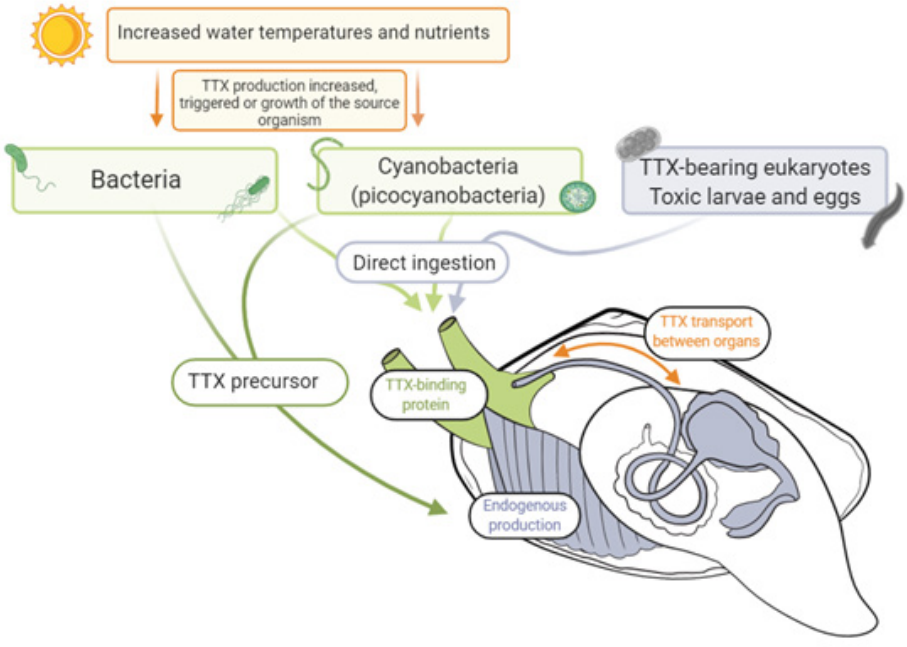

Fig. 1. Diagram synthesising the main hypotheses regarding the accumulation of tetrodotoxin in the different organs of the New Zealand clam Paphies australis (Created with BioRender.com)

australis [8]. Cyanobacteria are well known for producing a wide range of marine and freshwater toxins and this finding warrants further investigation.

Lastly, the possibility that wild *P. australis* could sequester and accumulate TTX from a dietary source was assessed. A novel method to artificially feed a controlled amount of TTX to bivalves was developed. The micro-encapsulation method incorporated humic acid so that the water-soluble TTX was bound to a solid substance. The main finding from this study was that *P. australis* can rapidly accumulate TTX from an external dietary source and concentrations were much higher (> 100 µg kg^{-1}) than the safe threshold in bivalves established by the EFSA (44 µg kg^{-1}) in under two weeks [9]. This further strengthens the hypothesis that bivalves most likely accumulate TTX from their diet.

Based on the key results from my Ph.D. thesis and in concert with previous studies on the subject, I hypothesize that *P. australis* are obtaining TTX from a dietary source and there are three main possible routes of accumulation which are summarised in Figure 1. My thesis has addressed several critical knowledge gaps in our understanding of the source of TTX in bivalves worldwide and I am open for future collaborations to solve this mystery.

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Continued from page 13

socio-economic consequences for fisheries and tourism.

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A European Imaging FlowCytoBot (IFCB) network

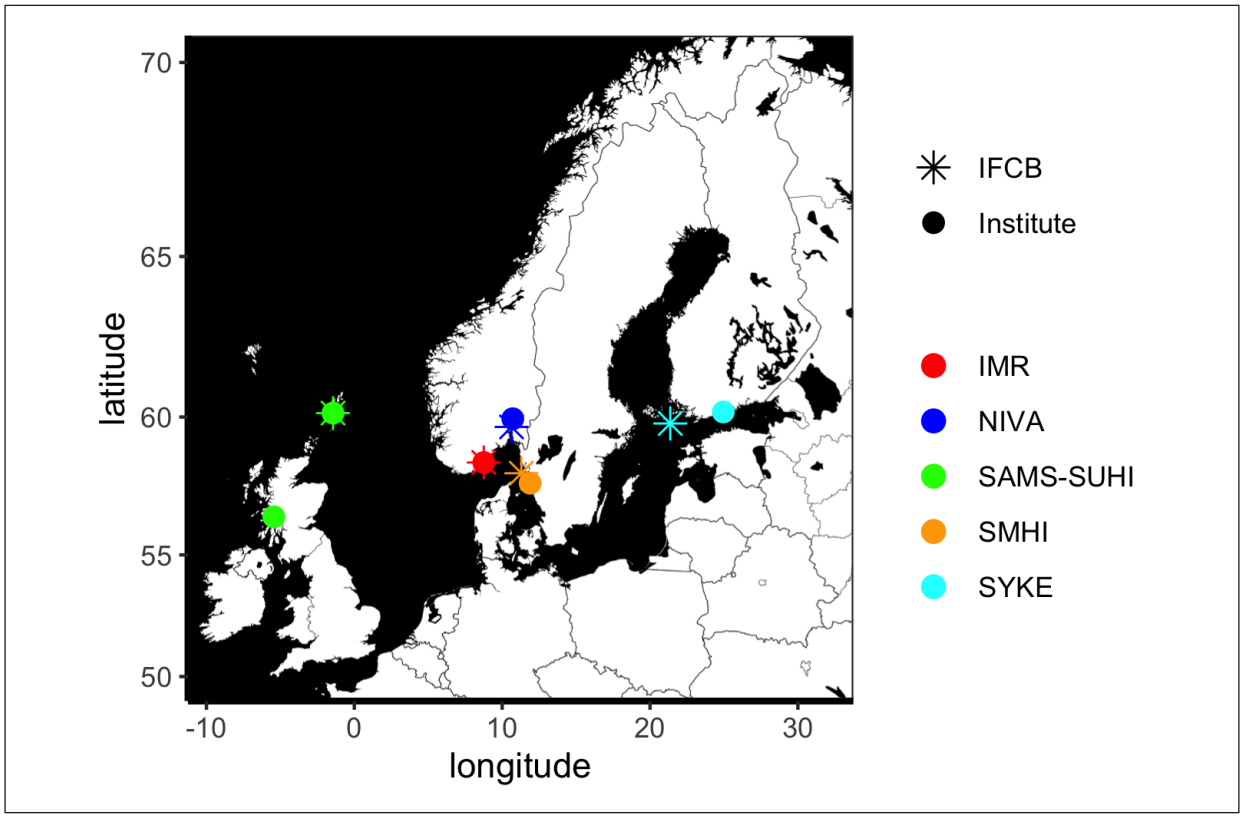

Fig. 1 Map showing locations of IFCBs operated in Europe until June 2021 and institutes operating the systems. Not all systems are fully operational. NIVA plans to operate one IFCB as part of ferrybox system on route Oslo-Kiel and SMHI plans to operate an IFCB during cruises with R/V Svea in the Baltic Sea and the Kattegat-Skagerrak. Acronyms: IMR (Institute of Marine Research, Norway), NIVA (Norwegian Institute of Water Research), SAMS (Scottish Association for Marine Science), SMHI (Swedish Meteorological and Hydrological Institute), SUHI (Shetland University of the Highlands and Islands), SYKE (Finnish Environment Institute).

The Imaging FlowCytobot (IFCB) [1,2] is a device for automated unattended phytoplankton analyses. A European IFCB Network was established in May 2021. Users of the instrument in Sweden, Norway, Finland [3] and the United Kingdom met virtually to discuss instrument handling and data processing. The basic premise is to help each other when problems occur and to join forces to develop software for automated identification of organisms. Regular virtual meetings are planned. The long term goal is to set up a European network for unattended automated phytoplankton analyses using platforms such as research vessels, ferrybox systems and fixed stations on islands or moorings. The IFCB can be part of early detection and early warning systems for harmful algae. Other European IFCB users are invited to join the group who will continue cooperation with IFCB users on other continents. The initiative for the European IFCB network came partly from *IOC-IPHAB Task Team on the Early Detection, Warning and forecasting or harmful algal events* and the *ICES-IOC Working Group on Harmful Algal Bloom Dynamics*.

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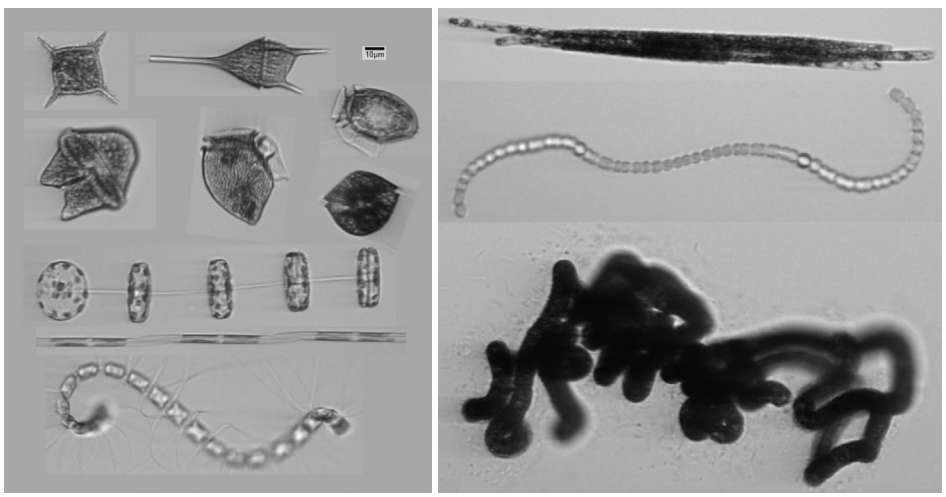

Fig. 2. Examples of images from IFCB. Left: Phytoplankton from the Swedish west coast. Right: Cyanobacteria from Utö Marine Research Station, Northern Baltic Proper.

Fifteenth session of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB)

In line with Covid-19 restrictions and to limit travel, for the first time since its establishment in 1991 IPHAB was held online (Fig. 1). The fifteenth IPHAB session was held between 23-25 March 2021. The mission of IPHAB is to assess progress, decide on priorities, and identify funding sources and opportunities for implementation of the IOC HAB Programme.

This programme is the only global intergovernmental effort to understand, manage and mitigate the harmful effects of algal blooms. The panel consists of 8 Task Teams, 5 Regional HAB networks and groups, 2 Expert Working Groups, 1 Steering Committee, and 1 Project group and works closely with the IOC Science and Communication Centre on HAB at the University of Copenhagen Denmark.

This session was the first IPHAB held during the UN Ocean Decade and in opening the session, Dr Vladimir Ryabinin, Executive Secretary of the Intergovernmental Oceanographic Commission and Assistant Director General of UNESCO addressed the delegates with a reminder of the importance of the work of this panel, the collaborative global work accomplished by our regional groups and topic task teams, and advances since the previous session in particular the completion of the Global HAB Status Report.

Among other topics, the delegates discussed the GlobalHAB Research Programme, Global Assessment of HAB Events, capacity building, a plan for improved research and management of ciguatera, improving HAB forecasting capabilities for more effective mitigation strategies, and a formulation/endorsement of specific objectives for regional activities.

Some major achievements reported, (some still in progress), included:

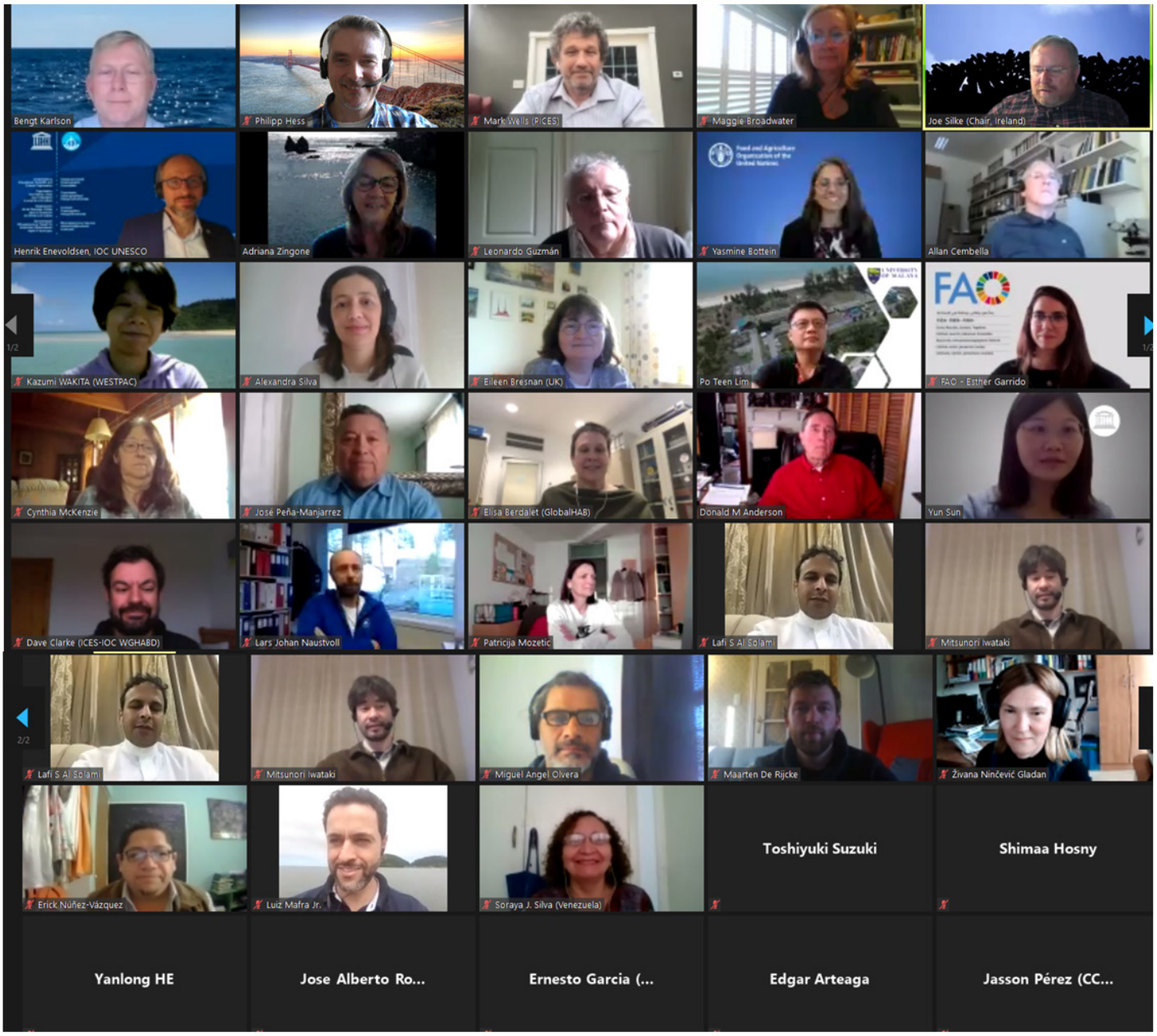

Fig. 1. Participants attending the XV IPHAB session 23-25 March 2021

- Results from the ICES-IOC Working Group on Harmful Algal Bloom Dynamics and ICES-IOC-IMO Working Group on Ballast and other Ship Vectors.
- Updates on the continued publication of the IOC Harmful Algae News.
- Implementation of four training courses.
- Growing development of the regional activities in Western Pacific (IOC/WESTPAC/HAB); the Caribbean (IOC/IOCARIBE/ANCA), South America (IOC/IPHAB/FANSA) and North Africa (IOC/IPHAB/HANA).
- Advances of the PICES HAB-Section and the GlobalHAB publication of "Guidelines for the study of climate change effects on HABs".
- Contribution to the development of a Technical Guidance Document for the Development of Early Warning Systems for Marine Harmful Algal Bloom Events to be published, as a joint publication of FAO, IOC-UNESCO and IAEA, as a FAO Fisheries and Aquaculture Technical Paper.
- Completion of the Global HAB Status Report and overviews on the IPHAB-IODE Harmful Algae Information System with HAEDAT and OBIS databases as providers of high quality information on HAB events, status and trends of HAB occurrence and assessment of climate change impacts.
- Advances achieved by the joint interagency strategy for Ciguatera, namely in testing and standards development and availability, and the publication of FAO-WHO Expert Meeting Report on Ciguatera.
- Developments under the joint IOC-SCOR GlobalHAB science programme such as the assessment of economic impacts of HABs in collaboration with NOWPAP, PICES and NOAA;
- Opportunities the UN Decade on Ocean Science for Sustainable Development bring to IPHAB for transformative science solutions for sustainable development of the ocean in relation to HABs and
- Development of activities and partnerships carried out by the International Society for the Study of Harmful Algae (ISSHA) to promote and foster research and training on HABs.

Commenting on the session, the chairperson of the panel Joe Silke (Ireland) said "IPHAB has continued a broad engagement between the science and management community of our activities, the strengthening of collaborations between IOC HAB groups in particular has led to sharing knowledge, guidance and capacity building; and further progress including implementation of early warning systems". He also stated "Further developments including the development of autonomous observing systems and molecular based approaches are bringing new HAB knowledge and the definition of future mitigation strategies"

IPHAB 15 took eight decisions and endorsed two recommendations concerning the work-plan and terms of reference. Work will continue on topics such as the regional task teams, early warning, database developments and global HAB status report, ciguatera strategies, desalination and HABS, monitoring of biotoxins and regulations, algal taxonomy and fish killing microalgae. These decisions were presented for the consideration of the IOC Assembly and were approved at its fiftieth session on June 18th.

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ICES-IOC WGHBD

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) held its annual meeting, online, from 20th to 23rd April 2021 where 40 participants attended. Dave Clarke, Marine Institute is the current chair for the new three year reporting cycle.

During the course of the meeting there were presentations and updates from WG members on the activities of related bodies and groups including IOC UNESCO updates (IPHAB XV UN Decade of Ocean Science (2021-2030), Harmful Algal News, training courses and the International Phytoplankton Intercomparison exercise) and an update on GlobalHAB Scientific Steering Committee activities. There was a series of presentations from WG members who are participating in a number of IPHAB Task Teams which include; and updates on the harmful algal information system (HAIS) and the first Global HAB Status Report.

The meeting also kicked off the new 3 year cycle of Terms of Reference (ToR's) with nine ToR's for the current 2021-2023 cycle. Highlighted ToR's include: a) Investigating the climate-driven changes in the distribution of planktonic and benthic HAB taxa (incl. cyanobacteria); b) Examining fish mortalities and the impact of sub lethal effects and the role of harmful phytoplankton as multi-stress contributors to fish morbidity; c) Molecular methods for the study and monitoring of HAB species and d) Implementation of automated observation systems for harmful algal bloom observations to improve early detection. Standing ToR's of North Atlantic area countries National Reports for 2020, and also on the updating of the Harmful Algal Events Database were also discussed.

The next WGHABD meeting is scheduled to take place from 5th to 8th April 2022. For more information contact:

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Maria Anna Faust
1930-2021

Dr. Maria Anna Faust (neé Spillenberg), aquatic microbiologist and research botanist emerita from the Smithsonian Institution, passed away on April 24, 2021, at the age of 91 from complications related to a stroke.

Maria Anna Spillenberg was born in Budapest, Hungary on April 21, 1930, with her twin sister Marta, to Anna Maria and George Spillenberg. Her childhood was spent in a small, close knit Hungarian village near Budapest. As a young girl, she endured the onset and duration of World War II. Her father, a medical doctor, was called into the Hungarian military and left his young family behind until after the war.

Maria received her Bachelor of Arts degree in 1951 from the Agricultural University of Budapest where she met Miklos Faust, the love of her life. They wed on July 17, 1954, and welcomed their daughter, Judit, two years later. The onset of the communist political system in Hungary provided many challenges which lead to the abrupt departure of Maria's family to Yugoslavia in 1957. They remained there in a refugee camp for 20 months awaiting asylum to the United Kingdom, Australia or the United States, whichever country would accept their petition first. Upon arrival to the United States, Maria, Miklos, Judit and Maria's parents worked toward a new life making their way from humble beginnings. The Faust and Spillenberg families valued education and felt it was a way of securing their futures. Consequently, Maria earned a master's degree in microbiology at Rutgers University in 1962, followed by a doctoral degree in aquatic microbiology at the University of Maryland at College Park in 1970.

Maria's earliest published work (Faust, 1969*) was on the effect of respiratory inhibitors and membrane-active compounds on bacterial motility followed by comparisons between bacterial and algal utilization of orthophosphate in estuaries. Her research interests lead to a postdoctoral fellowship at the Smithsonian Radiation Biology Laboratory (SRBL) in Rockville, MD in 1971 where Maria worked with Dr. Elizabeth Gantt. Maria accepted a position at the SRBL in 1973 but it was closed a few years later. Maria was reassigned and continued her work as aquatic microbiologist at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD. There her focus was on a series of prescient water quality studies. These included the relationship between land use practices and fecal bacteria in soils, organic carbon release by phytoplankton, transport of bacteria in temperate marsh sediments, tidal transport of microorganisms in brackish marshes and nutrient fluxes in tributaries of the Chesapeake Bay. Maria's dedicated work set the stage for subsequent environmentally relevant efforts to inform management decisions. One of her contributions at SERC was a pontoon boat duly named, the *Queen Maria*. This barge is still afloat and functioning in the same capacity it was designed for – a stable work platform for sampling on Muddy Creek and the Rhode River.

In 1983, at a juncture in her career, Maria attended an intensive three-week workshop in Advanced Phytoplankton Taxonomy. This was the third in a series of courses which started in 1976 upon the recommendation of the SCOR Working Group of Phytoplankton Methods that was organized in Drobak, Norway by the Marine Botany Section of the



Plate1: Field photos. A. Smithsonian's Field Station at Carrie Bow Cay (CBC), Belize 2009; B. CBC May 2001; C. Smithsonian's Field Laboratory at Ft. Pierce, FL July 2009; D. CBC May 2004 (front row: Marta Nicholas, Maria Faust, Pat Tester, R.J. Chrost; back row: Sabrina Varnam, Bert Pfieffer, Steve Kibler, Mark Vandersea); E. Celebrating Maria's birthday at the Smithsonian's Field Laboratory at Ft. Pierce, FL July 2009. (front row Maria, Pat Tester; back row Steve Kibler, Chris Holland, Mark Vandersea); F. Maria at the Smithsonian's Field Station at Carrie Bow Cay (CBC), Belize; G. Maria collecting dinoflagellates at South Water Cay, Belize, May 2002; H. Maria isolating dinoflagellates at CBC May 2004; I. Morning exercise, "Give me 10 more." CBC May 2003 (Sabrina Varnam, Mark Vandersea, Steve Kibler, Wayne Litaker, Maria); J. CBC sunset

University of Oslo. There Maria and her cohorts, many of whom would become leaders in their field, spent long days and some long nights under the tutelage of outstanding instructors, Grethe Hasle (diatoms) with assistance from Eric Syvertsen, Karen Steidinger (dinoflagellates) with assistance from Karl Tangen, Jahn Throndsen (flagellates), and Berit Heimdal (coccolithophores), with lectures from Barrie Dale.

From that time forward dinoflagellate taxonomy and ecology were Maria's major foci and she never looked back. In 1987 she joined the Department of Botany at the Smithsonian National Museum of Natural History, Museum Support Center (NMNH-MSC) in Suitland, MD where she remained until her retirement in 2009.

A series of studies on *Prorocentrum* (cysts, morphology, growth, photosynthesis, descriptions of nine new species) were published from 1985-1993. Much of her material and inspiration was from exploring the mangrove islands of Twin Cays, Belize, near the Smithsonian's Carrie Bow Cay Field Station on the Meso-American Barrier Reef system located fifteen miles offshore from Dangriga. Maria would return there over thirty times in her efforts to understand the taxonomy and ecology of tropical dinoflagellates more thoroughly. Work from 1994-1998 included her overview of benthic, toxic dinoflagellates and a review their life histories, broadening her interest to include *Ostreopsis* species and mixotrophic feeding by benthic dinoflagellates.



Plate 2: Professional. A. Maria Spillenberg 1953; B. Participants of the SCOR Working Group on Phytoplankton Methods organized in Drobak, Norway by the Marine Botany Section of the University of Oslo IOC Phytoplankton Course, 1983. Maria is in the front row, sixth from the right; C. Graduation from Rutgers University 1962; D. Phycological Society of America. Award of Excellence, Williamsburg, VA August 2004 (Wayne Litaker and Maria). E. Maria receiving PSA's Award of Excellence, Williamsburg, VA August. F. Smithsonian's Marine Science Symposium, Washington, DC 2007 (Maria and Wayne Litaker); G. Maria in New Caledonia 2008; H. Poster Session, SI's Marine Science Symposium 2007; I. Maria's office at the Smithsonian National Museum of Natural History, Museum Support Center in Suitland, MD, 2005 (Maria and Pat Tester); J. Maria in her lab at the Smithsonian National Museum of Natural History, Museum Support Center in Suitland, MD, 2005

Maria's paper on sand-dwelling dinoflagellates in 1995 described a new *Gambierdiscus* species that she named *Gambierdiscus belizeanus*. This was only the second species of what, at that time, was thought to be a monospecific genus. The only other described *Gambierdiscus* was the type species, *G. toxicus*, described in 1979 by Adachi and Fukuyo from the Gambier Islands in French Polynesia [1]. Her discovery would be followed in rapid succession by the description of three other new species found in the Pacific by Mireille Chinain in collaboration with Serge Pauillac of the Institut Louis Malardé, Tahiti [2]. One of these species, *G. polynesiensis*, would turn out to be the most toxic *Gambierdiscus* known to date. Maria's exquisite electron micrographs and her exacting organizational and record keeping skills in this study would prove most valuable a few years later.

The SEM stub containing preserved material for the GTT-91 isolate of *G. toxicus* from the 1999 study was deposited in the Type Collection of Dinoflagellates at the National Museum of Natural History, Smithsonian Institution. In 2009, when no live *G. toxicus* cells could be found, this SEM stub was used as the source for the redescription of the species and designation of an epitype [3]. This monograph described four new *Gambierdiscus* species and provided line drawings, high resolution light micrographs and SEMs of all extant *Gambierdiscus* species as well as sequence data that were deposited in GenBank. This comprehensive publication was subsequently selected for the Tyge Christensen Award for the best paper published in the International Phycological Society's journal *Phycologia* in 2009.

In her 40 year career, Maria published over 120 research papers. A notable publication, *Identifying Harmful Marine Dinoflagellates*, [4], is a taxonomic identification and reference guide of 48 harmful marine dinoflagellate species with descriptions of plate and thecal morphology and excellent scanning

Continued on page 22

Gertrud Cronberg
1937-2021



Phycologist Gertrud Cronberg has completed her odyssey.

A pioneer in the study of cyanobacteria and chrysophytes, limnologist professor emerita Gertrud Cronberg has left us. She worked at Lund University for more than 40 years. Gertrud was born in Malmö in 1937. Her interest in small organisms in water was sparked when she read H C Andersen's fairy tale "The Water Drop" as a child. This story describes the marvelous life in a drop of water from a pond and she acquired her own microscope from an early age.

Her father, Hans Hellsten, was head of the infection clinic at Malmö General Hospital, and had the official task of taking water samples from the pond Stora Pildammen, the drinking water source of Malmö. Gertrud often accompanied her father to the pond, and she gained the insight that a drop of water could also could contain organisms that caused life-threatening diseases. The relationship between aquatic microbes and diseases, an interest that originated from an early age, became a theme in her research activities throughout her life.

The Hellsten family spent their annual summer holidays in the Höör region of Sweden where they used to swim in Lake Ringsjön. Fifty years later, in a scientific paper on Lake Ringsjön, she described how the lake water in the 1940s was green from cyanobacteria and that she and her siblings got rashes. As a teenager she met the love of her life, medical student Stig Cronberg. After their marriage in 1960 they became the parents of seven children over the course of ten years.

In the late 1960s, Gertrud studied with the legendary phycologist Pierre Bourrelly in Paris. Bourrelly encouraged her to start studying chrysophytes since Sweden is a paradise of chrysophytes. Gertrud heeded his call. She became one of the first to use electron microscopy in Lund and produced thousands of unique images. Chrysophytes form species-specific resting cells that are hidden in the lakes' sediments. Gertrud became a pioneer in using these resting cells to map the historical and environmental changes in lakes as well as creating a systematic order among cysts from chrysophytes.

Along with the scientific importance, Gertrud's SEM photos with chrysophytes are amazing works of art from nature. Through this photographic art, Gertrud was invited to Oxford where she lectured on art in science.

In 1968, she began her doctoral studies which led to a dissertation and PhD in 1980. Her dissertation "Phytoplankton Changes in Lake Trummen Induced by Restoration" is one of the classic works describing the control of limnic ecosystems. In Lake Trummen, she and her colleagues performed pioneering experiments where the effects of fish on water quality were studied. They were the first to demonstrate that cyanobacterial mass development can be triggered by the addition of benthivorous fish. The results of these experiments inspired lake managers worldwide to reduce cyanobacteria in lakes by reducing benthivorous fish.

During several decades, Gertrud monitored the phytoplankton in hundreds of Swedish lakes. She focused on selected lakes with cyanobacteria blooms such as Lakes Ringsjön and Finjasjön. In the 1990s, the water quality of these two lakes were improved by the reduction of benthivorous fish. This management action was based on results from the experiments in Lake Trummen.

The presence of *Gonyostomum semen*, a nuisance alga belonging to Raphidophyceae, increased in a large number of Swedish lakes at the end of the last century. *Gonyostomum semen* is problematic because it produces a mucus that can irritate swimmers and clog the gills of fish and bottom-dwelling animals as well as water filters. Gertrud found this alga particularly interesting. In 2005, after decades of research, she revealed the complicated life cycle of *Gonyostomum semen*.

Gertrud's research activities attracted international attention and she conducted extensive research in waters at various latitudes. In Brazil, she participated in a WHO project focusing on Lago Paranoa, near Brasilia. Lago Paranoa was affected by heavy blooms of the cyanobacterium *Cylindrospermopsis raciborskii*. Using experiments, Gertrud succeeded in showing how the supply of nutrients triggered the development of cyanobacteria. Through this

research, and her teaching and supervision of technicians, Gertrud received the honorary title, "Mother of Limnology in Brazil".

In the early 1980s, she organized annual UNESCO-sponsored courses on tropical water resources for lake managers. This initiated a project on Lake Kariba, an impounded reservoir that was created on the Zambezi River. Gertrud built up a research station at the Zimbabwean lake side which has been appreciated by a large number of students and scientists.

West of Lake Kariba, the Okavango River forms the world's largest inland delta in the Kalahari desert. The hydrobiology of the delta has been explored under Gertrud's leadership. The algal flora of the Negril and Black River Morasses, Jamaica, the Namn-Guom Reservoir, Laos, and the lakes around Ho Chi Minh City in Vietnam also became parts of her field research.

Alone, or together with colleagues, Gertrud has discovered 91 species and varieties of algae and published approximately 100 scientific papers. Three algal species have been named after her; the chrysophytes *Synura cronbergiae* P.A.Siver and *Mallomonas cronbergiae* Piatek, the cyanobacterium *Chroococcus cronbergiae* Komárek & E.Novelo and the cyanobacterial genus *Cronbergia* J. Komárek, E.Zapomelová & F.Hindák, with four species.

During her expeditions around large parts of the world, Gertrud collected thousands of algal samples that were carefully studied and photographed. Gertrud was the co-author of a handbook on cyanobacteria, published by IOC-UNESCO and ISSHA in 2006, in which many of her beautiful images of cyanobacteria were presented. Her wide-ranging knowledge on harmful algae made her an important investigator when solving the cause of waterborne outbreaks.

She lived a life in wonder and curiosity with the constant passion for the diversity of life in a drop of water. Her work was characterised by professionalism, integrity and courage. Gertrud never hesitated to raise her voice against bullying or abuse of power.

Gertrud generously shared her knowledge with students and colleagues from all over the world. She was always keen to build up local skills in the countries she visited and therefore supervised a large number of master's and doctoral students from Asia, Europe and Africa. She opened her and Stig's home to them with warmth and her generous hospitality.

Gertrud is mourned by her husband Stig, seven children and families, twelve grandchildren, relatives and friends from all over the world.

We are many who will sincerely miss Gertrud and who feel a genuine and profound gratitude for what she has meant to us and to numerous others.

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Continued from page 20

electron and light micrographs. This contribution from the US National Herbarium has been widely distributed to and valued by students and researchers worldwide. It assisted in Maria's love of teaching. She freely shared her expertise and experiences with others by inviting them to visit her laboratory, sharing the research facilities at Carrie Bow Cay, Belize or spending time with them at the poster sessions at meetings. She was a strong supporter of the International Society for the Study of Harmful Algae and a member of the American Phycological Society, attending their meetings and contributing regularly. In 2004, Maria was honored by the Award of Excellence from the Phycological Society of America. Maria will be remembered for many things including her technically exquisite and artistic microscopy (SEM). But most of all she will be remembered for her old-world graciousness, her kindness, her wisdom and eagerness to share her knowledge and passion for dinoflagellates.

*See a full list of Dr. Faust's publications at *https://scholar.google.com/citations?user=VWu61XsAAAAJ&hl=en*

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ISSHA's Corner

The **19th International Conference on Harmful Algae (ICHA 2021)** will be held for the first time from 10th - 15th of October using an on-line format from La Paz, B.C.S., Mexico. We have received a total of 403 contributions divided between 9 plenary speakers, 244 oral presentations, 40 speed talks and 110 posters from 47 countries. Over 700 participants have registered for the conference!

The plenary talks will be given by outstanding researchers in different fields of harmful algae: Beatriz Reguera, Marie Yasmine Dechraoui, Michelle Burford, Christopher Whithead, Ernesto García Mendoza, Uwe John, Iwataki Mitsunori, Alexandra Worden and Jorge Mardones.

Several generous sponsors covered the registration fees of 77 participants from 13 countries.

- We will also have six special sessions which will be led different specialists:
- The Young Investigator Networking Session
- HAB Early Warning Systems Session
- Impacts of HABs on fish farms: Addressing industry and global insurance needs
- Control of Cyanobacterial Blooms
- NHABON-NE (National HAB Observing Network in the Northeast USA), a prototype node for a national HAB sensor network in the United States
- The presentation of a new book, 'WHO guidance on cyanotoxins' by Ingrid Chorus

During the conference, awards will be given to outstanding ISSHA members for their relevant and long-standing contribution in the study of harmful algae, to young scientists that have made a -break through- contribution in the field, as well as for the best oral and poster student presentation.

For more details on this conference visit our website: *http://www.icha2021.com/Secciones/inicio*



Elections for International Society for the Study of Harmful Algae (ISSHA) Officers and Council members

One of the benefits of the membership of the International Society for the Study of Harmful Algae (ISSHA) is the ability to vote for new Council members and Officers of the Society. Please take a moment to read the *candidate bios* and to vote using this link *here* by midnight La Paz, Mexico time on October 10, 2021.You will be required to login to the *ISSHA website* with your username and password. A password reset option is also provided.

The winners will be announced at the General Assembly meeting during the *ICHA2021 conference* on Monday, October 11, 2021. I look forward to "seeing" you at the Conference. Please vote!

Most sincerely,
Vera Trainer (ISSHA President)

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

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The 19th (1st Virtual) International Conference on Harmful Algae hosted from Mexico

The 19th International Conference on Harmful Algae (19 ICHA), was held in a virtual format for the first time, from the 11th - 15th of October, 2021 in Mexico. The Conference was hosted by the academic institutions IPN-CICIMAR and CIBNOR. Christine Band Schmidt chaired the local organizing committee with the support of members of the Mexican Society for the Study of Harmful Algae (SOMEFAN).

Initially, the Conference was planned to be held in the beautiful city of La Paz, Mexico, in October 2020, but due to the COVID-19 pandemic, it was postponed for one year. Innocently, at that point we still believed the virus would be under control in a few months and that we could all get back to our normal academic lives. However, as we all know this did not happen, and many of us are still working at home and in strict quarantine. At the start of 2021, we thought a hybrid event was possible. We believed that at least some of the colleagues would manage to travel to La Paz. Still, reality reached us when we realized that many of us, including Mexican colleagues, would not be allowed to travel to an in-person conference. Facing these facts, we had no other option than to hold a 100% virtual conference. This solution came with another reality: our lack of experience in logistics and technical details for organising such a task. However, the ISSHA council took this challenge to heart. Under the excellent guidance of our past president Vera Trainer we started monthly meetings in 2021 to discuss all of the details we needed to consider. The staff from Intermeeting (our conference organizer) also participated actively in these meetings since they were also facing the same challenge. Good communication and these productive meetings were an excellent way of working: they got us all on the same page, and between us, we made this Conference work.

The 19th International Conference was the second ICHA meeting held in Latin America and the first hosted by Mexico. We had a total number of 804 participants from 53 countries. The maximum attendance in the different sessions ranged from 46 and 401 participants, most of the sessions had an average around 100 participants. As always, plenary talks and special sessions had the highest number of participants, all with more than 200 participants. To our surprise, the virtual format allowed colleagues who usually cannot attend ICHA conferences due to the travel



The Virtual Conference Centre

unesco

Intergovernmental Oceanographic Commission

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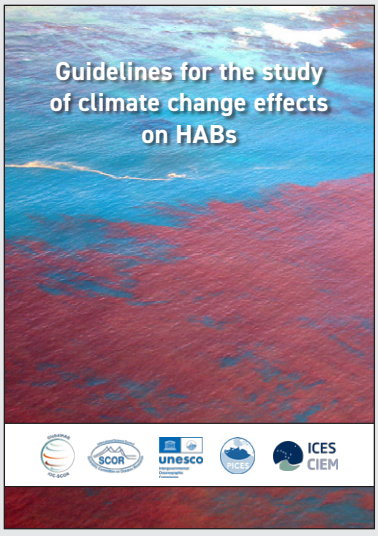

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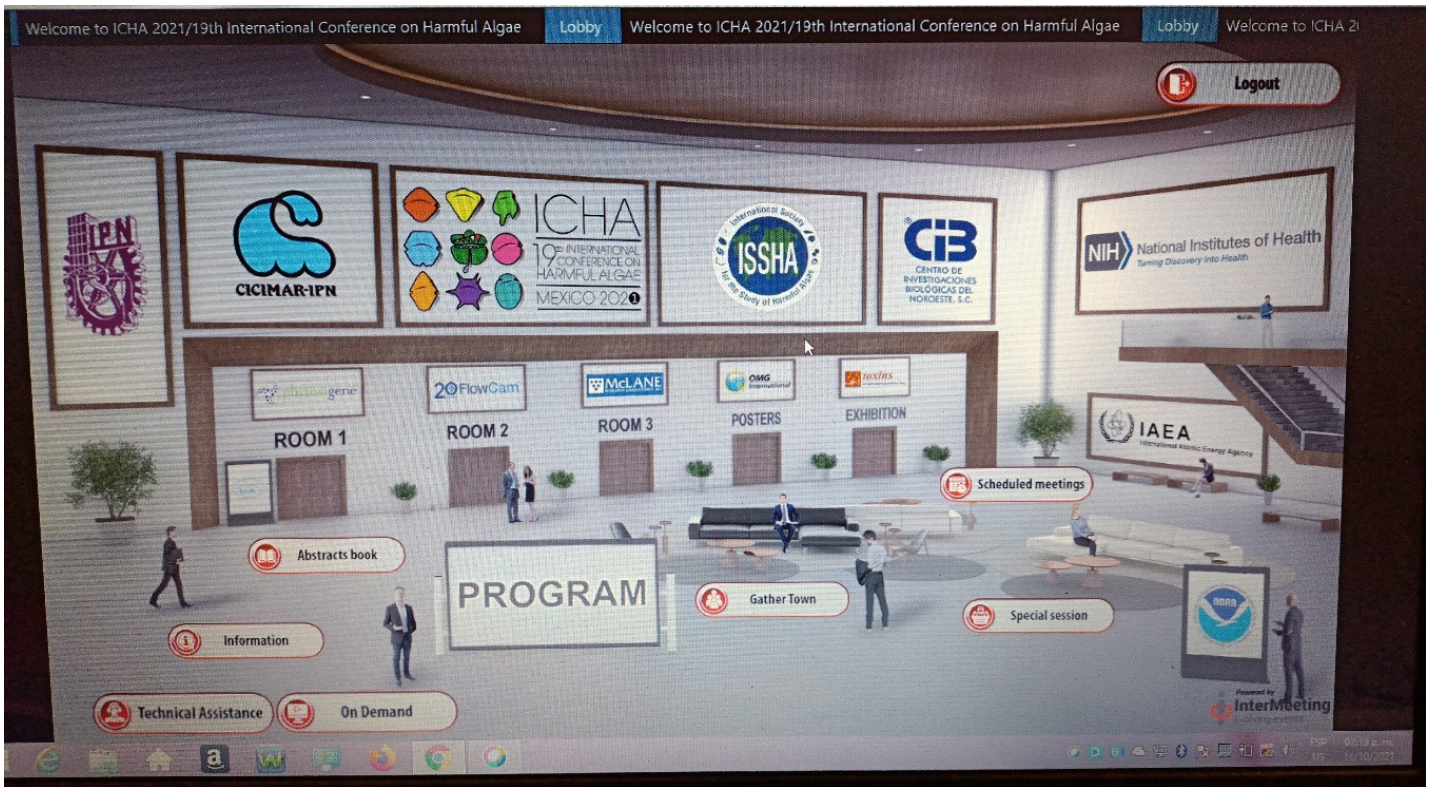

Lobby of the virtual ICHA 2021

costs to attend the meeting. The Conference started with two pre-conference sessions of the Young Investigator Networking Session coordinated by Lorena Durán, Carlos Rodríguez, Philipp Hess, and Harry Nelson. Previously, Lorena Durán and Carlos Rodríguez interviewed outstanding harmful algae scientists and longtime ISSHA members: Allan Cembella, Barrie Dale, Beatriz Reguera, Christine Band, Donald Anderson, Gustaaf Hallegraeff, and Vera Trainer. These interviews are available at: *www.youtube.com/channel/UCAjwNmXU-I9hBeWUw91k5w*.

The conference program was divided into two time slots to cover all the world time zones in the best possible way. The diverse topics covered were also divided between both time windows. However, this was not possible for a few topics due to the low number of contributions. Each time slot had three parallel sessions. For the first time in an ICHA conference, we had to face the challenge of sending our recorded contributions a month before the start of the Conference in order to have the platform ready on time. Recording the presentations was complicated for many of us since obtaining research results during 2021 was slower than in other years. Fortunately, all participants managed to send scientifically sound and very nice presentations.

The program could be downloaded to mobile phones or computers, and it indicated the time of the presentations in each specific time zone. We had five





Christine Band-Schmidt, Chair of the Local Organizing Committee. Welcome from La Paz, Mexico, the conference venue that could not be...



The Mexican scientists comprising the Local Organizing Committee

special sessions held live on Zoom that covered breakthrough topics on harmful algae from marine and freshwater environments: "HAB Early Warning Systems Session", "Impacts of HABs on fish farms: Addressing industry and global insurance needs", "Control of Cyanobacterial Blooms", "NHABON-NE, a prototype node for a national HAB sensor network in the United States", and the presentation of the new book WHO guidance on cyanotoxins. All sessions were led by outstanding experts and held at noon Mexican time to facilitate participation from all time zones. We knew this was complicated since, for some, it was very early in the morning and for others very late at night, but this was the best solution we found. ISSHA members were fully engaged, chairing nine plenary talks, 245 oral presentations, 40 speed talks, and 110 poster presentations. They also evaluated student presentations, elected the Yasumoto lifetime achievement awardees, and held the new ISSHA committee elections. After each session, virtual interactions among participants and presenters was accessible through live questions and answers. Also, informal meetings were possible on the platform "Gather Town." All presentations were available on the conference platform until the 30th of November 2021, which gave us the opportunity for the first time to see the presentations we missed.

Unfortunately, the popular ISSHA auction and the traditional social events could not be held this time, but they are still on the list of activities for future ICHA conferences. We look forward to *in-person* ICHA conferences again; however, after the organization of this Conference, we have learned that combining *in-person* and virtual conferences could offer all ISSHA members a broader opportunity to participate in these meetings.

It is essential to mention that this Conference could not have been held without the support of our generous sponsors: FAO, IAEA, SCOR, Flowcam, NIH, NOAA, McLane, Phytotoxigene, GlobalHAB, MDPI Toxins, COSCyT, Sumilab, RedFAN, and SOMEFAN. We greatly appreciate their support; it allowed us, among other things, to cover the registration fees of students and scientists from under-represented countries, as well as to hold several of the special sessions.

Thank you all for making this another great ICHA conference and for helping us hold the flame of ISSHA during complicated times. We very much look forward to seeing you all in person again.

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Scientific Highlights of the 19th International Conference on Harmful Algae

Summarizing all of the science from a conference is a huge and perhaps impossible task, but once done, it can serve as a reference for the future. We had five exciting and intensive virtual conference days where all the latest information in our field was presented. Undoubtedly, we cannot include summaries from all talks, and hence this overview focuses on some of the highlights. It would have been impossible to write these highlights without the incredible help of the session chairs and co-chairs. We thank all of them for their support!

Word cloud as a summary

We start this overview with a word cloud, which is a visual representation of the titles of the abstracts presented in the conference and provides the most prominent terms where larger font size means more counts (Fig. 1). So, the Word Cloud captures the essence of the 19th International Conference on Harmful Algae in one picture!

During our meeting, the most common words and the main topics were *management*, *bloom, HABs* and *toxins*, for which the latter came in other terms as well, like *toxic* and *toxicity*. *Shellfish* is also a prominent word, similar in coverage as *harmful*. The word cloud reveals a focus on dinoflagellate species, specifically on the genera *Alexandrium*, *Gambierdiscus*, *Dinophysis*, *Karenia*, but diatom genera, such as *Pseudo-nitzschia,* and freshwater *cyanobacteria* with *Microcystis* as prominent group, are also mentioned. The way we are now measuring diversity is using *genetic* and *molecular characterization* tools, which clearly also found their way in the titles and abstracts of the meeting. Several other words appear that can be related to each other. For example, *management* of *coastal* areas where *monitoring* of *species* and *events* are taking place.

Plenary talks

The plenary talks covered various topics, ranging from the taxonomy, toxicity, ecology, and management of harmful algal blooms across freshwater and marine habitats around the world. We heard about lessons learned from 40 years of research on the physiology, toxicity, and ecology of *Dinophysis* (**Beatriz Reguera**), the phylogeny and taxonomy of unarmed dinoflagellates in the Kareniaceae family (**Mitsunori Iwataki**), and the impacts of climate change on the eco-physiology of the harmful cyanobacterium *Cylindrospermopsis* (**Michele Burford**). Some other talks emphasized the role of species interactions, including gene expression, biosynthesis, and toxicity of HABs in response to copepods (**Uwe John**), and the intracellular interactions between

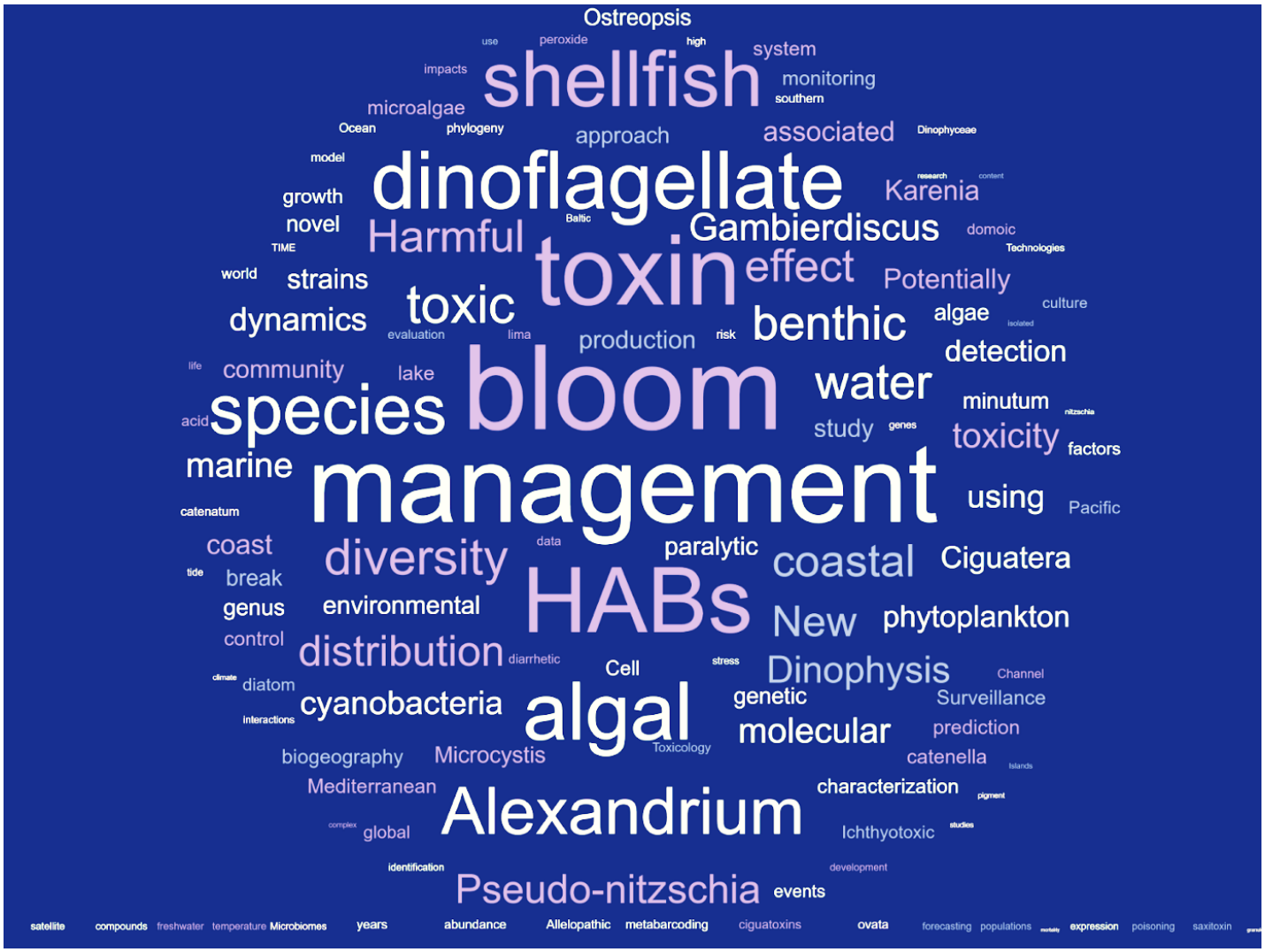

Fig. 1. Word cloud of the submitted titles and abstracts

predatorial protists, viruses, and bacteria (**Alexandra Worden**). Several plenary talks highlighted insights on HAB management, including emergent management strategies for monitoring, biotoxin detection, and human health risks associated with Ciguatera Poisoning (**Marie-Yasmine Dechraoui Bottein**); community partnerships in monitoring of HABs and shellfish toxins (**Christopher Whitehead**); examples about the importance of national networks to share HAB knowledge and promote communication, education and outreach (**Ernesto García-Mendoza**), and the consequences of eutrophication and extreme events on HABs and implications for salmon farming off the coast of Chile (**Jorge Mardones**).

1. Taxonomy - Who is out there?

A high diversity of benthic and epiphytic dinoflagellate flora in Australian tropical waters was reported, including *Coolia tropicalis*, *C. areolata*, *Ceratocorys malayensis*, *Prorocentrum lima* and *P. sculptile*, and also five new or currently undescribed species including one *Amphidinium*, one *Gymnodinium*, two *Ostreopsis*, and one *Prorocentrum* species (**Christopher Bolch**). A new species of the genus *Gertia* was described (**Kazuya Takahashi**), which is a unique peridinin-containing species in the Kareniaceae. In addition, there were several descriptions of new tropical dinoflagellates including two species of *Heterocapsa* (**Afiqah Hamilton Hanifah**), two species of *Alexandrium* (sub. gen. Gessnerium; **Nursyahida Abdullah**), a novel Suessiacean dinoflagellate (**Wai Mun Lum**) and new DNA sequence data for a diversity of gymnodinoid species (**Alexis Escarcega**).

Using cell immunolocalization techniques, cellular compartments that are involved in ciguatoxin (CTX) production and release in *Gambierdiscus australes* were identified. This finding suggests that the cell endomembrane system is involved in toxin production (**Giorgio Honsell**). Variations in toxin content and metabolomic profile were described for two genetically similar populations of *Gambierdiscus eccentricus* with different geographical origins along the coast of Brazil. Toxicity risks associated with *G. eccentricus* appear higher with warmer, clearer, and less turbulent coastal waters (**Luiz Mafra**). Moreover, CTX was shown to be retained for at least 3.3 months after fish are removed from a CTX source. This should be considered when developing models of CTX cascades between dinoflagellates and resident high site-fidelity fish and has implications for toxin analyses of fish that migrate away from CTX sources for long periods (**Clay Bennett**). A ciguatera poisoning survey along the west coast of Mexico showed that this illness is poorly documented and understood, particularly among medical professionals. This survey was the first step in developing effective means to inform physicians and the public (**Aramis Olivos-Ortiz**). Coupling of ecological and epidemiological data showed that the most significant respiratory impacts caused by *Ostreopsis* take place when blooms enter the late log phase, which is important information for protecting human health (**Elisa Berdalet**).

2. Distribution and diversity - Where are they?

There is no doubt about the high diversity and wide spread of HABs and toxins (many presentations) and molecular approaches reveal increasing diversity in HABs and toxin genes, but toxin genes and genotypes are not always related (**Cecilio Valadez-Cano**, **Emma Johansson**), and we are still asking ourselves if the frequency of HABs is increasing. A global monitoring metadatabase did reveal regional trends but reported no uniform global trend in HABs and increased occurrence could be linked to enhanced monitoring (**Gustaaf Hallegraeff**). In some specific cases, such as *Dinophysis*, a decadal distribution and associated shellfish toxins in the Regional Seas of Europe showed that while some subregions presented an increasing or decreasing trend for some species, no overall trend was detected at the genus level (**Philipp Hess**). A review of Ciguatera Poisoning (CP) global occurrences and trends was presented, which highlighted some major issues that currently hamper global efforts to capture the true burden of ciguatera. In this case, databases clearly reflect a significant weakness in data entry of CP events and *Gambierdiscus/Fukuyoa* spp. occurrence records, so data collated from scientific and grey literature are also important to understand global trends. The available time-series data on a limited number of CP endemic to countries/regions revealed that none of the studied areas showed an identical trend (**Mireille Chinain**). Longer time series on regional HABs were presented as well, which used ancient DNA and akinetes with over >9,000 and > 400 years records of dinoflagellates (Fig. 2) and cyanobacteria, respectively (**Linda Armbrecht**, **Steffaney Wood**).

3. Toxins and toxicology - What are they making?

An expansion of *Alexandrium pseudogonyaulax* in Northern European waters was shown, together with the global distribution of other known goniodomin (GD) producers. GD showed conversions and appropriate chromatographic techniques need to be applied in order to avoid artefacts. Preliminary results suggest that GD are not responsible for ichthyotoxicty of GD-producing species (**Bernd Krock**). A new data dependent LC-HRMS method was presented, which led to the discovery of several gymnodimine fatty acid esters and the detection of new gymnodimines through a backward analysis approach. Also, pinnatoxin G (PnTx-G) was for the first time detected in mussels in Sardinian coastal waters in 2016 (**Luciana Tartaglione**). The ichthyotoxicity of *Chattonella marina* was shown to depend on photosynthesis, especially related to the photoreaction stage (**Zhuoyun Xu**). Results from an experiment where *Daphnia magna* was exposed to cell free cyanobacterial spent medium from *Microcystis aeruginosa* highlighted that besides microcystin-LR, other secondary metabolites contribute to a negative impact of this cyanobacterium on *D. magna* survival and stress response (**Gorenka Bojadzija Savic**).

The phylogeny, lipid composition, pigment signature, ichthyotoxicity and growth of the fish-killing dinoflagellate *Heterosigma akashiwo* from Chilean Patagonia was highlighted. Further characterization of more Chilean isolates is needed to understand the precise ichthyotoxic mechanisms and environmental drivers that trigger massive bloom events in Chilean Patagonia (**Ana Flores**). A screening of the ichthyotoxic potency of *Karlodinium veneficum* strains against the rainbow trout fish gill cell line (RTgill-W1) showed that a

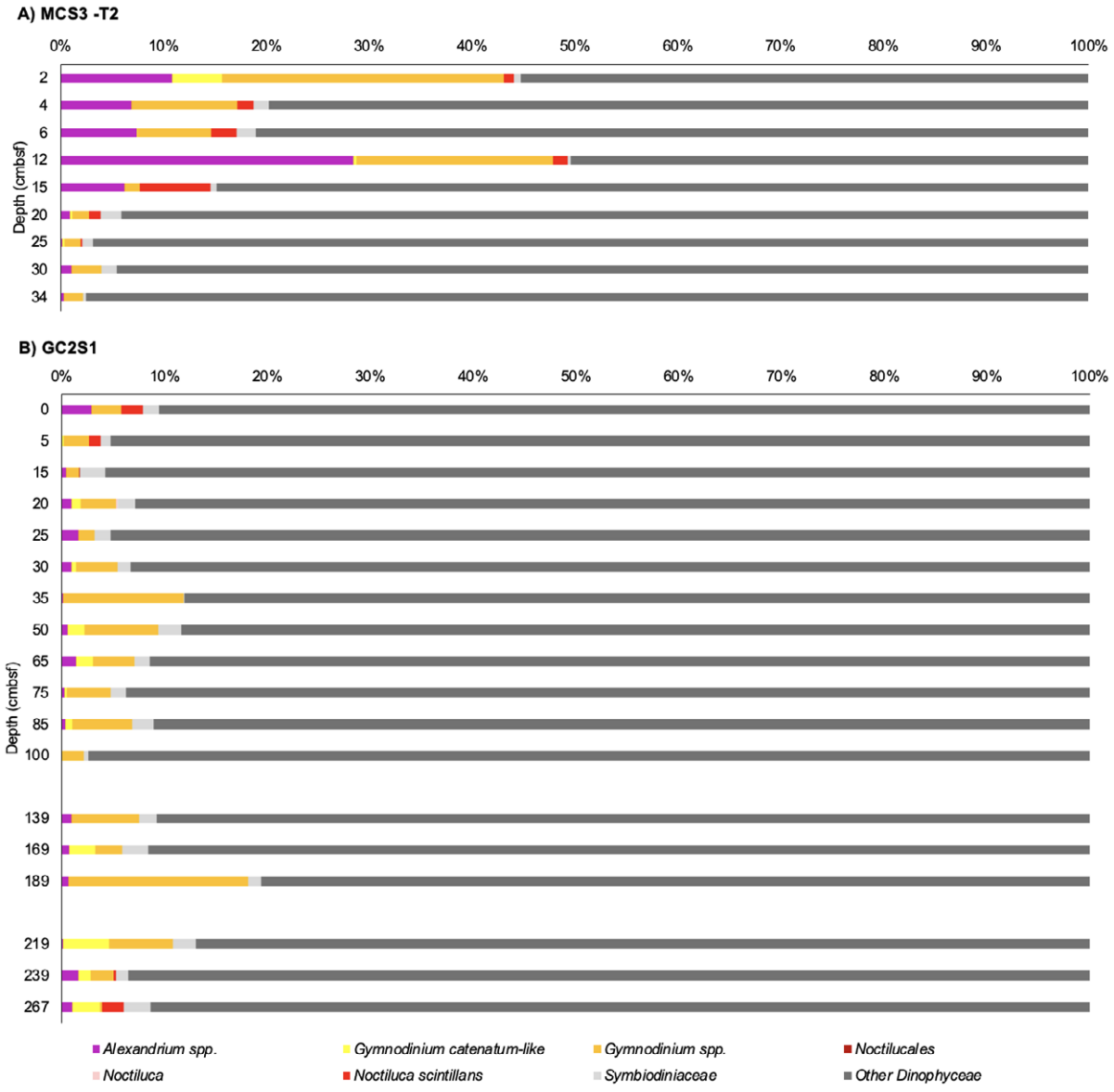

Fig. 2. Long-term history of HABs off Eastern Tasmania, Australia, using sedimentary DNA (courtesy of Linda Armbrecht)

French Mediterranean and a French Atlantic *K. veneficum* strain induced over 50% loss of cell viability. A high level of intra-specific variability of ichthyotoxic activity was observed among strains, and cytotoxicity of the intracellular and extracellular fractions were strain dependent. In general, the methanolic extracts were more cytotoxic than their corresponding extracts in the culture media (**Rima Beesso**). The emergent toxin pinnatoxin is shown to interact with nicotinic receptors (nAChRs), especially the muscle and neuronal (α7) subtypes, through binding and electrophysiological experiments. High-resolution 3D-imaging and in vitro competition studies on rat embryo sections revealed the specificity of [3H]-PnTx-G binding and its selectivity for muscle and neuronal nAChR subtypes (such as the α7 subtype). Results from the use of a human perfused cotyledon model and mass spectrometry analyses furthermore showed that PnTx-G crosses the human placental barrier, which indicates the potential adverse impact that exposure to pinnatoxins may have for human health (**Denis Servent**).

4. Species and interactions - What are they doing?

There were a number of presentations about *Alexandrium* life cycles including how cyst dormancy cycling controls *Alexandrium catenella* bloom termination in the Nauset Marsh estuary (**Michael Brosnahan**) and the degree day-based cyst germination rates and in situ germling production of this species (**Alexis Fischer**). These studies confirm with experimental data the usefulness of the approach (combining the effect of temperature and time) in life cycle experiments involving cyst germination, latitudinal differences, and the effect of global warming in phenological changes. The role of sexual reproduction, cyst dormancy cycling and germination rate in HAB development was also proved in the dinoflagellate *Alexandrium monilatum* in lower Chesapeake Bay (**Savana Mapes**). In the case of *Microcystis* blooms, the species was able to be successfully revived from the natural sediments in laboratory mesocosm studies (**Caitlin Romanis**), highlighting the success and pervasiveness of the genus stems from a meroplanktonic life cycle that features dense pelagic blooms in the summer and the benthic sedimentation of vegetative cells in the winter and the contrary. A cryopreservation method was developed that allows vegetative cells of thecate dinoflagellates (*Vulcanodinium rugosum*, *Alexandrium*

pacificum and *Durusdinium trenchii*) to be successfully frozen and revived with high viability (**Joseph Kihika**). This highlights the potential to have a microalgae diversity bank in the future.

Several presentations were focused on the intimate association between hosts and their microbiomes (**Christopher Gobler, Michèle Gourmelon,** Ávila **Andrés, Miguel Martinez-Mercado**) and parasite-host relation and genotype diversification for bloom species. **Antosiak** et al. showed how cyanophages infect freshwater *Aphanizomenon flos-aquae* attacking the carbon metabolism and in this way producing a decrease in the bacterial population. **Sassenhagen** et al. focused on infection experiments of diatoms obtained in spring bloom with eukaryotic unicellular parasites, where Chytridiomycota got the highest relative abundance after the diatom peak. The effect of four parasitoids acting on the dinoflagellates *Alexandrium pacificum* focusing on the parasitoid biological characteristics and possible bloom control was studied (**Boo Seong Jeon**). Overall, the studies presented interesting and cutting-edge approaches to the study of interactions between host and parasite or phages with implications for the physiology, ecology, and biological control of HABs. High diversity of parasitic chytrids in coastal areas affected by *Alexandrium* and *Ostreopsis* blooms was presented (**Alan Fernández Valero**). If these organisms are parasitoids or saprophytes is an open question, but there is no doubt that viruses, chytrids, and protists are impacting the physiology and ecology of HABs. Various examples of complex allelopathic interactions between HABs and competitors, grazers (Fig. 3) and parasites were also presented (**Marc Long, Sierra Cagle, Anna Junker-Olesen, Leyberth Fernández-Herrera**).

5. Management - What can we do?

Blooms of *Alexandrium catenella* were shown to not only contaminate shellfish in Alaskan waters with PSP toxins, but also commercially important fish (such as herring) which were found to contain PSP toxins with values several times above safety level. Highest concentrations were observed in the internal organs of the fish (**Steve Kibler**). At least 18 patients were hospitalized in Mirbat (Oman) in October 2020, after eating oysters (*Saccostrea cuccullata*) and mussels (*Perna perna*) that were

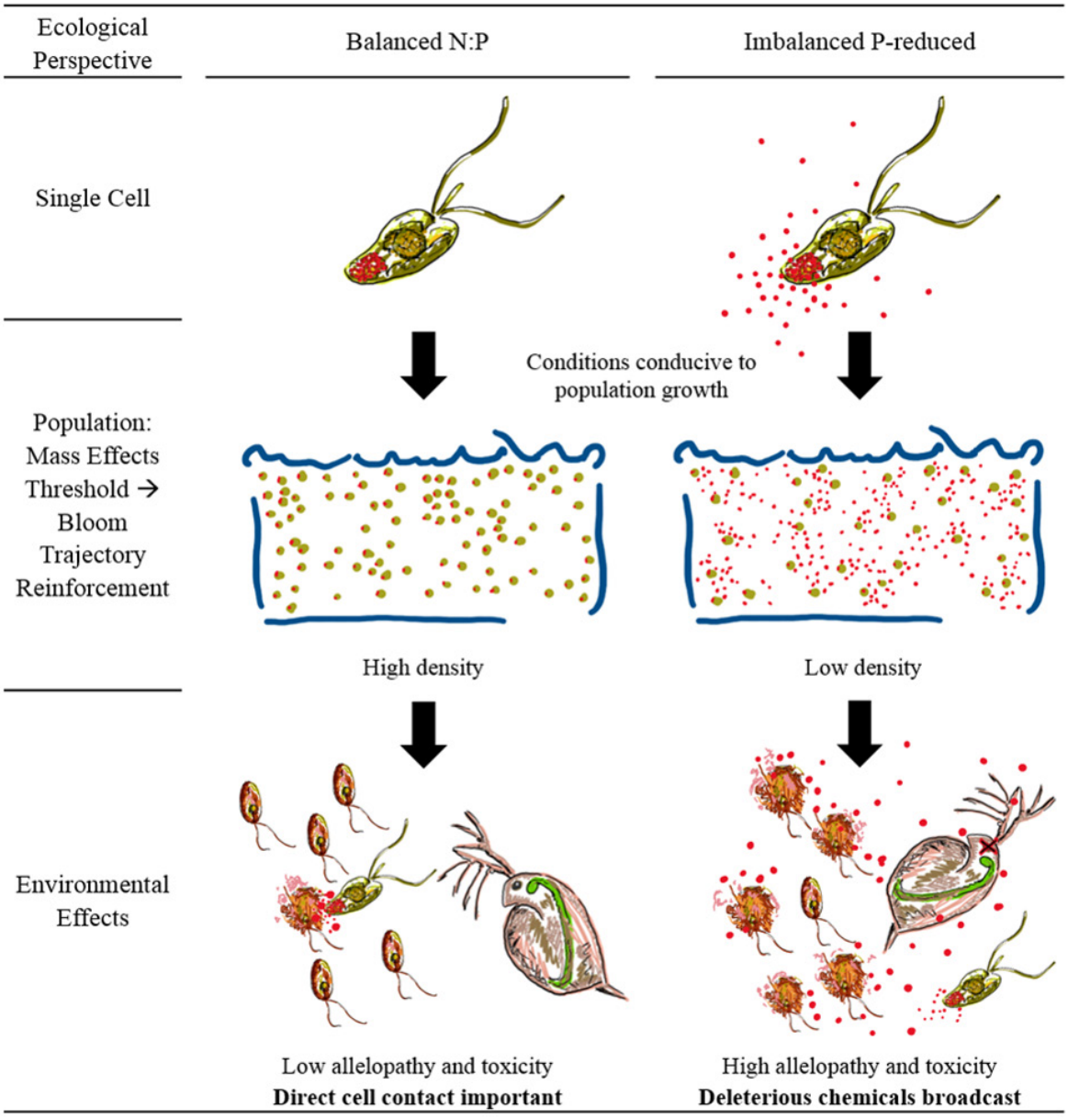

Fig. 3. Conceptual overview on the allelopathy driven interactions between Prymnesium parvum and Daphnia magna (courtesy of Sierra Cagle)

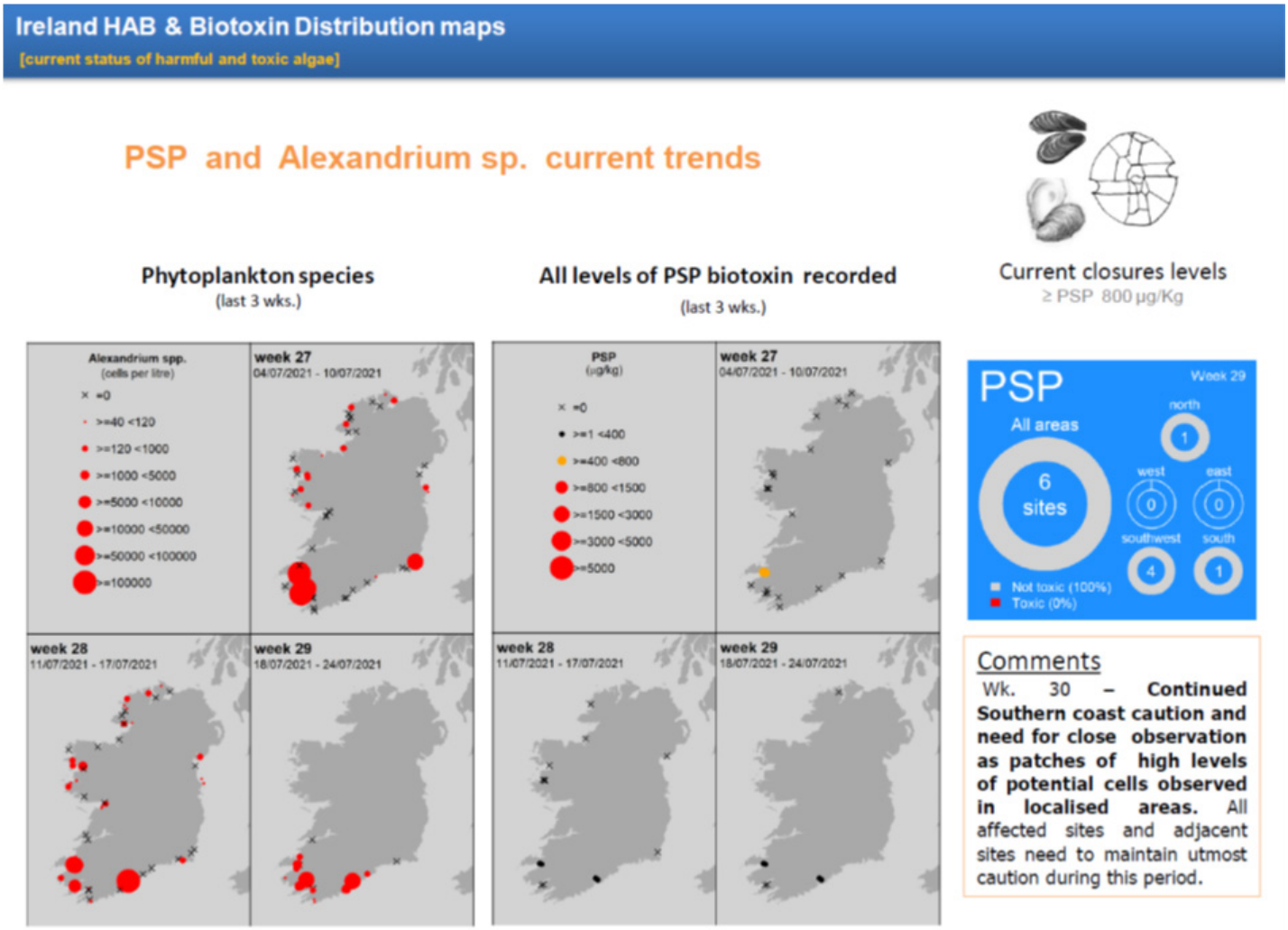

Fig. 4. Example from a weekly HAB bulletin reporting HAB events along the Irish coast (courtesy of Dave Clarke)

harvested along the coast. Analysis of seawater and oyster samples confirmed high levels of PSP toxins (**Margarita Fernández-Tejedor**). The design, implementation, and contents of HAB bulletins and early warning systems, including best practices guidelines on the development of weekly reports and forecasts, were reported for Scotland (**Callum Whyte**) and Ireland (**Dave Clarke**) (Fig. 4). Opportunities for improved forecasts of *Dinophysis* blooms in the Gulf of Mexico were shown with modelling approaches using temperature and prey size (**James Fiorendino**). More dedicated discussions took place in the special session on HAB Early Warning Systems chaired by **Aurelia Tubaro and, Marie-Yasmine Dechraoui-Bottein**.

Satellite surveillance of algal blooms in lakes and coastal waters was shown to have great potential for HAB monitoring. Particularly during COVID when human activity in the field was restricted, a new satellite *Copernicus Sentinel-2* with a pixel size of only 20 m, compared to 200 m in previously used satellites, increased the number of lakes that could be monitored for algal blooms many-fold (**Michelle Tomlinson**). For the assessment of the economic threat of diarrhetic shellfish poisoning (DSP), a financial-DSP-shellfish production model was developed for Scottish shellfish production. This model facilitated the investigation of the effect of DSP levels on shellfisheries closures and associated reductions in shellfish production in money equivalents. The model showed that a 1% increase in DSP toxin levels over the regulatory threshold reduced production by 1,080 tons, 15% of Scottish shellfish production (**Keith Davidson**).

A number of presentations showed that multi-sensor observations provide insights into bloom dynamics and raise new questions, especially about decoupling between cells, toxins, and shellfish. This was also highlighted in the special session on the national HAB sensor network of the United States (**Michael Brosnahan**). New developments were presented for the Imaging FlowCytobot, which showed continued operation for 6 months, handling over 60 samples a day covering size ranges of 5-150 µm (**Gregory Doucette**). Results from the Environmental Sample Processor (ESP) showed its applicability for high frequency sampling, with over 60 samples analyzed per deployment, with near real time monitoring of species and toxins using specific nucleic acid markers (**Nicolaus Adams**). Nanotube based electrochemical sensors were presented for rapid, sensitive and low cost analysis of HABs and their toxins (**Mònica Campàs**). Improved RNA detection of toxic species using magnetic microbeads and amperometric detection at screenprinted carbon electrodes were able to detect as low as five cells per liter for some species. This fast, simple, and cheap methodology can be integrated in easy-to-use portable systems (**Linda Medlin**).

Various mitigation strategies for harmful cyanobacterial blooms were presented. This included the payment

for lake ecosystem services and selection of environmental measures together with local farmers, which may reduce overall costs (**Claudia Wiegand**). A special session on the new WHO book 'Toxic cyanobacteria in water' provided advice and guidelines for the management of freshwater cyanobacterial blooms, including the need for system analysis to understand sources of nutrients (**Ingrid Chorus**). This was echoed in another special session on the control of cyanobacterial blooms, where various control strategies were also presented (**Petra Visser, Dedmer Van de Waal**). Such control methods may involve emergency methods, such as the use of hydrogen peroxide for selective suppression of harmful cyanobacteria. This was confirmed in a tropical reservoir, where cyanobacteria were suppressed and water quality improved (**Allan Santos**). Other talks described interaction of hydrogen peroxide with other factors, including reduced efficiency through interspecific protection by green algae (**Jef Huisman**), or limitation of these methods for high biomass blooms (**Eleni Keliri**). Another special session focused on the impacts of HABs on fish farms, and collaboration with the insurance industry was highlighted as an effective mitigation strategy to reduce losses due to planktonic fish-kills (**Mark Wells, Charles Trick**).

The last sessions of the meeting dealt with the wellness of coastal communities, where different experiences related to HAB impacts on society and its activities were shared. Dialogue and comprehension of the system were among the main approaches to achieve a better understanding of HABs and the importance of ocean communities. Different methods were presented including the use of social media and inclusive partnerships with participatory science for collaborative risk assessment (**Vera Trainer, Kazumi Wakita, Silvia Ramírez, Sergio Álvarez**).

Gracias amigos!

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Two local organizers and students from La Paz, B.C.S. Mexico at the closing of ICHA 2021 in La Paz. From left to right: Martín Yanez, Nadia Herrera, Christine Band, Ignacio Leyva (front center), Gema Villa and Leyberth Fernández.



Christine Band (the hostess) with her present, a beautiful carving from Prof Takayama, representing Gymnodinium catenatum, her main study topic. Present brought as a surprise to Christine by Nadia Herrera and other students of her.

ISSHA's Corner

The International Society for the Study of Harmful Algae (ISSHA) convened the 19th (1st Digital) International Conference on Harmful Algae (19 ICHA), held in La Paz, México, from 10th to 15th October 2021. The ISSHA Council had monthly meetings on-line between May and October to plan the Conference activities, Council elections, travel awards, achievement awards and the ISSHA General Assembly.

Travel Awards

There were applications for registration awards from countries all over the world to the ISSHA Travel Award Committee. Sponsors for the awards included the Scientific Committee for Ocean Research (SCOR), the National Oceanic and Atmospheric Administration (NOAA), the International Atomic Energy Agency (IAEA), the Food and Agriculture Organization (FAO) and private donations from ISSHA members. A total of 78 participants (including students, regular ISSHA members and managers) had their registration waived.

General Assembly, Council Elections, Closing Ceremony and Venue for the 20th ICHA 2023

The digital ISSHA General Assembly was held on Monday 11th October from 14:00 to 16:00.

The 13th ISSHA General Assembly was opened by President **Vera Trainer** and minutes from the previous assembly were approved. ISSHA members had voted on-line to renew Council officials and Executive members. Some had to step down after six years serving the Society in an executive position. Results of the elections were given by ISSHA Secretary. Officials stepping down were President **Vera Trainer** (USA), and Council members **Aurelia Tubaro** (Italy), **Ana Amorin** (Portugal), **Ingrid Sassenhagen** (France-Germany), **Keith Davidson** (UK) and **Marta Estrada** (Spain). Vera Trainer thanked all of them in the name of ISSHA for their years of service. Special thanks were given to **Marta Estrada**, council member and Chair of the Award Committee, for her years of dedication to the hard work of that committee, in particular coordinating all the student evaluations during the ICHA conferences to choose the Maureen Keller Awards. **Wayne Litaker** (USA) was the new elected ISSHA President.

Below is the new ISSHA Executive and Council for the 2021-2023 period (new members and old ones occupying new executive positions are in italics):

President
Wayne Litaker (USA)

Vice presidents
Po Teen Lim (Malaysia)
Dedmer Van de Waal (The Netherlands)
Secretary: Elin Lindehoff (Sweden)
Treasurer: Nina Lundholm (Denmark)
Past-president: Vera Trainer (USA)

Council Members
Philipp Hess (France)
Christine Band-Schmidt (Mexico)
Tim Harwood (New Zealand)
Luiz Mafra (Brazil)
Shauna Murray (Australia)
Esther Garcés (Spain)
Aifeng Li (China)
Marie-Yasmine Dechraoui Bottein (France)
Henrik Enevoldsen (Denmark)
Satoshi Nagai (Japan)
Ichiro Imai (Japan)
Rosa Figueroa (Spain)
Lorena Duran Riveroll (Mexico)
Ian Jenkinson (China, France)

Student Representative
Carolin Peter (Sweden)
Steffaney Wood (USA, student advisor)

The profiles of the ISSHA officers and council members can be found at *ISSHA Council*.

All details were planned in advance on line. Secret messengers, wearing COVID-proof masks, knocked on the door of the awardees at the right place and time. In this way, the prizes were awarded in real time and in direct connection with the digital venue of the event.



Linda Medlin was surprised at the time of the Yasumoto award announcement by her husband Dick Crawford with a Takayama carving of Alexandrium tamarense (mature cell and hatching cyst).

ISSHA Achievement Awards 2021

ISSHA 2021 Yasumoto Lifetime Achievement Award

Linda Medlin was the winner of the ISSHA 2021 Yasumoto Lifetime Achievement Award. The prize was in recognition for her outstanding contributions to the field of molecular biology applied to the study of marine phytoplankton diversity, monitoring of harmful algae and development of

molecular probes targeting HAB species (including their different life cycle stages), difficult to identify with conventional microscopy methods. More information about Linda's biography and publications can be found *here*.

Nomination letter of Linda Medlin to the ISSHA Council

"There are many, many reasons why Linda deserves this Award. Here we describe just a few. Linda has many accomplishments that qualify her for this prestigious award. Her long and productive scientific career has been crucial to the development of HAB research in Germany and Europe, and includes numerous contributions across the international community. Linda has a strong reputation in the fields of taxonomy and phylogeny. As an example, in a seminal 1988 paper with Mitchell Sogin, which has now been cited more than 2600 times, she published the 18S rRNA primers that are still used around the world for precise phylogenetic description of eukaryotic species and their evolutionary histories.

Linda, together with Chris Scholin, was instrumental in the development and application of species specific probes, FISH, and arrays for the rapid detection of HAB species. She initiated several workshops on FISH probe design and its application. Besides her German grants in HAB science and being the first and most productive scientist in German HAB science, she is also one of the foremost Euro-HAB scientists, as she led and initiated several successful HAB projects funded by the EU.

Besides being extraordinarily focused on her scientific career, she has always been known for her excitement and never-ending enthusiasm, and she is a great promotor of women in science! She has always fought for highly qualified women to make their careers based on their science and professional qualities. One thing that stands out is her dynamic role at HAB conferences; she was always active in keeping the scientific discussion running no matter what the topic. There was always the threat for the Chair to keep her controlled, allowing for no more than two brilliant questions, however Chairs, as well as the audience, benefitted by having Linda in the audience ensuring a high quality discussion among many different fields of HAB research. We have always been impressed by her deep and wide knowledge about nearly everything that matters in science!

Linda moved from the USA to the UK in 1983 and later to the AWI Germany (1991-2008). After being very creative and active during this period, she started in France (2009) at the CRNS Banuyls sur Mer.

She has been heavily involved in studies of the molecular ecology of many HAB species, establishing the backbone for studies on diatom phylogeny and leading several investigations on diatom evolution, and pushing genetic studies of a variety of toxic HAB species, often in collaboration with colleagues from all over the world. Many of Linda's PhD students and postdocs have moved into successful careers of their own, which has been a greater stimulus for plankton research in its own right. Her scientific productivity is exemplary, with about 200 peer-reviewed papers and more than 17,500 citations on a wide array of topics, in particular in the HAB field.

In summary, Linda Medlin is a creative and highly critical thinker with broad and significant impacts in HAB research, as well as major contributions to the educational aspects of our field. She has always been one of the most creative and productive members of our community, and has positively impacted the lives and careers of many people in HAB research. She eminently deserves the Yasumoto Award, and we therefore urge the Selection Committee to recognize her accomplishments and service with this honor. The timing would be perfect as Linda just recently retired from her long and impressive scientific career.

Nomination letter of Linda Medlin for the Yasumoto Lifetime Achievement Award 2021.

Presented by Marina Montresor, Stazione Zoologica Anton Dohrn, Napoli; supported and signed by 18 more senior HAB experts from Europe (11), North America (3), Asia (4) and Australia (1).



Sing Tung Teng with a carving of a tropical Synophysis from Prof Takayama.

The ISSHA 2021 Patrick Gentien Young Scientist Award

Sing Tung Teng received the *ISSHA 2021 Patrick Gentien Award* in recognition for his outstanding research about the diversity, systematics and erection of new diatoms species within the genus *Pseudo-nitzschia*. Species of this genus produce domoic acid, the neurotoxin responsible for the Amnesic Shellfish poisoning (ASP) syndrome.

The candidate nomination was presented by ISSHA members Profs Chui Pin Leaw (Malaysia), Stephen Bates (Canada) and 12 more senior scientists China (3), Japan (4), Malaysia (3), The Philippines (2) and Singapore (1).

Sing Tung began his research on the diversity of *Pseudo-nitzschia* species in the tropical Western Pacific region when he was working on his master degree. There was very little information about the causative organisms of ASP in this vast region. Four years after completing his PhD thesis under the supervision of Dr. Po Teen Lim, he had made impressive accomplishments. His sound knowledge of the taxonomy of pennate diatoms combined with his skills with molecular tools led him to the erection of several new species that included: *P. kodamae*, *P. sabit*, *P. bipertita, P. limii* (Teng et al. 2014, 2015, 2016). He also contributed substantially to the descriptions of *P. circumpora*, *P. batesiana*, *P. lundholmiae*, *P. fukuyoi*, *P. simulans*, *P. nanaoensis*, *P. chiniana*, *P. qiana*, *P. uniseriata*, and *P. yuensis*. He has been involved in the description of more than half (29 out of 53) the species of *Pseudo-nitzschia* in the tropical

waters of the South China Sea, a marginal sea of the Western Pacific (Teng et al. 2013, 2014).

Sing Tung Teng has demonstrated his training and dissemination skills by introducing a taxonomic grouping of *Pseudo-nitzschia* based on frustule morphology, which eventually developed into an interactive key to species identification and a database of *Pseudo-nitzschia* species.



Jose L. García-Corona

Maureen Keller Awards to best student presentations

Best student oral presentation **José Luís García-Corona,** from the European Institute for Marine Studies (Best, France) for his presentation about: "*In situ detection of the phycotoxin domoic acid in bivalve tissues: deciphering the subcellular mechanisms involved in its retention in the king scallop* Pecten maximus*"* co-authored by Caroline Fabioux, Hélène Hegaret, Elodie Fleury, Malwenn Lassudrie and Adeline Marzari.

José Luis is a marine biologist graduated with honors from the Autonomous University of Baja California Sur in 2014. He received the State Youth Award and the Science and Technology Medal in 2017 for his contributions to science development in Mexico. He obtained a Master of Science degree in aquaculture biotechnology from the Biological Research Center of the Northwest in 2018, where he developed alternatives to improve gonad maturation and oocyte quality in marine bivalves with commercial interest in Mexico. His research is mainly focused on the study of the physiological, functional, and genomic responses of marine bivalves with high-commercial interest that face changing environmental conditions. Since 2019, he is a doctoral researcher at the Laboratory of Marine Environmental Sciences of the European Institute of the Sea, in Brittany, France. His doctoral research, supervised by Dr. Caroline Fabioux and Dr. Helene Hegaret, aims to decipher the main physiological and molecular mechanisms involved in contamination and decontamination kinetics by domoic acid in *P. maximus*, comparing its response with other fast-DA depurator species.



Steffaney Wood

Oral Presentation, Honorable Mention was awarded to **Steffaney Wood**, from Scripps University, La Jolla (USA) for her presentation on: "*Cyanobacterial akinete distribution, viability, and toxin records in sediment archives from the Northern Baltic Sea*" co-authored by Anke Kremp, Henna Savela, Sultana Akter, Vesa-Pekka Vartti5, Saija Saarni and Sanna Suikkane.

Steffaney is a PhD student at Scripps Institution of Oceanography, UCSD in the Bradley Moore and Andrew Allen labs. She currently studies domoic acid biosynthesis in *Pseudo-nitzschia* diatoms.

Steffany began her career as a Fulbright-EDUFI Fellow at the Finnish Environment Institute Marine Research Centre, conducting research on harmful cyanobacteria blooms in the northern Baltic Sea. She continued with the same topic her MSc in in Microbiology and Microbial Biotechnology at the University of Helsinki, Finland and completed it in May 2020. The work presented at ICHA2021 was part of that research.

Steffaney is also a 2018 graduate of Davidson College where she majored in Environmental Studies with a Chemistry minor. During her time at Davidson, she was awarded a NOAA Hollings Undergraduate Scholarship and had the opportunity to work in Dr. Vera Trainer's lab. She credits this experience with fostering her interest in the ocean sciences and HAB research.



Taichi Ataka

Taichi Ataka, Kochi University, Japan, received the Maureen Keller student award for the best poster titled, "*Effect of adding macroalgal extracts on the growth of* Gambierdiscus scabrosus *and* G. silvae *isolated from Japan*", co-authored by Hiroshi Funaki, Haruo Yamaguchi, and Masao Adachi. Born in Japan, he pursued Bachelor of Agriculture and Marine Science at Kochi University and is pursuing his master's degree at Kochi University under the supervision of Prof. Masao Adachi. Koshi University is located in Shikoku Island, one of the warm-temperate areas in Japan where ciguatera poisoning (CP) has been sporadically reported. Taichi is very interested in clarifiying the mechanism of CP outbreaks in Japan including the clarification of the causative organisms and toxins for the poisonings in Japan. He is also interested in biological interactions in *Gambierdiscus* spp., in particular the interations between the macroalgae and *Gambierdiscus* and between *Gambierdiscus* and bacteria. He focused on the effect of fractions extracted from macroalgae on the growth of *Gambierdisus* in Japan and clarified that the addition of the extracts

from some species of Rhodophyta to the cultures significantly enhanced the growth of the two *Gambierdiscus* species that occur in Japan. He is now starting an investigation to clarify the effect of bacteria on *Gambierdiscus* growth in an attempt to elucidate the growth characteristics of *Gambierdiscus* in complex marine ecosystems.

Kochi University, Osone Kou 1519-19, Corpo IZUMI303 Nankoku City, Kochi Pr.



Marta Estrada with her presents (a Mexican "alebrije" carving and a conference T-shirt) with Elisa Berdalet as the local surprise messenger.



Teresa Silva

Teresa Silva was awarded with an honorary mention for her poster presentation, *"BHAB occurrence in the south coast of Madeira Island, Portugal: from 2018 to 2020"*, co-authored by Ana Amorim, Ana Sousa and Manfred Kaufmann. Teresa has a degree in Biology from the University of Aveiro and a MSc. in Molecular Biology and Genetics from the Faculty of Sciences of the University of Lisbon (UL), Portugal. In 2011, she joined the team of the Marine and Environmental Sciences Centre (MARE, UL) integrating several scientific projects focusing on phytoplankton ecology and chemotaxonomy. For her Masters degree', she investigated the physiological, toxin and genetic characteristics of *G. catenatum* populations occurring in the Portuguese mainland coast. She also studied the contribution of *G. catenatum* life cycle (cysts) in bloom dynamics. In 2016, she moved to Madeira Island (Portugal) to join the MPhytoLab (PI: Manfred Kaufmann), University of Madeira, where she is collaborating in several studies aiming to characterize the phytoplankton communities from seamounts and islands of the Macaronesian region, as well as to characterize the BHAB populations occurring in the Madeira Archipelago. She has participated in 8 national and international oceanographic expeditions in the Northeast Atlantic Ocean onboard research vessels. Currently, Teresa is working on her PhD project "Harmful benthic dinoflagellates from Madeira Archipelago in a changing ocean: ecology, systematics and toxicity" (Supervisors: Ana Amorim - University of Lisbon, Manfred Kaufmann - University of Madeira & Philipp Hess - IFREMER-France). Her main research goal is to characterize BHAB assemblages in coastal waters of Madeira Island, in order to assess risks and provide baseline information for the implementation of a sustainable monitoring.

Closing Ceremony

The closing ceremony was held on October 15th from 16:00 to 17:30. The Patrick Gentien (young scientist) and the best oral and poster presentations deserving the Maureen Keller awards were announced.

Ichiro Imai presented ongoing preparations and logistic information for those planning to attend the 20th ICHA conference to be held in Hiroshima, Japan, in 2023. The bid from Chile presented by **Leonardo Guzmán** to host the 21st ICHA 2025 in Punta Arenas, at the southern end of Magallanes, Chilean Patagonia, was chosen for the conference venue in 4 years' time. Finally, the new president **Wayne Litaker** expressed the gratitude of the ISSHA members and all participants in the 19th ICHA to the previous president, Vera Trainer and to the host of the Mexico conference, **Christine Band-Schmidt.** Both of them received a surprise messenger knocking at their office/home doors to give them a present in real time during the closing ceremony.

ISSHA President goodbye

Dear ISSHA members:
As many of you know, at the general glection held prior to the International Conference on Harmful Algae (ICHA2021) from 11-15 October 2021 (La Paz, Mexico), I stepped down as the president of the International Society for the Study of Harmful Algae (ISSHA), and welcomed your new president, Dr. Wayne Litaker. I want to take a moment to reflect on the accomplishments of the ISSHA Council during my tenure as president. First, we developed a new ISSHA logo and website. One of the motivations for the development of the new website, was the need to secure our membership list and all email addresses on a password protected site. The website also needed updating and a general refresh. The new ISSHA website has information on jobs, upcoming workshops, contains the *Proceedings of the past ICHA meetings*, and much, much more. Please take a moment to look at the *website* and feel free to contact your student representative, Carolin Peter (*carolin.peter@lnu.se*), with information about job postings, upcoming workshops, or other suggestions. Second, modest requests for funding for travel to international courses or workshops can be made by ISSHA members; students should take advantage of this opportunity. More details can be found *here*. Third, we have made sure that we acknowledge accomplished members of the ISSHA community, our *Trailblazers*. Any current ISSHA member can nominate one of their colleagues to become a Trailblazer by contacting any member of the current *ISSHA Council*.



Vera Trainer

Finally, but perhaps most importantly, we have adopted the policy that an early career scientist, an ISSHA student, will be nominated as a member of the Council. Our first ever student Council representative was Steffaney Wood (USA), who was recently replaced by Carolin Peter (Sweden). These students will receive support for their registration for the next ICHA conference as a thank you for their participation in Council meetings and assistance with website updates.

I was especially pleased by the attendance and enthusiasm shown by the entire ISSHA community at the first ever virtual ICHA meeting, chaired by Christine Band-Schmidt, with help from the local and international steering committees and the conference organizer, Intermeeting. We look forward to a hybrid (virtual and in-person) meeting style in 2023 in Hiroshima, Japan. As your past-president, I will continue to help the ISSHA Council and Wayne. I want to specifically thank Beatriz Reguera, now the past-past president of ISSHA for all of her help during my tenure. I couldn't have done it without you, Beatriz! I look forward to seeing all of you at future meetings! All the best for a healthy and productive 2022. Sincerely

Vera Trainer, ISSHA Past-President
National Marine Fisheries Service,
NOAA, Seattle, WA, USA



Hiroshima, Japan, voted as venue for the ICHA 2023

UNESCO Taxonomic Reference List of Harmful Micro Algae Editors Workshop 2021

On November 18-19th 2021, the nine thematic editors responsible for the UNESCO Taxonomic Reference List of Harmful Micro Algae and Henrik Enevoldsen from IOC-UNESCO gathered together in a hybrid format workshop, to discuss several aspects of this Register, which is a product of the IOC Harmful Algal Bloom (HAB) Program and the World Register of Marine Species (WoRMS). This took place in Hillerød, Denmark with a large physical attendance, supplemented with online attendance of a few editors and the WoRMS Data Management Team.

The nine thematic editors of the list are: Catharina Churro, Santiago Fraga, Mona Hoppenrath, Mitsunori Iwataki, Jacob Larsen, Nina Lundholm, Kenneth Mertens, Øjvind Moestrup and Adriana Zingone.

The goals of the workshop were: (1) discussing the future of the list, (2) discussing how the list can be improved, e.g. through the addition of new types of entries and links, (3) participating in a hands-on training on how to use the online editing platform provided by the WoRMS Data Management Team (DMT) which led to (4) bringing the list up-to-date and (5) nomination of a new chair of the HAB list.

The two days were very productive, both in terms of adding content to the Register, as well as discussing the way forward and the implementation of some initial decisions that were made during the meeting. A new addition to the website, currently under development will contain a list of species that cause non-toxic, harmful events.

During the meeting, the chairmanship of the group was passed on from Øjvind Moestrup, who has led the group for 24 years, to Nina Lundholm.

The organization of this workshop was supported by IOC UNESCO. The Data Management Team (DMT) is supported by LifeWatch Belgium, part of the E-Science European LifeWatch Infrastructure for Biodiversity and Ecosystem Research. LifeWatch is a virtual laboratory, which is used for different aspects of biodiversity research. The Species Information Backbone of LifeWatch aims at bringing together taxonomic and species-related data and at filling gaps in our knowledge.

Link for the list: *HAB Taxonomic Reference List*

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Book Review: A Catalogue of Phytoplankton from the Mexican Central-Pacific (in Spanish)

Authors: Hernández-Becerril, D.U., Barón-Campis, S.A., Ceballos-Corona, J.G.A., Alonso-Rodríguez, R., Rincones-Reyes, K.M., Becerra-Reynoso, R.T. & Arce-Rocha, G. 2021. Catálogo de fitoplancton del Pacífico central mexicano. Cruceros "MareaR" (2009-2019). B/O "El Puma". Universidad Nacional Autónoma de México. 254 pp. ISBN 978-607-30-4944-3

Our knowledge of the marine phytoplankton shows a strong bias towards the coastal areas of temperate seas, where the monitoring programs are mainly focused on harmful species. Consequently, there is a deficit of information on the phytoplankton of warm seas, and especially from the open ocean. Within the context of climate change, where tropical species expand their ranges of distribution, the biodiversity in warm seas requires more attention. However, we often only have the option of consulting classical monographs for each major taxonomical group (diatoms, dinoflagellates, coccolithophorids, etc.) with illustrations based only on-line drawings, and the nomenclature and classification is not updated. Fortunately, this new book contributes to filling this deficit providing an essential guide for phytoplanktologists, not only working on warm regions, but also in temperate waters because numerous species have wide ranges of distribution.

This book compiles the observations from research cruises carried out during 10 consecutive years in the Mexican Pacific. The transects were perpendicular to the coast, allowing, in addition to the oceanic species, to show the diversity of neritic species (i.e., *Alexandrium*). The authors combine net and bottle samplings, as well as different preservation methods, which allows a wide representation of phytoplankton, from the rarest species, to species with special preservation requirements such as coccolithophorids. We will find an underestimation of some groups such as the naked dinoflagellates, but this is inevitable because these fragile organisms are lost or distorted with the sample preservation. The result is a complete guide for the identification of warm-water phytoplankton, and also essential for phytoplanktologists working on temperate waters showing a trend to the "tropicalization". The text and micrographs reported 501 taxa: 257 dinoflagellate species, 195 dinoflagellate species, 35 coccolithophorids, and other groups. This is a record of the current species composition present in the region, and an essential tool to evaluate the future changes of biodiversity. The organization of the text, especially the combination of photographs from both optical and electron microscopy arranged in plates grouping the taxa based on their similarity, with distinct views of the individuals, allow comparisons. The high number of species included is remarkable which guarantees that in most of the cases the user will find the information required.



Phytoplankton is characterized by its extraordinary diversity, and phytoplanktologists are used to consulting bibliography from different sources and languages. The use of Spanish as language is not a handicap, and the profusion of photographs makes it a useful guide for any user regardless the language. This is a textbook for the phytoplankton identification courses organized by the authors, and the extensive glossary solves for the Spanish-speaking community any doubts on translation of the terminology to describe morphological characters. This is an essential resource for any person interested in marine phytoplankton, and it should be in every library that serves students and researchers in marine biology. Future editions are expected with additional information, including updated new records, and it is expected an extension with records from the Gulf of Mexico and the Caribbean Sea. The authors are commended for this effort in compiling their observations that facilitate the work of the identification and the formation of the phytoplankton observers.

Such a comprehensive study is the result of a joint effort led by Prof. Hernández-Becerril, who together with Prof. Rosalba Alonso, have largely contributed to the formation of a high numbers of phytoplankton experts that participate as co-authors, and also students that collaborate in the samplings and observations. Prof. Hernández-Becerril is one of the largest contributors to the knowledge of the biodiversity of warm-water phytoplankton in recent decades. His expertise with numerous taxonomical innovations (*Neocalyptrella*, *Proboscia*, etc.) is reflected in the extraordinary representation of typical warm-water genera such as *Asteromphalus*, but also other genera with a complex distinction of the species such as *Coscinodiscus*, *Chaetoceros* or *Thalassiosira*. Thecate dinoflagellates are largely represented with genera *Protoperidinium* or *Tripos*, but it is remarkable the high diversity of Dinophysales, a group where Prof. Hernandez-Becerril and his co-workers have proposed numerous taxonomic innovations. An effort was made to differentiate species that that many of us would simply pooled as *Phalacroma* cf. *rotundatum*. Two new combinations of little known dinophysoid dinoflagellates, *Phalacroma tailisunii* comb. nov. and *P. cyrtoptera* comb. nov. are proposed.

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Forthcoming Events

2022 UN Ocean Conference

GLOBAL ONLINE STAKEHOLDER CONSULTATION
Inputs to concept papers of interactive dialogues
Contribute your inputs to the concept papers of the interactive dialogues for the 2022 UN Ocean Conference! (see back page)

Deadline: 21 February 2022
Contribute your inputs here: *bit.ly/EConsultationOceanConference2022*

1. GlobalHAB WS: Modeling and Prediction of Harmful Algal Blooms see *https://oceanexpert.org/event/2573*

2. The IOC HAB Programme has launced its new website, visit *https://hab.ioc-unesco.org/* *The IOC HAB website has undergone a long awaited rejuvenation. You will find the well know features and information as well a new tool to search all previous HAN issues*



SAVE THE DATE

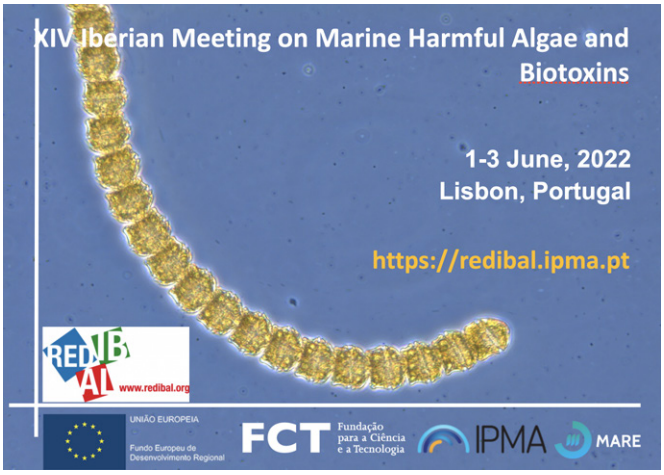
XIV Iberian Meeting on Marine Harmful Algae and Biotoxins

1-3 June 2022, Lisbon, Portugal

The XIV Iberian Meeting on Marine Harmful Algae and Biotoxins will be held in Lisbon, Portugal, from 1-3 June 2022, at the Magalhães Auditorium of the Portuguese Institute of Sea and Atmosphere (IPMA). This long-standing meeting (since 1990) brings together researchers and regulators to discuss all aspects of HABs, with emphasis on Iberia and the Macaronesia archipelagos (Canary Islands, Madeira, and Azores).
The meeting will focus on new challenges posed by HABs and related threats to human health, environment, and economy. Presentations will be organized in 5 main topics:

1. HABs and Climate change
2. Global and regional HAB trends: What have we learned from more than 30 years of monitoring in the Iberian Peninsula?
3. New challenges: new HAB species and emerging toxins
4. New approaches and technologies for detection of marine HABs and biotoxins in biological and environmental samples
5. Data science, management, and intelligent decision support systems.

Full information on the meeting *https://redibal.ipma.pt/*



GlobalHAB symposium on automated *in situ* observations of plankton

Aims and background

Harmful Algal Blooms (HABs) are affecting aquatic ecosystems and human societies.

Biotoxin-producing HABs species and species causing fish mortalities are problems for aquaculture, fisheries and also for tourism. HABs have caused mortality of marine mammals and pose a threat to human health. Ecosystem-disruptive HABs and highbiomass HABs may cause anoxia in deep water and some HABs result in a decrease in coastal water quality and fouling of beaches. High-frequency in situ observations of HABs and predators of HAB species are needed to be able to understand HAB dynamics, to develop predictive models of HABs and to produce well-founded warnings for HABs. In recent years, novel *in situ* instrumentation have been developed for automated high-frequency HAB detection in near-real time. In addition, instruments for observing grazers, i.e. microzooplankton and multicellular zooplankton have been developed. These instruments are now being adopted in research and piloted in monitoring programmes. Some of the instruments are becoming available commercially.

The aim of the symposium is to bring together experts on, and users of, automated in situ imaging systems to present methods, recent results and to share experiences.

Another aim is to carry out a comparison of results when analysing plankton communities quantitatively. Young scientists are particularly encouraged to attend the symposium and a special follow-on workshop for young scientists on data processing and report/article writing.

A hybrid symposium

Part of the symposium will be available on line. An evening session is planned for presentations made by participants not on-site. Presentations from the morning sessions will be recorded and published at an IOC web site, link to be communicated later. Training sessions in the afternoons will not be recorded, but instruction videos will be published on line.

Deadline for registration

15 March 2022

Pre symposium video conference

Virtual meeting Monday 30 May 2022 1600-1900 CET. All on site participants will be invited. Planning of hands-on training, intercomparison experiment etc.

Number of participants

On site max ~30 persons including lecturers

On line participation to the hybrid parts of the symposium max ~100 persons

Venue

Kristineberg Marine Research Station, Fiskebäckskil, Sweden

This is well-equipped field station is located at the mouth of the Gullmar fjord on the Swedish west coast, adjacent to the North Sea. More information is available at *https://www.gu.se/en/kristineberg*

Instruments

Imaging in flow instruments will be the main focus of the symposium. Instruments will include the Imaging FlowCytobot, the FlowCam and the CytoSense/Sub.

Symposium fee

The fee for on-site participation in the symposium includes costs for accommodation, food and bench fee. Cost per participant is 9000 SEK (approximately USD 1000 and €900).

Registration requires no payment. Payment can be made using credit card upon acceptance.

Financial support for participants

Funds are available for our sponsors to support a limited number of participants. Early career scientists can apply for support for travel costs and symposium fee when registering. The workshop coordinators will contact applicants to discuss potential funding prior to finalization of the participants.

Co-conveners

Bengt Karlson, SMHI, Sweden
Keith Davidson, SAMS, Oban, United Kingdom
Raphael Kudela, University of California, Santa Cruz, USA
Marina Montresor, Stazione Zoologica Anton Dohrn, Italy
Peter Tiselius, University of Gothenburg, Sweden
Lars Naustvoll, Institute of Marine Research, Norway
Elisa Berdalet, Institute of Marine Sciences (ICM-CSIC), Spain

Dates and deadlines

Deadline for registration 15 March 2022

Decision about participants early April 2022

Registration

Register through web forms at the SCOR web site: *https://www.gu.se/en/kristineberg*

www.neiwpcc.org/events/ushab11

#USHAB2022



LOGO CONTEST

For logo contest rules, please visit **our conference website.**

11TH U.S. SYMPOSIUM ON HARMFUL ALGAE

SAVE THE DATE: OCTOBER 23-28, 2022

We are pleased to announce that the 11th U.S. Symposium on Harmful Algae will be held on October 23-28, 2022 in Albany, New York, at the Hilton Albany. Local coordination is being led by NEIWPCC, New York State Department of Environmental Conservation, and U.S. Geological Survey with support from the U.S. National Office for Harmful Algal Blooms at the Woods Hole Oceanographic Institution.

The theme for the upcoming symposium is **"Science to Support Solutions from Shore to Shore"**. From freshwater to marine systems, the prevalence of harmful algal blooms (HABs) is a national environmental challenge, and solutions are needed. Celebrating this event in New York – the only state with Great Lake shorelines, marine coasts, and the diverse range of ecosystems between these extremes – creates an ideal setting to discuss progress in understanding algal bloom ecology and the solutions necessary to prevent and reduce HABs.

This conference is open to all registrants. Attendees will include national representatives and researchers from academia; state, federal, tribal, and municipal governments; the private sector; and watershed organizations.

Please **visit our conference website** periodically for the most current information on the conference.

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FOR GENERAL QUESTIONS ABOUT ATTENDING, SPONSORING, OR EXHIBITING AT THE 11TH U.S. SYMPOSIUM ON HARMFUL ALGAE, please contact Maryann Dugan @ **mdugan@neiwpcc.org**.

NEIWPCC COVID-19 POLICY

NEIWPCC is committed to providing an event that keeps all participants as safe as possible and promotes the well-being of our community. NEIWPCC requires all individuals who participate in the 11th U.S. Symposium on Harmful Algae to be fully vaccinated in order to attend. Please **visit our website** for more info.



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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 70:
April 30, 2022

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Harmful Algae News

An IOC Newsletter on Toxic Algae and Algal Blooms

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Mar Menor lagoon: an iconic case of ecosystem collapse



"Dead zone" illustrating the devastation after the autumn 2019 anoxic event in Mar Menor (Murcia, SE Spain). Photo: Javier Murcia Requena

Mar Menor (W Mediterranean, Spain) is the largest hypersaline coastal lagoon in Europe. In spring 2016, chl *a* concentration exceeded the average values (≤ 1 µg L^{-1}) reported for this variable since reliable measurements started in the 1980's [1] by two orders of magnitude. The high values of 2016 were unprecedented and pointed to a "phase change" in the eutrophication process of Mar Menor initiated many years before by a long history of anthropogenic pressures including urban discharges, intensive agriculture, livestock, dredging, construction of marinas, artificial beaches, etc.

In 2016 pollution was so severe that a collapse of the lagoon ecosystem was feared. Light penetration was reduced to values below the threshold required to sustain photosynthesis by benthic macrophytes, which were the main primary producers in the lagoon system that strongly influenced ecosystem functions and services [2]. This situation led to the mobilization of thousands of tons of carbon and nutrients that followed death and decomposition of the macrophyte meadows below the 2.5 m isobath (Fig. 1).

Ecosystem disruptive Algal Blooms

Analysis of the phytoplankton community during the acute eutrophication events confirmed the key role played by the cyanobacterial genus *Synechococcus* [3]. Similar events triggered by species of the same genus in other semi-enclosed systems around the world had been reported before. These microalgal events are known as 'ecosystem disruptive algal blooms' EDABs. Unlike other HABs developed in open coastal areas, they are produced by a large variety of small-sized phytoplanktonic species (cyanobacteria, pelagophytes, trebouxiphytes, eustigmatophytes, haptophytes, dinoflagellates) that share some common traits e.g. the capacity to use alternative sources to inorganic nitrogen, as well as being toxic and having low palatability for grazers [4]. The high availability of regenerated nutrients in Mar Menor combined with the disruption of the food web dynamics led to a massive bloom of *Synechococcus*. This bloom triggered a feedback process that lasted for months and deeply modified the system. The *Synechoccus* EDAB was the unambiguous signal that some

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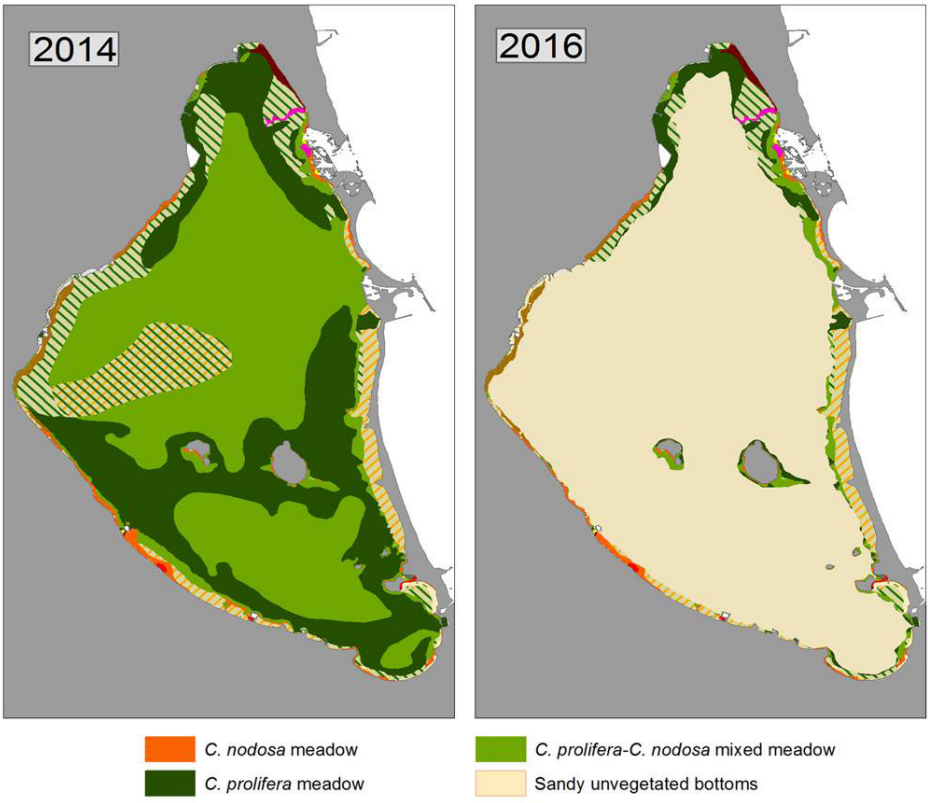

Fig. 1. Maps of macrophytes assemblages in Mar Menor lagoon before and after the 2016 anoxic event. Drastic changes in the distribution of the two dominant macrophyte species, the green alga Caulerpa prolifera and seagrass Cymodocea nodosa, are illustrated

thresholds, known in ecological theory as *tipping points*, had been crossed. Tipping points are levels of pressure beyond which the ecosystem can no longer cope with change, and suddenly shifts from one state to another (*ecosystem shift*), usually more deteriorated, but equally stable state [5].

How did this iconic ecosystem reach this condition?

In the first place, it is necessary to know the environmental framework of Mar Menor, a shallow (maximum depth 7m) hypersaline coastal lagoon with a catchment basin of 1,300 km^2 and a water renewal time of about 1 year [6]. According to available information on the semi-arid climate prevalent in the SE Iberian Peninsula, the only freshwater inputs to the Mar Menor are from runoff associated with a few torrential rains which occur every year (250 mm year^{-1}), and some more diffusive groundwater inputs. Recently developed models [7] indicate that the current input from runoff is quantitatively more important than that from aquifers. Nevertheless, the little and dispersed data available are not sufficient to provide reliable data for lagoon management.

1. Impact of the Estacio channel widening

In 1973, widening of the Estacio Channel, one of the five natural connections ("golas") between the lagoon and the Mediterranean, was carried out [8]. This public work, now considered a landmark of the anthropogenic transformation of the lagoon, changed the hydrodynamic regimen and led to a decline in salinity, from 50-52.5 in the early 1970's to the current values (42-48) which show an interannual variability determined by the frequency and intensity of the "DANAS" or "gota fría", i.e. intense cold spells bringing heavy rainfall. The increased exchange favoured the entry of some Mediterranean species and the decline of some others, members of the autochthonous fauna and flora. Water exchanges through the Estacio channel are now the main control of the lagoon hydrodynamics. One of the most favoured introduced species was the green macroalga *Caulerpa prolifera*, a well-known opportunistic macrophyte able to take up nutrients very efficiently and colonize extensive areas rapidly. This species has properties conferring the ability to outcompete native macrophyte assemblages, in particular meadows of the seagrass *Cymodocea nodosa* present in Mar Menor at least in the last decades. *Caulerpa* contains high levels of toxigenic secondary metabolites and contributes loads of labile organic matter to the sediments. Decomposition of this organic matter fuels anoxic processes and increased levels of reduced carbon, nitrogen and sulphur compounds.

2. Introduced macroalgal species and uncontrolled wastewater discharges

There is no argument about the increased primary production and deposits of organic matter sedimented in the lagoon during the last 60 years [9], but the negative impact of these changes on the ecosystem may have been overestimated, and conclusions have not been based on scientific evidence. A good example of this criticism concerns the supposed regression of native seagrass meadows, in particular *C. nodosa*, as a consequence of the expansion of *C. prolifera* [10]. Recent studies have confirmed that angiosperms did not decline during the decades preceding the collapse. Conversely, these studies provided evidence of the coexistence of the two macrophytes at least during the last forty years before the 2016 event [11] (Fig. 2) indicating a benthic primary production enhancement. Increased primary production supported by high availability of nutrients is an intrinsic feature of eutrophic ecosystems [12]. Therefore, man-made changes induced on the lagoon hydrodynamics were not the only factor that explained the expansion of *C. prolifera*.

Uncontrolled discharges of untreated wastewaters from urban and tourist developments took place years before the channel widening. In the 1990's, the wastewater treatment plan for Mar Menor was completed and wastewaters were no longer flushed into the lagoon (at the expense of being discharged into adjacent Mediterranean waters). Nevertheless, problems related with high nutrient inputs persisted or even increased due to the rapid development of intensive agriculture which started in the 1950's [13]. This kind of agriculture replaced the traditional drylands at the expense of aquifer overexploitation. The new agricultural practices marked another step towards deterioration of the lagoon [14]. Today, the irrigation area occupies 40% of the total drain-

age basin and is a major source of European winter vegetable production. But the transferred water resources, clearly insufficient to sustain such production, had to be complemented with aquifers that had suffered previous overexploitation and became brackish. These brackish aquifers needed treating in desalination plants which systematically diverted their nitrate rich (up to 600mg L^{-1}) waste into the lagoon. The intensive agriculture activities increased the nutrient load of the aquifers (ca 150 mg nitrate L^{-1}).

The fact that after decades of massive inputs of nutrients, these were not reflected in any apparent damage to the lagoon is paradoxical. At least until 2016, relatively clean waters and seabeds dominated by conspicuous communities of benthic macrophytes [15] were observed. In fact, different experimental approaches have provided evidence that before the collapse, macrophyte meadows played a key role in the control of man-driven nutrient inputs in the lagoon [16]. Benthic communities of filter feeder, in particular bivalve molluscs such as the flat oyster (*Ostraea edulis*) and the giant Mediterranean fun mussel, or Nacra (*Pinna nobilis*), that reached populations of 135 and 1.4 million individuals respectively [17, 18], may also have contributed to this control although specific studies supporting such hypothesis do not exist.

3. Alternative hypotheses

Another possible mechanism to explain the abovementioned paradox is stoichiometric imbalance of the main macronutrients (N, P, Si). For example, phosphorus is not very abundant in groundwaters, and its input to the lagoon mainly takes place by surface runoff through the watershed. Therefore, inputs of different nutrients are uncoupled in time and space. Intensive agriculture is a major cause of soil erosion and N and P transported by runoff have contributed to a sharp rise of nutrients in the lagoon in recent decades. In addition, climate change is expected to contribute to increase the frequency and intensity of extreme climate events.

As an example, during the 2019 DANA, about 60 million m^3 of water containing between 150 and 190 t of dissolved phosphate entered the lagoon [1] (Fig. 3). Phosphates are immediately taken up by the vegetation and/or deposited in the sediments, and only after resuspension and/or remineralization may become bioavailable again. There are other mechanisms, such as denitrification that may contribute to recycling but unfortunately, knowledge about biogeochemical cycles in the Mar Menor and its associated basin are practically non-existent. These cycles, together with the sources of the elements integrating them, the relative importance of each one and the entry routes and transformation processes are crucial aspects that need to be unveiled before taking measures to reduce the nutrients load and recover the ecosystem.

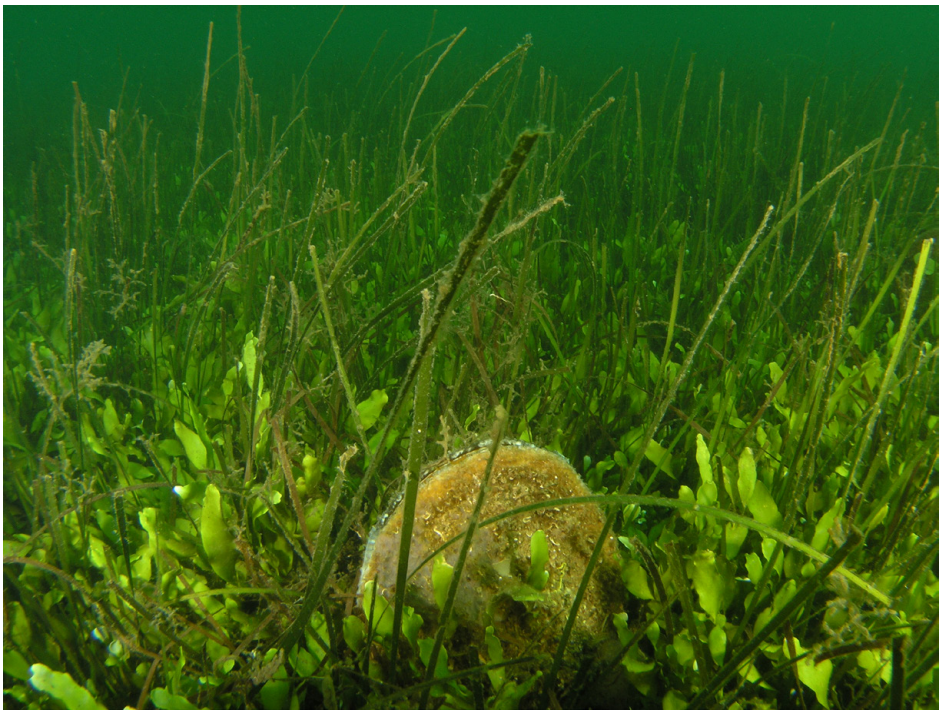

Fig. 2. Before the 2016 event, benthic macrophyte communities with dominance of seagrass Cymodocea nodosa (thin long leaves) and chlorophyte Caulerpa racemosa (wider shorter fronds) occupied large areas in Mar Menor. The giant fun mussel Pinna nobilis was a typical bivalve species in this ecosystem. Underwater photo taken in summer 2014 at 5 m depth in a site where the seagrass has completely disappeared. Photo: Juan M. Ruiz

A new alternative state?

As a result of the collapse in 2016, a large part of the lagoon resilience mechanisms and its ecosystem services were severely damaged. Eighty-five per cent of the macrophyte meadows extension and 95% of the giant mussel *Pinna nobilis* [2, 18], both with key roles in the control of nutrients and dissolved organic matter in the water column, were destroyed. The loss of these control mechanisms was clear evidence of the degree of deterioration reached (Fig. 4). Unfortunately, very little information is available about other components of the lagoon ecosystem to give a global evaluation of the disaster. Nevertheless, the reported losses agree with the growing feeling that the lagoon is nowadays a much more unstable system as compared with decades ago, more fluctuating and more vulnerable to environmental changes. In fact, since 2016 there has been a succession of phytoplankton blooms and high turbidity alternating with periods of clear water. But in contrast with the massive bloom of *Synechococcus* reported in 2016, blooms from recent years were dominated by the diatom genera *Nitzschia*, *Cyclotella*, *Cylindrotheca* and *Chaetoceros*, with the potential to trigger low oxygen events that threaten marine organisms as was the case during the mass mortalities of fish and other components of the wild fauna and flora in summer 2021. The first mass mortality event occurred in autumn 2019 (Fig. 5), although in that time it was forced by strong and persistent water column stratification after the massive discharge of freshwater into the lagoon (Fig. 3). These extreme events are characteristic of coastal areas in an advanced process of eutrophication. Nevertheless, despite their huge negative socioeconomic impact, little is known about the drivers and mechanisms behind the unreported high biomass HABs before the 2016 event.



Fig. 3. Satellite image (Sentinel 2) after torrential rainfall in September 12th and 13th in the Mar Menor watershed. Tons of terrigenous sediments, carbon, nitrogen and phosphorous are dragged by water runoff from agricultural lands into the Mar Menor lagoon (downloaded from https://www.copernicus.eu/)

Some of these events occurred without a previous increase in dissolved inorganic nutrients. This shows that these blooms are regulated by highly unstable biological processes, probably linked to the activity of other trophic levels, such as the bacterial control of the N cycle or benthos-mediated changes in vertical fluxes of nutrients. In any case, there is hardly any knowledge about the mechanisms behind the uncoupling of these processes. Understanding these processes is essential for a better assessment of ecosystem health to provide to decision makers

The foregoing indicates that a sound scientific knowledge of the Mar Menor and its associated basin ecosystem is a challenge that needs to be faced. Let's hope that the last disastrous events represent a tipping point for the authority's lack of awareness, and that it leads to a commitment to recover this unique ecosystem.

Acknowledgements

This study was supported by DMMEM project funded by the Spanish Institute of Oceanography IEO-CSIC for the long-term monitoring of the Mar Menor lagoon and its ecological assessment. We acknowledge logistical support



Fig. 4. Following the collapse, high biomass blooms of filamentous and mucilaginous algae are observed with increased frequency in periods of nutrient-enriched freshwater discharges. These algal masses, now common in spring months, suffocate benthic communities and promote sediment anoxia. Photo: Javier Murcia Requena

provided by President and Staff of the harbours Club Nautico Lo Pagán, Club Náutico La Puntica and Centro de Actividades Náuticas (San Pedro del Pinatar, Murcia, Spain).

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Fig. 5. The Mar Menor noxic event in September 2019 was so severe and extensive that bivalves from infaunal communities could not tolerate oxygen depletion in the water column (top) and fishes were not able to escape and died. Photo: Javier Murcia Requena



JuanMa Ruiz. Research Professor, IEO-CSIC

The Mar Menor Oyster Initiative, a strategy to prevent algal blooms in a eutrophic lagoon in Spain



Fig. 1. Map of study in Mar Menor, Murcia, SE Spain

Marine ecosystems are exposed to a wide range of pressures including water quality degradation, habitat decline, overfishing and climate change, in some instances leading to negative environmental, social and economic consequences. Different approaches can be taken to ameliorate the multiple human stressors altering the oceans, including the restoration of services which have declined through oyster restoration [1]. Oysters provide a variety of ecosystem services and are recognized for their potential to contribute to developing Blue Economies. From water quality improvement to sustainable maintenance of good habitat condition and coastal resilience, oysters can contribute to Nature-Based Solutions to restore impaired aquatic ecosystems [2]. Fossil records provide evidence that these essential services have been developed for millennia but bivalve stocks have decreased worldwide and with their disappearance, coastal ecosystems have also lost their ecologic and economic services [3]. Further declines in water quality and habitat serve as stressors to the bivalves that remain, which may lead to increased disease prevalence or feeding suppression, which can add to the loss of services provided.

Typically, coastal lagoons suffer from anthropogenic actions including the massive release of nutrients causing eutrophication (often associated with high biomass harmful algal blooms (HB-HABs). HABs are natural phenomena which may pose risks to human health, environmental sustainability and/or aquatic life due to the production of toxins or the accumulated biomass when increased production is no longer balanced by grazing [4]. In this last case, the high biomass from large algal blooms formed in eutrophic ecosystems may led to a cascade of negative ecological effects including seagrass mortality, fish kills and oxygen depletion [5]. For example, the harmful brown tides caused by the pelagophyte *Aureoumbra lagunensis* have led to seagrass die off and massive fish kills in Texas and Florida (USA) for their high concentration and persistence but not for the release of toxins [6]. Different ecosystems have suffered the effects of algal blooms, including the Mar Menor, a coastal lagoon suffering from severe eutrophication in Western Spain (Fig. 1). This is one of the largest hypersaline coastal lagoons in Europe with an area of about 135 km^2 and an average depth of 3.6 m (maximum 7 m). This lagoon ecosystem is particularly vulnerable to the impacts of human activity because it is a semi-enclosed body of water with a 1-year water residence time. It is particularly affected by the intensive tourism and agricultural development experienced in the area during the last decades, which led to the overlap of several environmental protection measures [7]. Before the anthropogenic influence on the lagoon increased, it was characterized by its oligotrophic and hypersaline waters (70-53 ppt) with the seabed containing sediment with seagrass. After receiving an excess of nutrients during the last three decades, the lagoon suffered from a massive proliferation of plankton in summer 2015, a sign of a eutrophication process that collapsed the entire ecosystem [8] (Fig. 2). Algal blooms recurrently appeared in the following years being *Synechococcus* sp. the most abundant species in August 2016, November 2017 and January

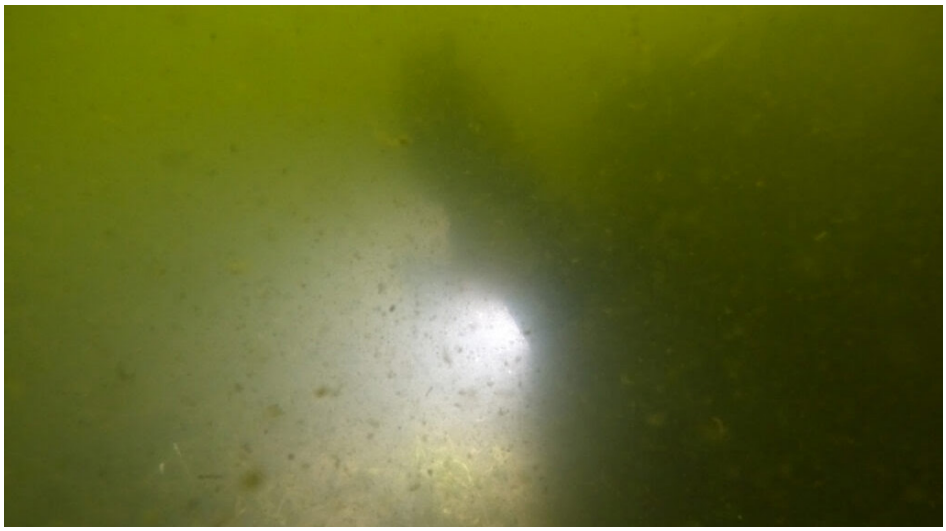

Fig. 2. High biomass microalgal bloom in the north basin of Mar Menor at 6 m depth. Photo ©P.García/ANSE on October 1st, 2021



Fig. 3. European flat oyster (O. edulis) in Mar Menor. Photo taken on February 25th, 2022

2018 with densities up to 6 x 10^6 cells mL^{-1} [9]. Mamiellophyceae also reached abundance peaks up to 4. x 10^6 cells mL^{-1} in 2016-2017 although this picoeukaryotic group was only present in sufficient abundance for quantification in summer or early fall [9]. The different recurrent algal blooms had cascading effects that ended in several mass mortality episodes and 70% mortality of seagrasses.

In an attempt to ameliorate the problems caused by algal blooms, and contribute to actions that must be put in place to decrease the introduction of nutrients to Mar Menor, a new initiative was launched in 2020 to use bivalves as a *Nature-based Solution* to filter the excess of phytoplankton in the water. Native European flat oysters (*Ostrea edulis*) (Fig. 3) had a population of 135 million oysters in Mar Menor in the late 80's but the last evaluation, carried out in 2006 reported an almost 10-fold reduction and the current status of the oyster populations in the lagoon is unknown [10,11]. With the oysters disappearing, the ecosystem services they provided were also gone. Knowing the historical natural abundance of the species in the area, and with the support of NORA (*Native Oyster Restoration Alliance*, *https://noraeurope.eu/*), the initiative aims to collect local oysters to use as broodstock, evaluate their filtration and reproduction potential, and restore the oyster seed obtained from an experimental hatchery, back to Mar Menor waters. Similar actions have been successfully implemented in lagoons in the USA, such as in Chesapeake Bay [12], but this is the first time to be proposed in a Spanish coastal lagoon. Recent funding has allowed the launch of *RemediOS* project (*https://remedio-sproyecto.wordpress.com/*), a proof of concept for the production of flat oyster seed with local broodstock from the Mar Menor. Several actions are taking place including the development of an experimental hatchery (Fig. 4) with scientific assessment and multiple outreach activities to engage the local community, fisheries and administrations associated with the initiative to help recover Mar Menor. The project collaborates with *ANSE* Foundation (*https://www.fundacionanse.org/*) to grow out the oyster seed in the Marchamalo salt flats, located in the south basin of the lagoon. The rationale of this project is to involve all interested stakeholders for successful large-scale restoration programs, which need public and political support, research, and outreach actions [13].



Fig. 4: Flat oysters in the experimental hatchery. Top: Broodstock; bottom: juveniles from hatchery larvae

Acknowledgements

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Multi-specific Harmful Algal Bloom in a Chilean Fjord: A dangerous phytoplankton cocktail

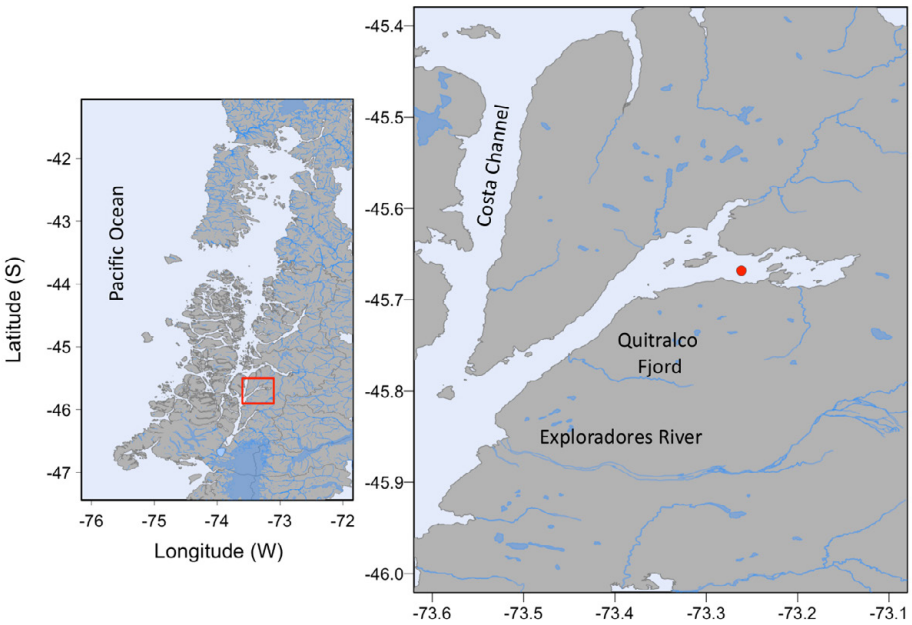

Fig. 1. Maps of study area showing: left, NW Patagonian fjords; right, Quitralco Fjord

Harmful Algal Blooms (HABs) in Southern Chile (Patagonian fjords) have followed the global trend of increasing reports and recurring negative impacts in the last decades [1,2]. Consequently, HABs in this fjord system have become a major problem that affects artisanal fisheries, aquaculture and tourist resources as well as coastal and inland ecosystems, and public health [3]. Recurrent events of Paralytic Shellfish Toxins (PSTs) producing species *Alexandrium catenella* pose the main threat to aquaculture and public health in Southern Chile [3]. Amnesic Shellfish Toxins (ASTs) events caused by the diatom genus *Pseudo-nitzschia* and Diarrheic Shellfish Toxins (DSTs) by endemic *Dinophysis* species (*D. acuta* and *D. acuminata* complex— producers of Okadaic Acid (OA) derivatives and Pectenotoxins, PTX) also threaten the Patagonian fjords ecosystem [1]. In addition, blooms of Yessotoxin (YTX) producers, such as the dinoflagellate *Protoceratium reticulatum,* recurrent in Southern Chile, have been associated with mass mortalities in Northern Chile [4].

In 2022, an intense summer bloom of toxic species, *Pseudo-nizschia* spp, *Alexandrium catenella*, *Dinophysis acuminata* and *Protoceratium reticulatum,* was detected in the southern fjords of NW Patagonia (Fig. 1A). The Quitralco Fjord, located south of the Aysén region, was the area most impacted (Fig. 1B). This diverse Harmful Algal Bloom (HAB) generated intense brown patches of water close to the head of the fjord (Fig. 2). In February 22, measurements of physical-chemical properties were carried out at a station inside Quitralco fjord to study fine-scale distribution of the HAB species. Vertical distribution (CTD profiles) of temperature (°C), salinity, *in vivo* fluorescence (equiv. µg L^{-1} Chl-*a*) and dissolved oxygen (mL L^{-1}) as well as water samples at 2m intervals from surface to 20 m (Niskin bottles) for microphytoplankton analyses were collected.

CTD profiles showed a stratified water column with salinity ranging from 26.8 to 29.5 and temperature from 13.5 °C to 10.5 °C between surface and 20 m (Fig. 3A). An intense subsurface chl-a maximum (SSCM) was detected from 3 to 5 m, with a maximum of 22.6 µg L^{-1} at 4 m depth (Fig. 3A). The phytoplankton community was dominated by *Pseudo-nizschia* (72%) and the dinoflagellate *Alexandrium catenella* (10%). Maximum cell densities of *Pseudo-nizschia* spp. (2 x 10^6 cells L^{-1}) and *A. catenella* (267 x 10^3 cells L^{-1}) were detected at 4 m depth, coinciding with the chl *a* maximum (Figs. 3B, C). High densities of *D. acuminata* (6.0 x 10^3 cells L^{-1}) and *P.*

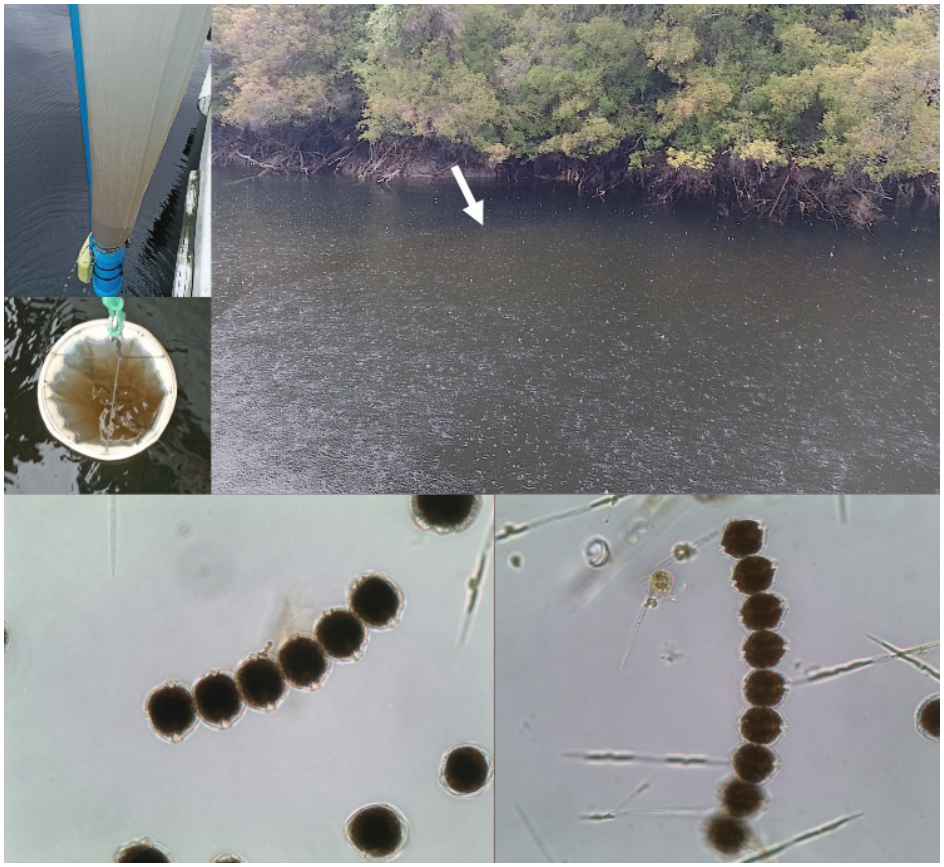

Fig. 2. Photographs of the red tide (upper panel) and light microscopy micrographs of Alexandrium catenella and Pseudo-nitzschia spp. (lower panel) from Quitralco Fjord

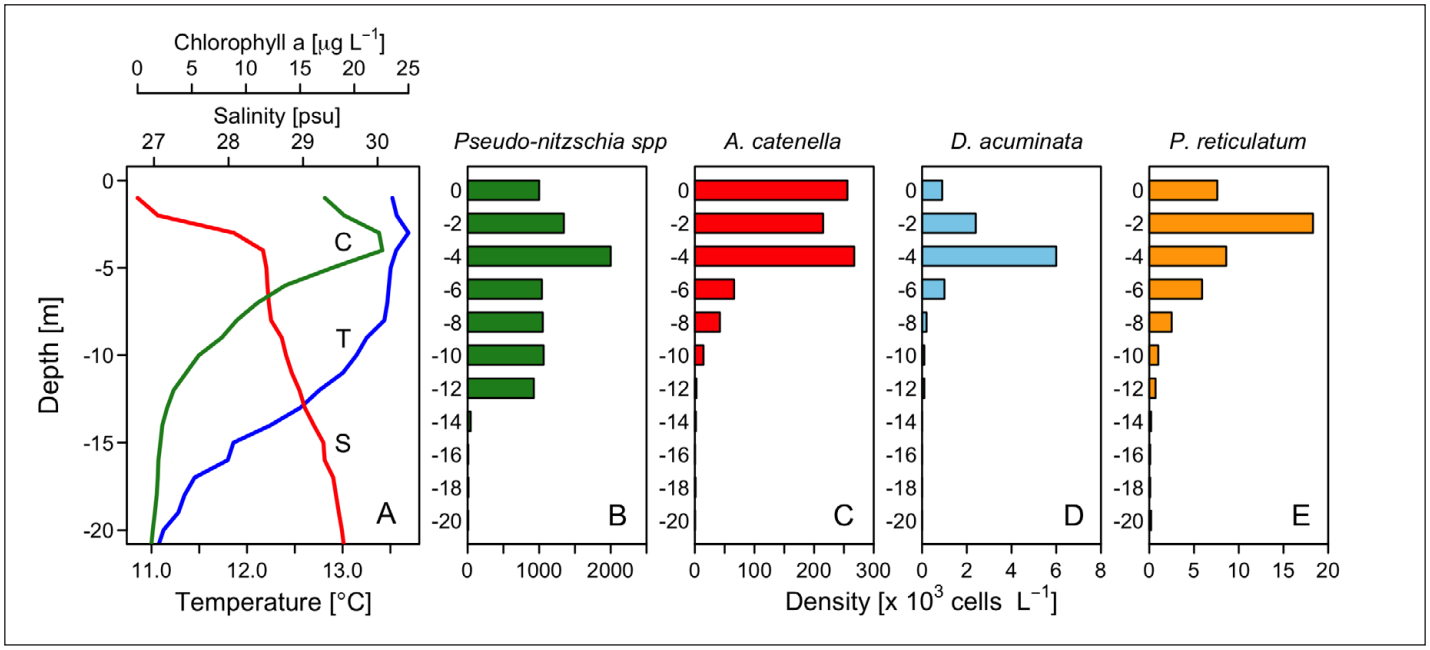

Fig. 3. Vertical distribution of A) temperature (blue line), salinity (red line) and chlorophyll a (green line); B) Pseudo-nitzschia spp; C) A. catenella; D) D. acuminata; E) P. reticulatum at a sampling station close to the head of Quitralco Fjord on February 22, 2022

reticulatum (18.3 x 10^3 cells L^{-1}) were detected in the same surface layer (Figs. 3D, E).

The bloom caused six human intoxications including two fatal cases and serious ecosystem loss. Fish mortalities (juvenile hake) were observed close to the head of this fjord. According to official reports from the Chilean Health Ministry Monitoring Programme record levels of PSP toxins (~14 x 10^3 µg STX eq. 100 g^{-1}) were found in clams (*Ameghinomya antiqua*) from Quitralco Fjord at the time of this event. The magnitude of these toxicity levels is similar to that recorded during the summer 2009 bloom of *A. catenella* [3]. Nevertheless, unlike the 2022 event the 2009 episode was caused by a monospecific bloom of *A. catenella* [5]. Although the Patagonian fjord and channel system have a high recurrence of HABs, multispecific blooms of toxic species are rare because each species exploit a different environmental niche. Thus, while optimal growth of *A. catenella* is observed in high salinities (~35) [6], *D. acuminata* and *P. reticulatum* occur in areas with high thermohaline stratification [7]. Different HAB species, even if belonging to the same genus, may exhibit distinct responses to changing environmental conditions [8]. This multispecific bloom gives a unique opportunity to determine the realized niche of these four toxic species and identify the optimal environmental conditions that they exploited during this event. *Pseudo-nitzschia* spp. showed a broader realized niche with greater tolerance to environmental conditions, in contrast to *A. catenella* that showed a smaller niche, being mainly restricted to the surface layer (0-4 m). *Dinophysis acuminata* and *P. reticulatum* showed a similar niche.

Considering the synoptic climate in the Chilean Patagonia (declining precipitation; 5-7% per decade and global temperature increase), these intense HAB events may become more recurrent in the coming years. Finally, the occurrence of this type of toxic phytoplankton cocktail may have synergistic effects and increase the risks for public health (human intoxications) as well as social and ecosystem impacts (invertebrate and vertebrate mortalities) and substantial economic losses.

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Red Tide Adaptation and Response Network (REARMAR): bridging local, scientific and policy knowledge for small-scale benthic fisheries in the northern Chilean Patagonia

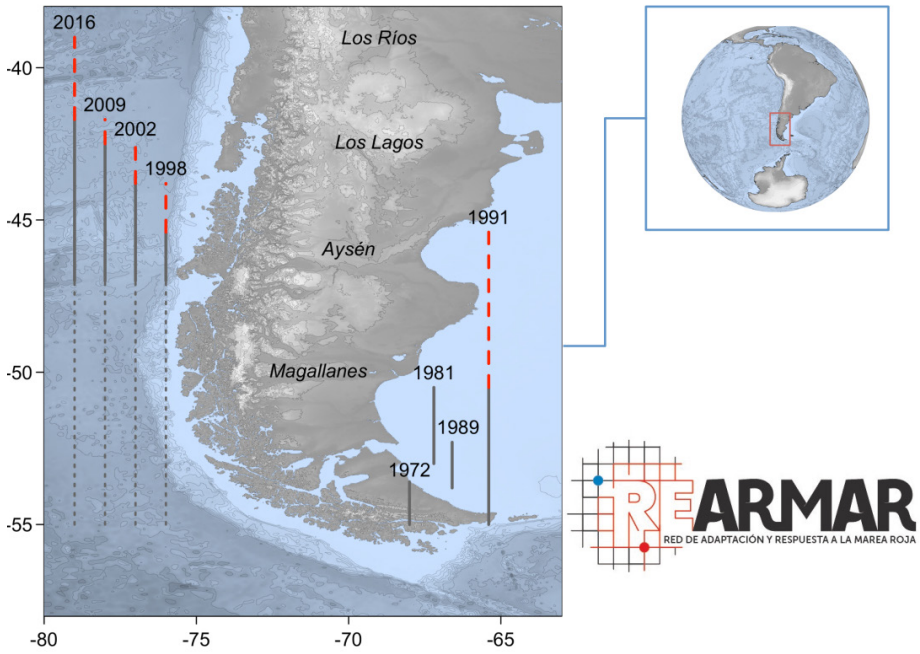

Fig. 1. Expansion of Alexandrium catenella-related PSP outbreaks recorded in the last four decades in Chilean Patagonia. Dashed lines indicate: red, the northward progression with respect to the last bloom; grey, the northward shift of the centre of gravity of PSP events distribution, from the Magellan to Aysén Region. Modified from Guzmán et al. [2].

Harmful Algal Blooms (HAB), particularly toxic blooms of the dinoflagellate *Alexandrium catenella*, have shown an impressive geographical expansion along the Chilean Patagonian coast in the last 50 years (Fig. 1). After the first confirmed fatal case of Paralytic Shellfish Poisoning (PSP) in Magallanes in 1972, *A. catenella* blooms have been reported to expand northward in the following decades at 44 Km per year. In 2016, the presence of toxic PSP events above regulatory levels expanded more than 230 km north from the latest known limit in Canal de Chacao (41°47'S) up to Mehuín (39°26'S) [1].

In Chile, various regulatory and policy initiatives have been put in place to address health and economic problems derived from HABs. In 1995, the "National Program for Prevention and Control of Red Tide Intoxications" was created under the Chilean Health Ministry. Ever since, permanent sampling of shellfish landings for toxin analyses has been carried out in key regional ports where PSP occurrence is confirmed. During extended closures, the government has often implemented subsidies to mitigate the economic effects of HABs on formally registered artisanal fishermen. In addition, 'risk' communication campaigns are frequently developed to protect fishermen and consumers from the potential threats of PSP on human health. The main foci have been to detect and prevent the sale and consumption of contaminated shellfish resources, and to mitigate economic impacts on fishers. Nevertheless, efforts to build long term adaptive capacities among resource users and managers have been limited.

More extended coastal areas affected by HABs imply increased numbers of impacted fishing communities. During the severe 2016 PSP event an estimate of 15,200 fishermen, divers and gleaners operating in the zone were affected by sanitary closures. High levels of toxins were detected in the flesh of key target species harvested, including clams (almeja, *Ameghinomya antiqua*), ribbed mussels (cholga, *Aulacomya atra*), mussels (chorito, *Mytilus chilensis*), hard razor clams (navajuela, *Tagelus dombeii*) and loco (*Concholepas concholepas*).

In what ways do HABs impact inexperienced fishing communities? Several interviews and workshops carried out in fishing villages or *caletas* affected by HABs in 2016 for the first time revealed that coastal communities were lacking the capacity to make sense of and cope with this new threat (Fig. 2).

- Fishermen alleged insufficient information and education about HABs. Public health teams and fishermen had no previous relationship when shellfish sampling started; hence, rapport had to be built from the ground up. Participants also expressed that the presence of HAB experts and researchers in the field when PSP toxin levels peaked —to exchange knowledge and experience—was missed; e.g. knowledge about toxin detection methods was lacking. In addition, existing communication channels between local



Fig. 2. Word cloud of experiences reported by workshop participants during their first HAB event in 2016 (larger font sizes represent higher use frequency).

fishermen leaders and fishery and health authorities were considered inappropriate. Several coordination problems arose when an authorized 6,000 loco landing could not be placed on the market because results of laboratory tests —detecting toxins slightly above the regulatory limit (80 ug STX eq. 100 g meat)— arrived too late to the *caleta*.

- Information and understanding gaps were quickly filled with rumors and beliefs about the origins and causes of HABs. Fishermen' discontent and frustration when their activity was forbidden led to their explanations and search for liable parties. In some *caletas*, people observed rare environmental signals the days before the official HAB alert, such as an increase of water temperature, intense brown patches, and invertebrate and seabird mortalities. The red tide event was therefore associated with human intervention and industrial pollution. An overall climate of suspicion and mistrust prevailed among fishing communities in their relationship with authorities and other local and regional parties.
- The arrival of HABs to their fishing zones, a dire threat to sustaining their livelihood, is considered to have increased the uncertainty to which they were already accustomed. In addition to changing conditions in climate, market and resource availability, the potential presence of PSP toxins in some of the economically important resources implies reduced stability and predictability. In some *caletas*, landing closures lasted over 18 months, negatively impacting families' income and traditional diet. This forced resource users to rely on or shift to other economic activities, such as farming and cattle raising, foresting, or professional diving. As a critical warning, benthic fisheries in the southern Aysén Region, where high levels of PSP toxins exist almost permanently, have dramatically declined in the last decades due to HAB effects. Therefore, shaping different future trajectories becomes an urgent need for fishers in northern Patagonia.



Fig. 3. During the "Red Tide Tour", fishermen leaders from Los Ríos Region visited the Health Service Laboratory in Puerto Montt, where shellfish samples are analyzed.

In 2019, the Red Tide Adaptation and Response Network (REARMAR) was created to address increasing disinformation, distrust, and uncertainty among fishermen organizations. The vision of REARMAR is to offer a learning platform to co-create relevant HAB knowledge and bring fishermen and scientific and policy actors together. Drawing on a social capital framework [3], weaving the network has included activities connecting different scales and closing existing knowledge gaps. Workshops were held to 1) nurture bonding (social glue) and bridging relationships (social lubricant) by exchanging people's experiences and concerns about red tides at the *caleta* level and also between *caletas* affected by HAB; 2) foster linking ties (social pipelines) between fishermen and researchers to share people's communications and discuss the state-of-the-art of scientific knowledge about HABs; 3) improve communication between fishers and sanitary authorities and to open the "black box" of PSP toxin analysis and detection. As shown in Fig. 3, the "Red Tide Tour" brought fishermen leaders from distant *caletas* in Los Ríos Region to visit the Health Service Laboratory in Los Lagos Region. They witnessed the procedure followed to detect PSP in samples taken from their shellfish landings, aiming at increasing trust and legitimacy among parties. REARMAR is also committed to science communication initiatives, such as micro documentaries [4], opinion columns, and online social platforms [5].

Conventional approaches to respond to HAB impacts have mostly focused on health problems, top-down command and control policies, and an early detection approach. More initiatives such as REARMAR are necessary to boost such efforts, and to enhance a fisheries management focus, bottom-up and participatory strategies, and proactive and adaptive approaches in Chile and elsewhere.

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An unprecedented harmful algae bloom in the beaches of Rio, Brazil

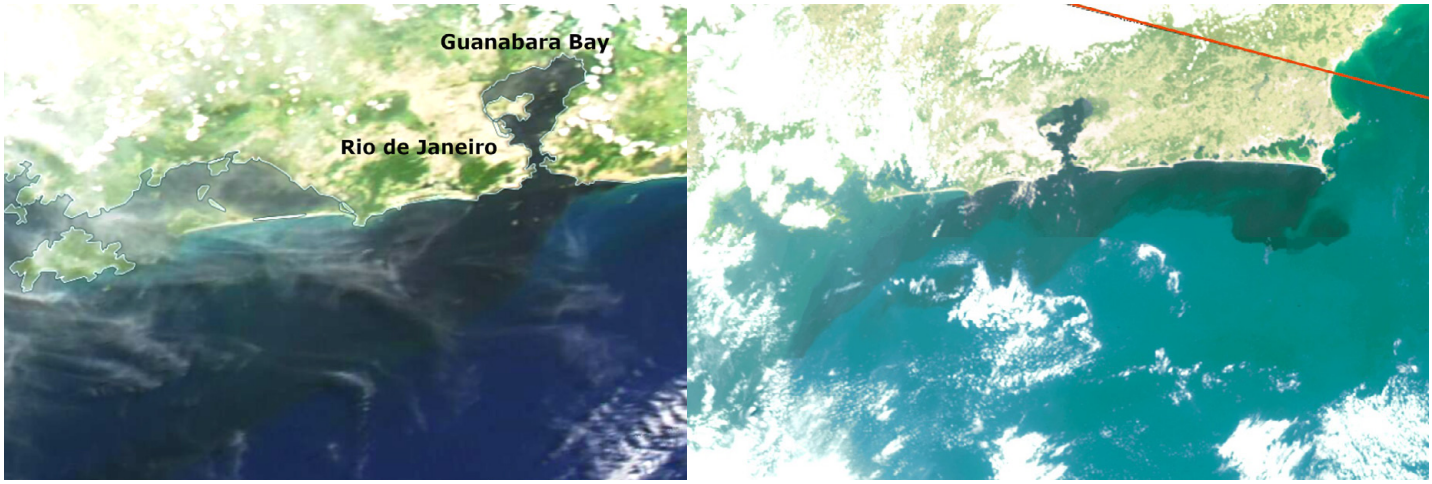

Fig. 1. Images acquired by Sentinel-3's Ocean and Land Colour Instrument – OLCI, on A) November 16th, 2021; B) December 5th, 2021. The dark water patch indicates the algal bloom. Source: Priscila Kienteca Lange, UFRJ

An extensive and long-lasting bloom tinted the waters of the famous Copacabana and Ipanema beaches in Rio de Janeiro city in late austral Spring 2021. The bloom was first seen on satellite images on November 3rd, 2021, and water samples taken on November 16th from the western part of the city of Rio (Recreio beach) revealed that the dark-red patches were dominated by the ciliate *Mesodinium rubrum*, together with the flagellate *Tetraselmis* sp. This flagellate is common in Guanabara Bay (GB) and a satellite image from 16th November 2021 showed dark waters coming out from GB towards the beaches of Rio (Fig. 1a).

Two weeks later (3rd of December), coastal currents transported the bloom easterly towards Arraial do Cabo, a scuba dive paradise, where the usually clear waters became dark brown. This massive bloom was evidenced in satellite images from December 5th, 2021 (Fig. 1b). The beaches of Rio city were dark brown with a pink foam during several days in December (Fig. 2). Water samples were taken from several beaches in Rio and from Arraial do Cabo for phytoplankton species identification and quantification and for pigment analysis by HPLC and are still under analysis.

Preliminary results showed that, on the 6th of December, filamentous cyanobacteria of the family Pseudanabaenaceae, *Tetraselmis* sp. and *Scrippsiella* sp. were abundant in Rio beaches. Pseudanabaenaceae are generally dominant in GB during spring and summer, while the dinoflagellate *Scrippsiella trochoidea* is often found in high abundances in the bay [1]. At Arraial do Cabo in the same day, the bloom was mainly composed by *Tetraselmis* sp., *Scrippsiella* sp. (Fig. 3) and one unidentified dinoflagellate species. The presence of the pigment gyroxanthin in samples from Arraial do Cabo indicated the occurrence of type two chloroplast dinoflagellates, probably of the family Kareniaceae. Between the 12th and the 18th of December, the water of beaches along more than 150 km between Rio and Arraial do Cabo were dark brown.

Patches of dark orange-glowing water (Fig. 4) were still observed in calm inlets in GB on December 21st. These water patches showed a diverse community of dinoflagellates (both auto- and heterotrophic) and the ciliate *Mesodinium rubrum*, along with small flagellates (Cryptophytes and



Fig. 2. Leme beach, Rio de Janeiro on the 10th of December. A) dark brown waters, B) pink foam. Photo by Silvia Nascimento

Tetraselmis). We speculate that these calm inlets could have possibly been the source of the massive offshore bloom, but further image and data analysis needs to be conducted.

The coast of Rio de Janeiro state is subject to coastal upwelling of the South Atlantic Central Water (SACW water mass) at Arraial do Cabo, driven by the occurrence of persistent Northeasterly winds from September to March [2]. The deep nutrient rich water fertilizes the surface ocean, where there is plenty of light for photosynthesis, supporting the increase in primary production. This productive water mass is usually dragged west (by the wind), often reaching the coast of the city of Rio in Spring and Summer. This phenomenon supports the diverse and abundant marine life and high fishery catch in Rio's coastal waters.

In 2021, unusual wet weather conditions occurred in September-October, with 6 cloudy weeks of constant rainfall, that probably contributed with nutrients to the coastal area. Off shore from the coastline, seawater was cold (upwelled) clear and blue. In early November, the weather became sunny. We hypothesize that the long day light periods, strong sunlight, clear nutrient-rich waters, and weak wind conditions led to this unprecedented bloom event.



Fig. 3. Scrippsiella sp. from a sample collected on the 3rd of December, 2021 at Arraial do Cabo. Photo by Lohengrin Fernandes

Harmful algae blooms have occurred in Rio and are usually seen in a couple of beaches when heat waves hit the city; but in 2021, instead of fading, the bloom spread. The sunny days and calm seas lasted for weeks. On late December, as the weather conditions changed, with more cold fronts from the South bringing rain and clouds, the bloom was slowly pushed towards the ocean.

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Fig. 4. Dark orange-glowing water in the inlet of Botafogo, Rio de Janeiro, observed on December 21st. Photo by Priscila Kienteca Lange

High Biomass Bloom of a dinoflagellate (*Scrippsiella* sp.) in a tropical estuary in northern Bahia State, Northeast Brazil

A bloom of *Scrippsiella* sp. was observed in the Rio Real estuary of Northeast Brazil (Figure 1A; 11º 18' 28'' S; 37º 16' 45'' W). According to the Köppen-Geiger climate classification [1], the climate of the region is characterized as humid tropical (AfA), with an average annual temperature ranging from 21ºC to 31ºC with a dry season (September-March) and a rainy season (April-August). The locality has a semidiurnal tidal regime with maximum range between high and low tide of 2.1 m [2]. The Rio Real estuary has a total area of 4,968.44 km^2, of which 2,567 km^2 is drainage area, 458.06 km^2 is perimeter and 110.7 km^2 corresponds to the main axis. The source area of Rio Real corresponds to 18.6% of its total area and is located within a region with a semi-arid climate. The surrounding vegetation is comprised of predominantly mangroves. Several activities are linked to this environment, such as subsistence fishing and shellfish harvesting, tourism, agriculture, subsistence cropping of various sorts and shrimp farming. The sediments found in the environment come from the Lagarto formation, Grupo Estância, and are composed of fine sandstones, clay, shales and silt.

Phytoplankton were sampled at eight stations along the estuary (Figure 1), following the salinity gradient, during four field campaigns — two in the dry season, January 2014 (22 mm of rain) and 2015 (10.1 mm), and two in the rainy season, July 2013 (241 mm) and 2014 (211.4 mm). Qualitative sampling was performed with subsurface horizontal hauls using a mesh of 30 µm pore diameter and the samples preserved with Transeau solution. Quantitative sampling was performed with a Van Dorn bottle and the samples preserved with 4% Lugol's iodine solution. Water samples were also collected and stored in one-liter plastic bottles refrigerated at 4º C for subsequent nutrient analysis: total phosphorus (TP), orthophosphate (PO_4+), total nitrogen (TN), nitrate (NO_3-), silicate (SiO_2) and chlorophyll a (Chl-a). Temperature, salinity, and dissolved oxygen (DO) were measured in situ with the aid of a HORIBA multiparameter probe. Cell counts were made using an Olympus CKX41 inverted microscope, with 400x magnification, according to [3]. The results are expressed as absolute density (cells L^{-1}).

Mean salinity for the four campaigns ranged from 3.6 (Station A8, upstream) to 27.8 (Station A1, downstream). Stations A1 to A3 were polyhaline, stations A4 to A6 were mesohaline, and stations A7 and A8 were oligohaline [4]. The estuary can be divided into three regions based on the ecology of the phytoplankton species collected: the lower estuary, represented by sampling stations A1, A2 and A3, is characterized by a greater abundance of marine, oceanic and neritic species; the middle estuary, comprising sampling stations A4 and A5, is characterized by a greater abundances of brackish species typically from estuarine environments; and the upper estuary, comprising stations A6, A7 and A8, is characterized by typically freshwater species. The bloom of *Scrippsiella* sp. (mean = 9.2 x 10^6 cells L^{-1}), which occurred in July 2014, was located in the lower and middle estuary regions from station A2 to A5 (Figure 1.A); these regions are close to urban concentrations and shrimp farms, which may have favored cell growth due to the input of nutrients with the greater continental runoff in the rainy season. Stations A1 and A6 to A8, did not have significant concentrations of *Scrippsiella* sp. The high concentrations observed resulted in low phytoplankton diversity compared to the other periods sampled.

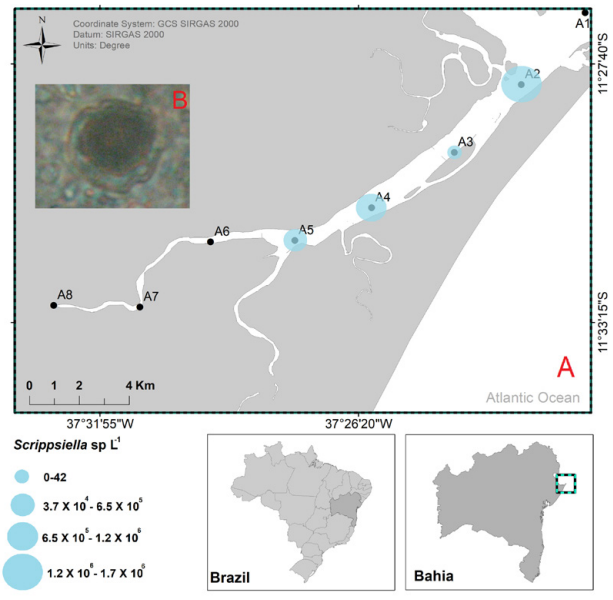

Fig. 1. (A) Location of the study area, NE Brazil, with sampling stations along the Rio Real estuary, showing overall (yellow to red color pattern) and Scrippsiella sp. (circle diameter) cells density (cells L-^{1}). (B) Image (400x) of specimen of Scrippsiella sp.

Acknowledgements

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Limnoraphis robusta bloom in Hanabanilla reservoir, central-southern Cuba

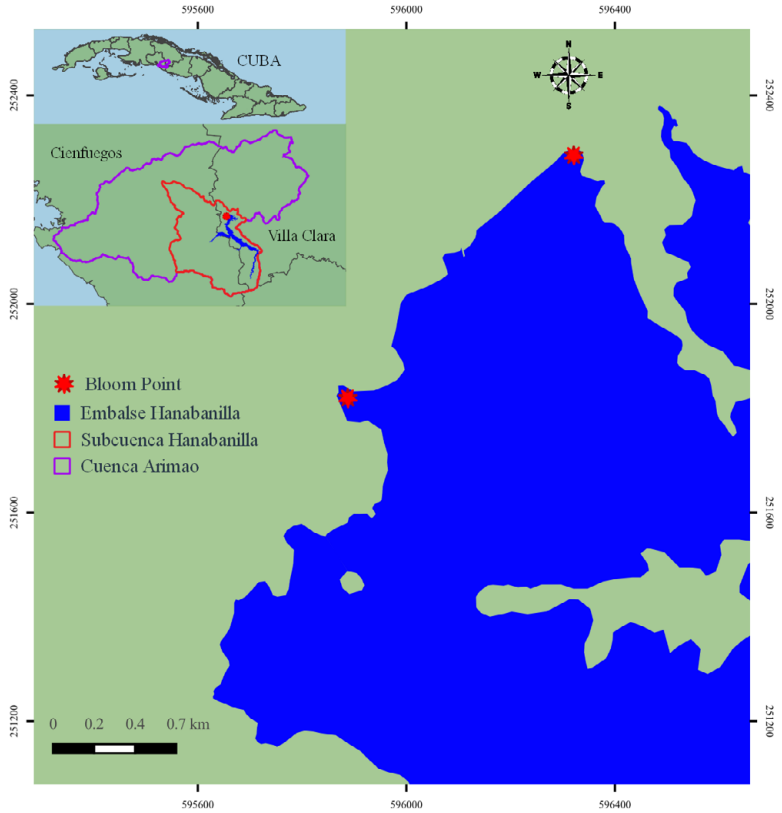

Fig. 1. Map showing the areas where the Limnoraphis robusta bloom occurred in Hanabanilla reservoir.

Harmful cyanobacterial blooms in freshwater ecosystems can form major water discolorations, threaten ecosystem functioning and degrade water quality for recreation, drinking water, fisheries and human health [1-3]. Cyanobacterial blooms have increased worldwide in the last years, and they are likely to expand further in coming decades owing to continued eutrophication, rising atmospheric CO_2 concentrations and global warming [3-4].

Hanabanilla reservoir is an artificial lake with mesotrophic to oligotrophic conditions, located in the mountainous region (364 m in height) of central-southern Cuba (Fig. 1). The reservoir occupies an area of approximately 19 km^2, an average depth of 1.2 m and a volume of 31 m^3; with a climate influenced by the two annual seasons: dry from November to April and rainy from May to October. Hanabanilla reservoir represents the principal source of drinking water supply in the Cienfuegos and Villa Clara provinces. Other uses include energy production (hydroelectric power) and recreational boating [5].

A bloom of *Limnoraphis robusta* (Parakutty) Komárek was identified in mid-February 2021 in Hanabanilla reservoir. Dense green mats were only noted in two areas of the reservoir (Figures 1-2). The bloom did not have noxious effects on human health or on other ecosystem organisms, although previous *L. robusta* blooms in the reservoir have been associated with small-scale deaths of juvenile turtles and aesthetic damage in the reservoir.

Morphological characters of *L. robusta* from Hanabanilla reservoir were in agreement with those observed during previous studies in Cuba and other tropical regions [5, 6]. Filaments ± straight or slightly curved, with free (or in clusters only in mass development) uniseriate trichomes enveloped by firm, thick, mucilaginous and colorless sheaths. Trichomes were 15–17 µm (mean of 16.1 ± 0.64 µm) wide, straight; cells were 4–8 times shorter than wide, green to green-brownish in colour, with aerotopes. Trichomes uniseriate, cylindrical, isopolar, usually not constricted at cross walls; end cells were widely rounded, without calyptra. Heterocytes and akinetes are lacking (Fig. 3).

Reproduction was by production of uniseriate hormogonia (Fig. 3), although they were very scarce, which indicate that *L. robusta* population



Fig. 2. Limnoraphis robusta bloom forming dense green mats in the reservoir.

from Hanabanilla was mainly in early vegetative stage. In contrast, some morphological characters which are indicators of later growth stage such as red-brownish trichomes and hormogonia were present in high abundance in a previous *L. robusta* bloom from Hanabanilla reservoir [5].

L. robusta occurred in high densities (average of 9.0 x 10^6 cells mL^{-1}, and chlorophyll-*a* of 18.9 µg L^{-1}), an almost monospecific bloom; similar to the previous bloom of this species in the Hanabanilla reservoir during 2014 [5]. Environmental conditions, such as high stability in the water column during the dry period, were favorable for the start of the cyanobacteria bloom although nutrient levels were below the detection limit ($N\text{-}NO_{2-}$ <0,005 mg L^{-1}, $N\text{-}NO_{3-}$ <0,01461 mg L^{-1}, $N\text{-}NH_{4+}$ <0,01 mg L^{-1}, $P\text{-}PO4_{3-}$ <0,05 mg L^{-1}).

Despite *L. robusta* not being a toxic species and there was no apparent damage in the reservoir during this event, blooms of this cyanobacteria could cause environmental problems in the future.

Acknowledgements

This study was funded by the Ministry of Science, Technology and Environment of Cuba (CITMA), and by the IWECO project (GEF/PNUD program).

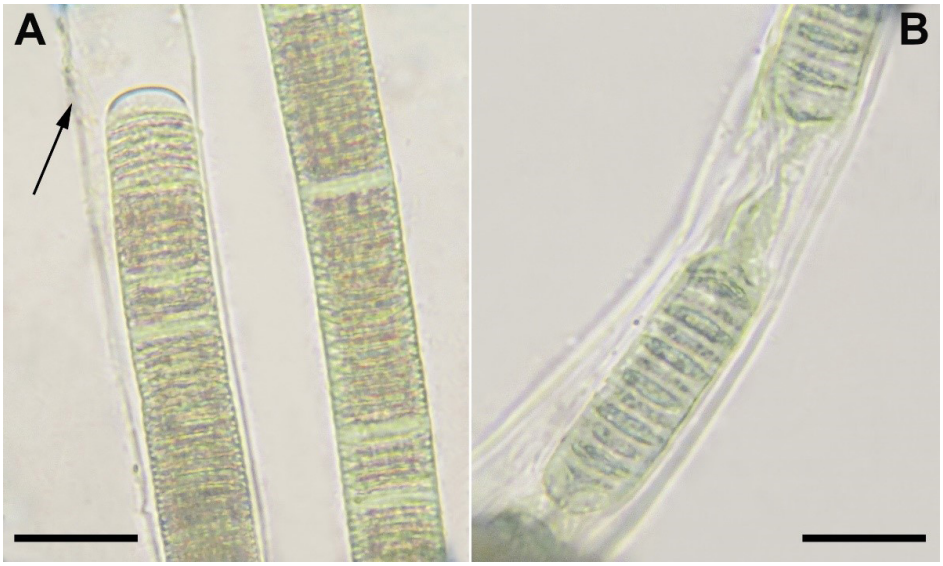

Fig. 3. Light micrographs of Limnoraphis robusta trichomes. A. Vegetative trichomes, arrow indicate the presence of a mucilaginous sheath. B. Trichome with hormogonia. Scale bars = 20 um.

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IOCARIBE HAB-ANCA launches the HAB Virtual Course in 360 virtual reality

This course is an immersive and interactive hands-on laboratory and sampling experience to learn about HABs. The App is a support for laboratory practices that encourages user autonomy, allowing them to learn at their own pace and anywhere.

The user only needs a mobile device and internet access to download the content. The App can be used in online and offline mode.

The course is designed in three learning paths: (1) Summary and Context; (2) Sampling and (3) Microscopic observation. Additionally, it includes the calculation of an aerobic mortality risk indicator – IRMA. This index developed to forecast the mass mortality of fish due to anoxia in a tropical coastal lagoon, corresponds to a eutrophication index, and is based on the concentration of three variables: dissolved inorganic phosphorus, chlorophyll and dissolved oxygen.

This HAB 360 course is a development led by IOCARIBE HAB ANCA, in alliance with the National University of Colombia, INVEMAR and the spin-off Nova Transmedia. It is a completely free course, for the moment only in Spanish. It can be downloaded from Google Play: "Florecimientos Algales Nocivos FAN".

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MixONET, a new SCOR Working Group # 165 on Mixotrophy in the Ocean

Traditional and contemporary methods in Biological Oceanography assume a false plant/animal dichotomy for plankton. This dichotomy has been the bedrock of marine science, operationally separating organisms into phototrophic or phagotrophic compartments wherein about half of Earth's carbon fixation and oxygen production are due to activities of microscopic marine phototrophic phytoplankton. These phytoplankton are ingested by microbial zooplankton which are then preyed upon by metazoan grazers etc. We now know that most of these protist phytoplankton can photosynthesise **and** ingest food while a third of the microbial zooplankton can photosynthesize through acquired phototrophy in addition to hunting prey thus contributing to **both** primary and secondary production. These protist plankton are now recognised as an important community of marine life – the mixoplankton [1-3] (Fig.1).

During the current UN Ocean Decade, marine researchers are faced with the challenge of answering how climate change will impact biology, productivity and carbon sequestration, food-webs, and allied ecosystems services in global oceans. Mixoplankton are at the heart of all these processes, for example, dominating production in various ecosystems (e.g., temperate summers) and supporting juvenile fish growth [3]. Biogeography studies have shown the ubiquity of mixoplankton [4,5]. Well-known phytoplankters that are actually mixoplankton include the coccolithophore *Emiliania huxleyi* [6]; the diverse bacterivorous phytoflagellates of the microbial carbon pump [7]; the toxin-producing dinoflagellates *Alexandrium* and *Dinophysis* whose blooms result in shellfish contamination and harvesting closures [8]. The traditional "microzooplankton" which are now recognised as mixoplankton include various plastidic ciliates, such as *Strombidium* and *Mesodinium* that support farmed and wild fish [9] as well as the green *Noctiluca scintillans,* a bloom forming dinoflagellate that harbours photosynthesizing endosymbionts. Ecosystem disruptive blooms of this green *Noctiluca* are leading to the collapse of the traditional phytoplankton-mesozooplankton link in the food-web in the Arabian Sea [10].

The base of the oceanic food-web is thus comprised of photosynthesizers that also eat and consumers that also photosynthesize, muddying the photo-autotroph/phago-heterotroph distinction that has dominated biological oceanography. This recognition where most oceanic primary producers cannot be analogized as miniature plants has led to a paradigm shift in trophic studies, and there is thus a critical need to evaluate how new sampling and monitoring methods and global plankton databases can be optimized for this new perspective. A cross-disciplinary team is required to address these needs; thus in 2021 we submitted a proposal for a working group to SCOR - MixONET.

The newly funded SCOR working group # 165 *Mixotrophy in the Oceans; Novel Experimental designs and Tools for a new trophic paradigm* (MixONET) (January 2022 – December 2024) is an international brainstorming effort involving experts with knowledge of ecophysiology and molecular biology of mixoplankton, new sampling and observation technology, biogeochemical cycling and ecosystem services (Fig.2).

Our overarching aim is to update biological oceanography to quantitatively accommodate the mixoplankton paradigm, and to evaluate which emerging methods and technology will enable accurate assessment of mixoplankton abundance and activities. MixONET will thus integrate the mixoplankton paradigm with traditional and novel methods of plankton research to provide tools for predicting the response of the ocean's biological communities and element cycles in the face of ongoing climate change to better understand how humanity can maintain healthy sustainable oceans. MixONET is co-chaired by Aditee Mitra (Wales, UK) and George McManus (USA); the membership (Fig.3) includes Beatriz Reguera (Spain), Helga Gomes (USA), Anukul Buranapratheprat (Thailand), Amany Ismael (Egypt), Áurea Ciotti (Brazil), Ahmed Al-Alawi (Oman), Fernando Unrein (Argentina), Hae Jin Jeong (S. Korea), KB Padmakumar (India), Koji Suzuki (Japan), Luciana Santoferrara (USA), Maite



Fig. 1. Artistic representation of example mixoplankton species. a: Lithoptera fenestrata, b: Strombidium conicum, c: Gymnodinium catenatum, d: Karlodinium veneficum, e: Akashiwo sanguinea, f: Alexandrium sp., g: Laboea strobila, h: Prorocentrum micans, i: Mesodinium rubrum, j: Pfiesteria piscicida, k: Heterocapsa triquetra, l: Tripos furca, m: Prymnesium parvum, n: Dinophysis acuta, o: Teleaulax amphioxeia, p: Noctiluca scintillans. Organism size is to scale; scale bar: 10 µm. Image credit: Claudia Traboni (2019) MixITiN project.

Maldonado (Canada), Mengmeng Tong (China), Michaela Larsson (Australia), Patricio Diaz (Chile), Robinson Mugo (Kenya), Tina Šilović (France).

The first meeting of the working group was held in silico (February 2022) with the second hybrid meeting scheduled to be held in Baiona (Galicia, Spain) in June 2022. Further details about the project and outputs will be available on the project website *www.mixotroph.org/mixonet*

Acknowledgements

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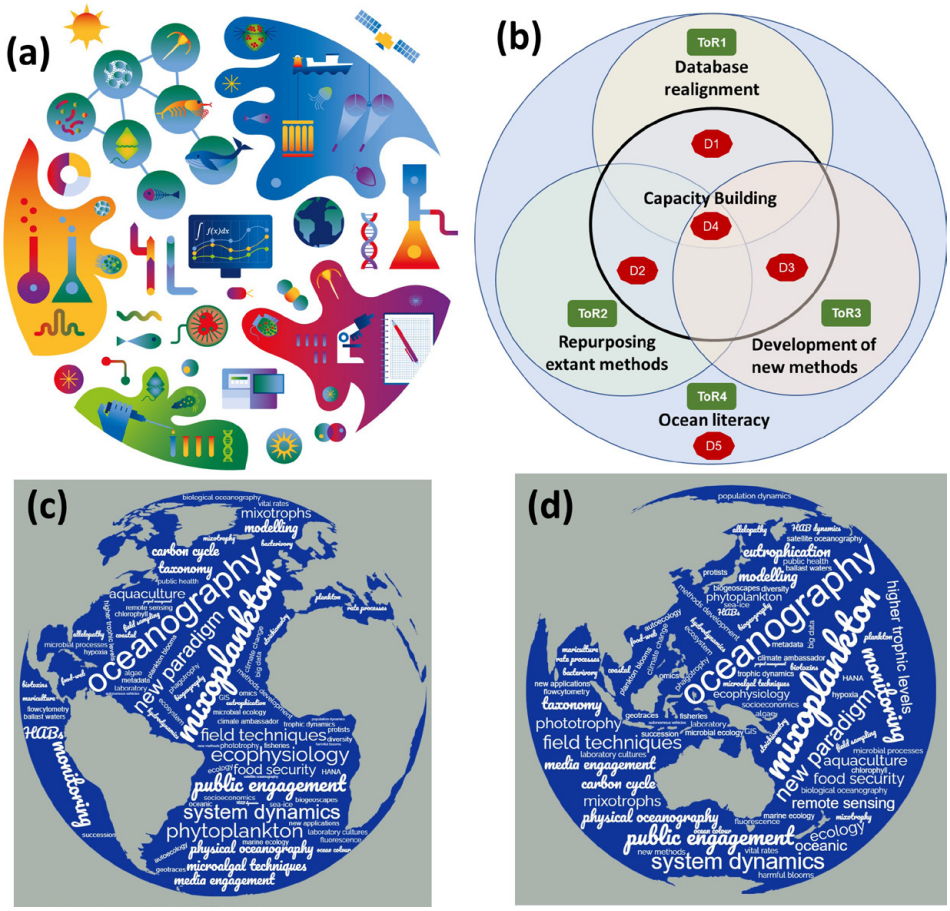

Fig. 2. Schematic depictions of the MixONET programme. (a) Integration of facets of mixoplankton science. Working clockwise from 12 o'clock these include field studies and monitoring, determination of rate processes, taxonomic and genomic analyses, chemical analyses leading to stoichiometric ecology, trophic dynamics, ecosystem services and sustainability/profitability. The centre denotes the focussing of all these activities through modelling for hypothesis testing and predictive purposes. © Aditee Mitra (b) The MixONET project envisions four major terms of reference relating to global databases, new and traditional methods of studying plankton, and ocean literacy. The Venn diagram shows the interconnectivity between the terms of references and deliverables for the project. (c) & (d) Word cloud created from the MixONET proposal to SCOR. From the relative sizes of the terms, their importance to MixONET can be inferred.



Fig. 3. MixONET team members come from 18 different countries and include early-career and more senior researchers. Some use traditional methods (e.g., microscopy) and others use new technologies (satellite sensors, flow cytometry, 'omics) in their research.

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GlobalHAB/EuroMarine Workshop on Modelling and Prediction of Harmful Algal Blooms

The typical harmful algal bloom is a regional- or local-scale phenomenon, a "perfect storm" of environmental conditions, ocean transport and mixing patterns, and microbial ecology. Because of this complexity, prediction of HABs is a grand challenge that requires multidisciplinary dialogue among physical scientists, biologists, computer modellers, and technologists, as well as community stakeholders and the government and industry end-users of prediction systems. The GlobalHAB/EuroMarine WS on Modelling and Prediction of HABs was held on 9-12 May 2022, at the University of Strathclyde, Glasgow, UK. **This 4-day workshop brought together 39 scientists from 17 countries for a combination of oral and poster presentations, round-table discussions, and tutorials,** in order to increase awareness of the range of modelling and observational tools that are in our community toolbox, and help scientists and technologists develop creative approaches to meeting the needs of coastal communities, governments, and industry worldwide.

Discussions consisted of:

- Engaging with stakeholders (facilitated by Aletta Yñiguez and Dave Clarke)
- Scalable solutions: bringing large-scale community tools to local applications (by Clarissa Anderson)
- Building blocks of early-warning systems (by Dave Clarke)
- Combining automated plankton observations and modelling (by Bengt Karlson)
- New directions in mechanistic plankton modelling: where do HABs fit in? (by Neil Banas, Onur Kerimoglu, and Bingzhang Chen)
- HAB model-observation systems for 2050: anticipating future societal needs and assessment tools (by Clarissa Anderson)

Tutorials consisted of:

- Getting started with machine learning using tidymodels in R (Johnathan Evanilla, Kasia Kenitz, and Bingzhang Chen)
- Satellite methods (Clarissa Anderson)
- Getting started with Individual-based modelling (Aletta Yñiguez)

Organising committee

- Representing GlobalHAB: Neil Banas (U Strathclyde, UK), Clarissa Anderson (Scripps/SCCOOS, USA), Dave Clarke (Marine Institute, Ireland), Aletta Yñiguez (U Philippines), Bengt Karlson (SMHI, Sweden)
- Local committee: David McKee, Bingzhang Chen, Paul Udom (U Strathclyde), Sofie Spatharis, Martin Llewellyn (U Glasgow), Keith Davidson, Dmitry Aleynik (SAMS)

Sponsors

The workshop was supported by GlobalHAB, NOAA's *National Centers for Coastal Ocean Science (NCCOS) Competitive Research Program (CRP)*, NOAA's *Integrated Ocean Observing System (IOOS)*, and *EuroMarine*. A follow-on, one-day event on Industry Perspectives supported by the Sustainable Aquaculture Innovation Centre deepened the engagement between workshop participants, the Scottish aquaculture industry, and companies building systems and monitoring technology to support that industry.

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Participants at the GlobalHAB/EuroMarine Workshop on Modelling and Prediction of Harmful Algal Blooms, Glasgow, UK, 9-12 May 2022.

Meeting of the GlobalHAB Scientific Steering Committee, Glasgow, Scotland, May 2022

On May 14th -15th, 2022, the Scientific Steering Committee (SSC) of the IOC-SCOR programme, GlobalHAB, celebrated its first hybrid meeting in Glasgow, UK, following virtual meetings throughout the Covid19 pandemic.

The GlobalHAB programme was launched in 2016 and the first SSC membership was partially *renewed* through a virtual meeting in May 2020. Since then, periodic meetings were held virtually in order to manage and make progress on implementing the programme's terms of reference and action items. Unfortunately, not all members of the SSC could travel to Glasgow, and therefore joined virtually (picture below).

The SSC meeting, co-sponsored by IOC-SCOR *GlobalHAB*, NOAA *National Centers for Coastal Ocean Science (NCCOS)*, the U.S. *Integrated Ocean Observing System (IOOS)* and the *EuroMarine* European Research Network, was held following the *Workshop* on Modelling and Prediction held on May 9-13, 2022 at the University of Strathclyde. The outputs of the workshop are currently being processed and reviewed and will be soon made available to the international community. After being postponed for the last two years during the pandemic, participants were delighted to finally attend the Workshop to present and discuss various aspects of modelling and prediction of HABs. A series of webinars were conducted in 2021 as a preparatory activity for this workshop.

The SSC reviewed the forthcoming activities that had been also postponed in the 2020-2021 period.. These activities include:

- The *GlobalHAB symposium* on automated in situ observations of plankton, to be held in Kristineberg Marine Research Station, Sweden, this August 2022, coordinated by Bengt Karlson.
- The Workshop on *Sargassum*, in preparation now, to be held in Mexico, coordinated by Brigitta van Tussenbroek and the GlobalHAB *Subcommittee* on *Sargassum*.
- The qPCR workshop organized by Raffaele Siano which aims at promoting the use of eDNA approaches in monitoring harmful algae and specifically the use of qPCR for species detection. The workshop will convey international experts on HAB qPCR approaches in order to foresee to which extent eDNA and qPCR can be used in HAB monitoring. Virtual meetings will be organised before the in person meeting in 2023.
- A workshop on solutions to control HABs in marine and estuarine waters co-organized by Vera Trainer and Marc Suddleson in coordination with PICES and ISSHA. This workshop will build on lessons learned from freshwater HAB control.

The SSC also analyzed the objectives of the *13 Themes* that structure GlobalHAB to identify new activities for the last period of the programme, i.e. from 2023 to 2025.

One objective of this new period is to engage the international community working on HAB research through *projects endorsement*. The GlobalHAB programme was implemented to serve scientists and resource managers covering the full breadth of marine and freshwater harmful algal bloom research. Endorsement by GlobalHAB can help foster international collaborations that are fundamental for the progress of our knowledge on HABs dynamics, their prediction and mitigation of their impacts. The GlobalHAB SSC has committed itself to increase communication with the international community by encouraging endorsement by GlobalHAB and by strengthening the links with IOC regional groups such as ANSA, FANSA, HANA, WESTPAC, etc.

Another objective is to consolidate information on freshwater HAB monitoring programmes, to better understand the global impact of freshwater HABs, data gaps, and trends.

Finally, GlobalHAB explored ways to actively participate in the *United Nations Decade of Ocean Science for Sustainable Development (2021-2030)* and contribute to the UN Sustainable Development Goals, a call for action by all countries – poor, rich and middle-income – to promote prosperity while protecting the planet.

The GlobalHAB endorsement application form available from the *webpage*. *GlobalHAB webpage (www.globalhab.info)* or on request from *yu.sun@unesco.org*.



Members of the SSC at the Merchants House of Glasgow and screenshots during the remote connection. Bottom image, from left to right, first line: Aletta Yñiguez, Elisa Berdalet, Heather Raymond; second line: Clarissa Anderson, Sun Yun, Malin Olofsson, Bengt Karlson, Neil Banas, Raffaele Siano, Dave Clarke. Po Teen Lim (top, right picture), Raphael Kudela (middle, right picture), and Marc Suddleson (not pictured) joined virtually.

The international community is invited to participate in the GlobalHAB programme, through seeking endorsement of relevant research, monitoring, and modelling activities

GlobalHAB APPLICATION FORM FOR ENDORSEMENT OF ACTIVITIES AND PROJECTS

To be completed in English and emailed to the Chair of the GlobalHAB SSC.

For further guidance consult the Chair and/or Vice-chair of the GlobalHAB SSC. Emails of the GlobalHAB SSC members can be found at http://www.globalhab.info/organization/scientific-steering-committee

Date:

1. PROJECT TITLE:

Planned duration of activity, from: to:

2. APPLICANT(S):

Principal Investigator name, title:
Address: Tel/Fax:
E-mail: Home page URL: (if applicable)
Other **key persons** (name, title and institution):
Contact person name and email:

3. PROJECT DESCRIPTION:

Include a brief summary or abstract and the relevant details of your project (maximum 5 pages).

Project web site (if applicable):
Contact person:

4. BENEFITS FROM GlobalHAB:

Please comment on
a) how the activity could benefit from endorsement by GlobalHAB
b) how the SSC might assist the activity

5. CONTRIBUTION TO THE GlobalHAB SCIENTIFIC OBJECTIVES AND TOPICS

Please specify how the project contributes to the Implementation of the objectives of GlobalHAB. The applicants are advised to refer to GlobalHAB Science and Implementation plan (available at www.globalhab.info) for write up in this section.

6. CONTRIBUTION TO coordination of HAB research

One of the missions of GlobalHAB is to foster international coordination and cooperative research to address the scientific and societal challenges of HABs (http://www.globalhab.info/about-us/goal-and-mission). Please, indicate how your project will contribute to it.

7. LINKAGES WITH OTHER PROGRAMMES:

Is the project part of a National Programme? Yes: ___No: ___ If yes, give title:

Is the activity part of, coordinated with, or affiliated with, other international/regional programs? Yes: ___ No. ___ If yes, give program title:

8. FUNDING
Has funding been obtained? Yes: No: (Prospective) source(s):

9. CONTRIBUTION TO UN DECADE OF OCEAN SCIENCE FOR SUSTAINABLE DEVELOPMENT 2030 AND UN SUSTAINBALE DEVELOPMENT GOALS (UN SDG)

Please specific how the project contributes to Ocean Decade challenges or outcome (https://www.oceandecade.org) and the UN SDG (https://sdgs.un.org/goals).
Tick one or more if appropriate, refer to Decade Implementation Plan for more information.

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Microbial life cycles in a changing ocean

Contributions that address the following topics are welcome:

- Diversity of microbial life cycles in different habitats and environments
- Regulatory and cell signaling mechanisms in life phase transitions
- Life cycle processes and genetic diversity. The role of sexual reproduction, clonal replication and dormancy in evolution
- 'Omics' resources providing new tools for the study of molecular mechanisms and regulatory pathways driving life cycle transitions
- Ecological implications of dormancy for stability of populations and communities in a variable environment
- Novel experimental approaches and advanced observation systems to study life cycle transitions in the environment
- Modelling approaches to life cycle studies on unicellular organisms

Organized by Miguel Frada, Anke Kremp, Marina Montresor, Sanna Suikkanen and Conny Sjöqvist



@Tvärminne Zoological Station (Finland)

10-14 October 2022

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The GlobalHAB mini-symposium on automated plankton observations

High-frequency observations of Harmful Algal Blooms (HABs) and grazers of HAB species are needed to be able to understand HAB dynamics, to develop predictive models of HABs and to produce robust warnings of HABs. In recent years, novel *in situ* instrumentation has been developed for automated high-frequency HAB detection in near-real time.

Automated plankton imaging-in-flow produces high quality images of phytoplankton larger than 5-10 µm including many harmful algae (Fig.1). There are four main approaches illustrated in Fig. 2: (1) Imaging-in-flow which is based on the morphology of organisms, (2) flow cytometry which is based on fluorescence properties (e.g., pigmentation) and scattering properties (e.g. size) of cells, (3) observations of bulk bio-optical properties (pigment based) of plankton communities, e.g. fluorescence, absorption and turbidity and (4) molecular methods. The mini-symposium was focused on the imaging-in-flow systems which give detailed information about HAB organisms, including some instrumentation which provided both imaging-in-flow and cytometric data. In addition, imaging-in-flow instruments for observing grazers, i.e., microzooplankton and multicellular zooplankton were included. These instruments are now being adopted in research and piloted in monitoring programmes. The aim of the mini-symposium was to bring together experts on, and users of, automated imaging-in-flow systems to present methods and recent results and to share experiences. Another aim was to carry out a comparison of results when analysing plankton communities quantitatively. Young scientists were particularly encouraged to attend the symposium. The mini-symposium/workshop on automated plankton observations took place at

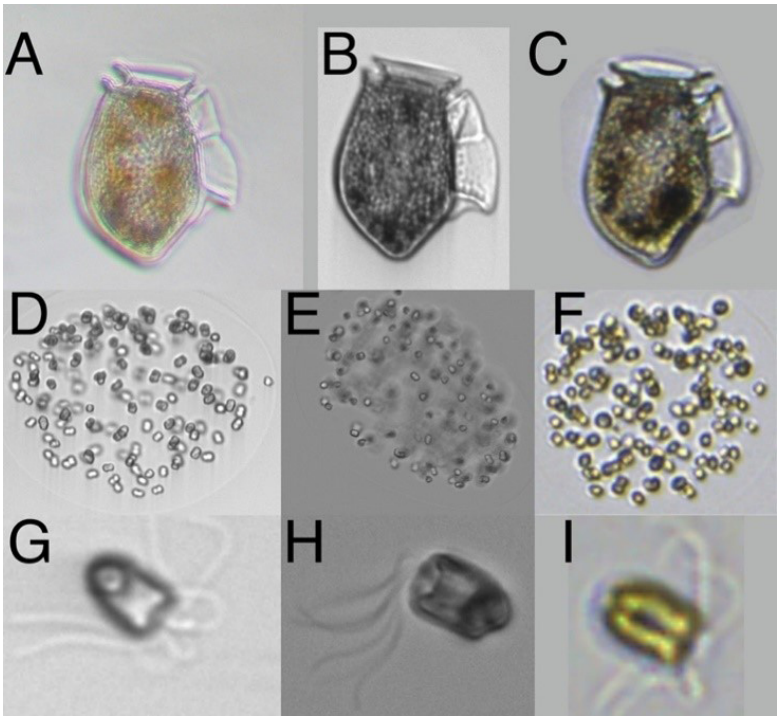

Fig. 1. Micrographs of phytoplankton observed during the mini-symposium. Scale is not consistent. A-C. Dinophysis acuta (field sample), A. Light microscope, photo: Mona Ring Kleiven. B. IFCB and C. FlowCam. D-E. Phaeocystis globosa (culture), D. IFCB, E. CytoSense/Cytobuoy and F. FlowCam. G-I. Pyramimonas sp. (culture), G. IFCB, H. Cytosense/Cytobuoy and I. FlowCam

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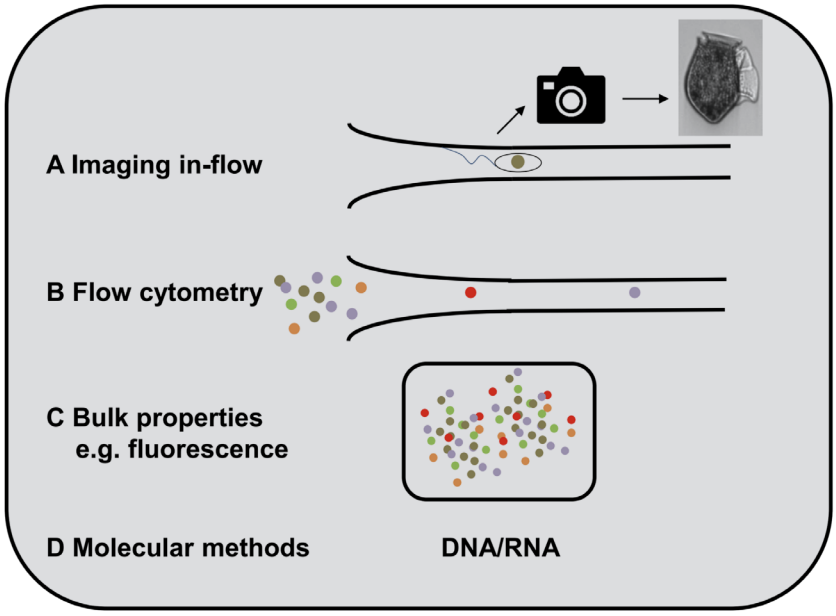

Fig. 2 Basic principles of methods of automated in situ observations of phytoplankton. A. Imaging-in-flow: Organisms are characterised based on morphology; cells are imaged. Camera is triggered by fluorescence or scattering. B. Flow cytometry: Organisms are characterised based on their light scattering and fluorescence properties (pigment content, target fluorochromes). C. The fluorescence or absorbance properties of all organisms in a certain volume of water give a signal of the bulk properties. D. Genetic material: e.g. in situ mini-laboratory for sandwich hybridisation (FISH)

the Kristineberg Centre in Fiskebäckskil, Sweden, 22-26 August 2022. The participants enjoyed nice weather and good company.

Instruments

Instruments from three manufacturers were demonstrated and used to analyse field samples and cultures (Fig. 3): 1. the Imaging FlowCytobot (IFCB, Mclane Research Laboratories Inc., Falmouth, USA), 2. the Cytosense/Cytobuoy (Cytobuoy Bv, the Netherlands) and 3. the FlowCam (versions 8400 and Macro, Yokogawa Imaging Fluidics, USA). The PlanktoScope was also presented, but this instrument was not available on site. There may also be other devices in development or available commercially. The instruments were operated in laboratories on land using field-collected and culture samples. The IFCB and the Cytobuoy are housed in water proof housings and can be operated *in situ*. These instruments are commonly operated in-flow-through systems in coastal locations or on ships, e.g., as part of Ferrybox systems. All instruments have strengths and weaknesses when compared to each other.

The IFCB and the Cytosense/Cytobuoy are true flow cytometers since they use sheath fluid to centre organisms in flow cells. The FlowCam is not a flow cytometer since water is simply pushed through the flow cell. This sometimes results in out of focus images. A unique feature with the FlowCam 8400 is that it produces colour images of the organisms while the other two instruments use grayscale cameras. In the FlowCam 8400 it is possible to change objectives with different magnifications. A high magnification results in images showing more detail but also in longer sample processing time. The IFCB images essentially all cells passing through its flow cell that are above a size threshold (~10 µm) and with a very high percentage of in focus images. The CytoSense/Cytobuoy produces images of only a subset of the cells passing through its flow cell. A large flow cell in this instrument makes it possible to cover a larger size range of organisms compared to the other instruments however a large flow cell may make it more difficult to centre organisms in the flow cell resulting in out of focus images. The Cytosense/Cytobuoy also use scattering properties and fluorescence fingerprints to characterize plankton. This makes it possible to quantify phototrophic picoplankton. The FlowCam Macro was used to count and characterize multicellular zooplankton.

The PlanktoScope *https://www.planktoscope.org*, a citizen science approach to automated imaging and identification of plankton, was presented during the mini-symposium. This is a low-cost alternative to the commercial instruments. The PlanktoScope can be used with objectives with different magnifications. At present, the PlanktoScope (version 4) is somewhat limited in functionality compared to the commercial alternatives and it may only be applicable to multicellular zooplankton and to larger HAB species at the moment. Future development may change the situation.

Images and automated identification of organisms

The instruments used produce high quality images of plankton larger than ~10 µm (Fig. 1). Smaller organisms are often difficult to identify automatically. Analysis of one sample often results in a few hundred or a few thousand images. To produce quantitative data the images are analysed using Artificial Intelligence machine learning (AI-machine learning). Supervised learning is applicable to HAB-organisms. This means that a phytoplankton identification specialist looks at many images and identifies the organisms. These annotated images constitute a training set for producing classifiers for automated identification of the plankton. The classifiers are used to analyse the main set of images acquired (Fig. 4). At present two main approaches are used for the AI-machine learning: 1. Random forest and 2. Convolutional Neural Networks (CNN). During the mini-symposium ongoing CNN-work in Finland, Scotland and USA were presented. There is also ongoing work on this approach elsewhere, e.g., in France.

Early warning systems

Systems for early warning of HABs using networks of imaging-in-flow devices were presented during the mini-symposium. Mike Brosnahan from Woods Hole Oceanographic Institution presented the HAB-Hub *https://hab-hub.whoi.edu* which includes a number of IFCB's operated on the east coast of the USA. Results are presented in near real time. Raphe Kudela, Kasia Kenitz and Clarissa Anderson (not present at symposium) presented the IFCB Network in California. Also, an emerging network in the Philippines was presented. In Europe an IFCB user network



Fig 3. Instruments used in the mini-symposium: Three Imaging FlowCytobot's (IFCB's) (top left) and two Cytosense/Cytobuoy's (top right), with their water proof housing removed during laboratory work, and the FlowCam 8400 (bottom left) and one FlowCam Macro (not shown) were used during the mini-symposium. The FlowCam Macro is shown in the photo. Bottom right: A PlanktoScope built by Maci Wigginton at Ocean & Earth Sciences, Old Dominion University. Source: www.planktoscope.org

has been established (Karlson, 2021, HAN 68, page 16) with IFCB's mounted in research vessels and in fixed locations. Felipe Artigas presented ongoing Cytosense work in the English Channel and the North Sea. Automated observations of phytoplankton is one task of the EuroGOOS Biological Observation Working Group (BIOWG).

Additional information

Due to space limitations only a selection of presentations and activities at the mini-symposium are mentioned here. Further reading recommended is listed below [1-7]. Most of the presentations made are available as pdf's on the GlobalHAB web site *http://www.globalhab.info/activities*

Some conclusions from the mini-symposium

1. Imaging-in-flow is a viable method for automated analysis of many harmful algae at the species or genus level.
2. High frequency sampling (~every 10-20 minutes) at fixed locations, on research vessels or on ships of opportunity (Ferrybox) results in very useful data.
3. Small species (< 10 µm) are difficult to identify using commercially available imaging-in-flow at present.
4. Very large species or colony forming species also pose a challenge since flow cells and tubes may be clogged.
5. The volume analysed (mostly 5-10 mL) is often too small to analyse rare harmful algae quantitatively. A solution is to pool data from several samples. The problem with a small volume analysed also applies to standard microscope methods, i.e., the Utermöhl method.
6. There is a need to join efforts in sharing annotated images of HAB-organisms and for producing classifiers for automated HAB analysis. There are initiatives in Europe and in the USA to accomplish this.
7. Citizen science initiatives for low-cost instruments such as the PlanktoScope are commendable.
8. Best practices are needed to establish routines for handling of instruments, images and data. At least two best practice documents about automated phytoplankton identification are at present in production, one in Europe and one in the USA.
9. **The successful application of these new instruments results from the intrinsic link between technology progress and phytoplankton identification specialists.**

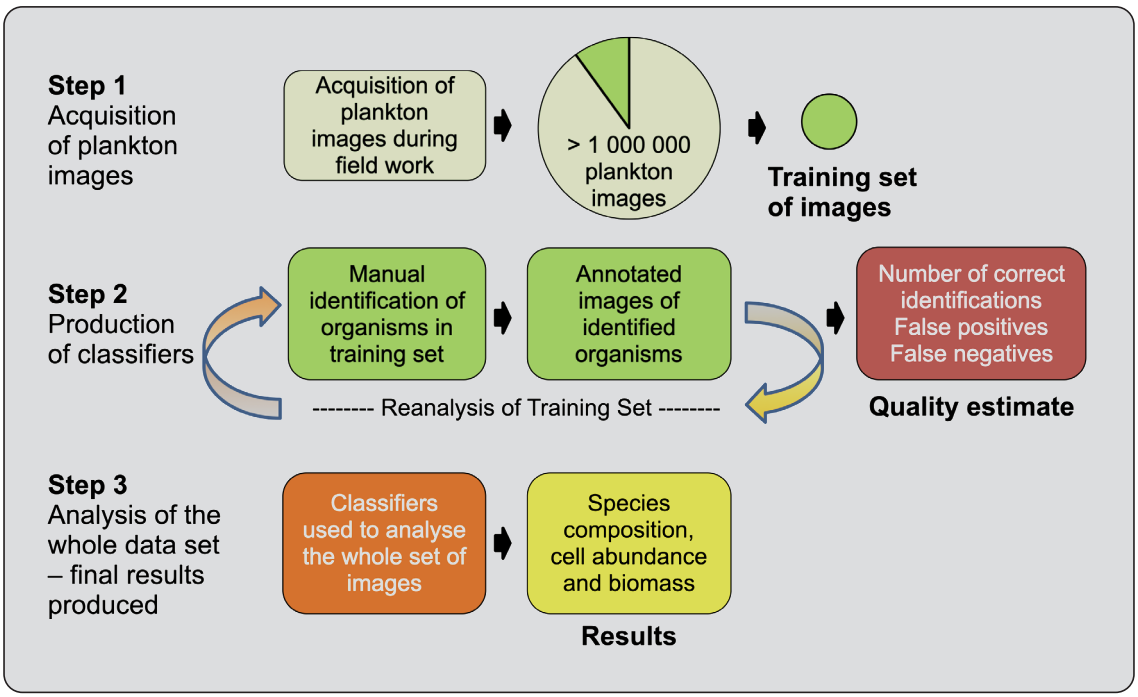

Fig. 4. The principle for using expert trained AI-machine learning for automated analysis of images acquired using imaging-in-flow instruments

Acknowledgements

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Fig. 5. Participants of the GlobalHAB mini-symposium on automated plankton observations

High-Biomass Harmful Algal Blooms (HB-HAB) in the Chilean Fjords System: *Lepidodinium chlorophorum*

Harmful Algal Blooms (HABs) have become a recurrent problem in Southern Chile (Patagonian fjords) during the last decades, responsible for large scale events affecting public health and the aquaculture industry, especially salmon farming. Although High-Biomass Harmful Algal Blooms (HB-HAB) have negative socio-economic impacts, most studies in Chile have been focused on toxin-producing microalgae [1]. Here, we describe the development of an exceptional HB-HAB of *Lepidodinium chlorophorum* during late summer and early fall 2020. This bloom covered a large part of the northern Chilean Patagonia extending from Aysén to the Chiloé, Inland Sea including the Reloncaví fjord and sound system (Fig. 1A; [2]).

The National Fisheries and Aquaculture Service (Servicio Nacional de Pesca y Acuicultura, SERNAPESCA) have reported the occurrence of *L. chlorophorum* cells since February 10, 2020 in the Chiloé, Inland Sea, with cell maxima up to $2x10^6$ cells L^{-1} in Reloncaví sound, between 6 - 19 April, 2020. This report coincided with the detection of high concentrations of chlorophyll-*a* (Chl-*a*; 50 µg L^{-1}) in the middle reaches of Reloncaví Sound between March 31 -April 15, 2020 (records from an oceanographic buoy at station 3 www.cdom.cl; Fig. 1A). On April 2, 2020, an oceanographic cruise was carried out in Reloncaví Sound to characterize the physical-chemical conditions associated with the *L. chlorophorum* bloom. A single CTD profile (0-50m) was carried out at station 2 and water samples were collected with high vertical resolution (every 2 m between 0 and 20 m) for phytoplankton analyses. In addition, surface water samples were collected for phytoplankton analysis in station 1 (Tenglo Channel; Fig. 1A).

The results confirmed the presence of *L. chlorophorum* in the surface layer, reaching a maximum of $6x10^6$ cells L^{-1} in station 2 at 4 m depth (Fig. 2A) coinciding with the highest Chl-*a* concentration (115 µg L^{-1}; Fig. 2B). This thin layer of *L. chlorophorum* was recorded at temperature and salinity ranging 13.5-13.7 °C and 30-30.5, respectively (i.e. above the maximum gradient located between 8 and 11 m; Fig. 2B). Furthermore, green surface filaments with densities of ~500,000 cells mL^{-1} of *L. chlorophorum* characterized station 1 (Fig. 1B, C), possibly due to a semi-confined water masses.

Fluviometric registers indicated weak rainfall during summer and early fall 2020 and; negative outflow anomalies (~200 m^3 s^{-1}; www.dga.cl) were observed for the Puelo River, the main freshwater input into the Reloncaví Fjord and Sound system

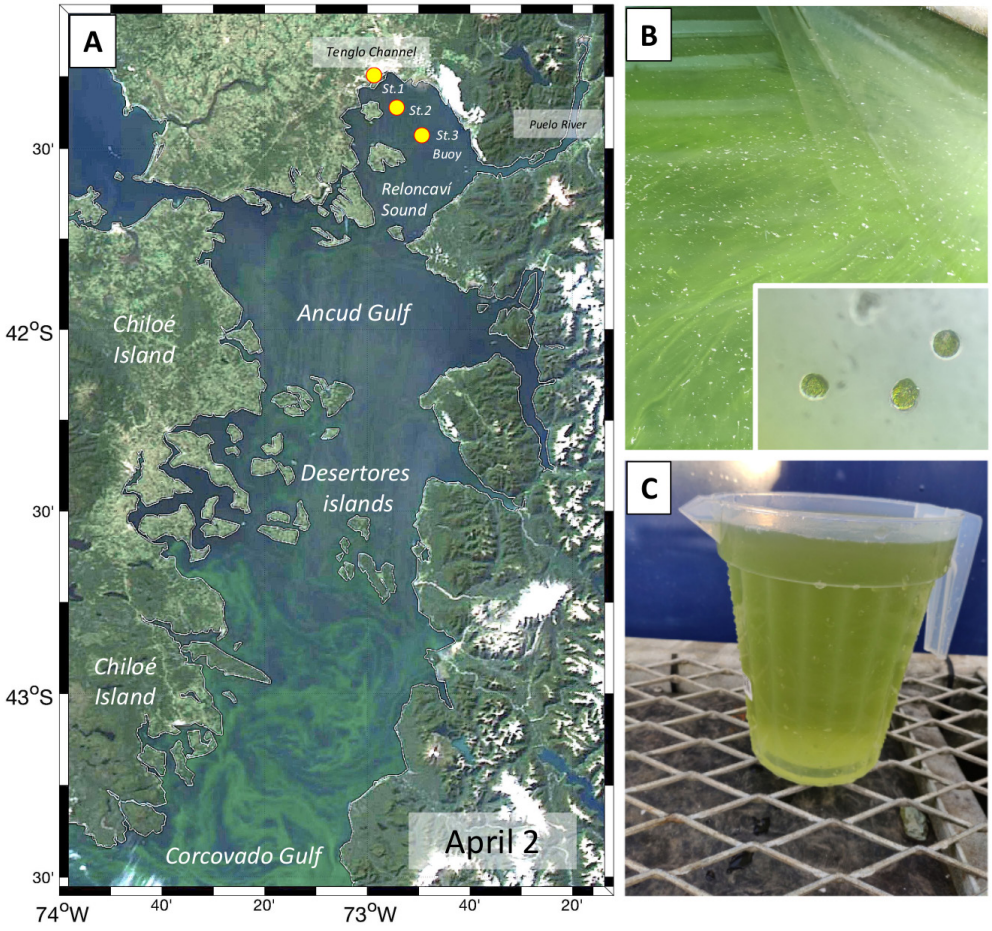

Fig. 1. A) True colour MODIS image of green filaments at the inner sea of Chiloé and the Reloncaví fjord and sound system. Oceanographic stations 1 and 2 and i~mar buoy (st. 3) are marked with yellow circles. B) Green filaments of L. chlorophorum in Tenglo Channel; C) Water sample from Tenglo Channel (st. 1).

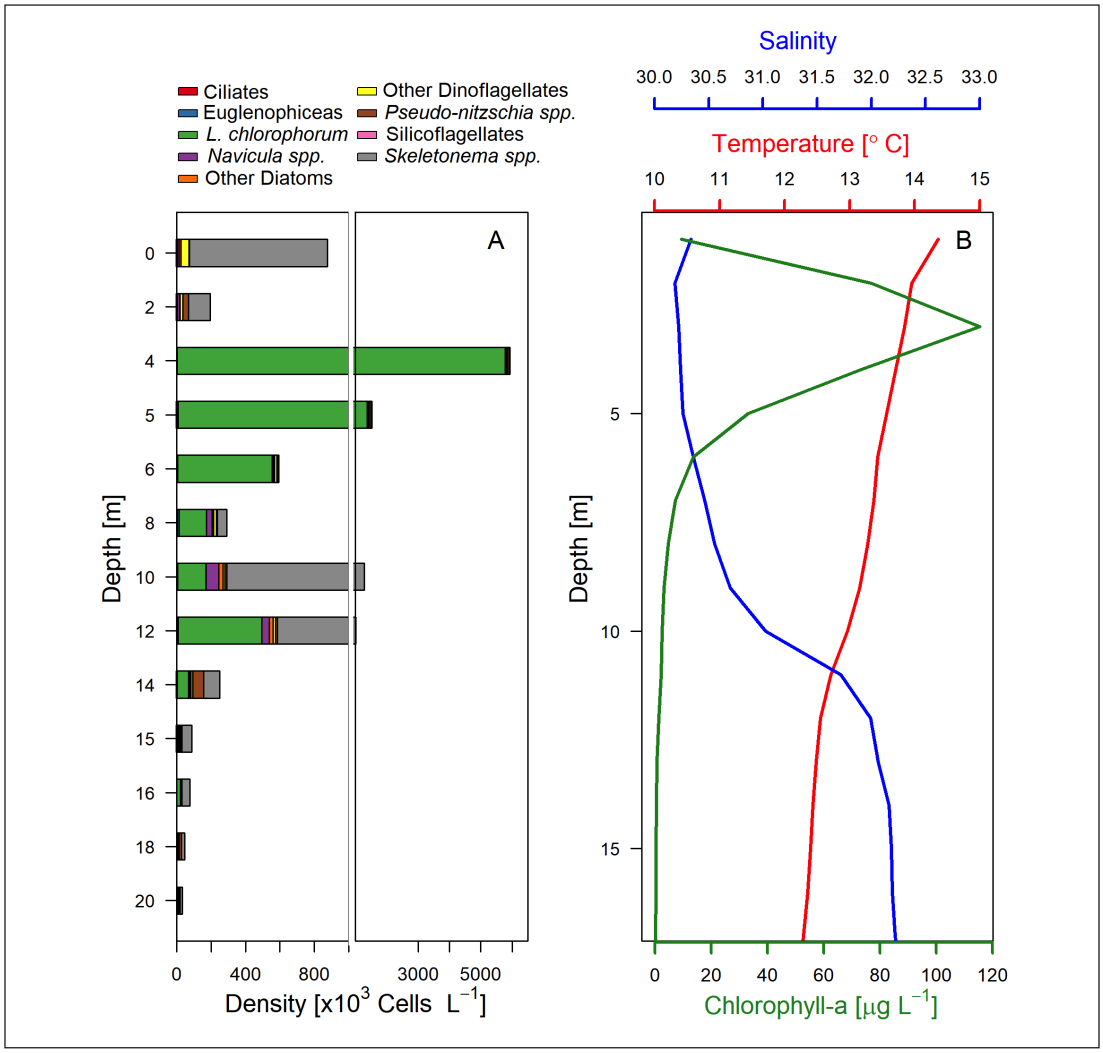

Fig. 2. Vertical distribution of A) phytoplankton cell counts, and B) temperature (red line), salinity (blue line) and chlorophyll-a (green line).

A similar event occurred in the same area during March and April 2003 (cell densities of 13x10^6 cells L^{-1} and Chl-a concentrations of ~1000 µg L^{-1}), causing a mass mortality of ~250,000 salmon [3]. Interestingly, these two events occurred with similar freshwater conditions and negative anomalies of river outflow. Similar events have been observed along the French Atlantic coast associated with an increase of nutrients, mainly ammonium [4,5] and resulting in a mass mortality of fish and shellfish [6,7].

We suggest the particular oceanographic conditions (especially high salinity), associated with reduced precipitation and weaker outflow from the Puelo River, favoured the proliferation of *L. chlorophorum* in Reloncaví Sound in 2020.

Although during 2020 there were no mortalities associated with this event, in March 2021, a bloom of P. chlorophorum in Reñihue Fjord, NW Patagonia (Los Lagos Region), caused a mass mortality of Pacific salmon (around 162 thousand fish) and losses close to 3.5 million US dollars.

This study highlights the need to pursue further studies for the understanding of non-toxic HB-HAB events, such as the *L. chlorophorum* events described above. These events represent a potential socio-economic, and environmental impacts, especially under current and future predictions of climate change including the decline of river discharges.

Acknowledgements

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Seaweeds of Rangitāhua hide toxic dinoflagellates



Fig. 1. Raoul Island (left), MY Dapple (right).

In November 2021 a team of scientists joined the Expedition Rangatahi to Rangitāhua/Kermadec Islands, a New Zealand territory approximately 1000 km north east of New Zealand. This was the first scientific voyage to Rangitāhua as part of the transformational MBIE Endeavour Programme, Te Mana o Rangitāhua, led by Ngāti Kuri and Auckland Museum. Using a holistic approach, the five-year funded programme combines indigenous knowledge and science considering both social and environmental elements. The project aims to understand the biodiversity and ecosystems of Rangitāhua from land to sea and identify indicators of ecosystem change to provide the tools for an iwi-led management of the Rangitāhua environment. The multi-disciplinary team spanned Auckland Museum, NIWA, University of Auckland, Massey University, and Manaaki Whenua. Research activities included selection of sites that will be regularly monitored during the programme, CTD profiles, ocean glider sampling and Triton Submersible testing. Scuba diving was a key activity with focus on community structure combined with environmental-DNA (eDNA) sampling and photogrammetry. The 18-day voyage to the Rangitāhua was on superyachts MY Dapple and The Beast and was made possible by generous philanthropic support (Fig. 1).

During the expedition 14 macroalgal samples were collected at Denham Bay Observation Site (GPS: -29.28472, -177.9536; Depth 8 - 16 m) for the isolation of epiphytic dinoflagellates. Macroalgae were shaken in tubes containing local seawater then removed and samples were transported back to the Cawthron Institute in Nelson, New Zealand, for isolation and culture of microalgae [1]. The macroalgal hosts were dried and kept as voucher specimens with subsamples preserved in silica gel for further molecular analysis. Dinoflagellates were isolated and the DNA sequence data prepared and analysed as described previously [2].

The dinoflagellate species isolated from samples collected at Denham Bay Observation Site are summarised in Table 1. *Gambierdiscus polynesiensis*, a

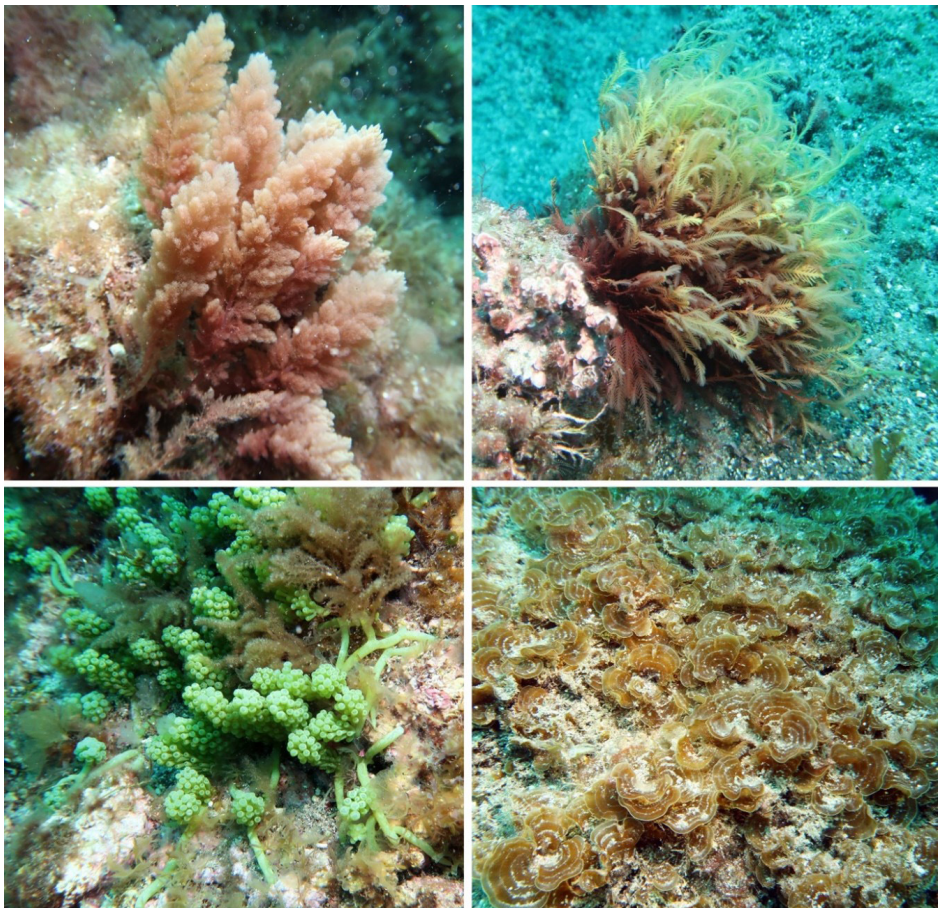

Fig. 2. Macroalgae hosting dinoflagellates: Asparagopsis taxiformis (top left), Delisea sp. (top right), Caulerpa racemosa (bottom left), and Distromium sp. (bottom right).

Table 1. Dinoflagellate species isolated from samples collected at Denham Bay Observation Site, Raoul Island, Rangitāhua Kermadec Islands, November 2021

Species	Macroalga host	CICCM code
Amphidinium carterae	*Dictyota* aff. *pfaffi*	ns
A. massartii	*Caulerpa racemosa*	CAWD377
Coolia canariensis	*Distromium* sp.	CAWD386, 387
C. palmyrensis	Cyanobacteria undetermined, *Distromium* sp., *Asparagopsis taxiformis*, *Caulerpa webbiana*	CAWD385
C. tropicalis	*Distromium* sp., *Caulerpa webbiana*	CAWD384, 388
Gambierdiscus australes	*Asparagopsis taxiformis*	CAWD381
G. polynesiensis	*Distromium* sp.	CAWD378, 380
Ostreopsis sp. 3*	*Plocamium* sp. *Delisea* sp.	CAWD383
Prorocentrum emarginatum	*Distromium* sp., *Actinotrichia* sp., *Dictyota* sp,, *Delisea* sp., *Plocamium* sp., *Galaxaura* sp.	CAWD392, 395
P. fukuyoi	*Dictyota* aff. *pfaffi*, *Distromium* spp. *Dictyota intermedia*	CAWD389, 390, 391, 393
P. hoffmannianum	*Dictyota* aff. *pfaffi*, *Caulerpa webbiana*	CAWD396
P. lima	*Dictyota intermedia*, *Dictyota* sp., *Ganonema farinosum*, *Delisea* sp., *Plocamium* sp., *Asparagopsis armata*	CAWD394

*CICCM: Cawthron Institute Culture Collection of Microalgae; *: Ref: [6]; not submitted to CICCM.*

known ciguatoxin producer, and *G. australes,* a maitotoxin producer, were isolated on previous expeditions [3] and were again isolated from samples collected on this voyage (Table 1). *Gambierdiscus honu*, which was first described from Rangitāhua [4], was absent. Associated dinoflagellates included species in the genera *Amphidinium*, *Coolia, Ostreopsis* and *Prorocentrum* (Table 1). Many of these isolates are now being maintained in the Cawthron Institute Culture Collection of Microalgae (CICCM).

The identification of *Gambierdiscus* species is of particular interest in relation to warming sea temperatures around Aotearoa New Zealand and the likelihood that these species will establish in New Zealand's sub-tropical north. This would bring with it the risk of ciguatera poisoning if established species produced ciguatoxins. In conjunction with isolating epiphytic dinoflagellates, metabarcoding of samples collected in Rangitāhua and Northland, New Zealand, will continue as a form of monitoring for such introductions [5].

The e-DNA samples collected from different habitats (macroalgae, turfing algae, barrens, and corals) have not been analysed yet. It is likely that further species of dinoflagellates will be identified indicating the need for further collections and study of toxic microalgae as well of poorly known macroalgae genera and undescribed species. In order to fully document the algal flora of Rangitāhua, the next voyage will be focusing on wider seaweed collections spanning different habitats, localities and depths and including the isolation of dinoflagellates from wider sampling.

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First isolation of toxin-producing benthic dinoflagellates from the Kingdom of Tonga, including the ciguatera causing genus *Gambierdiscus*

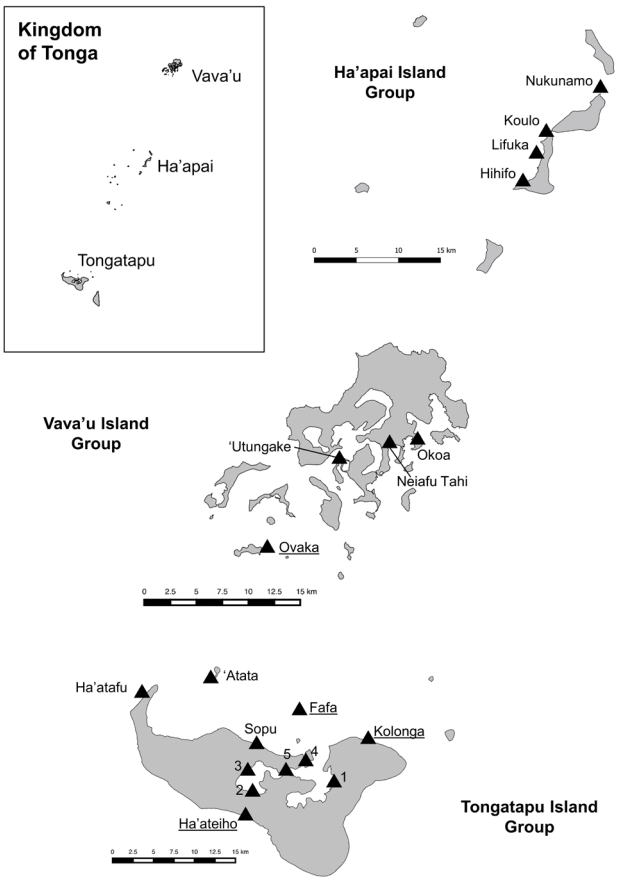

Fig. 1. Map of the Kingdom of Tonga showing study sites visited in June 2016 and 2017 across three island groups: Ha'apai, Vava'u, and Tongatapu. Sites where live isolates were obtained are underlined. Numbered sites on Tongatapu represent 5 sites sampled around the Fanga'utu lagoon.

Ciguatera poisoning (CP) is a serious and incurable human illness caused by ingestion of ciguatoxins (CTXs) in contaminated seafood [1]. CTXs are produced by *Gambierdiscus polynesiensis* (as definitively confirmed by liquid chromatography mass spectrometry; LC-MS) with other species displaying toxicity analogous to CTXs (reviewed in [2]). A number of targeted sampling efforts have documented the presence of at least twelve *Gambierdiscus* species across the northern and southern Pacific, including French Polynesia, Fiji, New Caledonia, the Cook Islands, Australia, Rangitāhua/ the Kermadec Islands, and New Zealand with a number of undescribed genotypes/ribotypes also requiring further investigation (reviewed in [2]). Other toxin-producing benthic and epiphytic dinoflagellate genera regularly found with *Gambierdiscus* Adachi & Fukuyo include *Ostreopsis* Schmidt, *Prorocentrum* Ehrenberg, and *Coolia* Meunier have also been documented across the Pacific [3, 4] and are of concern due to their human health, economic, and ecological effects. Harmful algal blooms have significant economic and health impacts in Pacific Island countries where fish consumption is part of the staple diet [5].

The Kingdom of Tonga is a southern Pacific nation where CP cases have been reported for at least 50 years [6]. There are very limited published records on the prevalence of CP despite local knowledge and awareness of the illness. It is likely that case numbers are higher than those reported due to difficulties in diagnosing CP and a lack of official reporting channels. Four previous reports have noted poisoning events associated with fish species from locations in Tongan waters [5, 6], with one reporting a total of 146 cases from Tonga between 2001 and 2005 [5]. Recently, four cases of CP were reported in New Zealand from people eating eel that had been brought back from Tonga; all four patients required hospitalisation [7].



Fig. 2. Artificial substrate samplers deployed in seagrass habitats in Tongatapu.



Fig. 3. (left) Phoebe Argyle working in in Fanga'utu lagoon, Tongatapu and (right) Neiafu Tahi, Vava'u.

Earlier reports of CP in Tonga indicate the presence of at least one toxin producing *Gambierdiscus* species, but there is limited information on the geographical distribution. During two comprehensive surveys in 2016 and 2017 (Argyle *et al*., in prep), samples of benthic dinoflagellates were obtained for the purpose of making live cell isolations to establish cultures for identification, toxin analysis and future research.

Sampling occurred during June 2016 and June 2017 sites around Tongatapu, Ha'apai, and Vava'u island groups, Kingdom of Tonga (Fig. 1). Five sites around Tongatapu were sampled in 2016 and in 2017 nineteen sites were sampled across all three island groups. Untreated samples for isolations were taken at low tide directly from seagrass and macroalgae (2016) or from artificial substrates (2017) within the shallow subtidal habitats (Figs. 2-4). Established isolates were identified to species level by using amplification of the D1-D3 region of the large subunit ribosomal RNA gene (LSU) as previously described [8].

In 2016, three *Gambierdiscus* and one *Ostreopsis* isolate were successfully established in culture. Two *Gambierdiscus* isolates were collected from *Sargassum* at Kolonga and were identified as *G. australes* and *G. honu* respectively (Table 1, Fig. 5a-b). Another *Gambierdiscus* isolate, *G. pacificus*, was collected from *Padina* from Fafa Island (Table 1; Fig. 5c). One *Ostreopsis* sp. 3 isolate (Fig. 5d) was collected from *Sargassum* at Ha'ateiho. Seagrass habitats were sampled at Sopu, Fafa, and Kolonga, but



Fig. 4. Study sites from top to bottom: Hihifo (Ha'apai), Fafa Island, and Ha'ateiho blowholes (Tongatapu).

no *Gambierdiscus* cells were successfully cultured from these samples even though they were visually present in the samples.

In 2017, one *Prorocentrum lima* and one *Coolia tropicalis* (Fig. 5e-f) isolate were successfully established into culture (Table 1). The *Prorocentrum lima* isolate was collected from mixed turf algae on Ovaka Island, Vava'u, and the *Coolia tropicalis* from seagrass (*Halodule uninervis*) at Kolonga. Phylogenetic analyses strongly supported the species identification of the dinoflagellates. Representatives of all isolates were deposited in the Cawthron Institute Culture Collection of Micro-algae (CICCM; Table 1).

Gambierdiscus isolates were analysed for selected algal CTXs, maitotoxin -1 (MTX-1) and 44-methylgambierone (44-MG) (using the method described in Murray, Boundy [9]; Table 1). *Gambierdiscus australes* (CAWD253) produced MTX-1 (8 pg cell^{-1}) and 44-MG. This is comparable to a previously described *G. australes* isolate from the Cook Islands (CAWD149; 8.3 pg MTX-1 per cell [10]. The *G. pacificus* (CAWD252) and *G. honu* (CAWD250) isolates also produced 44-MG, which is produced almost ubiquitously by *Gambierdiscus* species but has low toxicity compared to other dinoflagellate toxins [11]. None of the *Gambierdiscus* isolates produced any of the algal CTXs that were monitored (CTX-3B, CTX3C, CTX-4A and CTX-4B).

Ostreopsis sp. 3 (CAW249) did not produce any PLTX-like compounds, as determined using the on-column oxidative cleavage method (Selwood, van Ginkel [12]; LoD < 0.01 pg cell^{-1}). *Protocentrum lima* clade 3 (CAWD271) produced both diarrhetic shellfish toxins (DTX-1; 0.3 pg cell^{-1}) and okadaic acid (OA; 11.3 pg cell^{-1}) when tested according to McNabb, Selwood [13]. The *Coolia* isolate was not tested for toxin production in this study.

The species of *Gambierdiscus* found in this study have all been previously reported in other studies from the South Pacific (reviewed by [2]). *Gambierdiscus honu* was first described in 2017, and has been isolated from the Cook Islands, Rangitāhua/the Kermadec Islands, and Heron Island, Australia, in the Southern Great Barrier Reef (also reviewed by [14]). *Gambierdiscus australes* has been found in the Cook Islands, French Polynesia, the Kermadec Islands, and Hawai'i [2]. *Gambierdiscus pacificus* has also been found in the Cook Islands and French Polynesia, in addition to Kiribati and the Marshall Islands [2]. Obtaining new representative strains of *Gambierdiscus* species is important as there are wide species and strain-specific differences and plasticity in growth and toxin production across the dinoflagellates [2].

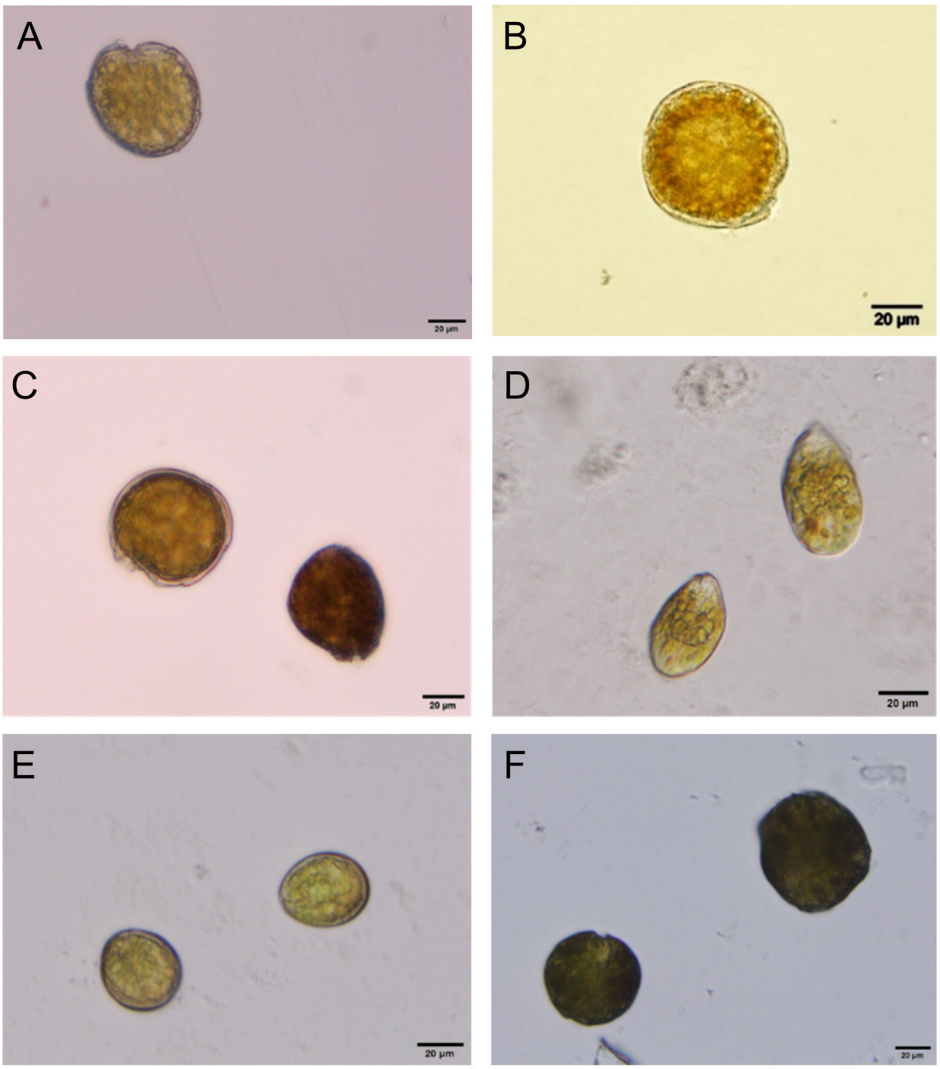

Fig. 5A-F. Light microscope images of representative cells of cultures made from isolates from around the Kingdom of Tonga in June 2016 and June 2017. **A** *G. australes* isolated from Kolonga (CAWD253), **B** *G. pacificus* isolated from Fafa Island (CAWD252), **C** *G. honu* isolated from Kolonga (CAWD250), **D** *Ostreopsis* sp. 3 isolated from Ha'ateiho (CAWD249), **E** *Prorocentrum* cf. *lima* isolated from Ovaka (CAWD271), and **F** *Coolia tropicalis* from Kolonga (CAWD277).

Co-occuring with *Gambierdiscus*, other harmful taxa including *Ostreopsis, Prorocentrum,* and *Coolia* may pose further risks to human and ecological health. Blooms of *Ostreopsis* are associated with human illness through aerosol inhalation or skin irritation, particularly in the Mediterranean [15], and through consumption of seafood that has accumulated palytoxins and/or ovatoxins [15]. *Prorocentrum lima* (as found in Tonga) is a toxin-producing species that can cause diarrhetic shellfish poisoning; the route of poisoning is through bioaccumulation, in the same manner as for CTXs leading to CP [16]. *Coolia tropicalis*, the species identified in Tonga, has demonstrated toxic effects, and a Brazilian strain has been shown to produce 44-MG [17]. Whether this species poses a risk to human health requires further investigation.

Harmful dinoflagellates are increasing their range, and it is possible that illnesses such as CP may become more prevalent [2]. Identifying toxic species, their distribution, and their toxin production and toxicity is the first step towards designing monitoring programs that may assist in mitigating harmful impacts. A wider survey of benthic dinoflagellates in Tonga shows that there is high species diversity and that *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* are widely distributed across the

Table 1. Dinoflagellate isolates successfully established into culture from the Kingdom of Tonga in 2016 and 2017. Details include collection site, macroalgae species the cells were isolated from, species identification of the isolates, toxin production, CICCM identification code, and species identification confirmation details based on the D1-D3 region of the large subunit ribosomal RNA. 44-methylgambierone (44-MG) represents presence/absence as this compound could not be quantified at the time of the study.

Species ID	CICCM Code	Site	Substrate or habitat collected from	Toxin production
Gambierdiscus australes	CAWD253	Kolonga	*Sargassum hemiphylloides*	8 pg/cell MTX 44-MG
Gambierdiscus pacificus	CAWD252	Fafa	*Padina* cf. *sanctae crucis*	44-MG
Gambierdiscus honu	CAWD250	Kolonga	*Sargassum hemiphylloides*	44-MG
Ostreopsis sp. 3	CAWD249	Ha'ateiho	*Sargassum polycystum*	not detected
Prorocentrum lima clade 3	CAWD271	Ovaka	Artificial substrate deployed in mixed turf algae (unidentified)	0.3 pg/cell Dinophysistoxin-1 Okadaic acid 11.3 pg/cell
Coolia tropicalis	CAWD277	Kolonga	Artificial substrate deployed in seagrass bed (*Halodule uninervis* dominant)	not tested

islands (Argyle *et al.,* in prep). Methods for sampling and identifying benthic dinoflagellates for monitoring purposes are still being validated [18], but it is envisaged that monitoring programs can eventually be established in Tonga to detect blooms and warn of the risk to humans of harmful taxa.

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Aotearoa New Zealand – Japan joint research project on mapping the predicted distribution of ciguatera poisoning-related benthic dinoflagellates after forecasted global warming

Aotearoa New Zealand and Japan are maritime nations with strong cultural, recreational, and economic connections to seafood. There is an increasing demand for stable production and supply of safe and secure seafood in both countries.

Benthic dinoflagellates are ubiquitous in marine ecosystems and several species can cause benthic harmful algal blooms (BHABs). Some of these BHAB species produce toxins which can contaminate seafood, impacting seafood production and supply. In particular, toxins produced by the genus *Gambierdiscus* cause ciguatera poisoning (CP), the most common non-bacterial seafood poisoning worldwide. Although CP is historically reported in the tropical/subtropical zones, climate change effects, particularly the warming of sea surface temperatures, could result in the range expansion of CP into new coastal waters including temperate regions. Based on forecasted global warming of both air and sea surface temperatures under the Representative Concentration Pathway (RCP) 8.5 scenario proposed by the Intergovernmental Panel on Climate Change (IPCC) [1], coastal waters around Aotearoa New Zealand and Japan could warm by up to 2 °C between 2050 and 2099, above current average temperatures [Briscoe et al. unpublished]. This level of ocean warming could alter the habitable range for CP-related genera, *Gambierdiscus* and *Fukuyoa*, in these regions. It is therefore essential to reveal the current distribution of these toxic genera/species and predict the likely 'future' distributions for CP risk assessments.

To contribute to the current and 'future' CP risk assessments in Aotearoa New Zealand and Japan, researchers from the Cawthron Institute and Kochi University initiated a joint research project in 2021, jointly funded for two



Fig. 1. A group photo taken during the project's meeting at Cawthron Institute, Nelson, New Zealand, in August 2022. Lesley Rhodes, John Pearman, Lucy Thompson, Sarah Challenger, Sarah Finch, Haruo Yamaguchi (top, from left to right), Dana Briscoe, Sam Murray, Tim Harwood, Goshi Araki, Hiroshi Funaki (middle, from left to right), Natsumi Nishikawa, Jacqui Stuart, Tomohiro Nishimura, Kirsty Smith, Cath McLeod, Masao Adachi, and Takumi Iijima (bottom, from left to right). Photo credit: Cawthron Institute, AgResearch, or Tomohiro Nishimura.

years by the Royal Society of New Zealand (RSNZ) and the Japan Society for the Promotion of Science (JSPS). The project title is 'Mapping the predicted distribution of toxic benthic microalgae after forecasted global warming in Japan and New Zealand'. The project aims to develop a pipeline for assessing the future risk of CP in both countries and has several research aims, including:

1. Create comprehensive distribution maps of toxic BHAB species utilising a review of previous research and new data from multiple sources including molecular phylogenetic, morphological, metabarcoding, chemical, and toxicological;
2. Determine the growth characteristics of key *Gambierdiscus* and *Fukuyoa* species;
3. Collate environmental data sets based on available modelled products by the Mercator Ocean's GLORYS reanalysis version 12v1 [2];
4. Create current spatial distribution maps of the target species through modelling;
5. Collate environmental data sets based on available modelled products by the IPCC and create 'future' spatial distribution maps of the target species by modelling based on the growth characteristics.

This research project will develop a new methodology pipeline for modelling the distribution of harmful algal species, that can then be applied to other taxa in Aotearoa New Zealand, Japan, and across the Pacific.

The teams had a virtual meeting/workshop in August 2021 due to the difficulty of travel during the COVID-19 pandemic, and finally met in person in August 2022 for a two-day hybrid meeting held at the Cawthron Institute in Nelson, New Zealand (Fig. 1). This meeting brought together over 20 attendees for oral presentations, workshops, round-table discussions, and facility tours of the three research centres of the Cawthron Institute.

The teams have published several papers from this project: metabarcoding of *Gambierdiscus* [3], growth characteristics of *Gambierdiscus* [4], a literature review of BHAB species in Japan [5], and the structural characterisation of maitotoxins [6]. The project will continue with further modelling and more publications.

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Human Dimensions of the Citizen Science Oceanography Program Operated by the Pacific Salmon Foundation, Canada

Citizen Science involves members of the general public taking part in certain scientific work (e.g. data or sample collection), often in collaboration with or under the direction of professional scientists. There is great value in this approach as it allows for the collection of data in a very cost-effective way as well as increasing public understanding of specific issues to help foster stewardship within communities. Citizen Science is becoming increasingly utilized in the scientific community and sharing lessons learned from operating long-term programs can be very informative to other community based initiatives.

The Citizen Science Oceanography Program (*https://sogdatacentre.ca/atlas*) run by the Pacific Salmon Foundation (PSF) has been operating since 2015 and currently (2022) is in its eighth sampling season. This program includes several dozen trained citizens from different communities throughout the Strait of Georgia, Canada. Sampling (physical collection of ocean water samples, nutrients and harmful algae) is done at approximately 55 stations on a regular schedule (about 20 times a year). Detailed analysis of the harmful algae and oceanographic conditions in the Strait based on the program's dataset can be found in [1]. The datasets collected through this program are some of the most comprehensive oceanographic data obtained from this area, and are publicly available via the Strait of Georgia Data Centre (*https://sogdatacentre.ca/*). Several years ago, this program was endorsed by GlobalHAB, an international program by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO.

After almost eight years of operating this program significant lessons have been learned, specifically related to operating oceanographic equipment, troubleshooting, communicating, and navigating unfavourable weather conditions. Overall, PSF has received tremendous positive feedback on both the methodology and longevity of the Program. We believe that the long-term success of this program is primarily due to our Citizen Science volunteers and their long-standing dedication and commitment.

All of the citizen science crew members are from local communities. They were initially selected because they have experience working on the water in some capacity (e.g. retired commercial fisherman, biologists, etc.) and own their own vessels (fuel expenses and a small honorarium are covered by PSF). Most of crews joined the program from the very beginning in 2015. An initial training on how to properly collect data and samples was carried out during a two-day workshop. Each team performed their first at sea sampling with a PSF biologist on-board for quality control. Each crew is accompanied by the PSF Program Coordinator about three times a year. Quarterly newsletters and regular updates via group e-mails and social media (*https://www.facebook.com/CitizenSciencePhytoplankton/*) are used to communicate updates or recent findings. An annual symposium is held to share citizen scientists' experiences and provide updates from the technicians and scientists who analyze measurements and samples.

For the 2022 annual symposium, citizen scientists were asked to take a survey to share and evaluate their experience with the Program. In the multiple choice questionnaire, all participants strongly agreed that they: have received adequate training, felt satisfied after sampling is done, and felt valued as a member of our team. Most of the participants (approximately 80%) felt that their work is important to the community and they were very likely to stay with the program for the next two years. It is clear that satisfaction with the program and feeling that their contributions make a difference, played a big role in high volunteer retention.

Citizen Scientists were also asked several open-ended, anonymous questions. The first one was – what was your motivation to join the program? Answers included:

- "I wanted to volunteer for a cause I cared about",
- "I am a marine biologist and wanted to put my skills to a useful scientific and community purpose. It was also a chance to go out in the marine environment again, something I had been missing",
- "Interest in doing something to help marine environment… Love of the ocean".

Most of the participants indicated that they were involved in other monitoring or work on the ocean for example: forage fish egg monitoring, eelgrass planting, and coastal waters monitoring surveys. Participants were also generally interested in ocean and marine life, particularly in fishes and marine algae. Survey participants were also asked if they learned any new skills or gained new knowledge. Respondents unanimously agreed with answers like, "Yes, absolutely", "Definitely – especially the technology side of our sampling", "Lots of new skills and knowledge, which I am grateful for", and "Working on the project has taught me more that I would have known". As for their favorite parts of being a part of the team, answers included:

- "contributing to important data collection",
- "knowledge that something is being done to better understand the marine ecosystem", and
- "contributing to something meaningful".

Finally, one piece of advice that the volunteers wanted to share is "Be open to new learning and adventures. The data we collect will make a difference".

There are a number of community-based harmful algae monitoring programs around the world. They fill in informational needs, enhance data baselines, improve bloom forecasting capabilities, increase awareness, and create new working relationships. Collected databases contribute to the current understanding of algae ecology and their impacts on marine life. From our experience, the success of the citi-











Cooperation scenes from the Citizen Science Oceanography Program, Strait of Georgia, Canada

zen science programs is the ongoing involvement of highly motivated volunteers, interested scientists who provide oversight and guidance, and continuing funding. With an apparent increase in intensity and duration of harmful algal blooms (HABs) and emerging threats, monitoring is becoming more important than ever. Establishing and maintaining long term programs will definitely benefit both scientific research and local communities.

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12th International Conference on Modern and Fossil Dinoflagellates

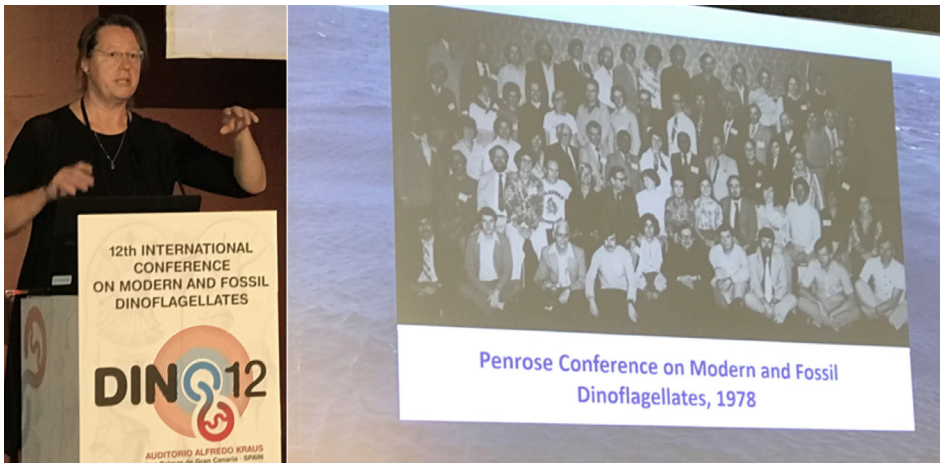

Fig. 1. Karin Zonneveld paying homage to the I Dino conference in 1978

Forty-four years after the Penrose Conference on Modern and Fossil Dinoflagellates (Fig. 1), the long awaited DINO12 conference was held 4-8 July 2022 at the Auditorio *Alfredo Krause* in Las Palmas de Gran Canaria (Fig. 2). The local organizing committee was chaired by **Emilio Soler Onís** with the support of the Canarian Observatory of Harmful Algae (COHAB) of The Canarian Science and Technology Park Foundation, University of Las Palmas de Gran Canaria .

DINO12 welcomed participants gathered (or joined remotely) for discussions on recent advances in the field of Modern and Fossil Dinoflagellates. The single session DINO12 format framed the topic of global warming while preserving the essence of the meeting in which the dinoflagellates and their cysts are the major protagonists. It provided a broad forum for basic and applied research including Climate Change, Ecology, Toxic Dinoflagellates and Biotoxins, Palaeoecology and Palaeoceanography, Mesozoic and Cenozoic Dynocyst Stratigraphies, Phanerozoic and deep time scales, Dinocyst Systematics, Life Cycles and Nutritional Strategies, Biodiversity and Systematics, Stratigraphy, Evolution, and Taxonomy.

DINO12's final presentation was an outstanding one presented by the Dale duo on *Ecological Signals in Dinoflagellate Biostratigraphy*. It brought together the richness of the fossil dinoflagellate records with concerns of modern environmental sciences using examples of studies in eutrophication, pollution and climate change. It was an elegant reminder of why the fossil and modern dinoflagellate researchers continue to meet in one forum.

Two workshops were also included in DINO12 activities. One was on Benthic HAB Sampling Methods (see separate article this issue) and Monitoring, Control and Management of Marine Microalgae. The DINO12 abstracts are available *here*.

Karin Zonneveld Center for Marine Environmental Sciences (MARUM) Geosciences Department, University of Bremen graciously agreed to host DINO13 in Germany. The conference venue and dates will be announced when plans are finalized. Contact Karin at *kzonneveld@marum.de* for further information.

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Fig. 2. Participants of the DINO 12 conference (Las Palmas de Gran Canaria, 4-8 July 2022) at the Alfredo Krause Auditorium

DINO 12 Workshop on Sampling Methods for Benthic Dinoflagellates

Unlike planktonic species, there is no standardized, or even agreed upon, quantitative method for sampling benthic dinoflagellates. During the workshop, methods traditionally used for collecting benthic dinoflagellates (BHABs) were reviewed and new techniques using artificial substrates were demonstrated for participants whose interests ranged from implementing cell-based monitoring programs to curiosity about quantification of BHABs. Unlike pelagic phytoplankton where monitoring can be achieved by collecting samples from a uniform medium (either by integrating the water column or at discrete depths), benthic dinoflagellates are found in a variety of complex habitats adhered to a variety of substrate types including algal turf, macroalgae, rocks, coral rubble, sand or seagrasses and artificial structures such as concrete seawalls and pilings. As with planktonic sampling, BHAB cells are patchy and can vary by an order of magnitude between sampling sites only a few cm apart. So, many factors confound the methods to quantitatively sample BHABs for monitoring purposes.

Key decision points for standardizing protocols for monitoring *Gambierdiscus* are how, when and where to sample. Each of these topics were reviewed with the participant's individual sampling requirements used as examples during group discussions. If you are interested in using artificial substrates for sampling BHABs, please contact Juan Fernandez Zabala (*jfernandez@fpct.ulpgc.es*) for more information and a prototype sampling device. He has an ongoing project to validate the use of artificial substrates in as many different areas as possible.

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Dino 12 Workshop, Las Palmas de Gran Canaria, Spain. Top: participants at the workshop; bottom, benthic dinoflagellates sampling

XIV Iberian Meeting on Harmful Algae and Biotoxins (REDIBAL)

The XIV Iberian Meeting on Harmful Algae and Biotoxins was held in Lisbon, Portugal, from 1st – 3rd June at the *Magalhães* Auditorium of the Portuguese Institute of Sea and Atmosphere (IPMA). The event was organized in a joint cooperation between IPMA and the Marine and Environmental Sciences Centre (MARE). It was a long awaited in-person meeting after two years of COVID-19 shutdown that brought together over 100 participants, promoting scientific debate and collaboration among Iberian scientists.

A total of 44 communications were presented (29 oral and 15 posters) distributed amongst 7 thematic sessions: *i*) microalgae and toxins time series, *ii*) toxins and bioactive compounds from marine microalgae, *iii*) toxins detection methods, *iv*) toxins in seafood, *v*) effects of toxins on marine organisms, *vi*) microalgae bloom dynamics and vii) ciguatera. A plenary talk by Don Anderson covered comparative studies of large-scale *Alexandrium catenella* blooms in temperate and Artic waters giving evidence for range expansion of this species under current warming climate trends. The abstract book is available for download in the conference website (*https://redibal.ipma.pt/livro-de-resumos-2022/*).



Redibal 2022 awards.Top panel. Lifetime Achievement Award to Teresa Moita and Santiago Fraga; middle panels, Young Researcher Awards: left, Elisabet Cruz, Best Oral Communication on Harmful Microalgae; right, André Patricio, Best Oral Communication on Marine Biotoxins; bottom panel: Celia Costas, Best Poster.

During the XIV Iberian Meeting on Harmful Microalgae and Marine Biotoxins there was also the opportunity to pay a tribute to M. Teresa Moita and Santiago Fraga, recently retired, in recognition for their inspiring and outstanding contributions to HAB research both in the Iberian context and worldwide. This series of meetings has also traditionally been an ideal forum for students and young researchers to present their work (see *http://redibal.org/* for details of previous Iberian Meetings). Following this tradition, a competition for young researchers best oral and poster presentations was held. Elisabet Cruz (University of Aveiro, Portugal) was awarded the best oral communication on "Harmful Microalgae" for her talk on "Transport pathways of *Dinophysis acuta*: from Portugal to Galician Rias Baixas"; André Patrício (University of Lisbon, Portugal) was awarded the best oral communication on "Marine Biotoxins" for his presentation on "Time-lagged correlation analysis of DSP toxicity across Portuguese shellfish producing areas, species and meteorological conditions"; Celia Costas (University of Santiago de Compostela, Spain) was awarded best poster for her work on "Early okadaic acid-induced ultrastructural alterations in the gut: role of serotonin". The awards were presented by M. Teresa Moita and Santiago Fraga.

Continued on next page



Group photo of participants of the XIV Iberian Meeting on Harmful Microalgae and Marine Biotoxins, Lisbon, 1-3 June 2022

ICES-IOC Working Group on Harmful Agal Bloom Dynamics Meeting

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) is an important forum for ICES (International Council for the Exploration of the Seas) and the IOC of UNESCO to review and discuss HAB events and to provide annual advice and updates on the state of HABs in the region. It also facilitates interaction between scientists working in diverse areas of HAB science and monitoring and provides a forum for the interchange of various approaches to HAB research. The group reports to both the ICES Science Committee (SCICOM) and IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB).

The WGHABD annual meeting was held 14-16 June at the Centre for the Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, UK. This hybrid meeting facilitated members and invited guests to attend in person, with the capacity for online remote access to discuss a variety of related topics including progress on the WG's current Terms of Reference, new activities and findings, and national reports of HAB events during 2021 in the ICES Atlantic Area region. The meeting was opened and participants welcomed by Prof Stuart Rogers, Chief Scientist, CEFAS. In addition to the review and summary of WG activities from the current three-year cycle of the nine Terms of Reference, there were a number of presentations, including: i) Distribution of *Dinophycyeae* with a focus on HAB species in the Baltic Sea and the Kattegat-Skagerrak based on meta-barcoding and microscopy (Bengt Karlson, Sweden) and ii) Update on satellite ocean colour discrimination of Harmful Algal Blooms (Peter Miller, UK)

Annual information on HAB events reported by the WG members is publicly available in the Harmful Algal Event database (HAEDAT *http://haedat.iode.org/*) and also through Harmful Algal Information System (HAIS *https://data.hais.ioc-unesco.org/*).

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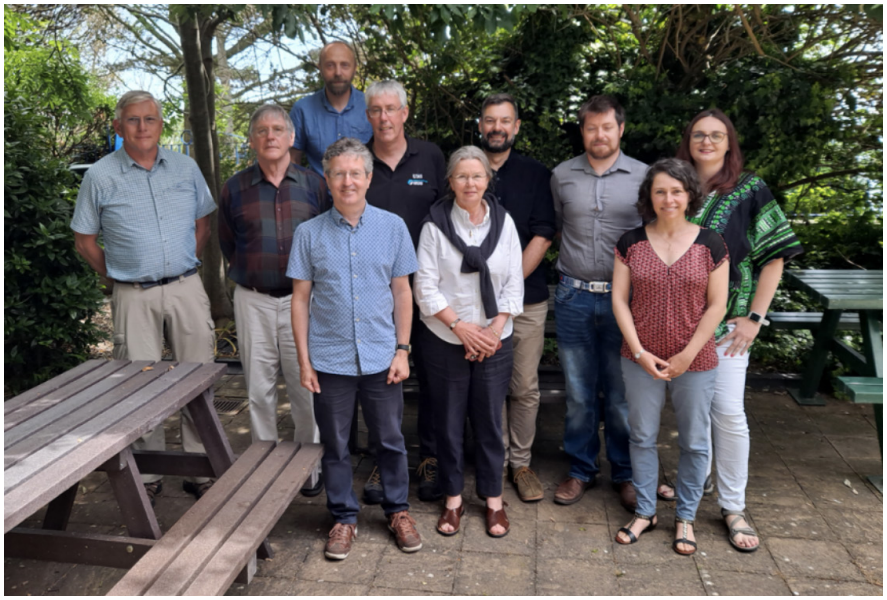

Participants in person at ICES-IOC WGHABD 2022 meeting

Continued from previous page

Finally, because art can bridge the gap between science and society, the Portuguese visual artist Diana Policarpo made a brief overview of her work on Ciguatera, which had already been presented in the Ocean Space TBA21–Academy Exhibition in Venice (*https://www.tba21.org/#item--dianapolicarpo-2317*).

The next REDIBAL Meeting will be held in the Canary Islands. See you in the Canarias in 2024.

Acknowledgements

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CARMINA

The **CARMINA Project** (CARibbean MIcro-algae respoNsible for ciguAtera poisoning: Diversity, Toxicity and Toxin Production) with 20 participants from 11 island nations and mainland Caribbean countries, France and the USA held its virtual kick-off meeting (Fig 1).

The countries both in and surrounding the Caribbean Sea, suffer significant impacts from ciguatera poisoning (CP). Historically, CP incidences have been higher in the northern and eastern islands of the Caribbean. There, the CP rates there are second only to those in the south Pacific Ocean. Seven species of the benthic dinoflagellate genus *Gambierdiscus* potentially responsible for CP are found in the Caribbean yet the microalgal species causing CP remain(s) unknown. The CARMINA project, co-funded by the *French Development Agency* (AFD) and the *French Research Institute for the Exploitation of the Sea* (Ifremer) aims at identifying the microalgae responsible for CP in the Caribbean region and will develop protocols for monitoring toxic species. Scientific collaborators also include the *French Food Safety Agency* (Anses) and the *US National Oceanic and Atmospheric Administration* (NOAA). Monitoring will rely on environmental screening using qPCR assays and passive sampling for dissolved toxins and biomarkers. Cell isolation and culturing will be core activities, as well as toxicity assessments and characterization of toxin profiles of *Gambierdiscus, Fukuyoa* and *Coolia* species occurring in the Caribbean Islands, Central and South America.

The partner countries of the CARMINA project were selected according to their geographic locations and involvement in existing networks of the *Intergovernmental Oceanographic Commission* (IOC) of UNESCO and the *International Atomic Energy Agency* (IAEA), two of the agencies already involved in the monitoring of the marine environment and the development of its resources.

The purpose of the kick-off meeting was multifaceted and served to bring together researchers and resource managers who have worked together in the regional networks *Algas Nocivas in Caraibe* (ANCA), the *IOC Regional Working Group on Harmful Algal Blooms in South America* (FANSA) and shared IAEA workshop sessions to increase their sampling and detection capabilities for harmful algae and their toxins.

Participants were surveyed to provide a current list of capabilities and laboratory capacities for sampling, taxonomy, cell isolation and culture (including high biomass cultures) and toxin detection methods. Lecturers provided short presentations on these topics bringing everyone up to date on new research and techniques. Subject-specific workshops will be developed to enhance current skills. In addition to the skills and capacities inventory, activity schedules with specific tasks and timelines were agreed upon. Quarterly Zoom meetings will be held to assess progress.

A Twitter account has been created: *@carmina_project* and an Instagram account is under consideration.

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Fig. 1 Participants at the virtual CARMINA kick-off meeting

Invitation to ICHA 2023 Hiroshima

Dear ISSHA members and colleagues,

Please save the date! The 20th International Conference on Harmful Algae (ICHA) will be held at the lovely Grand Prince Hotel Hiroshima, Japan from the 5th to the 10th of November 2023. The organizing committee of conference is looking forward to welcoming many in-person participants, but will have some options for virtual streaming of selected presentations. Stay tuned for more information that will be posted on the ICHA2023 Conference website (*https://icha2023.org/*) soon!

The hotel is located approximately 20 min from the downtown Hiroshima train station and about 1 hr from the Hiroshima airport. Several excursions will be available for Wednesday afternoon, including Miyajima – a world historical heritage site, a trip to historical sake breweries as well as Onomichi and Shimanami cycling and Taishaku-kyo trekking to view fall leaf colors. This is the most beautiful time of the year to visit Japan.

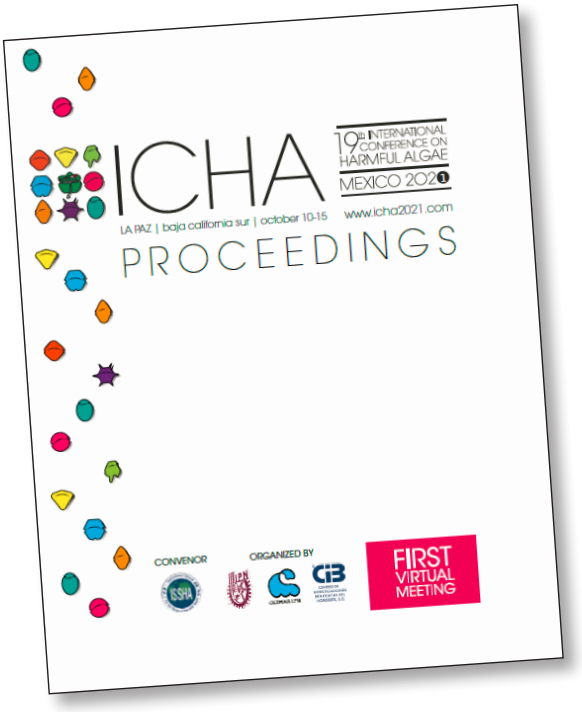
As the major host of the conference, ISSHA will support the following activities: student travel awards, achievement awards, and the popular ISSHA auction!

We are looking forward to welcoming you participants in Hiroshima. Please come and enjoy ICHA 2023.

Ichiro Imai
Chair of ICHA 2023
and members of local organizing committee


Venue at the Grand Prince Hotel, Hiroshima


Miyajima world heritage site



NEW ! The Proceedings of the 19th International Conference on Harmful Algae 2021 Mexico have been published. Each of the 47 contributions have been assigned a DOI identifier which can be found on the bottom of the first page of each manuscript. Eight case studies of Early Warning Systems for Harmful Algae are also included, based on a session, co-sponsored by FAO, IAEA and IOC, which was held at the virtual conference. Proceedings can be downloaded at this site:
https://issha.org/publications-resources/conference-proceedings/

The documents should be referenced as follows:
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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

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An IOC Newsletter on Toxic Algae and Algal Blooms

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Florida's West Coast *Karenia brevis* bloom – Spring 2023

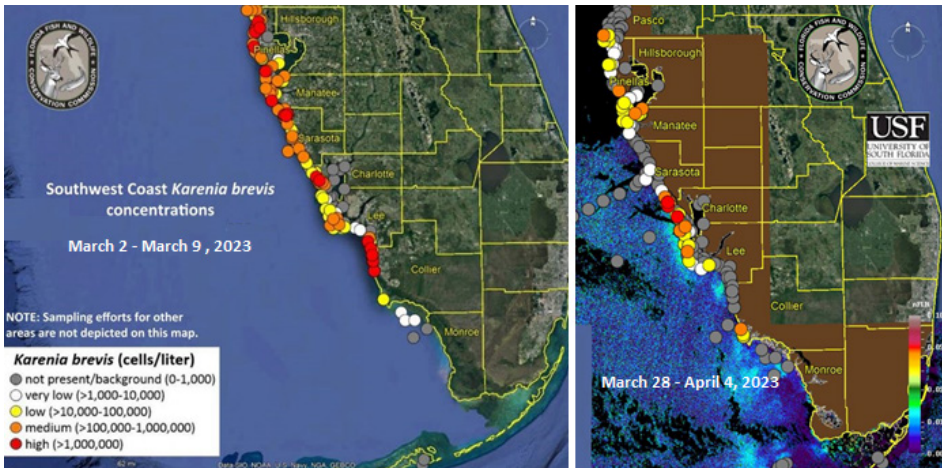

Fig. 1. Mapped Karenia brevis cell concentrations 26 days apart in March 2023. Note the offshore stations on 28 March to 4 April 2023 (gray circles right panel) have no K. brevis cells in surface samples. https://floridadep.gov/algalbloom

On the morning of 6 March 2023, the National Centers for Coastal Ocean Science (NOAA) issued an alert warning of "moderate to high risk of respiratory irritation" due to a *Karenia brevis* bloom along the southwest coast of Florida. This was no surprise to the many beachgoers who walk Florida's shorelines every day and frequently see dead fish washed up on the beaches during harmful algal blooms (HABs). The city or municipal government of the small towns along the west Florida coast routinely budget for beach clean ups during the nearly annual harmful algal blooms. But dead fish are especially problematic during peak holiday seasons or, in this case, spring break week(s) when schools recess and many visitors flock to Florida's wide, warm beaches. The "halo" effect of HABs can cause significant losses in revenue as the news, often exaggerated, is broadcast.

In an interview with the Washington Post (reported by A. Ajasa) Dr. Rick Stumpf (NOAA) noted that *K. brevis* cells often accumulate inshore in late summer and are pushed offshore to the mid shelf area of Florida's west coast) by winter winds associated with high-pressure systems [1,2] the persistent northerly winds do not occur *K. brevis* cells remain inshore. Stumpf speculates this is what happened in March 2023. It should be noted that while *K. brevis* blooms are known to occur at all seasons of the year, there are times when they are more frequent. High inshore cell concentrations are affected by wind patterns of frontal systems, hydrographic conditions like upwelling and in turn, nutrients upwelled to the surface waters [3]. Stumpf added "If you have a year where we don't get those persistent northerly winds pushing it out it [HABs] can hang around. And that's what's happening this year."

Florida's coast lines are some of the most closely observed marine areas. A number of State, Federal and non-governmental organizations and university programs contribute their resources to help forewarn citizens, public health officials, resource managers, property owners and tourists of conditions that are conducive to HABs (see below). These agencies also have willing volunteers who serve as citizen scientists and help crowd source data along vast stretches of the 1,062 km west Florida coastline. The most recent reports of the spring 2023 *K. brevis* blooms show a decline in the cell concentrations nearshore and no reports of surface

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Intergovernmental Oceanographic Commission

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concentrations offshore (Fig. 1). Perhaps 2023 summer vacation plans can go to plan after all.

As I reviewed the various sources of information on *K. brevis* cell concentrations, respiratory safety, bloom status, environmental information relevant to transport and landfall that are now publicly available to researchers, resource managers and the public alike, I am amazed at the technology. My thoughts go back to early November 1st, a Sunday morning in 1987. There were thousands of large, leaf-like dinoflagellates tumbling around in a Petri dish on the microscope stage. We did not know much about *K. brevis* in North Carolina before 1987. There had never been a red tide along our coast. But radio reports of coughing and sneezing by dogs and their owners at the beach caused me to learn, and quickly, about what was happening. I had never seen these dinoflagellate cells before and tentatively identified them from a photo in the Memoirs of the Hourglass Cruises [4]. Without being an expert taxonomist, one can tentatively identify *Karenia* (*brevis*) species and understand the potential consequences of having six million cells l^{-1} in the first samples. Later we learned there were no records of this species anywhere on the US east coast except for a few samples from below Jacksonville FL.

There was an urgency beyond being able to deal with questions from the public about what was happening on the beaches. Recreational shellfish had opened mid-October and the commercial shellfishing season was scheduled to start the next day, 2 November. My first call was to P. Fowler, a friend and colleague who worked with North Carolina Shellfish Sanitation. When I explained the situation to her, she looked for any information in her files on Florida Red Tides and joined me at the Beaufort NC NOAA lab. Time was passing quickly and we knew if notices to cancel commercial shellfishing were going to be signed and posted in time to be effective the next day, we needed to contact the laboratory directors immediately…..yes, on Sunday. Fowler called Dr. William Hogarth, Director of the NC Division of Marine Fisheries and Dr. Ford Cross, Director of the NOAA facility was advised. They must have been alarmed by the concern in our voices

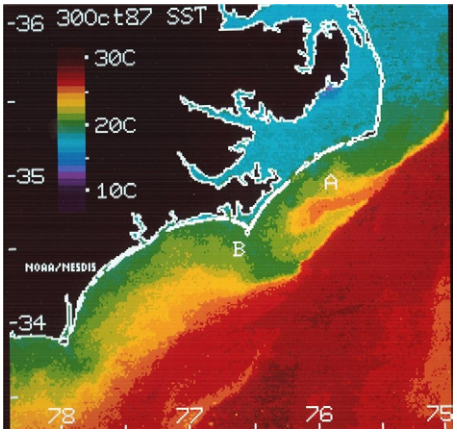

Fig. 2. A HAB event known as the North Carolina Red Tide occurred on November 1, 1987. Toxic Karenia brevis cells traveled from Florida via the Gulf Stream into the colder coastal waters near Cape Lookout, North Carolina. This resulted in a loss of ~$25 million (~$64 million in 2022 dollars).

because they joined us within 30 minutes to be updated on the findings. Notices to close commercial shellfishing were posted along the central NC coasts during the late afternoon of Sunday, 2 November. No toxic shellfish reached the market. Forty-eight recreational shellfishers were not so fortunate. They met the case definition for neurototoxic shellfish poisoning but their illnesses were generally mild and quickly resolved [5].

Monday morning, November 2nd Dr. Karen Steidinger tentatively verified our taxonomy and after the live samples arrived in Florida Fish and Wildlife Conservation Commission Lab in St. Petersburg FL, Tuesday, there was no longer a question. The North Carolina coast was fully involved in a massive *K. brevis* bloom that kept some of the state's 1,480 km^2 shellfish harvesting areas closed in until early May 1988. In trying to answer questions about where the cells had come from and how long the bloom would remain, we had strong support from Dr. Steidinger and her staff. But these were truly uncharted waters and *Karenia* proved to be more resilient and temperature tolerant than anyone suspected. Certainly, remotely sensed imagery would have been helpful, but NASA's Ocean Zone Color Scanner on Nimbus 7 launched in 1976 was not operational after 1986. Thermal imagery from the National Weather Service was not available but Dr. Steven Baig from the Coral Gables facility was able to provide a product he used for estimating the location and speed of the western wall of the Gulf Stream in the US South Atlantic Bight. This would be important for understanding the transport of cells from a bloom off Naples FL (24 August 1987) via the Florida Current and the Gulf Stream to NC by mid-October [6].

Soon our requests for imagery, even thermal imagery, reached Dr. Rick Stump's (NOAA) office and he was able to provide thermal imagery that showed the intrusion of the Gulf Stream into Raleigh Bay on 19 October. This warm filament continued to move shoreward and by 22 October was within 7 km of the shore east of Cape Lookout NC. This parcel of water remained intact for at least 17 days and inshore waters of both Onslow and Raleigh Bays warmed from less than 20°C to 22-24°C in about six days This warm, high salinity water mass served as a chemostat for transported *K. brevis* cells that moved down shore (south) from Raleigh Bay to Onslow Bay around Cape Hatteras NC in the coastal counter current into rich shellfishing areas (Fig. 2).

It seems easy as I write this now, but it took months and the efforts of many to put the parts of this transport event and subsequent HAB bloom together. However, the magnitude of this event served as the genesis of NOAA's Coast Watch program that provides open access satellite data at both regional and global scales as well as operational forecasts for harmful algal blooms.

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Minding the microbes: human pathogens and cyanobacterial blooms

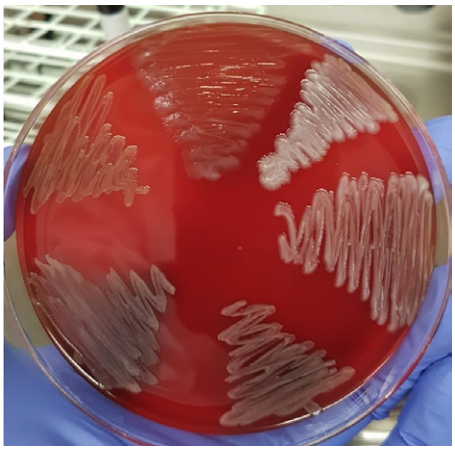

Fig. 1. Cultures of isolated bacterial colonies in blood agar

Although cyanobacteria are well known for their capacity to produce a wide range of toxins, little is known about the potential health effects from exposure to other biotic components of cyanobacterial blooms. Often, when people participate in recreational activities and/or live near to areas which experience cyanobacterial blooms, they may complain of non-specific health symptoms such as headaches, nausea and stomach issues [1]. However, with many of these symptoms, they are unable to be ascribed to the known actions of the many toxins and bioactive compounds produced by cyanobacteria. Consequently, it is possible that other, uncharacterised constituents of the bloom may be contributing to these observed adverse health effects. Furthermore, such constituents could also be present alongside the known toxins produced by cyanobacteria.

During toxin assessment of a cyanobacterial bloom in Florida in 2018, a pilot study was carried out to examine the presence of possible human bacterial pathogens associated with the bloom. Samples were removed aseptically and transported back to the lab on ice. Sterile subsamples of the bloom material were plated onto Blood Agar and incubated at 35°C. Serial culturing was performed to produce pure cultures of isolated bacterial colonies (Figure 1). Samples from 2 isolated colonies then underwent biochemical tests and identification using a Biomerieux Vitek2 Compact microbial identification system. The remaining bloom material was freeze-dried and underwent extraction and analysis of microcystins, anatoxin-a, cylindrospermopsin, guanitoxin and BMAA and isomers [2].

Microscopy showed *Microcystis aeruginosa* as the principal cyanobacterium present in the bloom material (Figure 2). Toxin analysis showed the presence of microcystin-LR according to UPLC-PDA and UPLC-MS at 4.6 mg/l. Co-occurring with this cyanotoxin was β-*N*-methylamino-L-alanine (BMAA) at 51 ng/l and a trace amount of guanitoxin according to LC-MS/MS and enzyme inhibition assay, respectively. Biochemical tests for the two isolated chemoheterotrophic bacteria showed them to be oxidase and catalase positive and negative for lactose fermentation, with one a Gram negative rod and the other a Gram negative rod with flagella. Assessment of the bacteria by the Vitek 2 identification system identified them as *Aeromonas sobria* and *Sphingomonas paucimobilis* with 93% and 98% probability, respectively.

Aeromonas sobria and *Sphingomonas paucimobilis* are known opportunistic pathogens and are ubiquitous in natural environments, including water [3,4]. Exposure to such bacteria is of particular concern for people with immunodeficiencies and underlying health conditions with *Aeromonas* having been shown to cause gastroenteritis and wound infections [5] and *Sphingomonas* often being associated with nosocomial infections due to contaminated water, solutions or instruments [6].

The presence of chemoheterotrophs and their potential influence on the toxicity of blooms has been inferred for some time. Often when people are exposed to cyanobacteria, generic symptoms are often reported such as headaches, sore throat, vomiting and stomach pains, as highlighted by army cadets performing kayak exercises in *Microcystis* scums on a freshwater reservoir [7]. Furthermore, based on the current knowledge of *Aeromonas* and *Sphingomonas*, these bacteria are capable of causing such symptoms [8,9]. Other recorded chemoheterotrophic bacteria connected with cyanobacterial blooms include *Vibrio cholerae* and associated outbreaks of cholera coinciding with microcystin-containing cyanobacterial blooms in Bangladesh [10]. Consequently, such chemoheterotrophic bacteria have the potential to drastically affect the adverse health effects possible from exposure to such blooms.

The chemoheterotrophic bacteria isolated and identified here could be responsible for the symptoms observed by the people of Florida in 2018 although the bloom material will likely contain many more bacterial species and genera than those that were culturable. A greater understanding of the contribution by and health risk from chemoheterotrophic bacteria in cyanobacterial blooms is required.

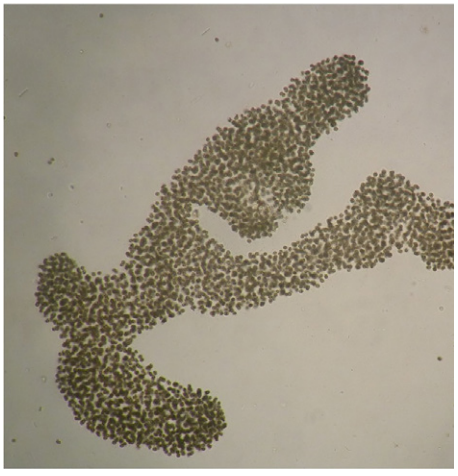

Fig. 2. Micrograph of Microcystis aeruginosa, dominant cyanobacterium in the bloom.

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Blooms of a red tide species *Tripos furca* in the aquaculture area of the Penang Strait, Malaysia

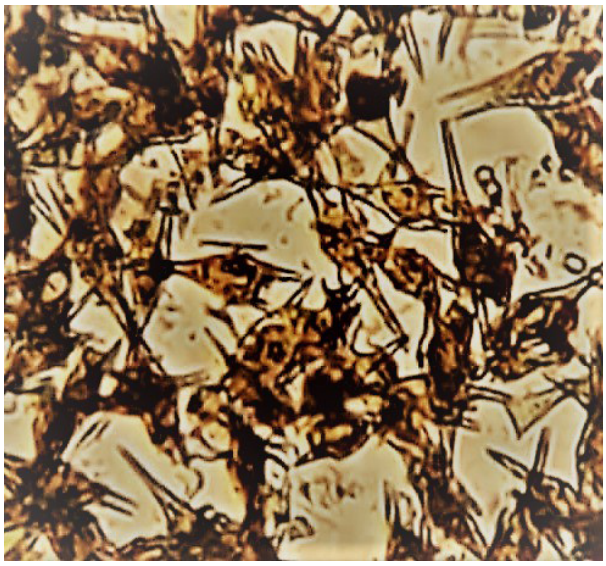

Fig. 1. Photomicrograph of Tripos furca from Aman Island

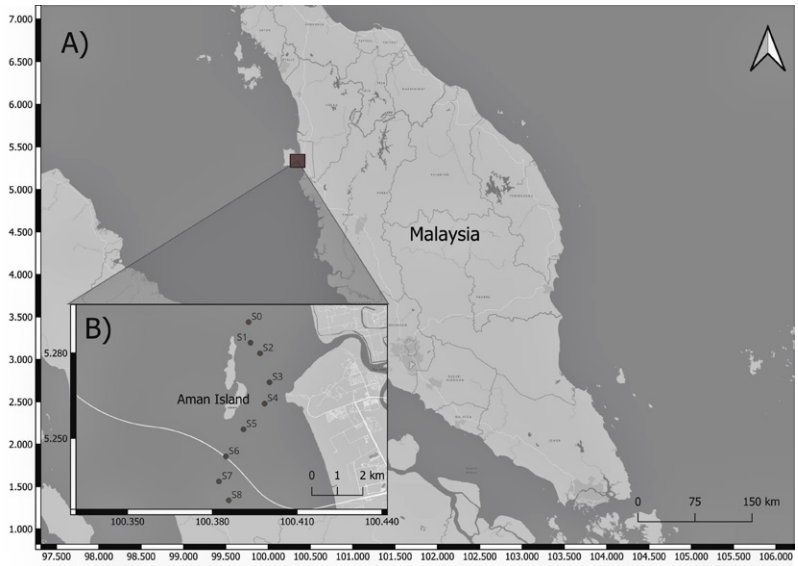

Fig. 2. Map of Peninsular Malaysia showing the location of Aman Island, Penang (A) and the sampling stations (B).

Blooms of the red-tide-forming dinophyte *Tripos furca* (Fig. 1) were observed along Aman Island in the Penang Strait in May 2022 (Fig. 2). The species caused dense red discolorations in the coastal water (Fig. 3). Bloom events of the species also lead to low concentrations of dissolved oxygen in the water, which promote massive fish kills due to either hypoxia/anoxia or damage to fish gills [1]. Blooms in the Penang Strait, fortunately, did not cause fish kills in the aquaculture area of Aman Island, as early warning notices had been issued and the local farmers had taken further action by harvesting before the blooms approached the farms.

Monthly field sampling conducted in the area showed that the blooms were recorded from May to August 2022 (Fig. 4). A previous study in 2012 reported the occurrence of the species in Aman Island, with the cell density reaching 76,500 cells L^{-1} [2]. In our study, the *Tripos furca* cell maximum of 1.2×10^6 cells L^{-1} was detected in June 28th 2022 (Fig. 4). Blooms were associated with water temperatures of 30.5–32.0°C, which describes their occurrence during the dry season. Blooms of this species have also been observed in salinity ranges of 26.5–30.5, while pH range was 7.5 - 8.5. Low dissolved oxygen levels were also detected in the surface water column, 4–7 mg L^{-1}.



Fig. 3. Red tide phenomenon in Aman Island.

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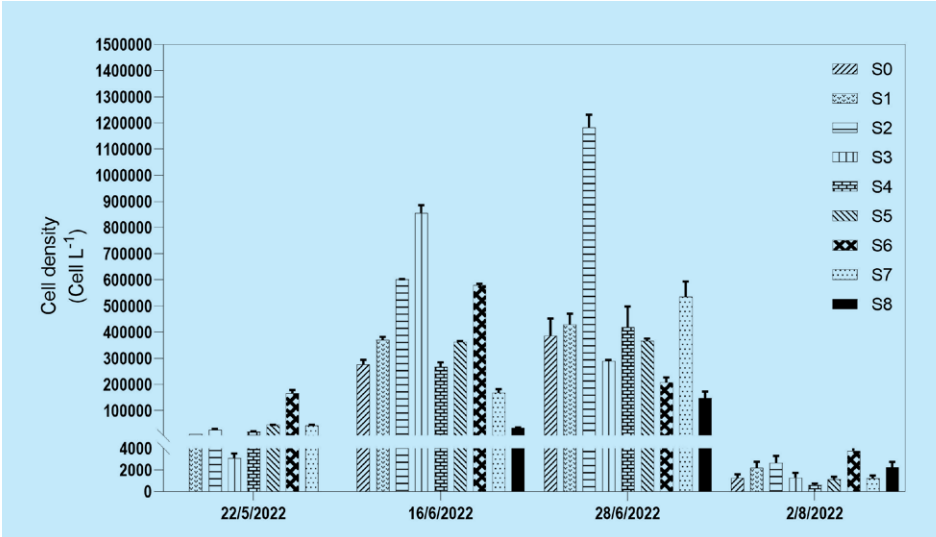

Fig. 4. Cell density of Tripos furca (cells L-1) between May and August 2022 in Aman Island.

A bloom of *Pyrodinium bahamense* in the port operations of a seafood processing plant, SE Gulf of Mexico

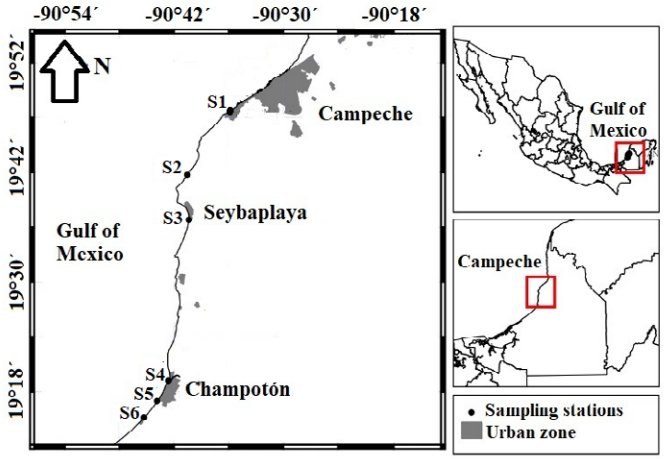

Fig. 1. Study area and sampling sites (S1-S6) in coastal waters of Campeche, Gulf of Mexico

The Mexican state agency CONAPESCA (National Aquaculture and Fisheries Commission) ranked the State of Campeche as seventh in the country in terms of fish catch volume with an active fleet of 3,401 artisanal fishing river boats and 26 plants along the coast providing processing capacity for fishing products. Most of the plants are adjacent to the landing areas, and the processed water is generally discharged directly into the sea without prior treatment. It should be noted that the wastewater discharge process is regulated by Mexican law. In 2005, the state agency for the protection of sanitary risks reported a bloom of a planktonic non-toxic dinoflagellate, *Blixaea quinquecornis*, in a sheltered port in the City of Campeche resulting in the discoloration of water.

As part of the monitoring program of harmful phytoplankton in areas with anthropogenic activities along the central coast of Campeche, six sites (depths of ca. 1 m) at three coastal localities (City of Campeche, Seybaplaya and Champotón; 19°17'52.55"-19°51'50.87"N, 90°30'47.37"-90°46'6.83"W) were sampled (Fig. 1). Sampling stations were chosen at the points of rainwater discharge or near the sites with anthropogenic impact and were monitored monthly from November 2021 to November 2022. Quantification of *P. bahamense* cells was performed according to the Utermöhl technique [1] The dinoflagellate *Pyrodinium bahamense* reached a maximum value of 1.2×10^6 cells L^{-1} in May 2022 at station S5 (Fig. 2), corresponding to port operations of a seafood processing plant. Salinity was 29.3, temperature 33.1°C, pH 7.9 and dissolved oxygen 9.1 mg L^{-1}. Discoloration of water was not observed.

Toxicity of *P. bahamense* in the subtropical waters of the West Atlantic was recorded in the coastal zone of East Florida [2], and there is recent evidence for toxic populations of *P. bahamense* in the coastal waters of Campeche, coupled with the presence of paralytic shellfish toxins in fish collected off the coast of Campeche [3]. Along the Campeche coast, *P. bahamense* is widely distributed, with marked occurrences in the dry (February to May) and rainy (June to September) seasons in 2005, 2008, 2010, 2019 and with abundances ranging from $3.0\text{x}10^3$ to $3.3\text{x}10^5$ cells L^{-1} [4]. It should be considered one of the key species with the potential for forming blooms in the study area. The high abundances of *P. bahamense* along this coast may be indicative of eutrophication due to fluctuations in environmental conditions caused by the intensity and type of human activities [5]. Near the northern coast of Yucatan Peninsula, from 2002-2011, the species was characteristic of the marinas of Telchac, Dzilám de Bravo and of the coast off Progreso, especially in the rainy season (F. del C. Merino-Virgilio, pers. comm.). The proximity of mangroves seems to favour the proliferation of the species. Therefore, it is essential to keep a record of bloom-forming planktonic species, with a special emphasis on harmful species that co-occur with production activities in coastal areas.

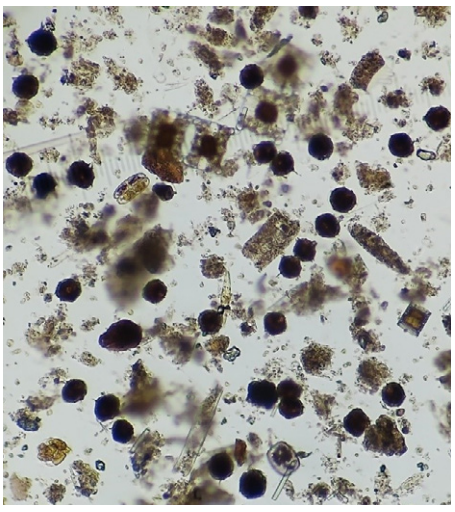

Fig. 2. Net haul of blooming Pyrodinium bahamense in the port operations area of a seafood processing plant, SE Gulf of Mexico, with abundant detritus characteristic of a coastal shallow zone

Acknowledgements

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The Sixteenth Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms (IPHAB-XVI)

The 16th session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms (IPHAB-XVI) was held at FAO Headquarters 27-29 March 2023. The Panel reviewed the actions completed during the intersessional period, noted the progress made and that several of the UN Ocean Decade challenges are being addressed. The Panel concluded that the Decisions and Recommendations of the Fifteenth session (March 2021) had been implemented highly satisfactorily within the available resources. The major achievements reported, some still in progress, include: (i) the results from the ICES-IOC Working Group on Harmful Algal Bloom Dynamics and ICES-IOC-IMO Working Group on Ballast and other Ship Vectors; (ii) the updates on the continued publication of the IOC Harmful Algae News; (iii) the implementation of three international training courses and several regional and in-country courses; (iv) the continued development of the regional activities in Western Pacific (IOC/WESTPAC/HAB); and the Caribbean (IOC/IOCARIBE/ANCA), the engagement, but shortage of resources, for South America (IOC/IPHAB/FANSA) and North Africa (IOC/IPHAB/HANA); (v) the advances of the PICES HAB-Section; (vi) the publication of the GlobalHAB "Guidelines for the study of climate change effects on HABs"; (vii) the publication of a Technical Guidance Document for the Development of Early Warning Systems for Marine Harmful Algal Bloom Events to be published, as a joint publication of FAO, IOC-UNESCO and IAEA, as a FAO Fisheries and Aquaculture Technical Paper; (viii) the continued compilation of data at all levels for the IPHAB-IODE Harmful Algae Information System with HAEDAT and OBIS databases as providers of high quality information on HAB events, status and trends of HAB occurrence and assessment of climate change impacts, and a toxin database linked to the taxonomic reference list via WoRMS; (ix) the advances achieved by the strategy for Ciguatera; (x) the developments under the joint IOC-SCOR GlobalHAB science programme such as the advanced modelling and automated in situ observations workshops (xi) the opportunities the UN Decade on Ocean Science for Sustainable Development brings to IPHAB for transformative science solutions for sustainable development of the ocean regarding HABs and (xii) the development of activities and partnerships carried out by the International Society for the Study of Harmful Algae (ISSHA) to promote and foster research and training on HABs.

The Panel took eleven decisions and endorsed two recommendations for the consideration of the IOC Assembly at its thirty-second session and of the FAO Committee on Fisheries (COFI). The decisions concern: (i) Regional HAB Programme Development taking into account the difference of support for the various groups and networks depending on whether they are affiliated to a regional IOC subsidiary body or not; (ii) the continuation of the Task Team on the Early Detection, Warning and Forecasting of HAB Events; with new terms of reference; (iii) the continuation of the Task Team on the development of the Harmful Algal Information System and a periodic Global Harmful Algal Bloom Status Report with new terms of reference; (iv) the continuation of the Task Team on a Global Ciguatera Strategy for Improved Research and Management with new terms of reference; (v) the continuation of the Task Team on Harmful Algae and Desalination of Seawater to formulate a proposal for a joint FAO WHO water safety risk assessment for drinking water coming from desalination plants; (vi) the continuation of the Task Team on Biotoxin Monitoring, Management and Regulations with new terms of reference; (vii) the continuation of the Task Team on Algal Taxonomy with new terms of reference; (viii) the continuation of the Task Team on Fish Killing Microalgae and Ecosystem Effects with new terms of reference; (ix) establishment of a Task Team on HAB Communication; (x) IOC-SCOR GlobalHAB beyond 2025; and (xi) the development of HAB Solutions (HAB-S) an UN Ocean Decade Programme Proposal.

Dr Philipp Hess (France) was elected as Chair and Dr Maggie Broadwater (USA) was elected as Vice-Chair. The Recommendations to the IOC Assembly encompass (i) the planned intersessional activities into a workplan and budget for the IOC HAB Programme 2024–2025; and (ii) the continuation of IPHAB with unchanged terms of reference.

Philipp Hess, Chair IPHAB &
Henrik Enevoldsen, IOC-HAB Programme Coordinator



ICES-IOC Working Group on Harmful Agal Bloom Dynamics Meeting

The joint ICES - IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) is an important forum for ICES (International Council for the Exploration of the Seas) and the Intergovernmental Oceanographic Commission of UNESCO (IOC-UNESCO) to review and discuss HAB events, and to provide annual advice and updates on the state of HABs in the North Atlantic area region. It also facilitates interaction between scientists working in diverse areas of HAB science and monitoring and provides a forum for the interchange of various approaches to HAB research. The group reports to both the ICES Science Committee (SCICOM) and Food and Agriculture Organization of the United Nations (FAO) -IOC UNESCO Intergovernmental Panel on Harmful Algal Blooms (IPHAB).

The group's annual meeting, in hybrid format, was held 20th – 23rd March at Stazione Zoologica Anton Dohrn, Napoli, Italy. The meeting was attended by delegates from USA, UK, Ireland, France, Spain, Portugal, Netherlands, Germany, Poland, Sweden, Norway, Belgium and Estonia and was opened by Marina Montresor and Adriana Zingone. Over four days, discussion and presentations covered a variety of related topics including progress on the 3rd and final year of the groups current Terms of Reference (ToR) for the 2020-2022 cycle, new and current project research studies, and three year national reports and new findings in relation to Harmful Algal Bloom events recorded during 2022 in the ICES Atlantic region. Time was dedicated to defining the working groups next set of ToRs for the forthcoming three year cycle.

In addition to the review and summary of the Working Groups activities from the current ToR cycle there were a number of presentations, including, 'Harmful Algae News update'; 'HAB Monitoring in the Gulf of Naples'; 'Marine phytoplankton monitoring by molecular approaches'; 'Update on Metabarcoding/eDNA in Nordic countries'; 'Update on eDNA EU projects (TREC, BioOcean5D, Obama-Nest) and ROME eDNA network'; 'Automated observations of harmful algal algae in the Baltic Sea and the Kattegat-Skagerrak using imaging flow cytometry'; 'Update on satellite ocean colour discrimination of HABs'; 'US National Harmful Algal Bloom Observation Network'; 'GES-4SEAS EU Project - Achieving Good Environmental Status For Maintaining Ecosystem Services By Assessing Integrated Impacts Of Cumulative Pressures'; 'Update on Tetrodotoxins in Netherlands' and 'Toxic bloom of *Prymnesium parvum* in the Oder River in 2022'.

The meeting was closed by Professor Chris Bowler, President, Board of Directors - Anton Dohrn Zoological Station where the next annual meeting is scheduled for April 2024, to be hosted by IFREMER, Nantes, France. For the next 3-year cycle, the working group will be co-chaired by Lars Johan Naustvoll (Norway) and Dave Clarke (Ireland). WGHABD, together with the ICES working groups for Phytoplankton and Microbial Ecology, and Introduction and Transfer of Marine Organisms are convening a joint theme session at the forthcoming ICES Annual Science Conference in Bilbao, Spain from 11th – 14th September on 'Integration of molecular tools for biodiversity, risk assessment, ecosystem advice within a changing climate'.

Information on HAB event occurrence in the North East Atlantic margin area is reported annually by the Working Group members and is publicly available in the Harmful Algal Event database (HAEDAT *http://haedat.iode.org/*) and also through Harmful Algal Information System (HAIS *https://data.hais.ioc-unesco.org/*).

Dave Clarke, WGHABD Chair



Workshop on an Early Warning System (EWS) for Harmful Algal Blooms (HABs) in Morocco

From December 5 - 8th, 2022 an IOC-UNESCO workshop on Early Warning Systems for Harmful Algal Blooms (HABs) was held in Casablanca, Morocco, hosted by the Institut National de la Recherche Halieutique (INRH). This workshop, funded through IOC-UNESCO by the Norwegian Cooperation and Development Agency (NORAD), was organized in the framework of the IOC-UNESCO project «*Detection and Early Warning Systems (EWS) adapted to Harmful Algal Blooms (HABs)*», in partnership with the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA). This project addresses HAB-related issues which impact seafood safety due to toxin contamination of shellfish, fish and crustaceans, and mass mortalities of marine organisms. Development of an early warning system (EWS) to help mitigate the impact of HABs is thus desirable to help protect against HAB impacts.

The workshop objectives were to identify stakeholder priorities and to train national experts on data management, imaging and predictive modelling to implement an EWS of HAB events in Morocco. Forty-five experts participated in this workshop (Fig. 1), which was attended by representatives of government agencies and other stakeholders in the aquaculture and shellfish processing industries as well as INRH scientists of the Marine Environment Monitoring Network (RSMM) responsible for the monitoring of HABs and marine biotoxins, INRH data analysts and IOC representatives.

The first thematic area dealt with the assessment of stakeholder priorities and requirements, current capacities, pre-existing and present gaps in knowledge and data acquisition, as well as limiting factors and resources in the establishment of an EWS in Moroccan coastal waters. Additional discussions took place on logistic issues related to the fulfillment of requirements for the laboratories to get an accreditation according to the ISO/IEC 17025 standard. Special interest was expressed about obtaining validation on analytical methods for toxin analysis and quantitative analysis of phytoplankton. Lectures and scientific presentations were given on different topics related to the HAB monitoring programme and EWS.

The second thematic area focused on training in *R* programming and predictive modeling systems that could be implemented for operational use to forecast the onset and duration of HAB episodes in Morocco. Also, training on the standardization of data from monitoring programs (levels of marine biotoxins in shellfish and abundance of harmful phytoplankton species in seawater) was provided to review adopted phytoplankton thresholds and potential forecasting models.

In Morocco, the monitoring programme in shellfish production areas, developed under the terms of the National Institute of Fisheries Research, Morocco (Institut National de Recherche Halieutique, INRH) is carried out in six regional centres (Nador, Tanger, Casablanca, Agadir, Laayoune and Dakhla) in compliance with the provisions of the Moroccan Ministry of Agriculture, Fisheries, Rural Development, Continental Waters and Forestry (MAPMDREF), order no. 1950-17 related to the sanitary classification of shellfish production areas [1]. The monitoring protocols meet national and international health requirements (in particular those implemented by the EU market). Monitoring for seafood safety is carried out using two complementary approaches: regular monitoring and alert monitoring.

Monitoring of the classified shellfish production areas is carried out to safeguard public health and environmental quality. This monitoring covers microbiological and chemical parameters (inorganic and organic contaminants), toxigenic phytoplankton and marine biotoxins, according to the established sampling plan. These areas are monitored to revise the sustainability of their

Continued on p. 9



Fig. 1. Participants in the IOC-UNESCO workshop on Early Warning System (EWS) for Harmful Algal Blooms (HABs) in Morocco. Casablanca, Morocco, December 4 – 8th 2022

HAB session during the Sixth Xiamen Symposium on Marine Environmental Sciences

A scientific session "Harmful algal blooms under changing climate: mechanisms, monitoring, and mitigation for a sustainable, safe and healthy ocean" was held during the Sixth Xiamen Symposium on Marine Environmental Sciences at Xiamen University, Xiamen, China, from 9 - 12 January 2023.

A total of 40 papers were accepted for this HAB session with 16 oral presentations covering the dynamics of HABs in the Pacific region. Topics spanned from ecological, physiological, and molecular responses of HAB species to biotic and abiotic drivers. The presentations also covered aspects of HAB monitoring, forecasting, and early detection using novel tools and instrumentation. Oral papers were presented in three three-hour sessions.

The session began with an invited lecture by Dr Satoshi Nagai, Japan Fisheries Research and Education Institute who shared his five years of research in predicting phytoplankton composition in Mombetsu, Hokkaido using metabarcoding and Artificial Intelligence technology. He illustrated how next-generation sequencing data were obtained from his study to clarify the seasonal dynamics of selected harmful algal species. He concluded that while the outcome of this application is promising, there are challenges to address before HAB prediction become possible.

Other highlights from the HAB session included the following:

Dr Kieng Soon Hii, University of Malaya, Malaysia, shared his research findings on current knowledge on the transcriptomic features of the toxic and non-toxic *Azadinium* and its implication in Azaspiracid biosynthesis.

Dr Shuwen Zhang, South China Normal University, China, demonstrated that the toxin production of *Pseudo-nitzschia* was enhanced by co-culturing *Pseudo-nitzschia* with *Noctiluca*, thus elucidating the interspecific interactions and successional mechanisms of HAB species.

Dr Monaliza Mohd Din, University Malaya, Malaysia, reported prolonged algal blooms in the inner Johor Strait that caused the formation of a hypoxic-anoxic dead zone, leading to an ecological disaster in the area.

Dr Yunyan Deng, Institute of Oceanology, China, described the active and inactive genes in resting cysts using *Scrippsiella acuminata* as the model species. Her findings enhanced current understanding of the molecular processes in dinophyte resting cysts.

Dr Jianping Li, The University of Chinese Academy of Sciences, China, highlighted the recent advancement of optical technology in real-time plankton monitoring and sea trial of instruments that.he has successfully deployed and how to overcome biofouling in the highly eutrophic waters of Daya Bay. He also explained how machine learning was adopted to identify plankton species in the samples, and the result was comparable to those obtained from the microscopic enumeration.

Dr Anouk Olleveir, Flanders Marine Institute, Belgium, introduced a video plankton recorder (VPR) approach to investigate the plankton compositions in shallow and turbid waters in Belgian coastal waters (North Sea). She further clarified the usefulness of the VPR despite limitations in shallow coastal waters and the detection of small plankton species.

As well as the above highlighted presentations, other remarkable HAB research findings, from oral and poster presenters in this HAB session can be found at *https://melmeeting.xmu.edu.cn/xmas/program_byday_poster.asp* (E-Posters) and *https://melmeeting.xmu.edu.cn/xmas/program_bysession.asp?id=7100075* (abstracts). This HAB session has shown the progress of HAB knowledge in the region and provided a platform to strengthen regional and international collaborations in addressing the expanding HAB issues worldwide.

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classification and update it whenever necessary according to reported results and the regulatory provisions of decree 1950-17 from the Moroccan Ministry of Agriculture, Fisheries and Freshwater and Rural Affairs (MAPMDREF) [1].

The potentially toxic microalgal species/genera identified in the framework of the Moroccan monitoring programme in shellfish production areas include: *Pseudo-nitzschia* spp, *Alexandrium* spp, *Gymnodinium catenatum, Dinophysis* spp, *Protoceratium reticulatum, Gonyaulax spinifera, Lingulodinium polyedra, Prorocentrum* spp, *Karenia* spp, *Ostreopsis* spp and *Coolia* spp. Monitoring of the three groups of regulated marine biotoxins (PSP, ASP and LSP) in shellfish products has enabled the detection of these toxins which in some cases exceeded the regulatory limits.

By the end of the workshop, participants expressed their satisfaction for the opportunity to exchange views on HAB management aiming to strengthen biotoxin protocols and alerts in Morocco. It was agreed that continuing to test a pilot EWS for HABs in Morocco is highly desirable, timely and relevant to everybody concerned.

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Red Tides Archives

In HAN 72 we introduce you to a new series of unedited contributions, rescued from red tide archives, which are relics from the days when the HAB community was starting to develop. The first is a transcription of an "After-dinner speech" by Tim Wyatt, HAN founding editor from 1992 to 2013, at the II International Conference on Toxic Dinoflagellate Blooms, Key Biscayne, Florida, 31 Oct - 5 Nov 1978.

"Let my people go" is a collection of extreme scenarios of high biomass events nowadays called Ecosystem Disruptive Algal Blooms (EDAB), combined with one of Tim's favourite exercises: to find a scientific interpretation of anthropological and mythological issues.

Let My People Go[1]

In classical times, the fringes of the Arabian and Red Seas were inhabited by the Ichthyophagoi or fish-eaters. Their descendants are the Shihuh and other tribes living along the coasts of Oman and Makran to this day. We learn from Strabo, from Diodorus Siculus, and from other ancient authors that these people lived exclusively on fish, that their camels too were fed on fish, and that their houses were built of the bones of fish (and whales) and roofed with the skins of camels. Diodorus tells us that these people disposed of their dead by laying them on the rocks and letting the tide take them. He commented that "by making their own interment a nutriment of the fish, they have a life which follows in singular fashion a continuous cycle throughout all eternity." The same life style has been noted by mediaeval and modern travellers, so that eternity in this case has lasted at least 2000 years. Vito Volterra in Italy [2] (and independently Lotka [3] in America) put this model into mathematical form in the early part of our own century, and by so doing, started the modern game of ecological modelling. 'The initial impulse for Volterra's studies was provided by the sardines of the Adriatic, just as the inspiration for Diodorus' remark was the sardines of the Ichtbyophagoi. A recent view of the models which have developed as a result of these early stimuli states that they have the "neutral stability of the frictionless pendulum" (p 50 in [4]). Translated from the idiom of modern physics, this simply means that they cycle throughout all eternity, or at least until someone switches the computer off. Quite rightly, in an age which has become aware that there may be limits to growth, modern ecologists have become very interested in the notion of stability, whether it exists in nature, and if so, how it is achieved. To balance this concern, I should like tonight to talk about ecological instability. As people at this gathering are well aware, nature does not always seem to behave in a stable fashion, at least over short time scales. If we could throw some light on the mechanisms whereby instability is initiated, we might at the same time learn something of the ways in which ecosystems normally maintain themselves in that state which we recognise as stable. Epidemiologists and those concerned with pest control face analogous problems, and it is not surprising that their models paralleled those already mentioned. Ross's studies of the epidemiology of malaria for example led to a model which is formally identical to Volterra's, and in fact preceded it slightly in time. Let us then look at some ecosystems in which the stabilizing mechanisms, whatever they be, have temporarily ceased operation.

Some Case Histories

In recognition of the fact that our hosts are Americans, I begin with that small lake in Wisconsin, Lake Mendota, made famous by the pioneering studies of Lindeman. Its story however begins about fifty years before Lindeman's, in July 1884. That summer 200 to 300 tons of perch died and drifted ashore around the lake. In the subsequent weeks myriads of flies appeared in the neighbourhood, and Superintendent Buckmaster of the State Insane Hospital noted that the gardens of that institution contained unusually large numbers of dead swallows and sparrows. It was later suggested in a report to the US Fisheries Commission that the perch had been poisoned by some natural event in the lake, and that the toxin was then transmitted to the birds by the flies.

A more recent and dramatic event of this kind occurred in 1968 near the Farne Island's which lie off the north-east coast of England. (The event is briefly referred to in Peter Ayres paper at this conference). Here a bloom of *Gonyaulax tamarensis* led to mortality of invertebrates and fish, and notably to the death of virtually all the shags nesting on the Farnes. Other fish-eating birds also died in large numbers, and there were 78 cases of PSP among the local human population.

Plagues of insects are an almost inevitable concomitant of any large accumulation of carrion. A very extensive mortality of fish following a red tide occurred in Walvis Bay in South West Africa in 1880, and was followed by flies. This is one of my favourite dead fish stories, and I take the liberty of quoting at length from the letter of Mr Koch in which this event was recorded. The letter was published by Gilchrist in 1914, and reads "On the evening of the 20th December 1880, some native fishermen procured eels, which were unknown. On the following morning these were again found, and other fish were seen, the natives, however, fearing to make use of them on account of the peculiar odour of the water. At nine o'clock of the same day, it being spring tide, Mrs Koch, along with the wife of Mr Bauman, the missionary, visited the beach and saw hundreds of living and struggling fish cast up on the sand by each wave and this continued for three more days. On Christmas' Eve it was almost impossible to proceed to the church on account of the odour, and it was scarcely possible to walk about without stepping on the dead fish. The *Alfred Lewis* was in the

1) Note from the author. In 1978, ecological stability was a central issue. Robert May [1] had questioned that complexity begets stability, and argued that ecosystems become less stable as complexity increases. The plagues illustrate that ecosystems can sometimes become extremely unstable. These days the plagues of Exodus would likely be attributed to climate change, pollution, or even some obscure injustice. Then, the deliverables, milestones, stakeholders were more discrete. Life was less macdonaldized



Image from the Egyptian Book of the Dead (c. 1275 BC)

bay at the time, but the water was so blood red and smelt so badly that she left for Sandwich Harbour where fewer fish had been cast up. Everything of a white colour became black, even the deck houses of the *Alfred Lewis*. After a few months the skeletons on the shore in the neighbourhood of the lagoon formed a pile five to six feet in height. Then began a plague of flies. It was only possible to drink a cup of coffee by keeping the hand in constant movement and it was only in September that it became bearable." The Walvis Bay region is well known for its fish mortalities, as indeed is the entire coast of South West and southern Africa. I shall refer to two other African episodes shortly. Less well known are the subsequent deaths of flamingos, baboons, and perhaps eels which succeed them.

This leads me to my next story, which is African with American connections, and the opportunity to get in a plug for a forthcoming paper. Captain Benjamin Morrell, an American sealer, reported seeing not less than half a million "dead seals on Possession Island in 1828 with their skins still on them". He described a similar occurrence at seal colonies in Luderitz Bay, 17 miles farther north, whilst living colonies were seen at the Long Islands on the open coast between the two sites. Morrell's veracity has often been called into question, but there are some indications that in this instance he was telling the truth. If this should eventually prove to be so, then a toxic dinoflagellate bloom seems a likely candidate to account for the mortality. I have argued Morrell´s case at some length in a paper which will appear in the near future [5].

The episodes I have mentioned so far are fairly commonplace, and most of you will have your own favorites in this genre. My next choice is more bizarre, and consequently less believable. The scene was Talara in northern Peru, January 1925, which then witnessed simultaneously a massive incursion of tropical water from the north - the famous El Niño current - and the first rains in 34 years. The combination was devastating, and since I cannot hope to emulate the fluid prose of Robert Cushman Murphy, I resort once more to quotation. The description is from his magnificent book on the Oceanic Birds of South America (1936, Vol. I, p 285). "Dead fish, mingled with untold numbers of birds, strewed the tide lines for hundreds of miles as the ocean turned sour, and thirty-five thousand tons of new guano were washed from the islands by falling water. The northern coves became choked with flotsam from engorged stream beds, and sea snakes, alligators, and strange lizards were cast upon unfamiliar strands. Mullets came out of the ocean to swim about in the squares and roadways of the petroleum towns, and, when the rains began to diminish, mosquitoes, and the 'caballitos del diablo' or dragon flies which prey upon them, were engendered in such swarms as had never been known." Murphy's use of the word caballitos seems particularly apt in such apocalyptic circumstances. The mixture of species is reminiscent of the Abominations of Leviticus.

More fantastic still is a series of events which occurred in the Saint Lucia Estuary in Natal in 1969. They were recorded briefly by John Grindley in 1970 [6]. Drought, evaporation, and withdrawal of water for irrigation apparently combined to produce very high salinities in the estuary, which killed fish, invertebrates and vegetation. This was in March. In July and August, during calm spells following rain, there were two separate outbreaks of red water dominated by *Noctiluca*. In July a plague of Chironomidae occurred, of a species favoured by enriched bottom substrates. These in turn led to a plague of tinyphid spiders which prey on midges. These spiders are orb-web weavers which disperse aerially. Finally, "In the Lester Point area the spiders' webs became so dense that plants and branches of trees were completely smothered and killed." Grindley proposed that the chironomid plague was caused by the reduction of predatory control of the aquatic larvae consequent on the events in March. The *Noctiluca* bloom is not obviously connected to the other plagues, but may be linked to the unusual climatic circumstances.

The next chain reaction I have chosen to mention occurred in the years 1847 to 1851, and its repercussions are still being felt today. I refer to the "Great Hunger", the worst of the Irish potato famines. The proximate cause of the famine - if we ignore the social and political angles - was a fungus, *Phytophora infestans*, which causes potato blight in the varieties then being planted. The sequelae to famine are too well known in our own time, and I do not need to spell out the ghastly details of suffering and pestilence. The population of Ireland in 1841 was between 8 and 9 million. Famine, typhus and dysentery probably killed at least a million, though precise numbers have never been established. Millions more fled the country - emigration is a euphemism for this exodus. By 1855, 3 million had gone, most of them to the United States. By 1891, following several less severe famines, the total population of Ireland was reduced to less than 3 million.

The Plagues of Egypt

Most of you are - I am sure - familiar with the plagues of Egypt described in Chapters 7 to 12 of Exodus, and as students of red tides will long ago have recognised that the first of these plagues

was a population explosion of some as yet unidentified algal species. I am not concerned here with the liturgical and dramatic aspects of the story, though these have of course ensured its survival. Why then should I be concerned with it at all? Many commentators have pointed out that all but the last of the plagues have parallels at other times, that they are in fact almost annual afflictions of Egypt, and that to pursue the matter further is a vanity. It is true enough that the Nile may appear red when the flood waters of Abyssinia bring down their mud, that great numbers of frogs and flies occur in some years when the river rises, and that locusts are a constant threat to crops in these arid regions. It is true too that the *khamsin*, the blistering wind, can carry dust and sand from the desert sufficient to obscure the sun. But it is easier for me personally to believe that the events recorded in *Exodus* were extraordinary, and that ordinary events of these kinds should become central to one of the great faiths of the world.

In ancient Egyptian belief, the Nile was formed by the tears of Isis, weeping for her husband Osiris who was slain by his brother Set. By means of Isis' tears "the country is converted into a sea, and nothing appears but the cities, which look like islands in the Aegean. At this season boats no longer keep to the course of the river, but sail right across the plain." This passage is taken from Herodotus, but would equally describe Egypt right up until the end of the nineteenth century. The Nile begins to rise in July and the flood is at its height in August and September. As it recedes, enormous shallow lagoons are left behind in the delta region. Cut off from the declining river, and, if connected with the sea, only weakly flushed by the small tides of the eastern Mediterranean, these lagoons form suitable environments for intense algal blooms. It is not unreasonable to suppose that in one such lagoon, perhaps a very large one, in the Land of Goshen, that part of the eastern delta occupied by the Israelites in pharaonic times, an algal bloom turned into a red tide. Parts of the water-courses themselves might also, as semi-stagnant conditions set in, develop blooms. If the Nile flood had been less extensive than normal, hyper-salinification might have occurred. We have already seen some of the scenarios which might follow such an event.

The Bible contains several versions of the plagues. The Exodus account has ten, which, in order, are (i) "and all the waters that were in the river were turned to blood"; (ii) "and the fish that was in the river died; and the river stank, and the Egyptians could not drink of the water of the river", "and the frogs came up, and covered the land of Egypt"; (iii) "and it became lice in man, and in beast; all the dust of the land became lice throughout all the land of Egypt." (according to my brother, who is a scholar of ancient languages including Old Testament Hebrew, the word here rendered as lice is probably gnats); (iv) "and there came a grievous swarm of flies and the land was corrupted by reason of the swarm of flies"; (v) "Behold, the hand of the Lord is upon thy cattle which is in the field, upon the horses, upon the assess, upon the camels, upon oxen, and upon the sheep: there shall be a very grievous murrain." Murrain is no specific disease of domestic animals; (vi) "a boil breaking forth with blains upon man, and upon beast". This despite the fact that murrain had already killed all the cattle! Blains are blisters or pustules; (vii) "and the Lord sent thunder and hail, and the fire ran along upon the ground; and the Lord rained hail upon the land of Egypt". This plague too killed animals and destroyed crops. Flax and barley were destroyed, but wheat and rye escaped; (viii) "and the locusts went up over all the land of Egypt and they did eat every herb of the land, and all the fruit of the trees which the hail had left"; (ix) "and there was a thick darkness in all the land of Egypt three days"; (x) "the Lord smote all the first-born in the land of Egypt." This too included the first-born (or simply chosen) of cattle.

Detailed examination of the text of *Exodus* has led to the belief that three different sources are combined here, no one of which mentions all ten plagues. These sources are labelled J, E and P, and the plagues of each are shown in the accompanying table. Further versions occur in Psalms 78 and 106. In my own reconstruction of the possible course of events, I have tried to account for all ten, since it is a relatively easy matter to delete some in order to comply with any one of the different versions. By this time, I am sure, you have all guessed what is coming, that several case histories outlined earlier can be linked together in such a way that a plausible ecological model of the ten plagues preceding the *Exodus* emerges.

A red tide broke out in the eastern delta, exacerbated perhaps by unusual climatic conditions either locally or in the distant headwaters of the Blue Nile. This must have caused mortality among invertebrates and fish, or have accompanied such mortality as in the case of the Saint Lucia estuary. In either case, the water would soon have become deoxygenated, beginning at the bottom. The surviving fish would rise to the surface and gasp for air, and eventually themselves die and be either cast ashore, or sink and further exacerbate the anoxia. Air-breathing aquatic insect larvae might of course survive, leading to plagues of mosquitoes, midges and other pests. 'The death of the bulk of the fauna would have intensified the success of emergence, and would yield a source of carrion in which flies could breed. Frogs, since most of their oxygen requirements are met through the skin, would be forced from the water, and would seek cool shady places such as houses. There is little difficulty then in accounting for the first four plagues (blood, frogs, gnats and flies). Lake Mendota, Walvis Bay and Talara provide modern parallels. There is similarly little difficulty with regard to plagues 5 and 6 (murrain and boils). Biting insects attacking both people and their domestic stock would in excess numbers soon lead to extreme irritation. Scratching the wounds would then provide sores, blains, an attractive environment for the non-biting dipterans and other insects exploiting the dead fish and frogs, and for the pathogens they harbour (murrain, boils). The next two plagues, hail and locusts between them destroyed the crops. Locusts are a sufficiently common affliction that we need have no qualms about them. The same unusual climatic conditions which allowed the delta. lagoons to develop red tide conditions, i.e. greater than average aridity, would cause dispersal of' locusts in search of food. Hail on the other hand is rare in Egypt, and a storm sufficient to damage crops significantly would consume the entire annual precipitation allowance of several decades. Hail (frost, snow)

could however be simulated by the gossamer and webs of aerially dispersed spiders, and there is little doubt that these and other insect predators such as dragonflies would have responded to the super-abundance of prey provided by earlier plagues. Each insect plague would in turn "darken the sky" and give rise to the ninth plague.

The tenth plague, the death of first-born (or chosen) presumably must have occupied a period of several months or more. The poor innocent Egyptians (it was Jehovah who hardened the Pharaoh's heart, not his own gods) were short of drinking water and food, and infected with all manner of pathogens borne by insects. They would have dispersed in search of help, and no doubt crowded into camps provided by the authorities. It would have been impossible to maintain standards of hygiene, the general level of morbidity would have been raised, and there would have been an exaltation of pathogenic virulence. In traditional societies, it is usually the first-born who are given the opportunity to avail themselves of privilege when a choice must be made due to limited family resources. In the circumstances imagined here therefore, they would have taken the brunt of the last plague.

Even if we concede that this chain of events is possible, however improbable, there still remains a serious obstacle. Why is it that only the Egyptians suffered from the plagues, whilst the Hebrews living amongst them were apparently immune? Perhaps if we are prepared to stretch credibility a little further, we can answer this question too. The first chapter of Exodus (Ex. 1: 7), tells us that "the children of Israel were fruitful, and increased abundantly, and multiplied, and waxed exceeding mighty; and the land was filled with them." and (Ex. 1: 12) "the more they (Egyptians) afflicted them, the more they multiplied and grew". The pharaoh tried to institute a number of measures, including infanticide, to curb their fertility, but these were unsuccessful. The Israelis were clearly *r*-strategists. Migration is characteristic of *r*-strategists in deteriorating habitats. Hence by labelling the Israelis *r*-strategists, we can account both for their survival in a rapidly changing environment, and for the *Exodus* itself.

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IOC Training Course

IOC Training Course and Identification Qualification in Harmful Marine Microalgae, August-October 2023

The purpose of the course, organized by the IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Denmark Copenhagen, Denmark, is to improve the taxonomic and identification skills of the participants for research purposes and for practical monitoring of harmful algal blooms. The course also includes a practical exam at the end of Part 2, with an IOC Certificate of Proficiency in Identification of Harmful Algae issued to participants who pass the exam..

The course, aimed at participants who have some years of practical experience in identification of microalgae includes 100 hours of teaching and is divided into two parts:

1. The first part of the course is an internet teaching programme on the Ocean Teacher platform giving general introductions to the various groups of harmful algae; this part is mainly for self-study and estimated to 40 hours of reading. Part 1 will be available on the 'International Oceanographic Data Exchange' (IODE) teaching platform 'Ocean Teacher' from August onwards;
2. The second part is a practical course in species identification including 60 hours of teaching. A microscope will be available to each participant during the entire period. Part 2 takes place from 15-26 October 2023

Participants

The course is aimed at participants who have some years of practical experience in identification of microalgae. The number of participants is limited to 16. If there are more applicants than available seats, priority will be given to applicants who have direct research or management responsibilities with regard to the occurrence of harmful algae.

Venue (Part 2)

IOC Science and Communication Centre on Harmful Algae, Department of Biology, University of Copenhagen, Denmark, c/o Danhostel, Lejrskolevej 4, 3400 Hillerød.

Application

Deadline for application is **1 June 2023**

Details on the programme, teacher, and practical information can be found the *IOC-HAB website*

Enquiries may be sent to Jacob Larsen, *jacobl@bio.ku.dk*

Forthcoming

International Workshop on Solutions to Control HABs in Marine and Estuarine Waters

The **International Workshop on Solutions to Control HABs in Marine and Estuarine Waters** is scheduled for October 21 and 22, 2023.

The workshop is sponsored by the Global Harmful Algal Blooms (*GlobalHAB*) Programme and the North Pacific Marine Science Organization (*PICES*) and will be held in conjunction with the PICES Annual meeting in Seattle, Washington, USA.

The workshop will specifically focus on HAB control methods that affect the target HAB organisms themselves, either killing them or removing cells and/or toxins from the water. There will also be a strong focus on methods that have been field tested. However, some advanced lab methods may be considered if strong evidence is provided about their suitability for use in the field. Lessons will be drawn from experiences in freshwater HAB control. Discussions will include effectiveness for use in marine systems, cost, scalability, regulatory hurdles issues and approaches for communicating with the public and stakeholders. The workshop will not focus on bloom prevention or HAB mitigation efforts to relieve the impact of blooms, such as monitoring and forecasting.

This workshop will be held in person with no opportunity for remote or absentee participation. Presentations are by invitation only and an associated poster session is planned. The venue is the Westin Seattle Hotel. Registration will be handled via the PICES-2023 Annual Meeting *website*. A user logon (pre-registration) must be established on the PICES website as a first step, at *this link*. Workshop attendees must register for the full meeting with a cost of $250 Canadian dollars (CAD) before June 15. After June 15, the registration cost will increase to $350 CAD. Students and invited speakers are offered a reduced registration cost of $50 CAD. Abstract submissions will be accepted online *here* until June 15 2023 with acceptance notifications coming about a month later. Financial support applications can be submitted *here* through June 15. (See *abstract* for affiliations and more details).

The organizing committee hopes to see you there!

Vera Trainer, Quay Dortch, Marc Suddleson, Pengbin Wang, Mark Wells, Natsuko Nakayama, Don Anderson, Heather Raymond, Hae Jin Jeong, H. Dail Laughinghouse



The International Society for the Study of Harmful Algae invites you to the **20th biannual International Conference on Harmful Algae** on November 5-10, 2023, at the Grand Prince Hotel Hiroshima in Hiroshima, Japan. The *conference website* is open for registration with abstract submission through 19th June 2023! Sign-ups for the banquet and excursions will be announced in April. Please note there are several conferences using the same name as ours (ICHA 2023) but these have absolutely no relationship with our official conference website (*https://icha2023.org*).

We aim to provide a safe in-person event for scientists, policymakers, and practitioners to share their knowledge and gather ideas regarding all aspects of marine and freshwater harmful algae. Limited online participation will be possible as well.

This conference provides a unique forum for collaboration, connection and sharing ideas. We hope that you will join us for this opportunity to connect with the global harmful algae community.

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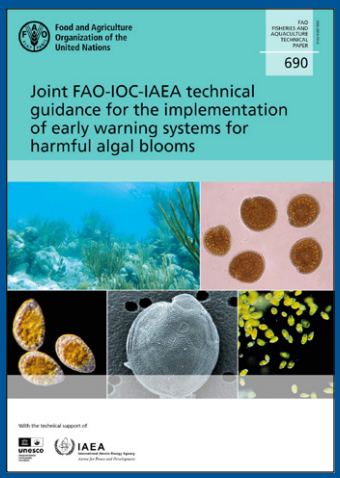

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

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Harmful Algae News

An IOC Newsletter on Toxic Algae and Algal Blooms

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Rapid response to a massive marine mammal stranding event associated with domoic acid poisoning in central to southern California

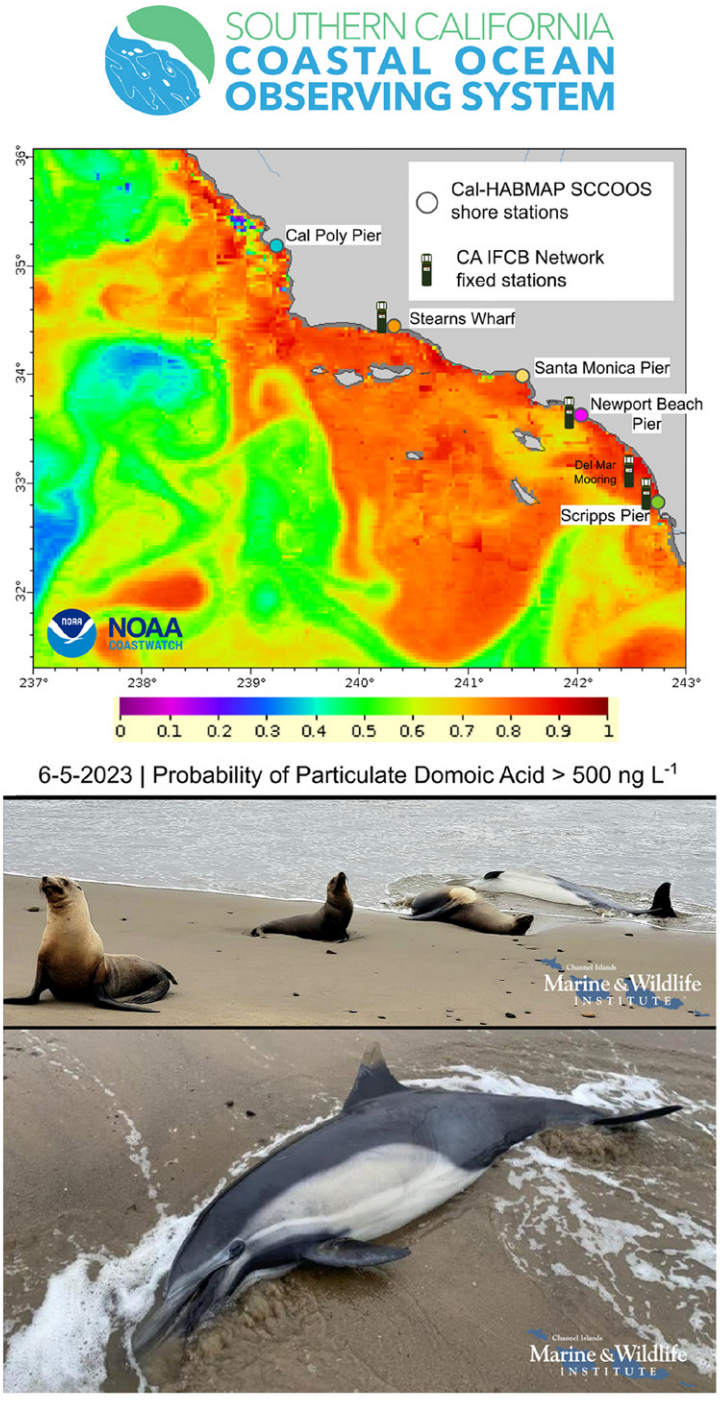

Fig. 1: **a)** *NOAA CoastWatch California-Harmful Algae Risk Mapping (C-HARM) system prediction of pDA risk (red = higher risk) on 9 June 2023; C-HARM tracked the nexus of the DA event from SLO to San Diego Counties. SCCOOS Cal-HABMAP and CA Imaging FlowCytobot (IFCB) Network stations are labeled with a circle and IFCB icon.* **b)** *(foreground) CSLs displaying signs of domoic acid poisoning and (background) a deceased long-beaked common dolphin. Credit: CIMWI;* **c)** *beached long-beaked common dolphin. Credit: CIMWI.*

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NEW! Book on Marine Benthic Dinoflagellates (*see page 4*)

Blooms of *Pseudo-nitzschia* are an almost annual occurrence in the California Current System [1, 2]. The last two years have seen sizable blooms with significant marine mammal impacts, but what has made 2023 stand out is the duration of the late spring-early summer bloom and the huge number of marine mammal strandings. California sea lions (CSL, *Zalophus californianus)* are well-known sentinels of domoic acid (DA) events, usually exhibiting distinctive behavioral signs of DA exposure [3,4]. Thus, the first few strandings of symptomatic CSLs at the end of May alerted marine mammal stranding response organizations to an impending problem. Only one week later, it was becoming clear that the dramatic increase in live and dead stranded CSLs and common dolphins (*Delphinus delphis)* throughout the Santa Barbara Channel region was likely due to DA poisoning. The tell-tale signs of the neurotoxin were prevalent in CSLs and other pinnipeds along popular beaches – disorientation, twitching, seizures, erratic behavior – as the event expanded to encompass the region from San Luis Obispo (SLO) County in central California to San Diego County in southern California over the next few weeks (Fig. 1). In fact, many animals displayed extreme aggression with at least seven reports of attacks, some serious, on beachgoers in the months of June and July ([5], CIMWI and PMMC communications).

The California Harmful Algal Bloom Monitoring and Alert Program (Cal-HABMAP) supported by the Southern California Coastal Ocean Observing System (SCCOOS) and the Central and Northern California Ocean Observing System (CeNCOOS) conducts weekly sampling at almost ten nearshore sites throughout California (Fig. 1), with a focus on enumerating the most problematic HAB taxa and analyzing samples in the laboratory for DA [6]. Cell abundance data are typically shared via the HABMAP email listserv a few weeks before toxin data become available in order to alert the community of potential bloom activity. While all the sites except Scripps Pier recorded a dramatic uptick in *Pseudo-nitzschia* spp. in mid-to-late May, only a few of those sites reported bloom levels of the larger, more toxigenic 'seriata' size class and those trends were not long-lasting, leading the HABMAP community to conjecture a possible ephemeral, nearshore bloom that rapidly advected offshore or a primarily offshore origin to the marine mammals DA-poisoning cases. The newly established California Imaging FlowCytobot (IFCB) Network operates robotic microscopes at Stearns Wharf (Santa Barbara), Newport Beach Pier (Orange County), and Scripps Pier (San Diego) for high-frequency, real-time plankton imagery and identification (Fig. 1), which would have been an ideal platform for evaluating nearshore dynamics. Unfortunately, three out of four IFCBs in the region were undergoing maintenance as this bloom developed. HABMAP sampling did capture a resurgence of bloom levels of the 'seriata' size class cells in the second half of June at Monterey Wharf (southern Monterey Bay), Cal Poly Pier (SLO), Stearns Wharf, and Santa Monica Pier (Los Angeles), suggesting a delay in onshore movement of the bloom or even a possible cycle of offshore advection followed by vertical delivery of the *Pseudo-nitzschia* populations onto the shelf via upwelling processes (Fig. 2). In fact, April-May values of the Biological-

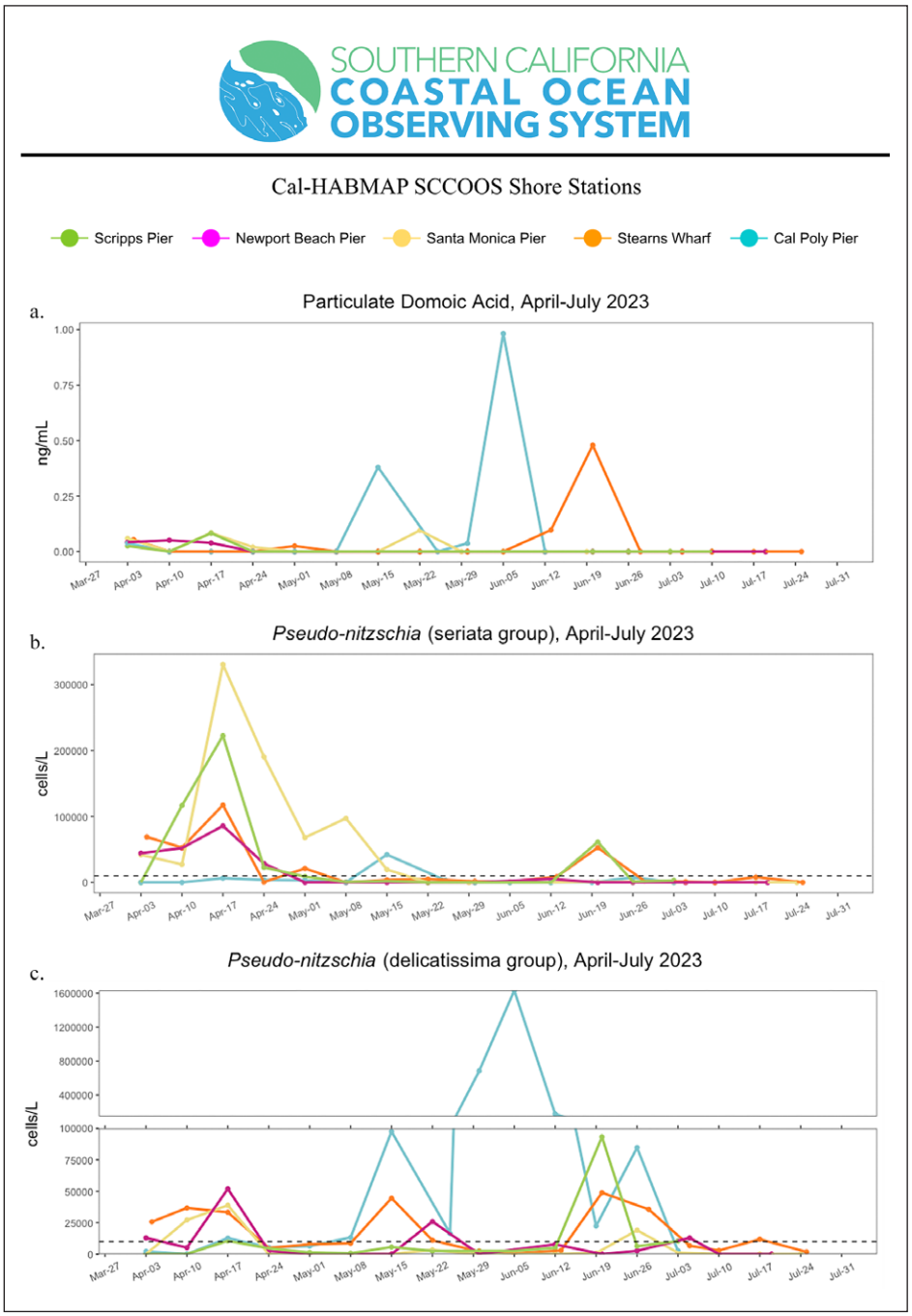

Fig. 2: Measurements from weekly California Harmful Algal Bloom Monitoring and Alert Program (Cal-HABMAP) sampling from May to July 2023 for **a)** *particulate domoic acid (pDA) concentration (ng mL^{-1});* **b)** *Pseudo-nitzschia 'seriata' size class abundance (cells L^{-1}) and* **c)** *Pseudo-nitzschia 'delicatissima' size class abundance (cells L^{-1}). The dashed line on Pseudo-nitzschia plots demarcates the 10,000 cells L^{-1} "bloom" operational threshold. Credit: SCCOOS.*

ly Effective Upwelling Transport Index (BEUTI; *https://mjacox.com/upwelling-indices/*) were anomalously high for central California the last two years, while hovering around average for southern California. This index tells us how much nitrate was likely available for primary production and is a strong indicator of bloom potential, suggesting that conditions in spring 2023 were conducive to supporting a prolonged bloom scenario.

The only synoptic, real-time view of the bloom available to researchers and the public was the California Harmful Algae Risk Mapping (C-HARM) System, a 1-3-day forecast produced by NOAA CoastWatch that predicts both the probability of *Pseudo-nitzschia* spp. blooms (above 10,000 cells L^{-1}) and high-DA surface water (>500 ng L^{-1}) at a 4-km horizontal resolution throughout coastal California [7]. C-HARM predictions homed in on a large swath of the central to southern California coast as an extreme hot spot for DA risk, highlighting the offshore potential for DA production and animal exposure (Fig 1). C-HARM predictions of DA risk have been shown to correlate well with CSL stranding records, but they are not well-suited for predicting DA risk in the most nearshore zones [7]. The HABMAP toxin data were not available until early July when they eventually revealed that there were elevated DA concentrations at Cal Poly Pier as early as May 15th followed by "event" levels of DA by June 5th, but by that date, the *Pseudo-nitzschia* population had shifted from the 'seriata' size class to the smaller, less toxigenic "delicatissima" size class (Fig. 2). This decoupling between cell densities and toxin concentration is a perennial struggle for predicting DA. The biological complexity related to the subtle differences in species, strains, the ratio of particulate to dissolved toxin, and variability in coastal to pelagic toxin vectors all confound our understanding of DA production and food web transfer.

These complexities are apparent when comparing the last two major events in our region. The CSL stranding numbers from the August 2022 DA event last summer were half those of 2023 (~500 vs. >1,000 animals) despite high DA in the water column in 2022 (J. Smith, unpublished data). For dolphin strandings, this year reached well over 100 cases as compared with ~30 in 2022, with many more pregnant females affected this year relative to other years. In addition, 10 live dolphin strandings were reported this year, which is rather rare. In terms of toxicity, the 2022 event resulted in some of the highest DA concentrations measured in animals – 500,000 ng/g in northern fur seal (*Callorhinus ursinus*) feces (R. Kudela, unpublished results). This year, dozens of dolphin samples collected by the Channel Islands Cetacean Research Unit (CICRU) and analyzed by the Northwest Fisheries Science Center's Wildlife Algal-Toxins Research and Response Network (NWFSC/WARRN-West, K. Lefebvre unpublished results) are yielding similar results, with a high of 296,538 ng/g DA in feces from a dolphin collected from Ventura County on 19 June 2023 and many others above 100,000 ng/g. On the human exposure side, there were no new recreational shellfish advisories associated with Amnesic Shellfish Poisoning (ASP) during the 2022 DA event. In 2023, the California Department of Public Health issued ASP advisories for bivalves that lagged shore station DA spikes and marine mammal strandings by almost a month, with the first alert posted on June 16th for Santa Barbara County. Interestingly, subsequent shellfish advisories issued for SLO and San Mateo Counties were due to the threat of Paralytic Shellfish Poisoning (*Alexandrium*/saxitoxin), not in relation to ASP. Given that the highest nearshore DA measured as part of HABMAP was at Cal Poly Pier, it is difficult to explain why no ASP advisory was ever issued for SLO County, although an annual mandatory quarantine for recreational mussel harvests is in place statewide to guard against ASP from May - October. In sum, there is 1) limited information on the conditions supporting DA bioaccumulation and depuration across a suite of bivalve taxa, 2) very little routine offshore sampling, and 3) significant seasonal and interannual variability in environmental conditions that trigger DA production [8, 9]. These remain knowledge and observing gaps for the community. Moreover, mammal and seabird life histories and foraging patterns in conjunction with toxic prey behavior [10] create a multi-dimensional tapestry that currently eludes forecasts of food web impacts.

Given this challenge to predict when and where animals will be most affected by a phycotoxin like DA, first responders at Marine Mammal Stranding Network (MMSN) organizations are often caught off-guard when strandings increase exponentially over the course of one week. When multiple MMSN partners are required to respond simultaneously, not only are volunteer networks strained but coordination between organizations becomes critical. The research community is accustomed to acting quickly to seek science funding, such as the NOAA NCCOS MERHAB Event Response opportunity. Indeed, co-authors have been awarded modest funding in both 2022 and 2023 to work with partners to collect offshore water samples, comprehensively characterize animal tissue toxicity and variability, and improve communication and data sharing across the marine mammal stranding network. Ships-of-opportunity are an essential component of event response when offshore blooms occur, with largely in-kind assistance from multiple non-governmental organizations providing our main characterization of offshore conditions. SCCOOS staff worked with the team to communicate daily with the MMSN, collating C-HARM predictions, news articles, and incoming data from the field into a web application. While rapid response funding is critical for assessing the causes and impacts of stranding events, it is not intended to directly improve staffing, supply, and volunteer shortages for response, rescue, and research activities, and the agencies and responders are also often underfunded to rapidly communicate within the network, other partners, and the general public. This highlights critical structural needs for improving MMSN efficacy through more sustained support and underscores the disconnect between the research community's efforts to understand bloom ecology and predictability and the sometimes loosely connected array of institutions and municipalities tasked with safeguarding protected marine life populations and the public.

What the California HAB response community has learned from two consecutive years with large marine mammal stranding events associated with DA poisoning is that coordination is not just necessary but must be comprehen-

sive, multi-agency, and transdisciplinary. A codified pipeline for communications and data distribution during DA events that is developed during non-event periods, and ideally refined during smaller events, is desperately needed for the California HAB, marine animal, and human health communities to more effectively address animal welfare and public safety concerns, as well as research priorities during future HAB events.

Acknowledgements

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NEW BOOK!

Marine benthic dinoflagellates – their relevance for science and society

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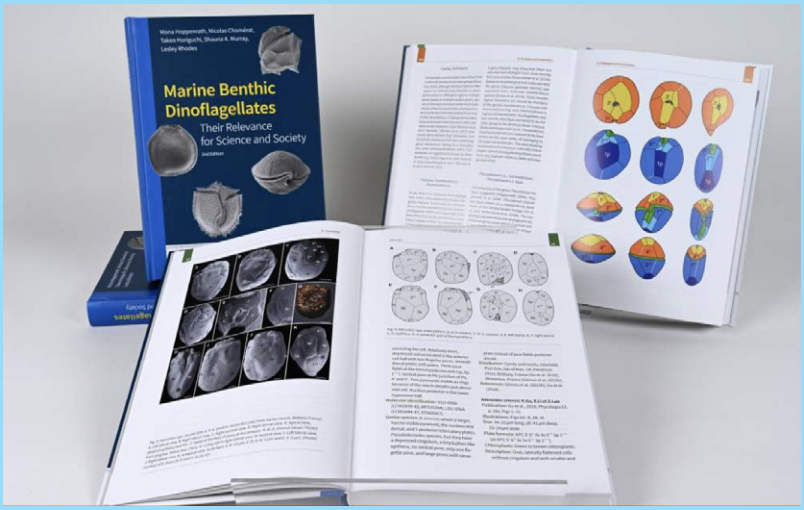

Northward expansion of the Atlantic waters may lead to increased presence of HAB species in the western Barents Sea

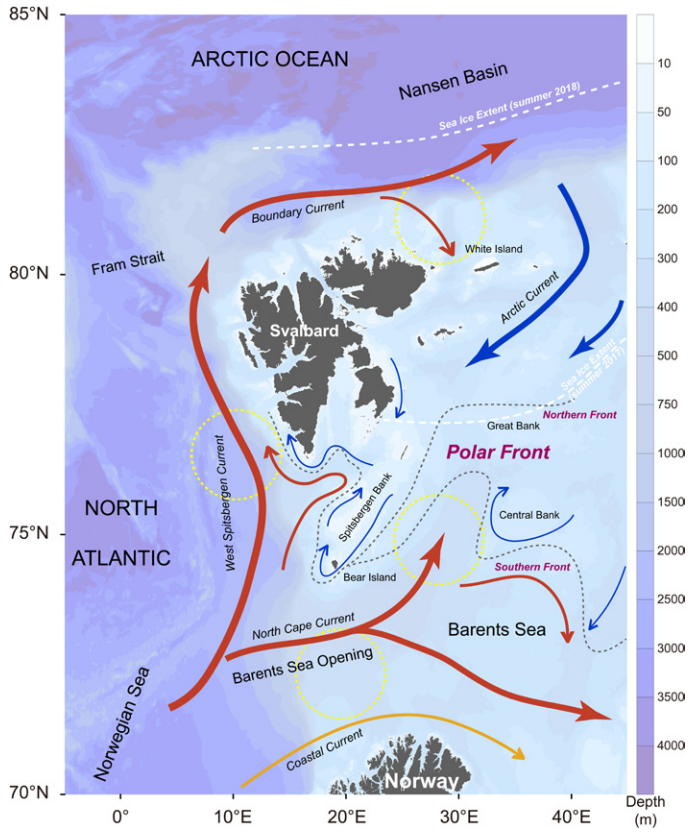

Fig. 1. Study region of the western Barents Sea with diagram of circulations [2] being superimposed on the topographical map (Yellow dashed line cycles indicate major areas of presence of HAB species).

The Barents Sea (BS) is one of the most productive shelf seas in the pan-Arctic region, with great wild-capture fisheries of cod, haddock and capelin [1] along with large populations of marine mammals and seabirds. Here we present an overview of Harmful Algal Bloom (HAB) taxa observed in a suite of research cruises in the western BS (Fig. 1) as a baseline for the monitoring of marine HABs in this region of the Arctic.

Two multidisciplinary cruises were carried out in summer 2017 (30th Jun – 8th Aug) and 2018 (11th Jun to 6th Jul). Discrete water samples of phytoplankton were collected at the surface, subsurface (chlorophyll maximum) and deeper waters (1% of the surface irradiance) using a multi-sensor CTD sampler. After 24 h settling of the Lugol's fixed subsamples in Utermöhl chambers [3], identification and enumeration of targeted HAB species were undertaken with an inverted light microscope.

A total of 85 taxa were observed over the two cruises, of which 24 taxa were harmful, with 15 taxa being potentially toxic. Four taxa (*Alexandrium*, *Dinophysis*, *Pseudo-nitzschia delicatissima* type and *Pseudo-nitzschia seriata* type cells ; Fig. 2) were observed with cell abundances above typical shellfish safety bloom thresholds of 40 cells L^{-1}, 100 cells L^{-1} and 5×10^4 cell L^{-1} [4]. Four other taxa (*Azadinium*, *Heterocapsa*, *Karlodinium* and *Prorocentrum cordatum*; Fig. 3) were observed, but with cell abundances below bloom values reported elsewhere [5, 6, 7].

Domoic acid producing *P. delicatissima* and *P. seriata* groups were observed to be widely distributed in the western BS from the Barents Sea Opening (BSO) across the BS shelf to the shelf break near Nansen Basin (Fig. 2e, f, g, h). The mean abundances of the *P. delicatissima* group reached up to 3.4×10^4 cells/L (maximum of 3.3×10^5 cells/L) and 8.7×10^3 cells/L (maximum of 4.6×10^4 cells/L) from all of the samples collected during summer 2017 and 2018, accounting for 94.1% and 89.0% of the total *Pseudo-nitzschia* abundances respectively. A large bloom (>5×10^4 cells/L) of the *P. seriata* group was recorded from one sample in the southern BSO in summer 2017 (Fig. 2g), with cell abundance reaching 1.6×10^5 cells/L. Although *P. seriata* group blooms were not detected in summer 2018 (Fig. 2h), relatively high abundances of this group were widely distributed far into the north across the BS shelf, probably due to the low sea ice extent (Fig. 1).

The presence of saxitoxin producing *Alexandrium* was very common in summer phytoplankton assemblages of the western BS (Fig. 2a, b), with frequency of blooms (>40 cells/L) of 36.0% and 37.5%, and maxima of 1.3×10^3 cells/L and 260 cells/L in summer 2017 and 2018 respectively. The okadaic acid producing *Dinophysis* also regularly presented in summer seawaters of the western BS (Fig. 2c, d), with bloom abundances (>100 cells/L) of 400 cells/L and 120 cells/L being recorded in the same location (east of Spitsbergen) of summer 2017 and 2018.

Of the other potentially harmful dinoflagellates observed (Fig. 3), the highest abundances of *P. cordatum* reached up to 3.5×10^4 cells/L (mean of 1.7×10^3 cells/L) and 4.6×10^3 cells/L (mean of 411 cells/L) in the waters southwest of Svalbard (Fig. 3g) and east of Spitsbergen (Fig. 3h) in summer 2017 and 2018 respectively. *Azadinium* (Fig. 3a, b), *Heterocapsa* (Fig. 3c, d) and *Karlodinium* (Fig. 3e, f) also regularly presented in the samples, with maxima of 1.4×10^3 cells/L, 2.4×10^3 cells/L and 560 cells/L in summer 2017, and 1.2×10^3 cells/L, 700 cells/L and 620 cells/L in summer 2018 respectively.

There were four major areas of presence of HAB species in the western BS (Fig. 1). Besides the area in the southern BSO, with a high risk of the toxic diatom *P. seriata* group, there were also three major areas (east of Spitsbergen, southwest of Svalbard and north of White Island) at high risk of toxic dinoflagellates. It is obvious that these four areas were closely associated with the northward expansion of the Atlantic waters [2] deriving from the North Cape Current, the West Spitsbergen Current

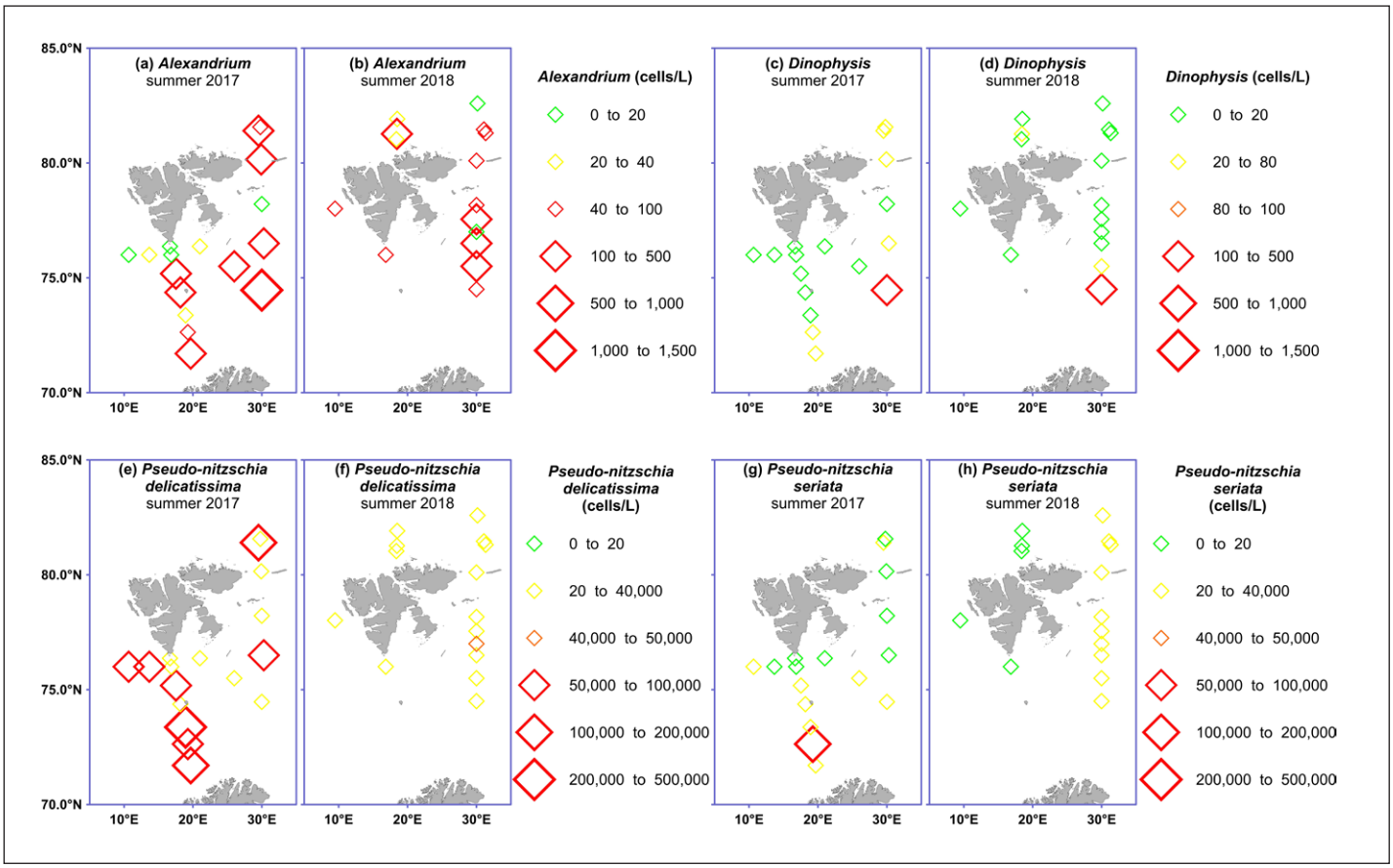

Fig. 2. Distributions of major HAB taxa (cell abundances above bloom thresholds) in the western Barents Sea in summer 2017 and 2018 (Diamonds display the maximum abundance that occurred in the sampling layers).

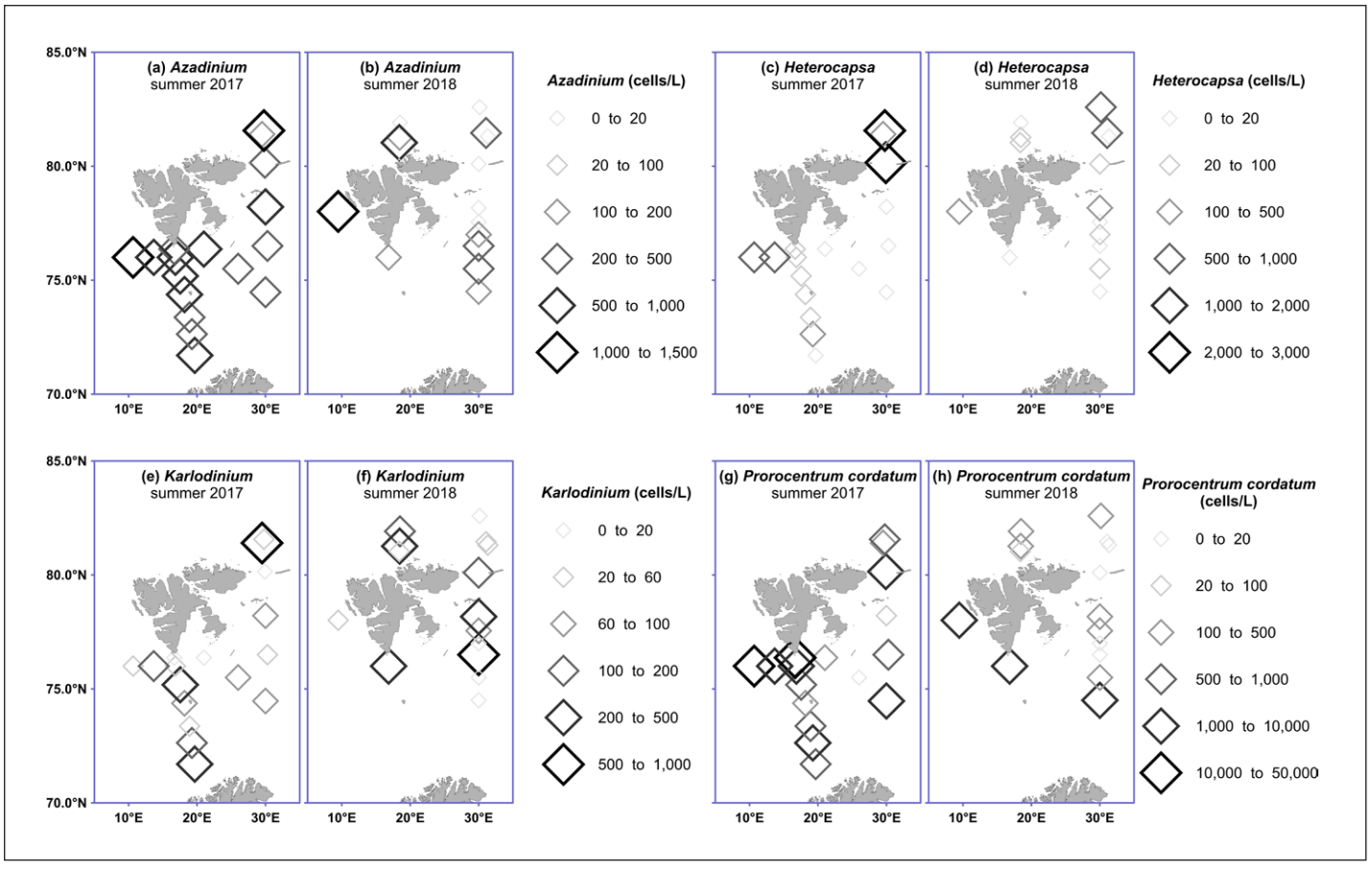

Fig. 3. Distributions of major HAB taxa (cell abundances below reported bloom values) in the western Barents Sea in summer 2017 and 2018 (Diamonds display the maximum abundance that occurred in the sampling layers).

and the Atlantic water boundary current. The encroaching warm and saline Atlantic waters in the BS and the Arctic provided an hospitable environment for the growth and persistence of those HAB taxa. Interestingly, some dinoflagellates (e.g. *P. cordatum*) recorded may behave mixotrophically [8] and could become an important component of the phytoplankton community in late summer or early autumn when the diatom cells began to die and sink out of the surface water allowing greater amounts of lights through.

The Arctic phytoplankton are experiencing borealization [9], a transition from polar to temperate regime. It is foreseeable that the presence of HAB species in the BS will continue increasing in light of the receding sea ice and northward expansion of the Atlantic waters. However, large uncertainty still remains about whether these species bloom locally or are being transferred from southern coastal waters. Field *in situ* evidence has been reported from the Alaskan Arctic waters [10], where the blooms of *Alexandrium* originated from both the transport by temperate Pacific waters and the local germination of resting cysts. Previous field investigation on dinocysts in the western BS [11] revealed the predominance of *Protoceratium reticulatum* cysts in the Atlantic-water influenced surface sediments. Although this yessotoxin producer presented in the waters east of Spitsbergen (summer 2017) and north of White Island (summer 2018), the cell abundances were quiet low (maximum of 40 cells/L) in the total communities.

HAB species, non-toxic or toxic, may lead to seawater discoloration, anoxia, mucilage or toxicity. Shellfish poisoning (of humans and marine mammals) and fish-kill events caused by HAB species are of growing concern worldwide. In the context of a warming ocean, '*Will the Arctic seas be a new HABs hotspot?*' is still a challenging question. Warming of Arctic waters, driven by both atmospheric heating and southern water inflows, may fundamentally altered the ecophysiological characteristics of those HAB species (being transferred or germinating from cysts locally), making them more suitable for a '*temperate state*' Arctic environment. This intrusion of HAB species to the Arctic phytoplankton may lead to cascading effect within the food webs and the entire ecosystem, and may generate potentially far-reaching ramifications to human and animal health through consumption of toxin accumulated seafood from wild fisheries, and from aquaculture should it become more established in the Arctic.

Increasing needs for field observations on these HAB species and toxins (including new or emerging) in the Arctic Seas are urgent, even though such campaigns are routinely performed due to logistic difficulties and lack of infrastructure. New monitoring tools, such as *AUVs* mounted with autonomous cell counters may offer the potential to capture the timing and distributions of these HABs, especially in the late summer or early autumn when dinoflagellates flourish and become dominant in the phytoplankton communities. In short, long-term field studies and modeling efforts should be undertaken now and in the future, to ensure a healthy Arctic marine ecosystem which supports services for the well-being of residents and indigenous people in the pan-Arctic region in a fluctuating climate.

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High-Biomass Harmful Algal Blooms (HB-HAB) in the Chilean Fjords System: *Prorocentrum micans*

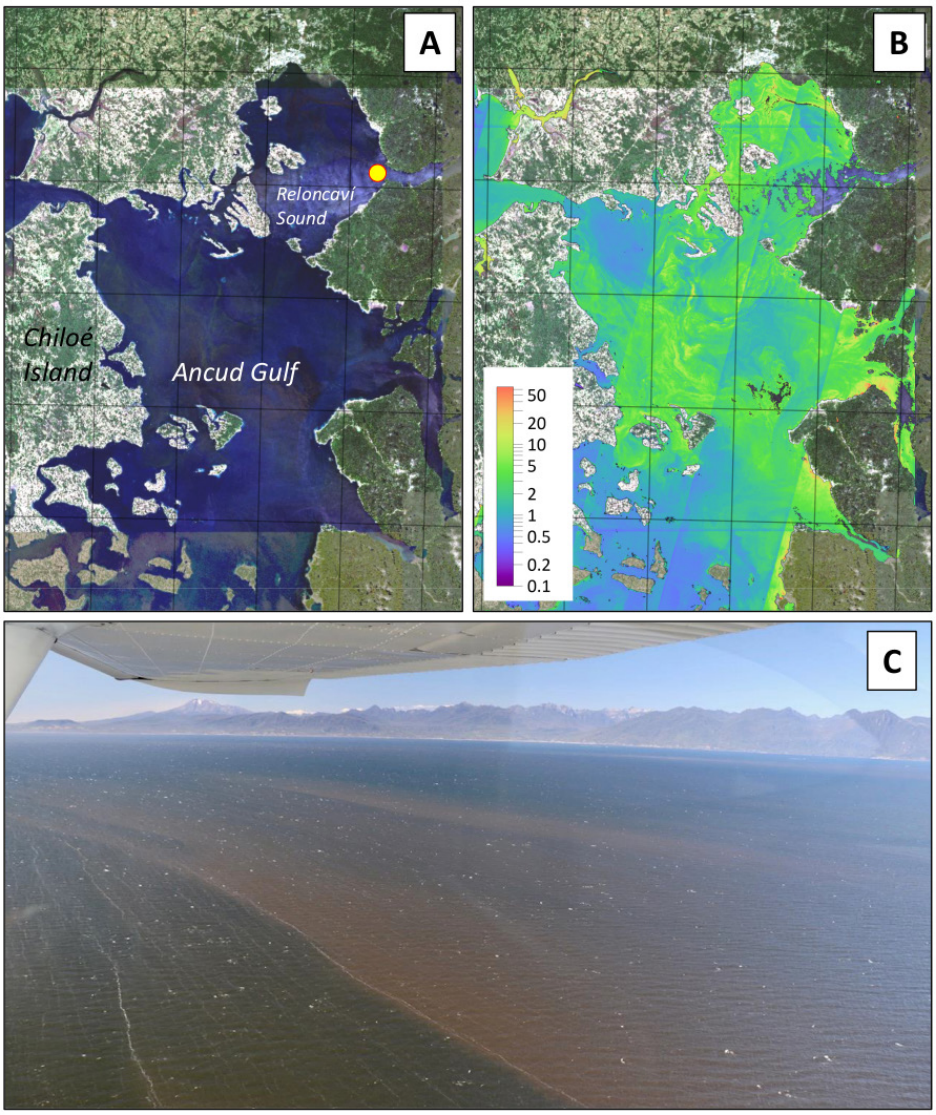

Fig. 1. Bloom of Prorocentrum micans in Reloncavi Sound, NW Patagonia, Chile. A) Location of the bloom; B) Copernicus Sentinel-2 Satellite image showing the distribution of Chlorophyll a (Chl a) in Reloncavi Sound, during the bloom; C) Aerial image of the bloom, showing extensive brown coloration of the surface layer of the sound.

Harmful Algal Blooms (HABs) are recurrent in Patagonian Fjord System. Although High-Biomass-HABs (HB-HABs) have socio-economic repercussions, most studies have focused on toxin-producing microalgae [1]. Two species that form HB-HABs are *Lepidodinium chlorophorum* and *Prorocentrum micans*, dinoflagellates associated with events commonly called "green tides" and "brown tides", respectively. Both species have been recorded in NW Patagonia where they have formed intense surface blooms with increasing frequency over the last years [2].

During the summer of 2022, an intense HB-HAB formed by the dinoflagellate *P. micans* occurred in NW Chilean Patagonia. Intense brown spots visible on the water surface and a strong 'gas' odour were reported at the end of February in the Reloncaví Sound and Ancud Gulf (Fig. 1). The Copernicus Sentinel-2 satellite images showed a colour change corresponding to the presence of intense brown patches on February 22 in the area, which was confirmed by the high chlorophyll *a* concentration (up to 50 µg L^{-1}; Fig. 1B) recorded.

Phytoplankton data from the salmon farming monitoring programme at Reloncaví Sound showed an intense bloom of *P. micans* from February 10 to March 10, 2022. The highest cell density of *P. micans* occurred on February 24, with values > 8.3×10^3 cells mL^{-1} measured at the surface (Fig. 2A). Oceanographic conditions recorded at the i~mar oceanographic buoy, located at the center of the Reloncaví Sound, were related to the temporal variability of *P. micans* cell densities through a T-S diagram showing a marked preference for conditions of narrow, high salinity ranges that fluctuated between 28 and 31 psu (Fig. 2B). Likewise, the highest densities were also recorded with high temperature values (16.5 – 19°C).

Microphytoplankton analysis of samples water collected on February 17, 2022 close to the mouth of Reloncaví Fjord showed a phytoplankton diversity of 35 species. Nevertheless, the phytoplankton community was dominated only by two species, the diatom *Leptocylindrus danicus* (>50.9%) and the dinoflagellate *Prorocentrum micans* (41.9%), with surface cell maxima of 1,195 cells mL^{-1} and 983 cells mL^{-1}, respectively (Fig. 3A). These high cell densities were consistent with a high chlorophyll a (chl-a) signal in the first 5 m of depth (~28 µg chl-*a* L^{-1}), associated with intense thermohaline stratification (Fig. 3B).

One of the environmental variables that has been described as a trigger for this type of event corresponds to the increase in the concentration of nutrients in the water column, mainly ammonium from the degradation of organic matter [3]. The increased N in coastal ecosystems has been driven globally by the intensification of aquaculture, massive land transformation, agriculture, and sewage. Until now, no massive mortalities of farmed salmon associated with HB-HAB of *P. micans* have been reported in Southern Chile. Nevertheless, the presence of intense areas of visible water discolouration in coastal areas of Reloncaví Sound, alongside the presence of an intense fetid odour, similar to decomposing organic matter reported by many residents in different localities in the area during the event, triggered a public alarm in the local communities, mainly in Puerto Montt city. This fetid odour is most likely linked to the emission of dimethyl sulfide gas or DMS which is the result of the decomposition of the dimethyl-sulfoniopropionate

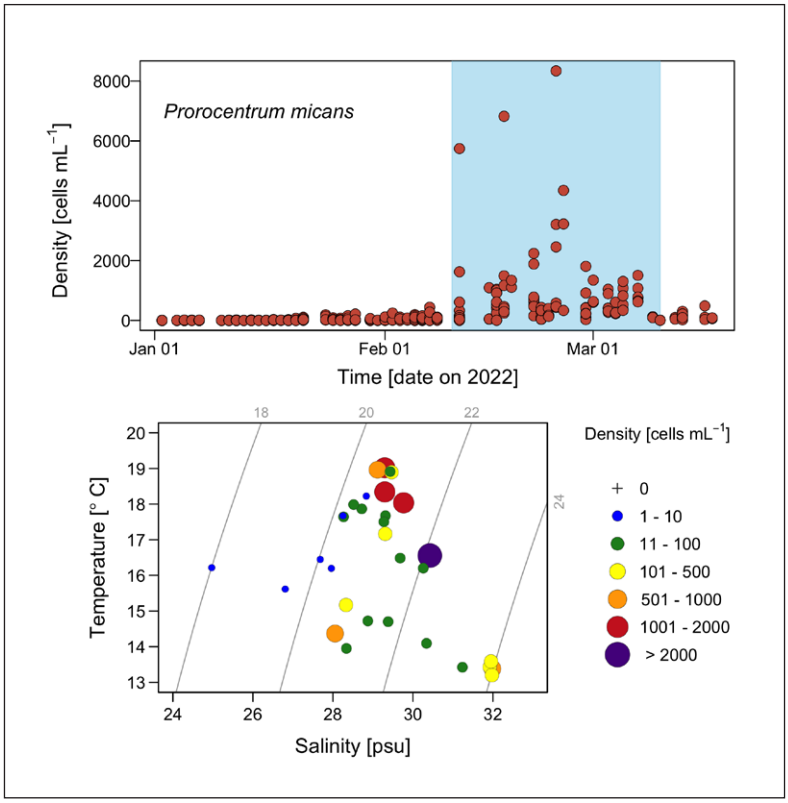

Fig. 2. Cell densities of Prorocentrum micans and their occurrence with temperature and salinity. A) Cell densities of P. micans, before, during and after the bloom; B) Cell densities of P. micans as functions of surface water temperature and salinity.

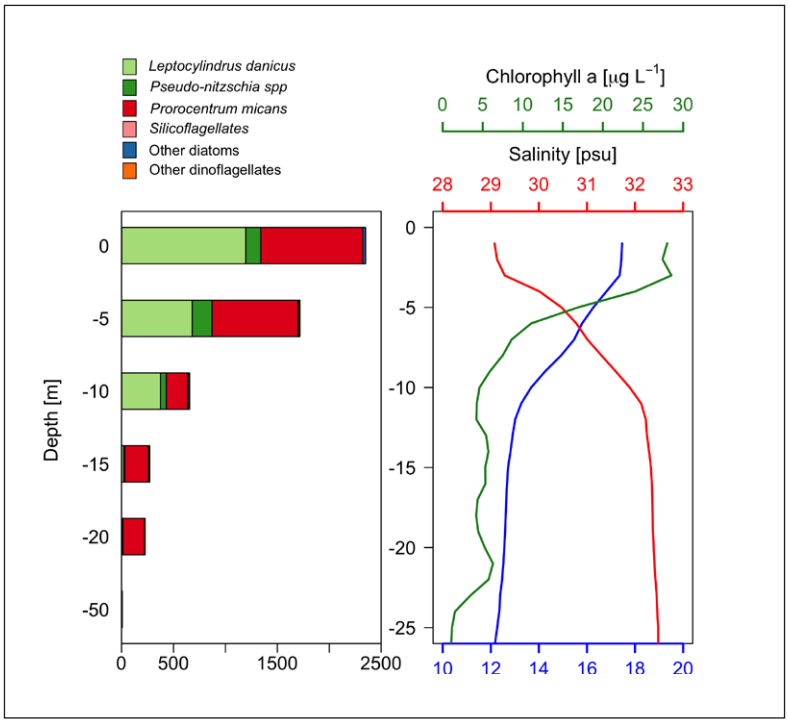

Fig. 3. Phytoplankton assemblage during the bloom of Prorocentrum micans at different water column depth (0–50 m), and vertical profile of temperature, salinity and Chlorophyll a in the water column (0–25 m).

(DMSP) produced by the bloom of *P. micans*, following grazing by higher trophic levels and lysis by microbes [4-5].

Recently, Diaz et al. [6] described HB-HAB events in Chilean Patagonia that are associated with dry summers and high solar radiation. Thus, considering the climatic trend observed in recent decades and the future projections for this area, the risk of increased occurrence and intensity of HB-HAB may increase. Consequently, the lack of knowledge about the basic aspects of the biology and behavior of both species (*L. chlorophorum* and *P. micans*) in coastal waters of southern Chile's fjord systems, as well as the critical levels (cell density) at which they begin to induce negative impacts on both the regional aquaculture industry and the public health, has made it difficult to implement timely management measures that would allow the mitigation of their negative impacts.

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Identification of Caribbean ciguatoxins from benthic dinoflagellates advances knowledge on toxin chemistry and analysis

Ciguatera poisoning (CP) is a serious food-borne illness caused by the consumption of seafood from endemic tropical and sub-tropical regions, including the Pacific, Atlantic and Indian Oceans, and the Caribbean Sea, that contain highly toxic ciguatoxins (CTXs) that are present at very low levels [1]. Ciguatoxins are produced by *Gambierdiscus* spp. and transferred through the marine food web following consumption by reef herbivores. Microalgal species responsible for the production of CTXs in the Pacific Ocean (P-CTXs) were identified in the last decades of the 20th century, facilitating the production and purification of P-CTXs from culture, analytical method development, and leading a deeper understanding of the metabolism, trophic transfer, and toxicology of P-CTXs[2, 3].

Ciguatera poisoning in the Caribbean is associated with a class of CTXs that were initially isolated and identified in fish from the region. Lewis *et al.* first identified Caribbean CTX-1 (CCTX1) in the late 1990s[4]. Since that time, no additional C-CTXs were definitively identified, nor had an algal source of C-CTXs been confirmed with certainty, although *Gambierdiscus* spp. were suspected to be involved based on *in vitro* bioassays. It has since been shown that C-CTXs occur in fish not only in the Caribbean Sea, but throughout the tropical Atlantic Ocean and even in the Mediterranean Sea[5]. However, the lack of an identified algal source, and the difficulty of producing sufficient purified C-CTXs from fish, has impeded progress on the toxicology, analytical chemistry, and ecological risk assessments of this toxin group, and has hindered the development of effective mitigation strategies for CP management in these regions.

In an attempt to address these issues, an international collaborative project, CiguaPIRE, was initiated in 2017 funded by the US National Science Foundation with parallel support from the Norwegian Research Council, Center for the Environment, Aquaculture and Fisheries Sciences (UK), and the National Research Council of Canada[6]. The project includes global partners from the US, UK, Canada, Norway, and Australia, along with partnerships with a large group of both sovereign island nations and dependent island territories throughout the Caribbean and Western Atlantic. The overall objectives of this global initiative were to examine the dynamics and persistence of toxigenic benthic dinoflagellates and their metabolites in reefs around the globe to better understand the production and fate of C-CTXs. To enable this, it was necessary to develop improved analytical and purification methods for CTXs to allow identification and sensitive detection of an array of novel toxins in algae, invertebrates, and fish.

The initial focus of chemical research within CiguaPIRE included isolation of C-CTX1 from fish, and use of this semi-purified material for method development and *in vitro* metabolism studies that led to the identification of novel glucuronides of C-CTX1 [7]. The availability of C-CTX1 also led to the confirmation of the metabolites C-CTX3 and C-CTX4 as the C-56 reduction products of C-CTX1, through a series of chemical transformations combined with LC–HRMS comparisons with extracts from naturally contaminated fish [8]. Furthermore, the production of isotopically labelled C-CTX1[9], and development of methods for selective boronate extraction of C-CTXs (and a range of P-CTXs) from fish extracts, hold promise for improving analysis time while reducing the impacts of potential matrix effects and interferences[10].

The improved LC–HRMS method

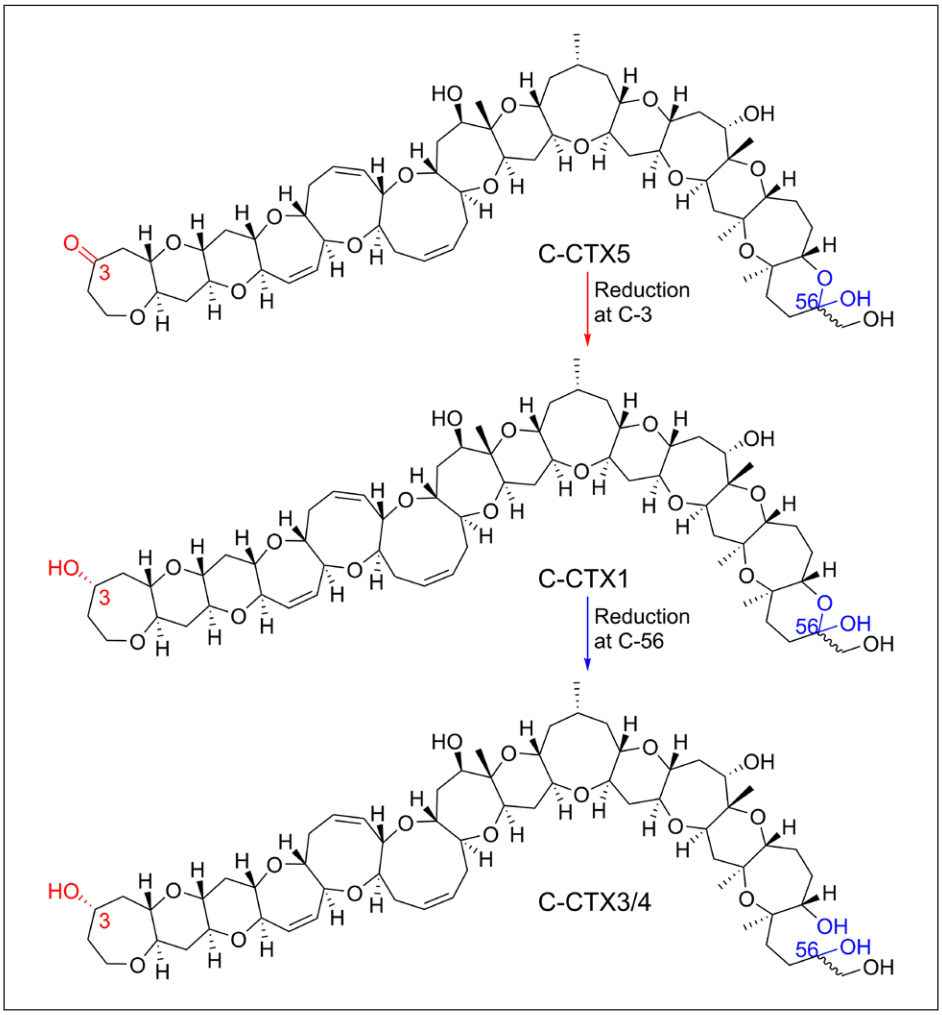

Fig. 1. Structures of the new algal precursor, C-CTX5, and its relationship to C-CTX1 and C-CTX3/4 previously identified in fish.

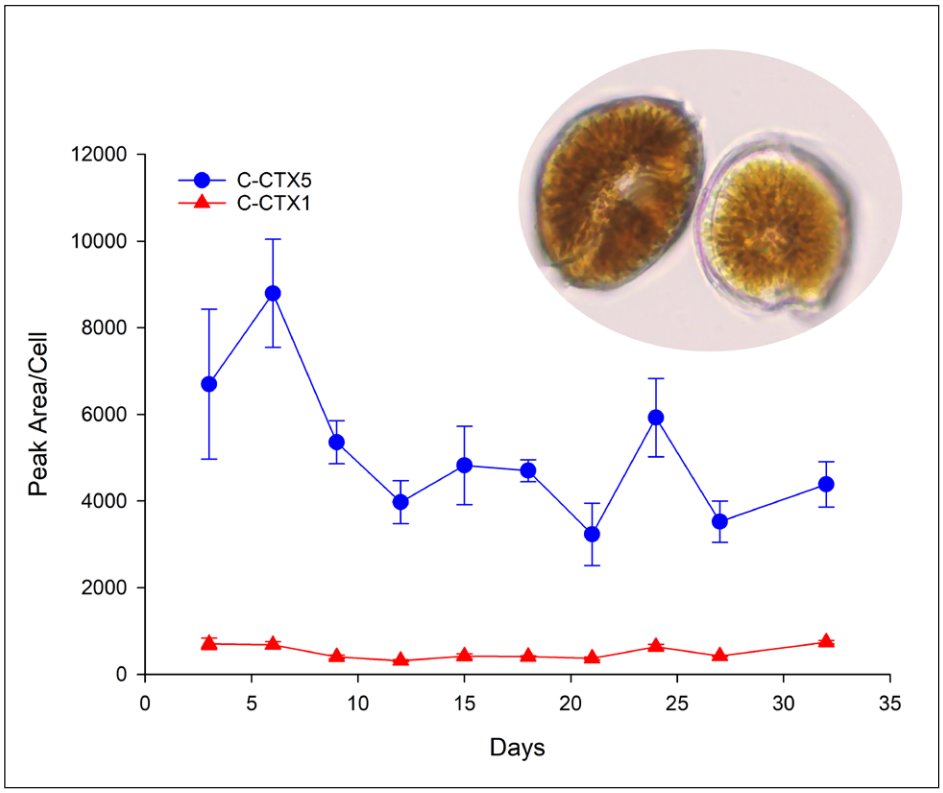

Fig. 2. Toxin content per cell (peak area per cell) grown in L1 medium at a salinity of 35‰ over 32-days (error bars: standard deviation of three culture replicates). Inset: Light microscopy image of Gambierdiscus silvae cells in culture (×400 magnification)

was used to identify a putative C-CTX1 analogue, named C-CTX5 (Fig. 1), in established cultures of *Gambierdiscus silvae* and *G. caribaeus* from the Caribbean Sea[11]. The chemical transformation and isotopic labelling procedures developed in the project for C-CTX1 and C-CTX3/4 were applied to C-CTX5 to unambiguously show that it was 3-oxoC-CTX1. It was then demonstrated that C-CTX5 was biotransformed to C-CTX1 by fish liver microsomes, showing that it is a precursor to the C-CTX1 found in fish. In a semiquantitative study, C-CTX5 was shown to have slightly lower ciguatoxin-like activity than C-CTX1 in an *in vitro* mouse neuroblastoma assay (N2a-MTT), suggesting that it has qualitatively similar sodium channel activity to other C-CTXs. These findings are significant and pave the way for a wide range of studies that were previously not possible.

Initial efforts focused on large-scale culturing of the strain of *G. silvae* (1602 SH-6) that was confirmed to produce C-CTX5 along with lower levels of C-CTX1, with the goal of purifying sufficient material for structural elucidation and studying the stability of the compounds. Over the time course of large-scale culture C-CTX production per cell was consistent (Fig. 2). Large-scale culturing and subsequent isolation of C-CTXs will allow structural confirmation by nuclear magnetic resonance spectroscopy and enable production of small amounts of much-needed quantitative reference materials. Progress in these aspects is vital for subsequent development and validation of *in vitro* bioassays, immunoassays and LC–MS-based methods for C-CTX analysis. Application of such methods in conjunction with previously developed sample clean-up techniques will greatly improve the tools available to the scientific community for understanding and managing the risks presented by C-CTXs in the marine foodweb.

Acknowledgements

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Exploring the current and historic health of lakes in Aotearoa-New Zealand

The biggest survey of current and historic lake health in Aotearoa-New Zealand is nearing completion. The ambitious Lakes380 Programme (*www.lakes380.com*), co-led by the Cawthron Institute and GNS Science, kicked off in 2017 with the lofty target of sampling 10% of the lakes in Aotearoa-New Zealand. The project involved sampling and analysing lake water and surface sediments to understand contemporary lake health. Lake sediment cores were also collected and analysed to provide lake histories dating back more than a thousand years (prior to human arrival in Aotearoa-New Zealand).

The team recently released the results from their national-scale survey of over 300 lakes [1] and subsequent water quality modelling for all the lakes in Aotearoa-New Zealand. This revealed that nationally, 45% of the lakes in Aotearoa-New Zealand are estimated be in poor or very poor health (Fig. 1). More gripping are the regional variations observed, with over 80% of lakes in the North Island in poor health or worse, and in some regions of the country this reaches 90%. In summer, many of these lakes in poor health will experience severe cyanobacterial blooms that have the potential to produce toxins. This information can also be viewed in an online app (*https://lakes380.upshift.co.nz/*).

By studying the lake sediment cores, the team has been able to track the history of cyanobacterial blooms, showing that in most lakes this is a recent occurrence (50 years) [2]. The research indicates that this is related to land-use intensification (which can lead to nutrient enrichment through fertiliser run-off and sediment in-flows) and the introduction on non-native fish (which can dramatically alter food webs). The development and application of molecular methods to detect organisms that do not leave fossilised remains in lake sediments have been invaluable in this endeavour. In some cases, teasing out the drivers of lake health degradation has only been possible through weaving together paleoecological studies of lake sediment cores with additional pieces of information such as Indigenous knowledge [3] or data on algal ecological niches [4]. Despite the challenges, proper understanding of the key drivers of degradation are critical for guiding effective restoration strategies.

Another significant focus of the project was working with Māori (the Indigenous people of Aotearoa-New Zealand) to understand more about the relationships they have with their lakes and to further empower them in their lake restoration aspirations. A digital story platform was created to document and share these experiences (*https://www.lakestoriesnz.org/*). The team also developed a virtual reality platform to transport people to a remote lake in the top of the South Island (Lake Moawhitu) and allows them to travel backwards and forwards in time (*https://lakes380.com/he-reo-no-te-puehu/*). Through this medium, people can see how changes in the landscape surrounding the lake have impacted the water quality and the creatures and plant that live in it. The virtual reality platform was co-designed with Ngāti Koata – the iwi (tribe) who have territorial rights for Lake Moawhitu and a long

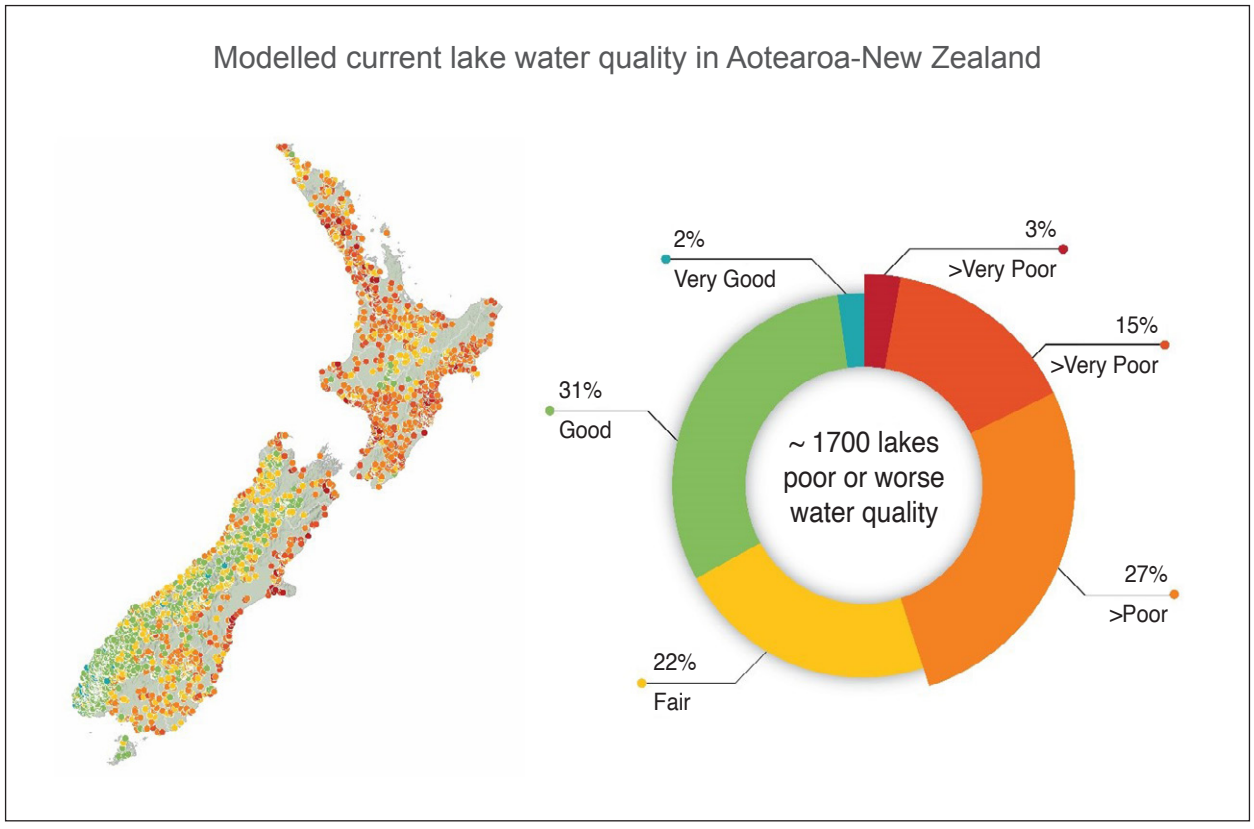

Fig. 1. Data from 'Our lakes' health: past, present, future' – www.lakes380.com

Virtual reality example - Lake Moawhitu (Rangitoto ki te Tonga/d'Urville Island)

Examples from a three-dimensional virtual reality platform created in 'Our lakes' health: past, present, future' in partnership with Ngāti Koata and Department of Conservation. www.lakes380.com/he-reo-no-te-puehu/



history with the lake – allowing their knowledge and history to be woven into the experience, and their restoration vision for the lake to be communicated with their people.

The Lakes380 Programme has provided a strong foundation of knowledge on the current condition and history of lakes in Aotearoa-New Zealand. The team is now shifting their focus to revitalising lakes in Aotearoa-New Zealand, through the development and implementation of holistic approaches to ensure that resources are efficiently targeted. This will require the development of new tools that can guide effective lake restoration, and strategies to engage communities in the multi-generational journey to rejuvenate the precious lake ecosystems in their regions.

Acknowledgements

This research was funded by the New Zealand Ministry of Business, Innovation and Employment research programme - Our lakes' health; past, present, future (Lakes380; C05X1707). We thank all members of the Lakes380 team for field assistance (*www.lakes380.com/about-the-project/the-team*). We acknowledge the support of regional authorities, iwi, Department of Conservation and landowners nationwide for their assistance with sampling, accessing sites and guidance throughout the programme.

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Expansion of the Cawthron Institute Culture Collection of Microalgae (CICCM)

The CICCM is housed at the Cawthron Institute in Nelson, Aotearoa New Zealand, and in recent years has seen an increase in the number of sub-tropical species maintained [1]. The curated collection (Fig. 1) is partly government funded (through the Ministry of Business, Innovation and Employment) and partly funded by the Cawthron Institute.

Since the last article in HANews [2], the collection has increased to nearly 700 isolates, half of which are cyanobacteria and which are cryopreserved. In the last two years the regions represented have expanded to include polar diatoms and dinoflagellates (in particular *Polarella*) from Antarctica. Diatom species have also been isolated from sea ice brine, seawater, and samples collected from the sea ice/platelet interface. Doctoral student Jacqui Stuart travelled to Antarctica to collect these samples for a portion the 2022 Science field season with funding from the Antarctica New Zealand Sir Robin Irvine Doctoral Scholarship program. During her time in Antarctica Jacqui also assisted with work from two research projects, one led by NIWA scientist Dr. Natalie Robinson in collaboration with Prof. Ken Ryan from Victoria University of Wellington (VUW) assessing the sub-ice platelet ice using novel sampling equipment developed at NIWA. The second research project was led by Dr Andrew Martin (VUW) and Prof Ken Ryan (VUW) investigating light sensitive bacterial communities within sea ice. During the field season time was set aside for Jacqui to collect live microalgal samples from multiple sites with different ice conditions. These isolates will be used to understand the impact of changing environmental conditions on these species, and assess how this may influence the ecosystems that rely on them. The 2022 Antarctic field season was exceptionally challenging with the late development of sea ice and harsh weather causing snap freezing of some of the sampling equipment. Even with these challenging conditions Jacqui thoroughly enjoyed her experience and feels exceptionally privileged to have camped on sea ice, seen the last sunset of the season and stood in the shadow of Mt Erebus, a truly humbling and life changing adventure.

To make enquiries about microalgae in the CICCM contact Sarah Challenger through the Cawthron Institute website: *https://www.cawthron.org.nz* › ciccm.

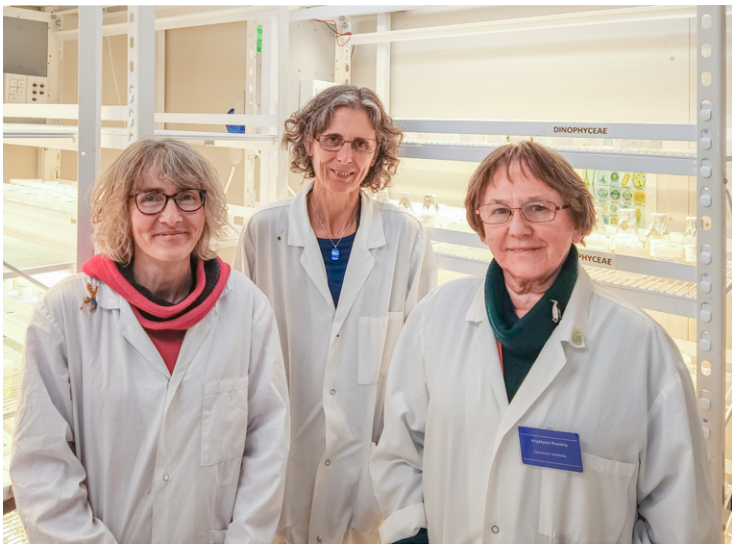

Fig. 1. Curation of the CICCM is caried out by (from left) Sarah Challenger, Lucy Thompson and Krystyna Ponikla.



Fig. 2. Expedition to Antarctica, 2022. Top: Jacqui Stuart on the ice (image J. Stuart); Bottom: Jacqui Stuart and Prof Ken Ryan measuring the thickness of the ice (image: Svenja Halfta, NIWA).

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HABs in ASLO 2023



The Association for the Sciences of Limnology and Oceanography (ASLO) held its annual meeting from 4 – 9 June 2023 in Palma de Mallorca, Spain. ASLO 2023, the first post-covid in-person ASLO meeting in Europe, under the general theme "Resilience and Recovery in Aquatic Systems", drew around 2400 attendees from 72 countries.

Harmful algal blooms, with six theme sessions, had a strong presence. Topics included mechanisms of HAB development; advances in monitoring and mitigation with better prediction (improved models) or direct treatment to eliminate the toxic cells; and a good component of next generation tools used to answer questions that were so far unapproachable. In this HAN issue we present the highlights of two successful sessions. One of these sessions was dedicated to the "Mixoplankton", the new term used to define planktonic protists conventionally considered as "phytoplankton", which can trap live food like a micro-zooplanktonic organisms. The other session focused of dinoflagellates of the genus *Karenia*, in particular *K brevis* the agent of multiple noxious effect in the Gulf of Mexico.

The editors

The Mixoplankton Paradigm in Planton Ecology

The past decade has seen the recognition of diverse plankton functional groups in marine ecology, beyond the classical phyto- and zooplankton. The mixoplankton paradigm overturns the traditional idea that all plankton can be analogized as "little plants" (phytoplankton) or "little animals" (zooplankton). In reality, many, perhaps most, planktonic organisms have the metabolic capability of synergistically employing both phototrophy and phagotrophy; because of this mixed trophic mode, they have been dubbed "mixoplankton". The base of the marine food web is thus not as traditional science describes, raising profound questions about how we project climate change and allied anthropogenic events in reshaping the marine ecosystem and thence how we manage resources. To address such challenges, *SCOR Working Group 165* – '*MixONET*' was recently formed. This ASLO session (Mixoplankton: The New Paradigm Testing the Resilience of Our Science in the UN Ocean Decade), consisting of *12 oral and 1 poster presentations*, focused on the overarching aspiration of MixONET to extend the mixoplankton paradigm beyond blue-sky research to real-life applications such as environmental monitoring, management, policy making, and education (Fig. 1).

Mixotrophy, as the coupling of photo-osmo-mixotrophy (i.e. photosynthetic organisms that also take up dissolved organic matter such as vitamins, urea, amino acids, etc.), has been researched within microalgae, and especially associated with Harmful Algal Bloom (HAB) events, for decades. The new mixoplankton paradigm incorporates into the food web those protist plankton that engage in photo-osmo*phago*-mixotrophy, using uptake of particles to supplement their nutritional needs. In the contexts of autecology and community ecology, there are major differences between the photo-osmo-mixotrophic (i.e. phytoplankton such as diatoms) and the photo-osmo-phago-mixotrophic (i.e. mixoplankton) microalgae (talk by **Aditee Mitra**; "Mixotroph or mixoplankton: what's in a name?"). Mixoplankton actively hunt, kill and eat, thus acquiring nutrients and directly impacting trophic dynamics through removal of prey/competitors.

Various exemplar protistan 'phytoplankton' and 'zooplankton' species such as the food web supporting *Tripos* and *Stromibidium* species, the ecosystem disruptive bloom forming green *Noctiluca scintillans*, and the HAB forming species within the *Alexandrium*, *Prorocentrum*, *Dinophysis*, and *Prymnesium* genera, are all mixoplankton (**Luciana Santoferrara**; "Global distribution of marine mixoplankton based on morphological observations and metabarcoding"). Some mixoplankters (e.g., *Alexandrium*) have their own chloroplasts while others need to acquire phototrophic capability by retaining plastids from ingested prey items (e.g., *Dinophysis*) or by hosting symbiotic phototrophs (e.g., green *Noctiluca*). Tracking mixoplankton distributions, spatially and temporally, based on morphological identifications or DNA sequences is now facilitated by the recently launched *Mixoplankton Database (MDB)*. The MDB is currently being cross-referenced with the IOC-UNESCO Taxonomic Reference List database of over 200 HAB species to create a reference for the many HABs that are mixoplankton. Importantly for science, in moving away from a plant-animal dichotomy in marine ecology the term mixoplankton provides concise clarity. The MDB provides a tool for us to redefine the core underpinning of marine science, to perform keyword searches, to differentiate database entries, model descriptions, and to reflect the biogeochemical and ecological consequences.

Outputs from climate change models underpin various environmental management policies. Yet, climate change models are still based on the outdated phytoplankton-zooplankton dichotomy while HAB events are typically predicted using modelling frameworks that focus on physics rather than biological interactions. Mixoplanktonic activities would be expected to have far reaching impact on higher trophic levels and thence ecosystem services. Many efforts are underway to understand these



Fig. 1. Oral communications presented at the ASLO-2023 Session SS018 on Mixoplankton in Plankton Ecology.

trophic interactions and incorporate them into models of aquatic food webs. The importance of light and nutrient availability in mixoplankton succession and thence in ecosystem structure and functioning has been shown using a substantial dataset from 98 freshwater tropical lakes (**Mariana Costa**; "Ecosystem size drives patterns and control mechanisms of mixotrophs success across tropical lakes: A large-scale assessment of the grand écart hypothesis"). Incorporation of functional diversity of protists under the mixoplankton paradigm within 3D coupled biogeochemical models has provided a better understanding of the impact of environmental stressors on plankton trophic dynamics in a regional bay off the UK coast with aquaculture facilities (**Molly James**; "Addressing the challenges of modelling ecosystem services in 3D under the mixoplankton paradigm").

Various studies have shown the importance of considering impact of environmental stressors on the ecophysiology of mixoplankton and consequent implications for the overall plankton community structure and higher trophic levels. For example, laboratory experiments suggest that the "fish-killer" *Prymnesium parvum* could have a higher feeding efficiency when provided particulate matter compared to live prey (**Clémence Boucher**; "Mixotrophic growth of the ichthyotoxic Golden algae, *Prymnesium parvum*, under different sources of phosphorus"). Species of the cosmopolitan genus *Prorocentrum* have been shown to use a remarkable feeding strategy by crafting globular mucus traps of up to 120 µm diameter to capture and immobilize potential prey ranging from bacteria to *Teleaulax* (**Urban Tillmann**; "Mucus trap assisted mixoplanktonic activity in *Prorocentrum*") (Fig. 2).

Dinophysis spp. is a globally ubiquitous genus that produces lipophilic toxins resulting in diarrhetic shellfish poisoning (DSP) events and closures of shellfisheries. However, current protocols in monitoring programmes are poorly designed to sample these low density non- constitutive mixotrophs, and very often their ciliate prey organisms are destroyed with fixative or mechanical damage. (**Beatriz Reguera**; "Key parameters and procedures to improve monitoring and forecasting of a rare and specialized protist - *Dinophysis* species"). Acquired phototrophy in *Dinophysis* spp. is supported by plastids from the *Teleaulax*/*Plagioselmis* cryptophyte clade. However, due to mis-match in size, *Dinophysis* cannot capture cryptophyte cells directly but retains chloroplasts from its prey, the ciliate *Mesodinium* spp., which also retains cryptophyte plastids. While the plastids remain active in *Dinophysis*, its control of their division and function is limited. Due to the deleterious impact of *Dinophysis* blooms on ecosystem services, the transfer of functional chloroplasts within the cryptophyte-*Mesodinium*-*Dinophysis* complex has been the basis of various studies. More recent studies have focused on the impact of climate change and environmental stressors on this complex. Laboratory studies suggest that changes in ocean carbonate chemistry would not have any significant effect on the cryptophyte or *Mesodinium* spp. (**Per Juel Hansen**; The combined effect of pH and dissolved inorganic carbon concentrations on

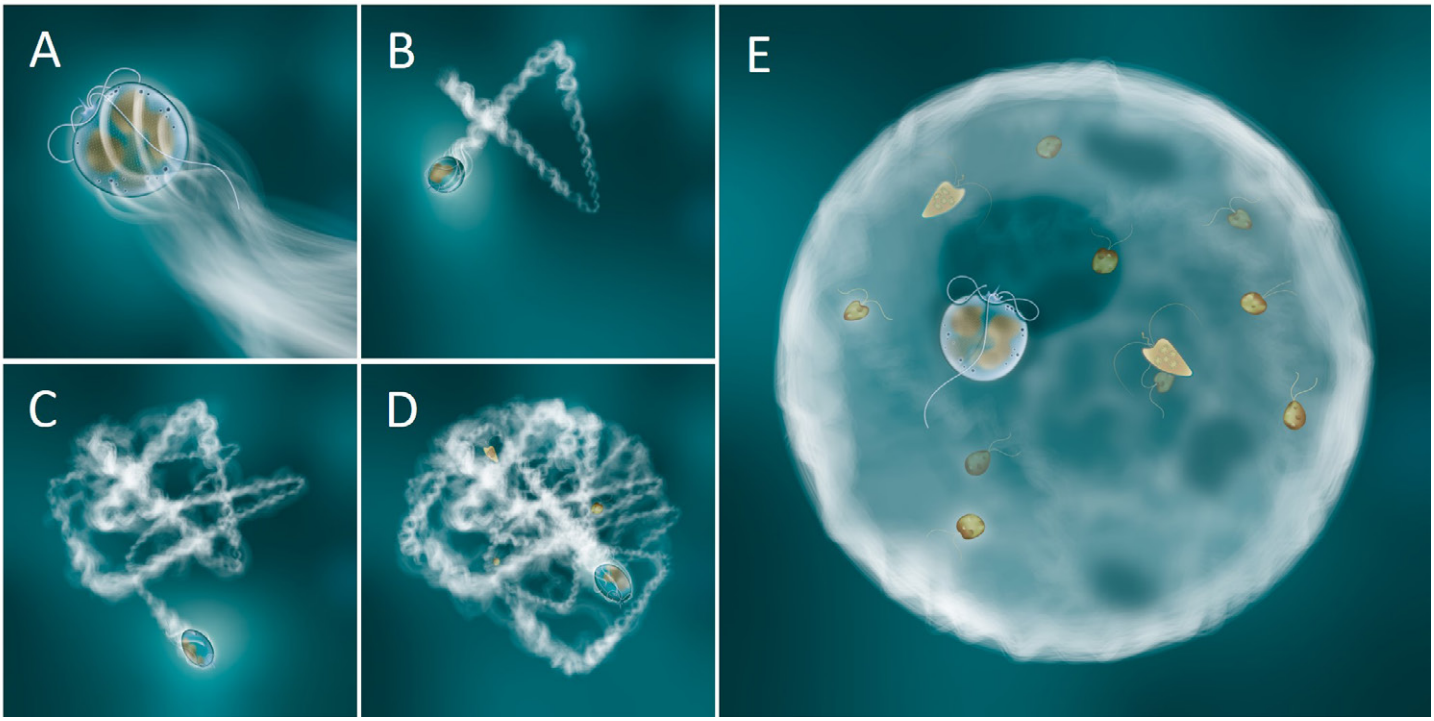

Fig. 2. Suggested sequence of events leading to the formation of the mucus trap by Prorocentrum pervagatum. (A) Solitary cells (cell length ca. 13µm) swim in a spiralling style, releasing mucus from the large pores. (B–D) Under certain conditions, most likely of very low turbulence, the spiral swimming style coupled with random changes in direction lead to a process of mucus deposition and physical pushing by the Prorocentrum cell that leads to the formation of a hollow mucus ball. As the mucus ball takes shape other particles, and notably motile potential prey, become stuck to the outside surface. Towards completion of the trap, with the Prorocentrum now located within the hollow ball near one of the few remaining openings, the continuing rotational swimming of the dinoflagellate draws water and particles in through the large opening (a "rotating position"), with water exiting through the porous mucus walls, leaving particles trapped on the inside of mucus ball. Trapped prey are immobilized by a combination of chemical (allelochemical) and physical (mucus threads) means, and may then be consumed (E). When the trap is vacated, the decaying remnants then sink, while the Prorocentrum builds another trap. Image from [1]

the plastidic ciliate *Mesodinium rubrum* and its cryptophyte prey). However, low light levels of different wavelengths are expected to alter the competitive advantage in the ability to acquire and maintain plastids by *Mesodinium* and *Dinophysis.* Promising results with last generation transcriptomic tools showed differences in nuclear encoded genes in *D. ovum* controlling the synthesis of phycoerythrins. This difference enables *D. ovum* to photoadapt and may prove useful to prepare molecular probes against the two species (**Sarah Garric**; "Photoacclimation of Cryptophyte plastids of *Plagioselmis prolonga* in their hosts *Mesodinium rubrum* and *Dinophysis acuminata"*). In contrast, *Strombidium rassoulzadegani*, another non-constitutive mixoplankton, can retain and use plastids from chlorophytes and cryptophytes, but appears to grow wholly heterotrophically on dinoflagellates (poster by **George McManus**; "Laboratory studies with the model ciliate mixoplankter *Strombidium rassoulzadegani*").

The response of the different mixoplankton species to climate change and thence prediction and detection of HAB events is a formidable challenge for scientists and managers. Methods (field, laboratory, modelling) developed for phytoplankton and zooplankton need to be adapted for mixoplankton science - where required, new methods need to be developed. For example, the widely used dilution technique was developed for studying grazing on phytoplankton by zooplankton and in its current form cannot account for mixoplanktonic activities where a single-celled organism employs photo-phago-mixotrophy synergistically (**Guilherme Ferreira**; "Acknowledging mixoplankton within dilution grazing experiments"). Laboratory studies have shown that chlorophyll, used as a proxy for phytoplankton biomass in traditional dilution experiments, is inadequate for mixoplankton and that this technique needs to be modified to consider diel feeding rhythms in the experimental design.

A methodology that has been employed successfully in monitoring the mixoplanktonic HAB *Dinophysis* and could potentially be adapted for monitoring other protist plankton is the Imaging FlowCytobot (IFCB) (**Lisa Campbell**; "Continuous automated imaging flow cytometry for early warning of the mixoplankton harmful algal bloom forming *Dinophysis ovum"*). The IFCB, combining flow cytometry with video technology and machine learning, can identify and enumerate not only the HAB *Dinophysis* but also its source for acquired phototrophy – the ciliate *Mesodinium*. Discrimination between different "species" of the *Dinophysis acuminata* complex, with identical ribosomal and mitochondrial gene sequences, is also crucial in monitoring programmes, in particular in regions where one of the species has a much higher toxic potential than the other (i.e. Southeast US coasts). The automated sampling program deployed at the Texas Observatory for Algal Succession Timeseries (TOAST) has enabled early detection for HAB species, especially *Dinophysis ovum*. Furthermore, these long-term data from TOAST have been integrated into a mechanistic model describing the interactions between the mixoplanktonic *Dinophysis* predator and its prey (**James M. Fiorendino**; "Modeling the effect of prey size on *Dinophysis* bloom dynamics"). Simulations using this model framework showcase the importance of consideration of the ecology and physiology of the microalgae as well as their interactions with their prey and predators.

Given the growing interest in both HABs and mixoplankton, this session was undoubtedly a harbinger of many to come in future meetings.

Acknowledgements

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Research on *Karenia brevis* blooms in the eastern Gulf of Mexico

The widespread distribution of the toxic dinoflagellate *Karenia* has significant impacts on coastal communities across the globe. Therefore, the goal of ASLO Session SS088 *Advances in Understanding, Prediction, and Monitoring of Toxic Karenia (Dinoflagellate) Blooms Around the Globe* was to highlight global research on the activity and impacts of this toxic genus.

There were six presentations and four posters of *Karenia* research presented during Session SS088. All focused on elucidating environmental factors and microbial interactions that influence bloom longevity and the success of mitigation tactics potentially used to combat blooms. Most session abstract submissions were focused on research related to *Karenia brevis* activity and impacts in the eastern Gulf of Mexico (GOM) (Figs. 1-2)

K. brevis blooms have occurred in the eastern GOM almost every year for at least the past century, likely much longer, and local bloom monitoring programs have collected data for nearly 50 years.

In the last 5 years, two *K. brevis* blooms have lasted longer than 12+ months, resulting in a determined focus by scientist and stakeholders to understand *Karenia* bloom dynamics in this ecologically and economically important area.

Statistical techniques demonstrated that the North Atlantic Oscillation was a regional climatic stressor that positively correlated with increased *K. brevis* bloom severity in the GOM. The associated warmer temperatures and higher precipitation may result in environmental conditions favourable for *K. brevis* bloom expansion (**Tristyn Bercel**). Rain events were shown to deliver dissolved organic matter (DOM) via urban stormwater runoff and atmospheric deposition with both stimulating *K. brevis* culture growth and have the potential to promote bloom duration (**Amanda Muni-Morgan**). Riverine estuarine systems are also known to deliver DOM, specifically humics and tannins, to the GOM. Short-term incubation experiments using DOM extracts from three Florida rivers resulted in a larger growth response when compared to controls. It is believed these compounds are supplying additional nitrogen to the bloom (**Molly Dent**). Based on historical data, cold fronts are weather events that can contribute to bloom decline, but seemingly only when multiple cold fronts occur in proximity of each other. Observations from a single meteorological cold front that decreased sea surface temperatures by 2°C showed that while cells were stressed, it did not result in bloom termination (**Chloe Manley**).

This session also included projects focused on microbial community dynamics associated with blooms. Primers were developed for the identification of both *K. brevis* and *K. mikimotoi* in recent 2021 and 2023 blooms in the GOM. In 2021, *K. mikimotoi* was found at its highest concentrations when the seawater temperature was highest (33.5°C) demonstrating that these blooms are not monospecific and intragenus interactions need to be included in bloom dynamics models (**Sarah Klass**). To understand how an individual *Karenia* cell interacts with its microbial consortia within its own microenvironment, termed the phycosphere, a protocol is actively being developed for tandem microscope and microosmotic pipette systems to isolate the pico-to nanoliter volume surrounding individual cells. Subsequent DNA extraction and whole genome amplification is used to identify the taxonomy and functional composition of the phycoshpere microbial community, providing information that can be used to understand what role the prokaryotic community plays in supporting the health of a single *Karenia*, and potentially their role in bloom health (**Carly Moreno**). Through isolation and characterization of thirty bacterial strains from GOM bloom seawater, there is evidence of symbiotic and antagonistic bacterial activity. It was found bacterium *Mameliella alba* CE5 supports *K. brevis* growth while *Croceibacter atlanticus* CE21 inhibits *K. brevis* growth. Based on genome sequencing, *M. alba* may provide critical vitamins and cofactors while *C. atlanticus* may synthesize polyketide algicidal compounds. This work highlights that *Karenia* bloom health can be impacted by the bacterial community members (**Cong Fei**) (Fig. 3). GOM derived viruses were also shown to lyse *K. brevis* cultures. Metagenomic sequencing



Fig. 1. Aerial photo of 2022 K. brevis bloom offshore Cayo Costa Florida, USA in the eastern Gulf of Mexico. Darker water contains the bloom, the white "line" separating the different water colours is congregated dead fish as a result of brevetoxin poisoning. Image courtesy of Florida Fish and Wildlife Conservation Commission

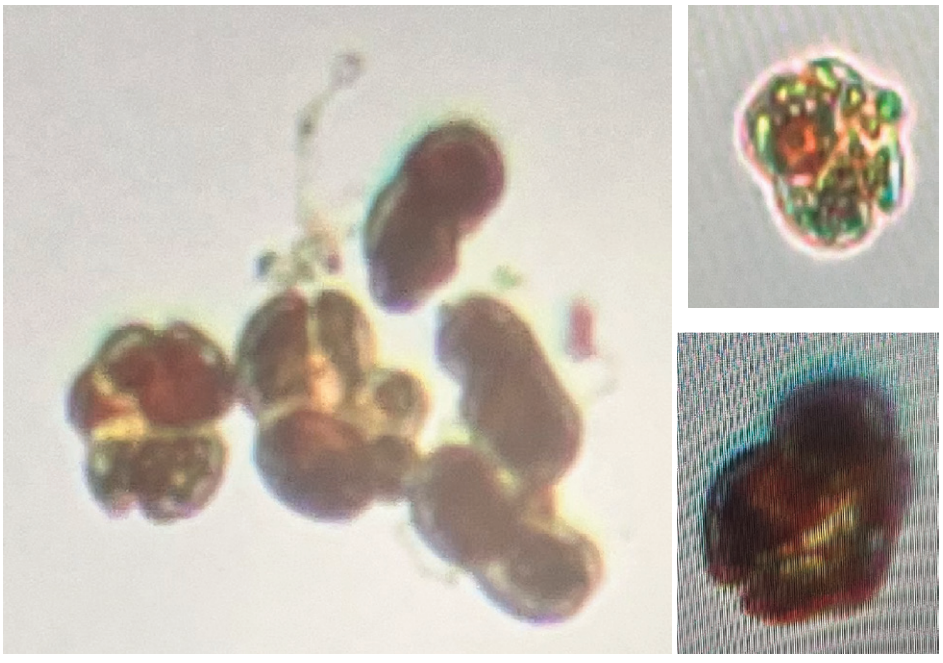

Fig. 2. Light microscopy micrographs (400x) of Lugol's preserved Karenia brevis cells from the 2023 bloom. Samples collected offshore Sanibel Island, Florida, in February 2023. Image courtesy Amanda Muni-Morgan.



Fig. 3. Cong Fei presenting work on symbiotic and antagonistic bacterial interactions with K. brevis during ASLO 2023 Session SS088.



Fig. 4. Anne Booker presenting efforts to find natural marine viruses capable of lysing K. brevis during ASLO 2023 Session SS088.

analysis indicates some of these marine viruses are in the giant virus phylum Nucleocytoplasmic Large dsDNA Virus that are known to only infect eukaryotes. There are currently no described viruses that use *K. brevis* directly as a host, indicating novel giant viruses may be additional microbial community members capable of influencing bloom health (**Anne Booker**) (Fig. 4).

The large ecological and economic impacts from *Karenia* blooms in the GOM prompts the desire for mitigation techniques. The goal of *Karenia* mitigation is to clear blooms in a way that does not hurt the local environment. Laboratory incubation experiments conducted found that curcumin was the most effective tested compound at reducing *K. brevis* photophysiology and cell counts across four strains. Clay was the second most effective tested compound at disrupting photosynthesis by reducing quantum yield of Photosystem II and relative electron transport rate (**Miah Manning**). In a second mitigation study, laboratory *K. brevis* cultures were incubated with fifteen different mitigation compounds and the resulting brevetoxin concentration were measured after 48 hours. Most of the mitigation compounds resulted in either no change or an increase in the brevetoxin present in the incubation. The exception being high doses of the macroalgal species *Gracilaria lemaneiformis* that eliminated 100% of *K. brevis* cells and 61% of total brevetoxins after 48 hours (**Cynthia Heil**).

In conclusion, ASLO Session SS088 showcased progress in understanding *K. brevis* blooms in the eastern GOM. The research demonstrated how both environmental factors and microbial interactions influence blooms, and highlighted compounds that could be used for bloom mitigation. The resulting insights are crucial for managing bloom impact on this ecologically sensitive region.

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Second UK Harmful Algae Group Meeting - Aberdeen 2023

Linda Lawton and **Christine Edwards** from the CyanoSol Research Group at the School of Pharmacy and Life Sciences in Robert Gordon University (RGU), Aberdeen, Scotland, hosted the 2nd meeting of the UK Harmful Algae Group on 13th & 14th June 2023. They were delighted to welcome international experts from industry, academia and Government bodies who shared, discussed, and highlighted the latest developments, initiatives and strategies relating to HABs.

Guest speaker (sponsored by the Microbiology Society), **Philipp Hess**, Director Research Unit PHYTOX, IFREMER, kicked off the meeting with perspectives from the French HAB Research Network, PHYCOTOX, which was the inspiration for the formation of the UK Harmful Algae Group. The presentation also highlighted developments with emerging HABs and toxins between 2018 and 2022, including updates on brevetoxins, tetrodotoxin, ovatoxins and pinnatoxins as well as the current global status of *Dinophysis*, *Pseudo-nitzschia* and *Alexandrium,* and their associated toxins. An overview of the proposed Harmful Algal Bloom- Solutions Programme (HAB-S) from the IOC UNESCO Intergovernmental Panel on HABs (IOC IPHAB) was also presented. **Janina Costa** from the Sustainable Aquaculture Innovation Centre (SAIC) also funded by the Microbiological Society, gave an overview of SAIC's work including a range of academic-industry related SAIC supported projects such as "Mitigating microbial hazards – eliminating HABs risks in salmon farms", a project led by RGU, partnering the Scottish Association for Marine Science (SAMS), OTAK, Bakkafrost and Queens University Belfast.

Early warning mechanisms

Gavin Tilstone from Plymouth Marine Laboratory presented Sentinel-3 products for detecting signals of eutrophication and HABs in the French-English Channel (S-3 EUROHAB). These products were demonstrated by Tilstone in a separate workshop during the meeting and can be explored at *www.s3eurohab.eu/portal/*. **Peter Miller** (Plymouth Marine Laboratory) presented approaches for early warning of HAB risk using satellite ocean colour and Lagrangian particle trajectories. The pros and cons of satellite ocean colour for HABs alerts were presented and the value of merging these dataaets with particle trajectories in two case studies along the coast of England to providing improved interpretation for early warning of HAB risk. This process is now running operationally.

First year DATALAB/Marine Directorate of Scottish Government PhD student **Gary Groves** (University of Highlands and Islands (UHI)/SAMS) gave an overview of the first UK experiences using Imaging Flow CytoBot (IFCB) technology for HAB detection in active aquaculture sites in the Shetland Islands in Scotland. This technology holds great potential and the first year of the project focused on overcoming logistic and technical challenges associated with deployment.

Detection methods

Presentations on new detection methods came from **Soumya Palliyil**, Scottish Biologics Facility, University of Aberdeen who reported on the development and application of recombinant antibody fragments (scAb) for the detection of microcystins and cylindrospermopsin in water and tissue samples in ELISA or lateral flow format (Fig. 1). **Bob Hatfield** (CEFAS) presented his work on Nanopore Sequencing for "fast and dirty identification of *Alexandrium* anywhere" and delivered a workshop on this technology following the meeting.



Fig. 1. Cyanobacterial bloom collection in Rambakan Oya reservoir, Sri Lanka for toxin testing using scAb based ELISA (photo credit Malinda Dissanayake).

Occurrence of HABs and toxins

Andrew Turner from CEFAS reported on recent impacts of HABs on animal health in the UK looking at the impacts of cyanotoxins on dogs and cattle and results of analysis of pelagic and benthic fish species for algal toxins. A detailed study of the common Sunstar, *Crossas-*

ter pappossus (Fig. 2), from diverse sites around the UK showed all contained PSP toxins, some as high as 12,000 µg/kg whole organism even though there was no association with phytoplankton and the toxin profiles were unique and dominated by decarbamoyl saxitoxin suggesting alternative producers, possibly symbiotic bacteria.

Eileen Bresnan from the Marine Directorate of the Scottish Government gave an overview of Scotland's changing coastal environment and implications for plankton and Harmful Algal Blooms. Results from *Marine Climate Change Impacts Partnership* and work from *Oslo/Paris Commission Quality Status Report* were presented which showed widescale changes in the plankton community in UK and European waters. Data from Scottish Coastal Observatory (SCObs) highlighted changes in physical, chemical and biological properties of Scotland's coastal waters which are associated with changes in phytoplankton community structure (Fig. 3). This presentation was supported by "Harmful Algae in a Policy Context" delivered by **Martin Lilley** from the Marine Evidence team (DEFRA) discussing the need for scientists to engage with policy makers.

Mitigation

Early career scientists from the CyanoSol Research Group presented multiple strategies for the removal of toxic cyanobacteria. **Jane Moore** presented proof of concept data on the use of biologically enhanced biochar (BEB) from industrial waste such as coconut shells, for the destruction of *Microcystis* and microcystins, demonstrating the potential of nature-based water treatment solutions. Hydronation scholar, PhD student, **Indira Menezes** demonstrated the efficiency of UV-A irradiation for destruction of 6 strains of *Microcystis aeruginosa.* Carlos Pestana presented "A paradigm-shift in water treatment: In-reservoir UV-LED driven TiO_2 photocatalysis for the removal of cyanobacterial – a mesocosm study". Cyanobacteria were reduced by 85% after 7 days of treatment using TiO_2 immobilised on recycled glass beads combined with 365 nm UV-LEDs in mesocosms in a Brazilian drinking water reservoir, however experimental challenges during field work included piranhas!



Fig. 2. The common Sunstar, Crossaster pappossus (photo credit Karl Deane, Cefas).

In summary, the meeting covered a wide range of topics and provided an excellent opportunity for early career researchers and extensive networking during breaks with posters and a pub meal and everyone is looking forward to the next one that will be hosted by CEFAS in Weymouth in 2025.

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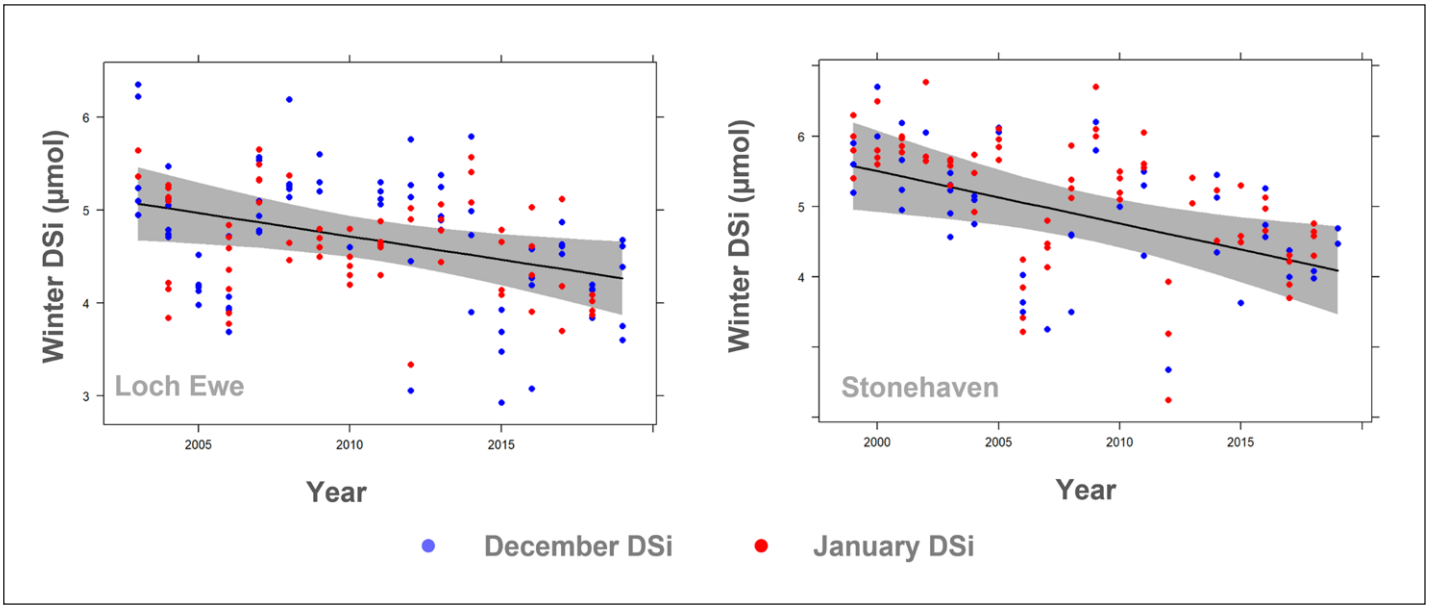

Fig. 3. Winter (Dec, Jan) dissolved silicate (DSi) concentrations from the Scottish Coastal Observatory monitoring sites at Loch Ewe (west coast) and Stonehaven (east coast).

Gary Jay Kirkpatrick
in memoriam

On August 6th, 2023, a memorial service to celebrate the life of Gary Jay Kirkpatrick (April 13, 1952 – July 25, 2023), was held by his family, friends and colleagues in Sarasota, Florida.

Gary was an engineer, a marine scientist and an otherwise unclassifiable person of tremendous impact, deep moral purpose and radiant kindness.

Gary's life story is an extraordinary example of how the freedom of independent thought and respect for interdependent reality can combine to make a luminous whole more than the sum of its parts. He was a person who found awe, and let it change him.

Gary was one of four brothers raised in Bradfordwoods, Pennsylvania, in a creative, tight-knit family where love of animals, a sense of wonder, unmeasured curiosity and mechanical skills were encouraged and embraced.

In 1961, the sportswriter Myron Cope, known as the "voice of the Pittsburgh Steelers," heard about a family out in Allegheny County building their own airplanes, sports cars, television sets, and go karts. The Kirkpatricks, people said, looked like a regular family: the father, Robert, who went by Kirk, had a nine-to-five; the mother, Henrietta— Heni for short — raised their four boys, George, Bob, Doug, and Gary. But they had pet raccoons, and they sold wooden bowls, and well — you just had to go see them. In the resulting feature in *This Week* magazine, "Keeping Up with the Kirkpatricks," their house — which Kirk built himself in a single year out of fir, glass, and sandstone — is described as a "three-bedroom, umpteen-workshop lodge-type," a place where "nothing seems to be planned, but just about everything seems to happen."

In Cope's vision of the Kirkpatrick home, a motor gets bought, and an airplane gets built; a plump, domesticated raccoon named Specs eats a breakfast of swiss cheese and bananas, and Heni tends to hundreds of orchids. "Activity is contagious," Kirk insists, before recounting the time Bob backed his (homemade) sports car into Kirk's (homemade) plane — but Heni thought he'd run over George — and everyone screamed so loudly that Gary, who was playing in the woods half a mile away, thought something was horribly wrong, and came running. They laughed about it, eventually.

The article is a charming snapshot of a creative, tight knit family, during the last year that all four boys lived at home.

During high school, Gary had enrolled in the Civil Air Patrol, following in the footsteps of his father, a WWII veteran of the Air Force, and his brothers, all three of whom attended the Air Force Academy. After graduation, he joined the Air Force, serving as a fighter pilot until a crash convinced him that life on the ground working as an electrical engineer would be safer. The change was fine with Barbara, his soulmate and wife of 49 years, who he had met when she, too, was a cadet serving in the Miami Civil Air Patrol.

After leaving the Air Force in 1980, a number of very fortunate events happened as Gary turned his attention to the future. During graduate school at North Carolina State University in 1982, he was introduced to Dan Kamykowski in the Marine, Earth and Atmospheric Science Department who later became



Fig. 1. Gary Kirkpatrick and Dan Kamykowski, 1988



Fig. 2. Gary (right) with Jim Hillier and their first Slocum glider (Mote Marine Laboratory, 2005)

his mentor. In Gary, Dan saw someone who had the skills to build the tools he would need during a promising, productive career.

For those of you who did not come to marine science until the 21st century, a bit of explanation is useful: Earlier days required a great deal of its researchers. To understand the ocean, you need physics, chemistry, and biology, and beyond that, none of the tools that you *needed* to study the ocean actually *existed*, so you also needed to have some engineer in you. In 1982, Gary had everything on that list. He was also intellectually curious, open-minded, and collaborative — a genial, calm person you wanted on your watch at sea.

The fall of 1990 found Gary at Mote Marine Laboratory in Sarasota, Florida. And in a stroke of luck, there were no phytoplankton ecologists at Mote. Gary started his work there with a broad purview, over a wide variety of organisms, in both marine and freshwater environments. By 1992, as red tide surged along Florida's southwest coast, he narrowed his focus to the organism that would eventually be known as *Karenia brevis*, named for one of his and Barbara's co-researchers, naturalist Karen Steidinger of Florida's Fish and Wildlife Conservation Commission.

At Mote, Gary developed the Breve-Buster, the first automated red-tide detector, along with the process it used to detect *K. brevis* — called fourth derivative analysis, which is now a widely-used absorption spectra classification technique. He also built SUPA, "self-contained underwater photosynthesis apparatus," an algal laboratory that could be lowered into the water.



Fig. 3. Gary in a FL Red tide meeting in St Petersburg, FL 2007 talking with Jan Landsberg



Fig. 4. Gary and Oscar at the Underwater Glider Workshop 2019.

But he wasn't done yet. He developed FlowThrough CDOM, a method of measuring organic matter in waterways and, working with Robert Currier, Barb (an Ed.D. studying the human-health impacts of harmful algal blooms in her own work), Gary developed HABScope, a low-cost microscopy system that enables the Red Tide Respiratory Forecast.

Talking with Gary was freeform: We'd move from fourth derivative mathematics to hard-core cell biology to the engineering of gliders in the same conversation; his was an intense curiosity, always wanting to know more about how something worked, and why. He was humble; he wore his intelligence with modesty. He was the last person on the planet who would ever try to show up another person for being smarter, and he was never motivated by trying to be the first person with the answer. In our relationship, I was the cheerleader; Gary was the one who always wanted a little bit more data. We would toss around crazy ideas, and between that push-and-pull, that dance of enthusiasm and rigor, somehow they always seemed to get some momentum, and a lot of them became real things that people actually use.

Gary worked at Mote from 1990 to 2022, publishing more than 80 papers, raising his own money, managing his own team, and collaborating with hundreds of scientists at dozens of institutions. He was also an incredible mentor who helped to support and guide the careers of numerous students and young colleagues.

With his electrical engineering skills and understanding of both physical and environmental science, Gary was able to move beyond concepts.

He built real tools. Most importantly, Gary built our field.

Submitted by Oscar Schofield. Adapted from the eulogy written by Gary's niece, Barbara Bourland, and delivered by Oscar Schofield, at Gary's memorial service on Aug. 6, 2023. You can honor Gary by performing a random act of kindness. As a living-donor kidney transplant recipient, Gary would also encourage you to become an organ donor.

The Sherkin Island Marine Station Long-term Phytoplankton Record

HAB-related science at Sherkin Island

One clear sign of the aging process is when the editors of the IOC Newsletter *Harmful Algae News* invite you to submit "a piece" for their historical archive series. This time, the invitation coincided with the recent publication of what is arguably the most extensive phytoplankton record to date (*https://doi.org/10.15468/crt6sc* and *www.sherkinmarinedata.ie*). This allows me to pay tribute to some remarkable HAB-related work that started before the well-funded establishment of the active research field we enjoy today: it has been quietly soldiering on until now, unfunded, and largely under the HAB community radar.

I first became aware of this work in 1986, when the director of Sherkin Island Marine Station (SIMS), Matt Murphy, stopped by my office at the University of Oslo and asked me if it was true that I knew something about "red-tide". I must have convinced him that there was at least some truth in that. After a brief discussion he said "if I provide round-trip airfare and accommodation for 12 scientists from anywhere in the world, could you help select them and help organize an international conference on "The problems of toxic dinoflagellates in aquaculture"? Neither of us realized that this was the start of a great enduring friendship and scientific cooperation. (Perhaps somebody told him of my highest regard for voluntary service?)

Like many of our generation, Matt and his late wife Eileen were surely inspired at least in part by Racheal Carson´s book *Silent Spring.* With no formal scientific training, they nevertheless established the Marine Station in 1975 on a little island almost at the southern tip of Ireland, and embarked on a life-time ambition to record as much as possible of the marine and terrestrial wildlife in the surrounding area and to monitor the inevitable future changes it faced. Details of the station and its research activities including surveys of the rocky shore, terrestrial plants, birds, otters and insects may be found at *www.sherkinmarine.ie*.

The phytoplankton survey is unique in all its main features, on a global scale, and as with all SIMS work was funded on an extremely low budget without any government assistance, and carried out by young volunteer scientists. I believe this survey, with unusually dense sampling of the water column within a restricted coastal area over time, together with associated environmental data, arguably produced the largest and most comprehensive record to date of phytoplankton assembled from one area of the marine realm. Many researchers, and perhaps all funding agencies may be surprised by this claim – but the details speak for themselves.

The long-term phytoplankton survey

The phytoplankton programme started in response to a "red tide" event along the southwest coast of Ireland in 1978. It evolved through an initial exploratory phase, with particular focus on the toxic species threatening the local shellfishery, and the station hopes to publish data from these and subsequent toxic events at a future date if possible. However, realizing the need for long-term monitoring of the whole phytoplankton for harmful algal blooms (HAB) to be considered in their ecological context, the survey of the whole phytoplankton reported here was established. This covered 12 station (Fig. 1). Station B8, closest to the Marine Station, was designated as a reference site to be sampled most frequently (a total of 980 sampling trips between 1978 and 2014), and stations B2, B5, B6, S1, S2, S3, and S4 were established to provide core information for the long term monitoring record (1980-2014). Four additional stations were subsequently added: B3 (from 1985 onwards), B4 and B7 (from 1986), and B9 (from 1988). B3 and B7 were re-established from two sites pre-

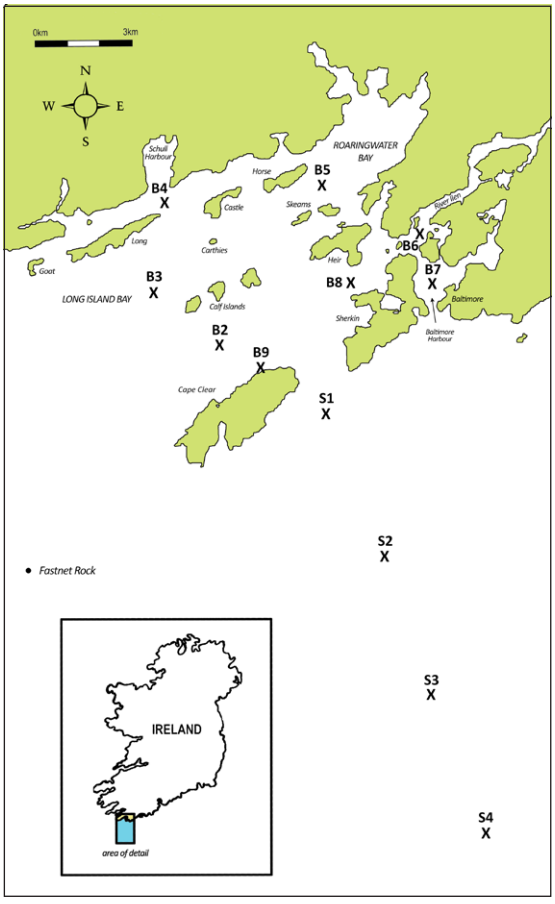

Fig. 1. Sherkin Island. Stations surveyed

viously sampled during the initial stages of the survey and records from those early years are included in the dataset.

The survey aimed to sample stations approximately every 12 days, subject to weather conditions, between April and October each year. Discrete water samples were taken by Nansen bottle for analysis at depth intervals of 0, 2.5, 5, 10, 15, 20, 25, 30 and 50m where water depths permitted at each station. When possible, a vertical plankton net haul was taken at all stations to cover the water column between the sampled depths. Water temperature and salinity were recorded for each sample, and Secchi disc readings were taken for estimating water transparency at each station.

The dataset includes standardized phytoplankton counts of all identifiable species together with recorded location, date, water depth, water temperature, salinity, conductivity, Secchi disc estimate, tide height at time of sampling, sea conditions and weather conditions. Standard air temperature records from the nearby manual weather station at Sherkin Island Marine Station covering the same time period offer comparisons with the marine records for climate-related studies (Met Éireann : The Irish Meteorological Service (online) c.2021 [accessed 2021 Sept 17] *https://www.met.ie/climate/available-data/historical-data*).

In all, the unique dataset presented here comprises a long-term record from 1980 to 2014 encompassing over 34,000 samples and at least 440,000 counts of individual phytoplankton species, together with over 34,400 seawater temperatures, and 28,000 salinity measurements. Over 350 phytoplankton taxa were recorded, identified to species where possible with reference to literature available at that time. Anyone who has successfully applied for a project to carry out the equivalent of even any small part of this programme will appreciate this amazing achievement. It is interesting (to say the least!) to attempt to guess what the cost might be to carry out such a programme through the established science funding systems. A "back of an envelope calculation" would quickly suggest millions of dollars – unrealistic, and beyond the most ambitious scientist´s imagination for a grant proposal. Nevertheless, these results show a level of complexity in natural variation that challenges our ability to separate this from the humanly-induced environmental damage everyone is concerned about (Dale & Murphy 2014). Can we know what is happening out in the ocean without detailed monitoring? It strengthens my belief that an annual budget on the order of that NASA and all the other countries spend on space exploration is needed if we are to really understand the oceans and their environmental problems. If that is impossible, then at least it should be recognized, and publicized.

Other HAB-related activities at Sherkin Island – International Conferences

1st International Conference/workshop on The Problems of Toxic dinoflagellate Blooms in Aquaculture

At the time of writing, the HAB community is looking forward to celebrating the 20th International HAB Conference, to be held in Japan in 2023, and it is interesting for me to reflect on the year of the first of these meetings, also in Japan, in 1987. I was not able to attend that meeting, but 1987 stands out in my memory, as I am sure it does for others who were there, as the year of the 1st Sherkin International Conference/workshop: *The Problems of Toxic Dinoflagellate Blooms in Aquaculture,* resulting from my first meeting with Matt Murphy, Director of SIMS. Matt realized the great potential for aquaculture to offer local jobs for young people in coastal Ireland who otherwise had to move away to find employment, many immigrating to other countries. HAB was emerging as the major obstacle for developing this potential. Little was known about "red tides", and as elsewhere just the mention of such an event was enough to put consumers off all marine food. Traditional fisheries suffered economically, and insurance premiums for aquaculture start-ups were prohibitive.

We assembled a mixed group of fifteen speakers to share their science from different countries, followed by a workshop to discuss where we were with "red tides" and what was needed to progress further in understanding them. The conference was attended by a few invited scientists other than the speakers, and was open to local politicians, fishery representatives and insurers who were interested. The speakers included a couple of oceanographers and a mixture of both established and up and coming HAB scientists. Most are now well-known contributors to the field: Dan Baden (who also presented a paper from Karen Steidinger), S. Fraga, I.R. Jenkinson, G.M. Hallegraeff, T. Okaichi, Max Taylor, Alan White, and Charlie and Clarice Yentsch. Apart from the science, the highlights included the spectacular arrival by helicopter of the Minister for the Marine for the opening ceremony, and a magnificent banquet put on by the chefs from the best hotels in Cork using seafoods donated by local fisheries.

Among the recommendations from this meeting were:

1. The need for a newsletter to update scientists and aquaculturists worldwide on the outbreaks of "red tides". This prompted SIMS to publish "The Red Tide Newsletter", quarterly from 1988 to 1992, the forerunner for others up to today´s IOC (UNESCO) Harmful Algae News.
2. The need to maintain training for phytoplankton taxonomists – at that time seemingly a "dying breed". The International Phytoplankton Training Course run from the University of Oslo, Norway, was facing an uncertain future at the time. Henrik and his Copenhagen team have certainly answered that call well!
3. The need to bring "red tide" scientists, aquaculturists, insurers and local politicians together to help resolve start-up difficulties for this emerging industry. Including local politicians proved particularly valuable – they asked pertinent questions one does not normally expect in scientific discussions. SIMS sent invitations to the 2nd International Conference to all the local councils in Ireland inviting them to send representatives, and many did.

2nd International Conference/workshop on The Problems of Toxic dinoflagellate Blooms in Aquaculture

The second conference, in 1989, began with a workshop on HAB species taxonomy, with the world authority on the genus *Alexandrium*, Enrique Balech from Argentina invited to share his wealth of



Fig. 2. From left: Matt Murphy, Vera L. Trainer, Enrique Balech, Karen Steidinger. Sherkin Island 1989.

experience from over 50 years studying phytoplankton worldwide. Among the other well-known HAB scientists were Karen Steidinger, Vera Trainer, Gustaaf Hallegraeff, Malte Elbrächter, and Santiago Fraga (Fig. 2). A highlight of the conference was the entrance of a horse into the lecture hall which Matt Murphy promptly grabbed by the mane to escort back outside. The main topics and discussions of the conference centered on the worldwide occurrence of HAB, and problems for aquaculture, with presentations from insurers, managers, and regulators of the aquaculture industry. To my knowledge, this was the first, and perhaps only such meeting where scientists, and both fishing and insurance industries and politicians discussed HAB together?

Publishing "The Genus Alexandrium Halim (Dinoflagellata)" by Professor Enrique Balech

It is appropriate for me to include mention of one of the particularly noteworthy results from the second conference at a time when we mourn the recent passing of Karen Steidinger, since she was very much involved. While Prof. Enrique Balech was with us we learned that he was preparing a manuscript for a book on *Alexandrium*, but was uncertain about publication possibilities. Matt said the Station was honoured to publish it, and Karen accepted responsibility for helping Balech complete the work. Karen, who regarded Balech as a mentor (Fig. 3), employed a Spanish translator to produce an English version from which Karen´s colleague, Beverly Roberts completed the published version. "The Genus *Alexandrium* (Halim) Dinoflagellata" by Balech is an essential reference for anyone working on *Alexandrium* worldwide. Both Karen and Matt wrote moving tributes to Balech in the book, and Matt said that publishing this great man's work and making it available to the scientific world was a privilege for the Station. A limited number of copies are available for purchase from SIMS at 40 Euros inclusive postage, and here I wish to acknowledge the generous spirit of a German colleague who willingly bid twice that much at an ISSHA conference auction.



Fig. 3. Enrique Balech and Karen Steidinger. Sherkin Island 1989. Photo by Santiago Fraga

Concluding remarks

I hope this little view into SIMS work will inspire others. Some of the young volunteers gained invaluable experience that helped them on to a career in our science, and readers will recognize some well-known HAB researchers among the list of volunteers in the background information to the published survey data. There is still a pool of young, well qualified students without relevant job opportunities who could benefit from the sort of chance that SIMS offered, and it is questionable if an alternative way can be found to producing sorely needed adequate long-term data. Unfortunately, SIMS could not continue the phytoplankton survey after 2014, but I could wish that someone somewhere could take up the challenge. Maybe some philanthropic foundation, or even a funding agency willing to think outside the box could be persuaded to raise a little seed money to start such an effort?

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ISSHA's corner

Get Ready for the Upcoming ICHA20 meeting

The ICHA meeting, 5-10 November in Hiroshima Japan (*https://icha2023.org*) is nearly here. For those of you who are attending, please remember to book your room and register for the Wednesday afternoon excursions and the Gala dinner. There are still a variety of accommodations available to choose from. The afternoon excursions include Iwakuni City located in Yamaguchi Prefecture featured the renown Kintaikyo Bridge and the restored Iwakuni Castle; trip to Kumano brush, one of Hiroshima's traditional craft of brush making; a visit to Saijo one of the three most famous Sake brewing areas in Japan; visit to scenic port town of Onomichi noted for its scenic beauty, old temples and seasonal flowers; and the shrine Island of Miyajima, a world heritage site with Itsukushima-Jinja Shrine, Misen Mountain and friendly deer.

Prior to the Gala dinner, people will gather at Grand Prince Hotel, for a pre-party at the "Hiroshima Gokoku Shrine" , located near Hiroshima Castle. Here, light refreshments and welcome drinks will be served, and participants will enjoy a samurai performance and ken-dama. Afterwards, participants will move to "Hotel Granvia Hiroshima", the main venue for the dinner near JR Hiroshima Station, where they will enjoy a seated buffet-style dinner and enjoy "Kagura", a traditional performing art of Hiroshima. For those brave souls who wish, following the Gala dinner, there will be an optimal drinking session in "Nagarekawa", the famous downtown area of drinkers' heaven in Hiroshima City where the participants will enjoy a variety of establishments.

ISSHA Auction

This meeting will also see the resumption of the long tradition of holding an auction to raise funds to support student travel to the next conference. This is a great way to spend time with colleagues and having fun bid on interesting and fun items. To further contribute to the festivities, drinks will be available for purchase. Items can include pottery, games, toys, liquors, clothes, books, etc. All proceeds go to supporting student travel for the next conference. If you plan on donating an item, please send a picture (jpg or png preferable) and a short description to the following email address, preferably several weeks or more ahead of the meeting. This information will be used to produce a slideshow of auction items. You can drop donated items off at the information booth adjacent to the reception area upon your arrival at the venue. Alternatively, if you ship them ahead of time, kindly utilize the address below for shipping purposes. We kindly request that you forward an email to the designated contact person to ensure a streamlined process. This email should include your name, shipping address, and pertinent item details. Also, to exempt import tax, please mention on the shipping invoice that the item is "gift", and the estimated value of the item should be less than 10000 JPY (For items more than this value, please at first contact us by email).

Contact person and email address: Dr. Satoshi Nagai, *nagai_satoshi44@fra.go.jp*

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Looking forward to seeing you at the auction on November 9th from 19:30 at the room Setouchi 1 & 2.

Once the meeting concludes, a summary of the meeting will be published in HAN. We hope to see you at the meeting.

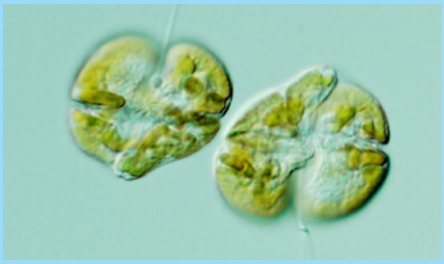
Wayne Litaker (ISSHA President)
Ichiro Imai (Chair), and Toshiyuki Suzuki, and Satoshi Nagai, Members of the Local Organizing Committee





Top: Hiroshima Gokoku Shrine
Bottom: Kagura, a traditional Hiroshima performing art

Announcement for a forthcoming Special Issue of the newsletter dedicated to the life and work of Karen Steidinger



As an admiring early colleague of Karen, I was asked to help announce the editors´ intention to produce a Special Issue celebrating her wonderful legacy. There was such a warm spontaneous response from so many people as word spread of her passing: ex colleagues or students in international taxonomy courses in Europe, American close colleagues or simply people knowing her work and loving her personality. Karen´s closest colleagues held a very moving tribute to her in Florida on September 9th, and the editors wish to follow this with a Special Issue marking the respect and admiration for Karen held by the broad international HAB community and ISSHA membership. Publication date will be announced later, and in the meantime a long-serving ISSHA member and ex-President (2004-2008), Pat Tester – in collaboration with Jane Landsberg, has accepted the invitation to assemble written contributions and photos and be Guest Editor for the proposed Special Issue. I look forward to the Special Issue – I am sure the ISSHA community will produce a worthy tribute to one of its earliest pioneers.

Barrie Dale, University of Oslo, Norway

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline to submit material for next issue
December 1, 2023

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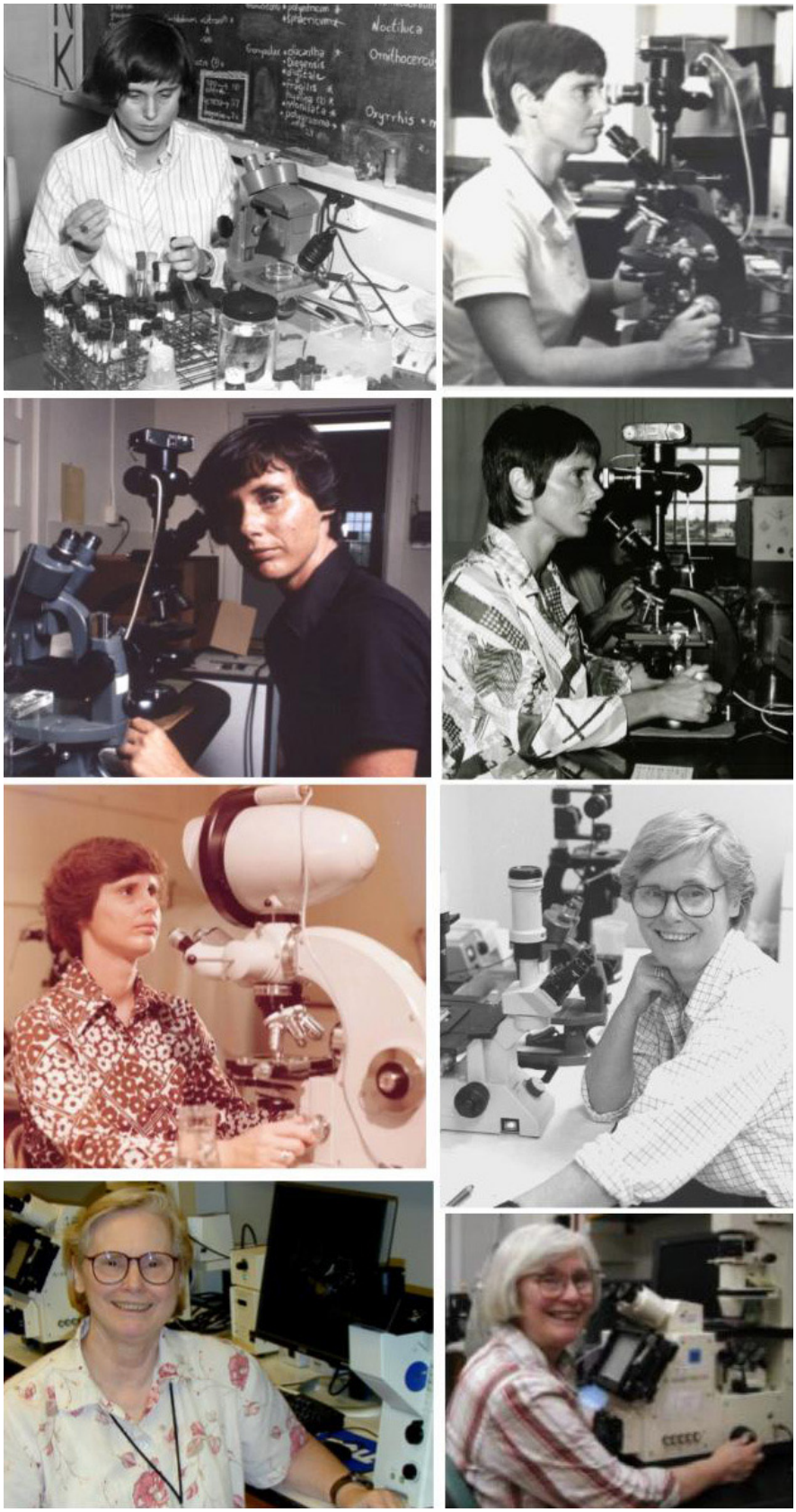
Karen A. Steidinger

The Legacy of a HAB Pioneer and Visionary



Karen at the microscope through the decades (1960s onwards). Photo credits: Fish & Wildlife Research Institute, Florida Fish & Wildlife Conservation Commission.

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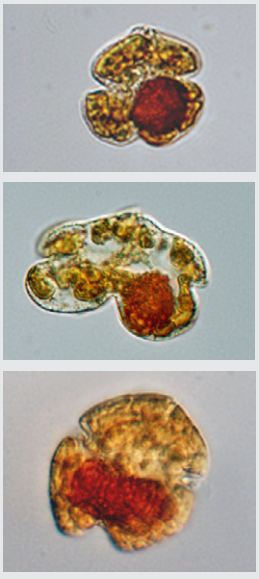

Karenia species from the Gulf of Mexico. 1. K. brevis; 2. K. papilionacea; 3. K. selliformis. Credit: FWC-FWRI-HAB Group

A Short History of Karen's Career: A Taxonomist, Ecologist, Administrator, Teacher, Mentor, and Friend



Fig. 1. Photo of Karen (then Director of Marine Research, former FMRI) taken in the late 1980s. Photo credit: Fish & Wildlife Research Institute, Florida Fish & Wildlife Conservation Commission.

Dr. Steidinger's contributions to and her impacts on phycology and oceanography spanned almost six decades, beginning with her work at the Florida State Board of Conservation's Marine Research Laboratory and continuing through completion of her undergraduate degrees and her subsequent positions as marine biologist, supervisor, and then chief administrator (Fig. 1) of the Florida Marine Research Institute (FMRI, research arm of the then Florida Department of Natural Resources, [FDNR]).

Karen's initial professional appointment as laboratory technician for the Florida Board of Conservation was in 1963. In slightly more than a decade, she had risen to Laboratory Supervisor. She was appointed as Chief of the Bureau of Marine Science and Technology in 1980 and in 1983 became Chief of Marine Research for the Marine Research Laboratory (FDNR, renamed FMRI in 1988), serving in that role until 1993. Her forethought to codify a research mandate within the fisheries management organization and publish reports and in peer reviewed journals was seminal. Karen oversaw 165 staff at the St. Petersburg main facility and at five field laboratories, while managing an $8 million budget [1]. During her administrative tenure in the mid-1980s, Dr. Steidinger together with Dr. Peter Betzer (College of Marine Science, University of South Florida) negotiated a fixed capital outlay (greater than $10 million) from the State of Florida for construction and outfitting of a new building that was completed in 1994 (shared with USF). In 2002, "In recognition of her four decades of dedicated service to the State of Florida, her exemplary scientific career in harmful algal bloom research, and her inspirational role as mentor and ally to colleagues at the Florida Marine Research Institute and throughout the world…", the 250-seat auditorium was named by the Florida Fish and Wildlife Conservation Commission (FWC) in her honor. (In 2004, the FWC incorporated FMRI into the Fish and Wildlife Research Institute [FWRI]) [1].

Hired on in 1963 to the then Florida Board of Conservation's Marine Research Laboratory to assist with red tide studies, Karen spent the remainder of that decade identifying phytoplankton in Florida's marine waters, but she focused her attention on *K. brevis* because of the severity of the 1963 red tide event (Fig. 2). Her early publications were reported in a series of technical reports produced by the Marine Laboratory of the Florida Board of Conservation [2-8].

Recognition of Dr. Steidinger's insight on and contributions to the systematics and oceanography of red tides and her influence on this discipline were recognized "early on" by her colleagues. In 1972, she coordinated and directed a research team addressing red-tide outbreaks and their entrainment off the west coast of Florida, and their subsequent transport to the east coast via the Florida Current and Gulf Stream system. This work, published in *Limnology and Oceanography* in 1975, emphasized the importance of physical forcing and transport in bloom occurrence and proliferation. Within the manuscript, the use of satellite thermal imagery for detecting "frontal systems" associated with *Karenia* blooms initially was described [9].

At the convening of the first red tide conference in Boston, MA in 1975, Dr. Steidinger presented a paper, "*Basic factors influencing red tides*", where she



Fig. 2. Karen in 1964 pointing to her hand-drawn chalk illustration of a Karenia brevis cell. Photo credit: Fish & Wildlife Research Institute, Florida Fish & Wildlife Conservation Commission.



Fig. 3. Ecology and Oceanography of Harmful Algal Blooms – ECOHAB: Florida. 1997–2002 funded through new legislation by the National Oceanic and Atmospheric Administration (NOAA) and the U.S. Environmental Protection Agency. Twenty-three scientists from 13 institutions collaborated in innovative science that broke ground for the follow-on program from 2002–2007. NOAA's Monitoring and Event Response (MERHAB) program supported the Eastern GOMx Sentinel Program that built capacity for enhanced harmful algal bloom monitoring and response.
1999 collaborator photo (bottom row [left to right]: Bill Hemme, Ruoying He, Merrie Beth Neely, Frank Muller-Karger, Rich Pierce, Greg Doucette, Gabe Vargo, Bob Weisberg, John Paul). (Middle row [left to right]: Heather Penta, Gail MacAulay, Jason Lenes, Cindy Heil, Karen Steidinger, Shelly Paraso, Susan Cook, Jan Landsberg, Kristen Lester, Danyelle Spence, Rachel Merkt, Mary Culver, Fran Van Dolah, Pat Tester, Paula Houhoulis, Brian Bendis, Brad Penta, Leanne Flewelling, Dan Kamykowski). (Back row [left to right]: Gary Kirkpatrick, Peter McGuire, John Walsh, Jack Fournie, Brad Pederson, Mike Henry, Dave Millie, Paul Bissett, Oscar Schofield, Kevin Sellner). Photo credit: unknown.

first put forth the concept of sequential bloom phases for ted tides – initiation, growth, maintenance (plateau), and transport. Within this seminal presentation, she discussed the life cycle and emphasized the importance of physical and chemical forcing (particularly that of salinity) for constraining the dispersion of harmful algal blooms like *Karenia*. This recognition that blooms possess multiple, sequential phases, during which distinct forcing phenomena predominate, serves as the basis for our understanding of the roles that nutrient requirements and environmental forcing have in red tide development [10]. This theory has been the basis for myriad contributions from physical chemical oceanographic studies to increasingly complex predictive models of the progression of blooms.

Such utilization of "cutting-edge" research tools to assist studies of red tides and the factors and/or mechanisms that transport them has been a recurring theme in Karen's work. In 1981, she worked with a former student, Ken Haddad (who, in 1993, succeeded her as Director of FMRI) to examine *Karenia* bloom development in relation to the Loop current off the west Florida continental shelf. This historic work subsequently was published in *BioScience* [11].

Dr. Steidinger's influence on Florida's red-tide research culminated with the planning and administration of the federally mandated program, ECOHAB-FLORIDA (1998–2003). Beginning in 1997, she recruited, assembled, and coordinated more than 20 scientists from numerous academic, state, and federal institutions to work together toward a common research goal (Fig. 3). She subsequently assisted representatives from the United States Congress (House of Representatives) in compiling information concerning harmful algal blooms and hypoxia for the preparation and subsequent authorization of legislative funding (Harmful Algal Bloom and Hypoxia Research and Control Act, or HABHRCA (HABHRCA 1998; embedded in Public Law 105-383).

From the administrative- and policy-filled days as Chief of Marine Research, Karen always sought scientific 'renewal' by looking through a microscope, whether it was her work with Linda Walker in which the entire life cycle of the first toxic dinoflagellate was documented (specifically, gamete formation within the vegetative cell to meiosis and production of the chain of four vegetative cells in *Alexandrium monilatum*) [12], to training and inspiring participants in the prestigious International Advanced Phytoplankton Course (APC, convened periodically, first in 1976 at the University of Oslo, Norway and then in 1985 onwards [for the most part] at the Stazione Zoologica Anton Dohrn in Naples, Italy) (Fig. 4a) at the Gulf (of Mexico) States Regional Harmful Algae Taxonomy Workshops (2006, 2007, 2009); at the Binational U.S.–Mexico Phytoplankton workshops, Mexico (2009 x 2, 2011 x 2); at the EPA/GOMA Harmful Algal Bloom Taxonomy Training Workshop, Florida Keys (2011); the International Marine Phytoplankton Identification Workshops, Plymouth, U.K. (2012, 2014); or lastly in 2016 and 2017, at the U.S. Harmful Algal Bloom Taxonomy training course held at Bigelow Laboratory for Ocean Sciences (Fig. 4b). The Bigelow course



Fig. 4. A. Karen was Instructor on dinoflagellates for the International Advanced Phytoplankton Courses (APC, Italy) 1985–2005 (May 1998, Naples, Italy, taken at the 7th APC).



Fig. 4. B. She was an instructor for the U.S. Course on Harmful Algal Taxonomy, East Boothbay, Maine, 2016–2017. This course at Bigelow Laboratory for Ocean Sciences has been renamed in her honor. Photo credits: A. unknown B. Bigelow Laboratory.

has been renamed in honor of Karen's contributions to taxonomy and taxonomic training. As of 2023, it is now known as the Karen A. Steidinger Marine Harmful Algae Taxonomy Training Course [13].

From the time of Dr. Enrique Balech's recommendation (in 1983) of Dr. Steidinger as a faculty member of the International APC, she treasured the time spent with the most promising of the next generation's phytoplankton systematists. In truth, this was probably her greatest contribution to science; throughout her career, she freely mentored and trained (both 'young' and 'old') scientists, so they could experience the same love for science that she did.

Clearly, Dr. Steidinger's professional contributions have impacted numerous phycologists and oceanographers; however, her influence extends far beyond the red tide arena, and into science policy and administration. She administered programs as diverse as fisheries ecology, shellfish sanitation, endangered species, and coastal and marine resource assessment. Her foresight in the use of technological tools for addressing habitat and resource management issues led to the development of state-of-the-art GIS capabilities to map habitat and overlay biological data so environmental interactions could be more easily identified and understood. The GIS facility at FWRI subsequently was enhanced by significant data storage and retrieval capacity. With such a technological base and under Karen's leadership, five-year plans were developed by the Institute to assist the defense and protection of crucial habitats and resources [1].

In 1997, Karen answered the call from the UNESCO's Intergovernmental Oceanographic Commission (IOC)'s Science and Communication Center on Harmful Algae to establish an international organization to foster and promote research and training programs and co-sponsor meetings at the national and international levels. The International Society for the Study of Harmful Algae (ISSHA) resulted from Karen's nearly single-handed efforts, and she served two terms as Society's president. Today, ISSHA meets biennially with 400–500 attendees and is a fitting legacy among her many contributions to the harmful algal community.

Scientists cognizant of Dr. Steidinger's scientific achievements and those fortunate enough to work with her held Karen in very great esteem. In 2000, Danish researchers honored her by renaming the genus of several *Gymnodinium* taxa (including *G. breve*), to *Karenia* [14]. In short, she became the gold standard in matters concerning *K. brevis* as well as other harmful algae.

Karenia G. Hansen & Moestrup, *gen. nov*. Dinoflagellata inarmata cum fucoxanthin et/aut 19'-hexanoyl-oxy-fucoxanthin et/aut 19'-butanoly-oxyfucoxanthin pro pigmentis principalibus accessoriisque. Nucleus sine loculis in involucro et sine capsula. Canalis anticus rectus. Unarmoured dinoflagellates whose major carotenoid is fucoxanthin,



Fig. 5. Karen holding the Karenia brevis carving by Dr. Takayama presented to her at the Xth International Conference on Harmful Algae (ICHA) 2002, St. Petersburg Beach, Florida. Photo credit: Don Anderson.

19'-hexanoly-oxyfucoxanthin and/or 19'-butanoly-oxyfucoxanthin. Cell nucleus without nuclear envelope chambers and nuclear capsule. Apical groove straight.
ETYMOLOGY: Named after Karen Steidinger in recognition of her many contributions to dinoflagellate research.
TYPE SPECIES: *Karenia brevis* (Davis) G. Hansen & Moestrup *comb. nov*.
BASIONYM: *Gymnodinium breve* Davis (1948, p. 358).
SYNONYM: *Ptychodiscus brevis* (Davis) Steidinger.

Since this time, some twelve additional *Karenia* species have been renamed or newly identified in honor of Karen. Two other species of dinoflagellates were named after her by colleagues (*Lissodinium steidingerae* Carbonell-Moore 1993, and *Prorocentrum steidingerae* Gomez et al. 2017).

In 2001, the Fish and Wildlife Foundation of Florida honored Dr. Steidinger with the Louise Ireland Humphrey Award (given to FWC employees) and in 2003, she was bestowed an Award of Excellence by the Phycological Society of America. This award recognizes phycologists who have demonstrated sustained scholarly contributions in and impact on the field

of phycology over their careers. This was clearly demonstrated by Karen's sixteen *J. Phycol* publications and service on the journal's editorial board from 1990–1992, 2005–2007 and 2014.

Karen and the Florida Harmful Algal Bloom community hosted the Xth International Conference on Harmful Algae in her hometown of St. Petersburg, Florida in 2002 just prior to her retirement in July 2003 (Fig. 5). Karen continued to work with the staff of FWC's FWRI and many other colleagues with the same kindness, respect and understanding that were her hallmark. In 2008, at the 13th International Harmful Algae Conference in Hong Kong, ISSHA awarded Karen the Yasumoto Lifetime Achievement Award (Fig. 6). Karen's broader environmental protection efforts were also recognized in 2008, when she was awarded the U.S. Environmental Protection Agency's Gulf Guardian Award for "individuals…that are taking positive steps to keep the Gulf (of Mexico) healthy, beautiful and productive."

After retirement, until 2013, Karen served as a Harmful Algae Specialist at the University of South Florida Institute of Oceanography on contracted and special projects such as the taxonomy of *Pyrodinium* and benthic, phagotrophic dinoflagellates; instruction of dinoflagellate systematics within various workshops; assisting scientists in the identification of dinoflagellates; serving as a steering committee member for workshops; preparing and reviewing manuscripts; and facilitating Florida's Harmful Algal Bloom Task Force Public Health Technical Panel. Karen also continued working at FWC from July 2011 in a part-time capacity.

In 2013, at the 7th Symposium on Harmful Algae in the U.S., Karen was recognized for 50 years of service to the State of Florida, a very productive career that not only extended beyond the study of harmful algae, but formed a programmatic foundation for one of the premier state government marine biology research facilities in the world. Her legacy lives on to this day. Karen continued working for FWC until ill health in 2022 prevented her from continuing.

Dr. Steidinger authored or co-authored more than 120 manuscripts in scientific journals, book chapters, and books. As recently as 2018, she co-published two book volumes with Dr. Esther Meave del Castillo, entitled "A Guide to the Harmful Algae of the Gulf of Mexico", freely accessible on the internet [15]. Moreover, she served as editor/associate editor of numerous contributions and professional journals.

As well as the breakthroughs noted earlier, Karen described or co-described ten dinoflagellate species including four genera of dinoflagellates (*Cryptoperidiniopsis*, *Luciella*, *Pfiesteria*, *Pseudopfiesteria*) new to science (Table 1); she was the first to use the term "seed beds" in relation to the initiation



Fig. 6. The International Society for the Study of Harmful Algae's (ISSHA) 2008 Yasumoto Lifetime Achievement Award winner, Dr. Karen Steidinger (Hong Kong), presented by then ISSHA president, Dr. Pat Tester. Karen's award was based on her contributions in and our understanding of bloom dynamics, and included taxonomy, physiology, ecology, life cycles, and how data can be used to mitigate the negative impacts of HABs on fisheries and public health. Photo credit: unknown.

Table 1. New dinoflagellate species and genera described or co-described by Karen

Species	Authority	Reference
Alexandrium balechii	(Steidinger) Balech	[16]
Bysmatrum subsalsum	(Ostenfeld) Faust & Steidinger	[17]
Cryptoperidiniopsis brodyi	Steidinger, Landsberg, Mason, Vogelbein, Tester & Litaker	[18]
Karenia papilionacea	Haywood & Steidinger	[19]
Karenia selliformis	Haywood, Steidinger & Mackenzie	[19]
Luciella atlantis	Mason, Litaker, Reece & Steidinger	[20]
Luciella masanensis	Mason, Jeong, Litaker, Reese & Steidinger	[20]
Pfiesteria piscicida	Steidinger & Burkholder	[21]
Prorocentrum texanum	Henrichs, Steidinger, Scott & Campbell	[22]
Pseudopfiesteria shumwayae	(Glasgow & Burkholder) Litaker, Steidinger, Mason, Shields & Tester	[23]

of HABs and recommended benthic dinoflagellate cystmapping: and she showed that many small gymnodinioid dinoflagellates were actually armored and heterotrophic or mixotrophic, a fact previously unknown. Karen's ecological foresight and breadth of knowledge lead her to hypothesize for the first time, for example, that brevetoxins transferred through the food chain could play a significant role in marine mammal mortalities [24], a fact that became evident in multiple marine mammal mass mortalities in the decades since. Karen's noteworthy scientific contributions are documented here, but it is her students and colleagues who remind us that her greatest legacy was her kindness, respect, understanding, and encouragement. Thank you, Karen, thank you.

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Remembrances: Memories of Karen's Colleagues, Students, Co-workers and Friends

Some of Karen's HAB colleagues and friends wanted to memorialize their memories of her. The following remembrances are in no order by author or date but serve to illustrate Karen's influence on a few of those she met, taught and worked with.

Message to Florida Fish and Wildlife Conservation Commission (FWC) Fish and Wildlife Research Institute (FWRI) staff from FWRI Director, Gil McRae (June 12, 2023)

It is with deep and profound sadness that I report the passing of former Florida Marine Research Institute Director and Senior Research Scientist Dr. Karen Steidinger. Karen passed away peacefully over the weekend comforted by her partner of more than 30 years, former FWC Research Scientist Dr. Jan Landsberg.

For those of us that knew and worked with Karen, her passing leaves a void that cannot be filled, but we can take a small measure of solace in celebrating her tremendous legacy. When Karen began her career in the early 1960's the playing field was far from level for women scientists; in fact, it was unclear if there was a playing field at all. Through her hard work, perseverance, and innate ability to adapt to the needs of the day, Karen not only leveled the playing field but changed the rules of the game. She was a pioneer in the field of harmful algal bloom research, particularly related to the Florida red tide (*Karenia brevis*) which was named in her honor in 2001. She was an inspiration and mentor to countless colleagues, especially women, over her more than 60-year career and her evolution from a groundbreaking woman scientist to a world class researcher who happened to be a woman speaks volumes about her legacy.



Fig. 1. FWRI Director Gil McRae with Karen at her retirement from FWC/FWRI. Evening dinner, 27 June 2003, St. Petersburg, Florida. Photo credit: Fish & Wildlife Research Institute, Florida Fish & Wildlife Conservation Commission.

Her enthusiasm and humor were infectious, and her collaborative approach to science and rigorous attention to detail formed a solid foundation on which we continue to build to this day. Karen helped establish the first state sponsored marine laboratory in Florida, setting the stage for the latter incorporation of research as a core function of FWC in the Florida Constitution and providing an example for public service science we strive to achieve in our work at the FWC Fish and Wildlife Research Institute.

I see and feel Karen all around me (Fig 1). I have the honor of occupying an office she used to work in within the FWRI St. Petersburg Headquarters that she made a reality. I see her in every scientific program where a focus on relevant, objective, high quality science allows us to serve the public in a manner that honors her legacy. Most of all, I see and feel Karen in the eyes and hearts of a large group of fantastic women scientists I have the great privilege of working with on a daily basis. Free from the tedious dissipation of energy that comes from just proving that you belong, these scientists continue to change the rules of the game.

Please take a quiet moment today to appreciate Karen's legacy and voluminous contributions to science and society. She will be dearly missed, but her legacy lives on.

Message to FWRI Harmful Algal Bloom team from Kate Hubbard, Director, FWC Center for Red Tide Research (June 25, 2023)

It is impossible for one person (or twenty people!) to capture all the dimensions of Karen, who truly was a multifaceted gem, and a mentor and friend to so many of us. As I have been trying to process this loss, I wanted to share some of the unique things about Karen and her insights into science that impact our existing program.

Karen was one of those rare people that was comfortable thinking across all scales, from the tiniest minute microscopic detail to global processes, and connecting those dots. She had a unique vision for what was needed to help truly evaluate red tide based on her observations and research. We are quite literally living out those dreams and insights, pushing to address similar questions to those she raised in papers and reports decades ago, but in new ways and at new scales, moving research forward to ask new questions. We could not do that without the extensive field and research program that she built and that you all have helped grow and sustain. Karen is why we are here at a state research institute, working with a huge network of partners, on all aspects of harmful algal blooms. There is no other place like this, in terms of technology, precision, timeliness, and reporting. She provided taxonomic training to many researchers across the world, the US, Florida, and at FWRI, through archives of teaching and culture material developed and augmented over time. She helped blur the line between monitoring and research, something that we continue to try to embrace and improve upon Karen was never one to back down from a challenge. Her work on *Pfiesteria*, Kareniaceae diversity, dinoflagellate taxonomy, and leading a research institute focused on Florida's most pressing fish and wildlife issues (just to name a few challenges!) often required a strong hand and decisive leadership, and she did not shy away from taking a sometimes-controversial position when supported by research or lack thereof. She also carefully considered how to achieve the most harmonious outcome. She had a special way of lightening difficult situations

with her easygoing communication, true interest in your background and family (if you were interested in sharing), and frequent laughter (something I hear in my head whenever I think of Karen). She prioritized supporting each other to do more together. Her unique perspectives and willingness to listen, learn, and provide solid feedback and support on a personal level will be dearly missed. She cared so much about her colleagues and their families (including pets), and her partner Jan (and their pets). She sent holiday cards to a large group of staff, funny gifts to help celebrate milestones, and shared a plethora of calendars each New Year from charities. She truly defined her own style of leadership that recognized the importance of the person behind the science, which continues to serve as a model.

Karen continued to monitor the growth and development of our program and never stopped sharing her research questions and feedback. Her delight in hearing about many of the advances –IFCBs, trace metal work, antibody development, HABs in the Indian River Lagoon, the US HAB Taxonomy course (and FWRI's involvement), Task Force developments, the recent *Karenia papilionaceae* bloom in New Jersey and particularly the efforts focused on understanding the life cycle and population dynamics of *Karenia brevis*—was tempered by the recognition of the challenges in responding on so many different levels. After one call, she sent this message: "Thank you for taking the time to update me on what was going on in HAB, it's challenging but you all are up to it. Doing all the right things and particularly resourceful! Karen fully embraced her own scientific evolution throughout her productive 60 years career, and she never stopped learning new things and incorporating new dimensions into her research and way of thinking. Karen also told me recently that she dreamt of dinoflagellate cysts and was thrilled that we found DNA signatures of *Karenia brevis* in sediments. "But what does it mean?"

My heart is with Karen and her loved ones, and with those of you feeling this loss right now. Although we can never fill the tremendous gap that she leaves, we can keep her alive in our hearts and brains, in our contributions to HAB research, in how we treat each other, and every time we say *Karenia*.

Remembrances from Karen's coworkers (Leanne Flewelling, Kate Hubbard, and HAB staff) at the FWRI, Florida Fish and Wildlife Conservation Commission

Karen the Scientist

Karen began her career in 1963 – more than 60 years ago – the entirety of which was spent at what is now the Fish and Wildlife Research Institute. Over the decades Karen has made major contributions to the fields of phycology and oceanography, primarily focusing on the taxonomy, life cycles, and ecology of dinoflagellates. She made innumerable lasting friendships, scientific advances, and trained many generations of scientists. Karen is why we are here at a state research institute that works on all aspects of harmful algal blooms. There is truly no other place like this.

Karen helped blur the line between monitoring and research. For *Karenia brevis* specifically, she provided the framework for understanding bloom phases and life cycle dynamics; spearheaded efforts to use satellite and in situ technologies in bloom detection; and contributed to our understanding of the importance of numerous forcings on bloom dynamics and the need for interdisciplinary evaluation and forecasting models. She astutely asked critical questions and mapped out complex hypotheses decades ago that have progressed, but that were so on point, comprehensively laid out, and ahead of their time, we are still working on them today.

Karen displayed an uncommon openness to collaboration with countless individuals and organizations. It's rare to find a single person who can immerse themselves so completely in the most minute details of a microscopic organism, but who can also step back, envision and convey the broader picture, and effectively bring together all of the people and the pieces needed to conduct large-scale interdisciplinary research integrating chemical, physical and biological routes of inquiry to understand HAB bloom dynamics. Our website links to numerous public-facing tools for monitoring and tracking blooms that were created by a range of partners. These tools, which we use every single day, are just a few of the outcomes of her interdisciplinary foresight and determination to provide relevant scientific information to anyone who might want it.

Karen's breakthrough work on*Pfiesteria*, Kareniaceae diversity, dinoflagellate taxonomy, and no doubt leading this research institute often involved controversial issues that required a strong hand and decisive leadership. Karen could be stubborn when the situation called for it, and she was never one to back down from a challenge, but even here, she was graceful, kind, and guided by reason. Karen fully embraced her own scientific evolution throughout her productive 50+ year career, and she never stopped asking questions, learning new things, and incorporating new dimensions into her research and way of thinking.

Karen the Teacher

Karen was by nature a teacher who freely and generously shared her knowledge and expertise, as many of us have experienced first-hand. As a teacher of national and international taxonomy classes and a mentor to countless young scientists, Karen was creative, patient and kind. She directed individual students through tailored courses on the taxonomy of dinoflagellate species, taking the time to provide technical background and hands-on side-by-side instruction, at the same time managing to make it a truly enjoyable and fun experience. Today, her teaching legacy lives on in the newly-renamed Karen A. Steidinger Marine Harmful Algae Taxonomy Training Course offered annually at Bigelow, as well as the ongoing International Phytoplankton Taxonomy Courses that she played such a prominent role in for so many years.

This year we also launched the Steidinger Scholar Internship program in honor of Karen. This is FWRI's first paid internship program. The goal of the program is to provide opportunities to 1st and 2nd year college students of all backgrounds to explore careers in fisheries and wildlife research through experiential learning. Our first cohort this summer got to learn about not only harmful algal blooms, but also sea turtles, alligators and crocodiles, freshwa-

ter fisheries and social science research. It was successful and rewarding – for both the students and our scientists. There's a poster here today that shares some of the students' feedback on what they got out of their time with us. We hope to grow this program to serve as a lasting legacy of Karen's enthusiasm for developing young scientists.

Karen was a mentor to many young scientists, even those who did not necessarily share her love of the microscope. She was encouraging and supportive, helping folks find their paths and then gently (or not so gently) starting them on their own scientific journeys. She offered her insights, helped forge professional connections, and always modeled the highest form of ethics and regard for others.

Karen's research interests combined with her many collaborations placed her at the forefront of HAB research and monitoring and through this, she developed a unique set of experiences and achievements. The significance of her contributions to the field has been marked by multiple lifetime achievement awards, and even the naming of the genus *Karenia* after her. The venue we all sit in now was designated the Karen A Steidinger Auditorium 20 years ago. And perhaps less broadly known, but incredibly meaningful to us here, are the FWRI HAB group's 22 and 31-foot catamaran Research Vessels respectively named *Miss Karenia RV Steidinger*.

Karen the Friend

On a more personal level, Karen is largely responsible for the caring, collegial, and staff-centric culture we enjoy at the Institute. Many of us that had the good fortune to interact with Karen on a daily basis were a bit in awe of, and perhaps intimidated by, Karen when we first learned of her. However, with just one conversation, that intimidation quickly dissipated into the beginning of a long and unique relationship. She had an amazing sense of humor, was quick to laugh and even quicker to smile, and was genuinely interested in what you had to say. When you passed her in the hall, she greeted you as though you were exactly who she wanted to talk to at that very moment – and she made you feel like you were the most important person in the room.

She never ceased to amaze by remembering your birthday, remarking on a career milestone, or bringing you a gift from her travels abroad. Her generosity was always on full display, and clearly extended beyond the bounds of her personal relationships. Every year Karen would receive literally dozens of calendars from the many charities she supported, and she shared them each year at our section holiday potluck. The trick was to get there early to get the best ones!

Karen made a special point to connect with her friends each Christmas, leaving holiday cards on our desks or mailing them across the globe. This small gesture had a big impact on those of us on the receiving end, and when they didn't come last year, the world noticed – sending messages from afar asking about her well-being.

Karen truly defined her own style of leadership that recognized the importance of the person behind the science, which continues to serve as a model and one of her many long-lasting legacies. We continue to see Karen every day, in our hallways, work, our lives, and every time we think of *Karenia*.

Adriana Zingone, Naples, Italy

My memories of Karen Steidinger are mostly linked to the Advanced Phytoplankton Course (APC). In 1983 she was a student at APC3 in Drøbak (Oslo) and since APC4 in 1985, she joined the faculty of the Course in Naples till APC 11 in 2015 (Figs. 2). Meeting her during APC4 was a turning point in my approach to dinoflagellate taxonomy and identification: by teaching me how to



Figs 2A & 2B. Karen at the APC 3, September 1983, held in Drøbak, Norway. Happy Birthday, Karen. Let's eat cake! Photo credits: Adriana Zingone.

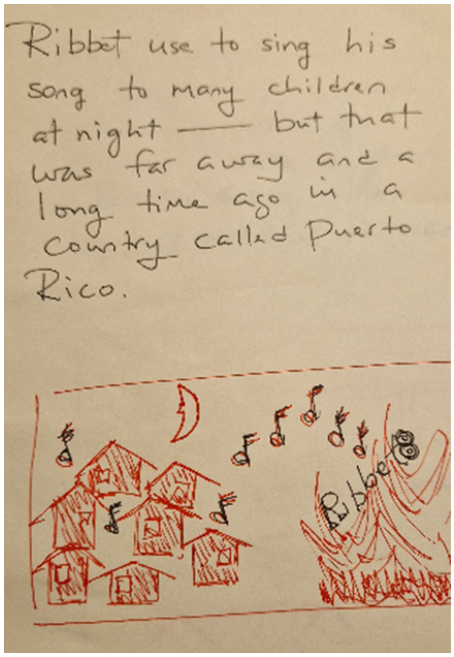

Fig. 3. Karen's playfulness was fully expressed in the little ditty about Ribbet. Photo credit: Adriana Zingone.

use a toothpick to squash theca under a coverslip she opened a world to me, providing me with a new weapon but also teaching me to be confident in my potential to identify those little things.

Since then, each following course was a new chance to learn new things about dinoflagellates but also to enjoy her company, her contagious enthusiasm, humoristic vein and boundless generosity. It was dangerous to tell her that you liked something she had, she would give it to you immediately. At each meeting she would bring gifts and sweets, take nice pictures, write poems, and make all efforts to share her vast knowledge and experience with everybody. Students were so fond of her lively lessons and so amusingly challenged when they found some mysterious dinoflagellates and Karen offered them a toothpick, accompanied by the order 'squash it!'

Among the sweetest memories of Karen is the story she invented for my daughter Laura, who was less than 2 years old at the time of APC 5 in 1990. It was about a small green frog from Puerto Rico, Ribbet, who was hidden in a towel above Laura's bathtub, and would sing songs to cheer up Laura who was frightened. The story was written in a booklet made of bent sheets, with funny illustrations drawn in black and red pen (Fig. 3). Several years ago, I reminded her about the story and she asked me for a copy, as she wanted to write a book

with other stories she had written over the years…Who knows if she ever did that.

Santi Fraga, Vigo, Spain

Since I met Karen in the Advanced Phytoplankton Course in Drøbak, Norway, in 1983 she became a reference and a friend for me. Later during a Spain-USA Cooperative program (see next section) I had the opportunity to work with her. I remember once I was not able to identify a species, so I asked her. Her answer changed my mind when she answered me: "It could be a new species". I started to think as a taxonomist, not just to identify species. Ever since, we met again many times in conferences or project meetings where our friendship was increasing. The last time we were together was at a project meeting in Sweden in 2007. I remember that after the meeting we took the same train and I helped her with her very heavy baggage and recommended her to bring her heavy papers in pen drives that were lighter. We took the train by the first door we found, and soon after we realized that we were in the silent department, and we were not allowed to talk. Then, as two children, we start with a nervous laugh because we should be silent. The picture is from that meeting, where four of the participants of that first project met again twenty years later (Fig. 4) in another project (EU Project SEED 2005-2008), coordinated by Esther Garcés. Nice to remember her (Karen) laughing and happy.

Beatriz Reguera & Isabel Bravo, Vigo, Spain

Karen in Galicia. In 1985 Spain had not joined yet the European Union. Galicia, in the Northwest corner, was one of the poorest regions, accessed by twisty and dangerous mountain roads. Still, extraordinary coastal resources in Galicia (northern limit of the Canary Current upwelling system) had attracted the attention of eminent scientists. Striking red tides in Ria de Vigo in the1950's had been a source of inspiration for Ramón Margalef and his seminal Mandala. But the red tides topic was forgotten until October 1977. That autumn, a severe paralytic shellfish poisoning (PSP) outbreak, the first report of *Gymnodinium catenatum* in Europe, made nearly 200 mussel consumers ill and put the Galician shellfish exports in check. A nation-wide programme was established in record time in 1978, to monitor phytoplankton and environmental conditions on different parts of the Spanish Atlantic and Mediterranean coasts. All beginners with meagre grants at the time on the IEO, including Santi Fraga and myself, have to acknowledge *G. catenatum* for getting out first contract.

In 1984, attracted by the news of a new laboratory being built in Vigo and rumours of potential cooperation projects with the Americans, I moved to the IEO Centre in Vigo. That summer, Santiago Fraga, PI of the phytoplankton group, was preparing the proposal for a study on *Toxic Dinoflagellates in Galician Coastal Waters*". The counterparts were Don Anderson (WHOI), PI of the American side, Karen Steidinger (then Florida Department of Natural Resources) and Clarice Yentsch (Bigelow Laboratory for Ocean Science). The proposal was approved and funded (Grant CA-8411/089; 1985-1988) by the US-Spain Joint Committee.

Karen Steidinger and Clarice Yentsch had their first project trip to Vigo in September 1985. We were very excited but also very concerned about what the Americans were going to find. The new laboratory had not been completed yet and the facilities in the old one were appalling: crowded in an old flat facing the fishing harbour, with heavy traffic, soot coming through the loose window-frames and a permanent deafening noise from seagulls overflying the fishing vessels. Then, that bad reputation the Americans had of being fierce competitors, only worried about their determination to lead new publications. At least we had the small but very efficient RV *J.M. Navaz.* Meeting Karen and Clarice was a great surprise that overcame all our prejudices. Karen was so welcoming and approachable, it was like meeting an old friend or relative willing to teach you her kitchen recipes. And she had the virtue of making things sound easy and simple. We went (Isabel Bravo and myself) on a boat survey to the Cíes Islands by the mouth of Ría de Vigo, the site of spectacular bioluminescent blooms of *Noctiluca* in summer, and took samples from the intertidal lake in the main Island in addition to net tows and bottle samples. Back in the lab with Karen we lost our fear about single cell micro-pipetting to establish monoalgal cultures and laid (without knowing it) the cornerstone of what would become one of the most important Culture Collection of Harmful Microalgae in Europe. While Isabel was isolating her first strains of *Prorocentrum lima*, Clarice came screaming with excitement at the sight of the orange colour autofluorescence ofn a cell of *Dinophysis acuta.*

The following year (1986), Isabel Bravo went to Karen's lab as a visiting scientist. In her own words: "I remember Karen very fondly. My memory is of a very determined woman with a great capacity for work. With her I discovered the world of benthic dinoflagellates. Her help and kindness were invaluable. Above all, I remember the ease and



Fig. 4. Isabel Bravo, Santiago Fraga, Karen and Don Anderson, July 2007, in Lund at a coordination meeting of the EU project SEED (2005-2008) project: Life cycle transformations among HAB species, and the environmental and physiological factors that regulate them. Photo credit: Santi Fraga.



Fig. 5. Members of the old and new ISSHA Executive and Council pose at the Jumbo Conference Banquet around the 2008 Yasumoto Award, Karen Steidinger. From left to right, front row: Ichiro Imai, Gustaaf Hallegraeff, Beatriz Reguera, Pat Tester, Karen Steidinger, Rita Horner, K.C.Ho; back row: Lincoln MacKenzie, Greg Doucette, Hak-Gyoon Kim, Henrik Enevoldsen, Don Anderson, Ian Jenkinson, Øjvind Moestrup, Nina Lundholm, Barrie Dale, Jennifer Martin, Jane Lewis and Eileen Bresnan. Photo credit: Brian Lapointe.

courage with which she approached new study objectives. She took me in at her home in St. Petersburg in 1986, during my two-month stay at the Florida Marine Research Institute, where she was also my tutor. During this time I had the pleasure of knowing her great kindness and great generosity on both a personal and professional level."

Years later I (Beatriz) had the opportunity to see Karen in action on two occasions. First in 2002, when she was chairing the Scientific Steering Committee for the 10th ICHA Conference in Saint Petersburg, Florida. Karen had to deal with the chronic discussion preceding every ICHA steering committee meeting: "Shall we continue publishing ICHA proceedings?" Karen led the discussion swiftly and tipped the balance to the traditional series of proceedings (later interrupted in two following conferences) and with a "Why not, I do not see any problem, if they are going to be too many contributions, let's make them 3 instead of 4-pages long", she gave way to the thickest ICHA proceedings ever edited. The second occasion was at Havreholm, Denmark, spring 2006. In that meeting, with energy and pragmatism, Karen was the person who established the basis for the functioning of the International Society for the Study of Harmful Algae (until then a utopia): the Council structure was laid, Committees formed for different tasks, duties distributed and the statutes set forward. Finally, we all had the opportunity to congratulate her in Hong Kong, 2008, when she received the Yasumoto Award (Fig. 5)

Karen's criteria always prevailed, as those of a High Priest, because it was easy to perceive she was always looking for the common good, and without a speck of arrogance. In summary, she was *the authority*

Quay Dortch, Washington, D.C.

(Tribute to Karen Steidinger given in introductory remarks for National HAB Observing Network Community (NHABON) Webinar #8 Observing needs for *HAB Events and Response: Regional Highlights*. June 14, 2023 by Quay Dortch (Fig. 6), NOAA Senior HAB Scientist and Co-chair of Steering Committee for NHABON of Practice)



Fig. 6. Jan Landsberg, Don Anderson, and Quay Dortch having dinner with Karen in Sarasota, Florida. Photo credit: Don Anderson

"I want to welcome everyone to our 8th NHABON Community of Practice Webinar. We will have four talks on Event Response and then a question and answer period. There must be a lot of interest as we have a record number of registrants. Before we start, I would like to take a minute to note the passing of Dr. Karen Steidinger, whom many consider to be one of the founders of HAB science. She was a dinoflagellate taxonomist, who focused on *Karenia brevis*, which after several changes of name early on was named after her. However, her science became much broader than *Karenia* or taxonomy, focusing on trying to understand and manage multiple HABs. She was also for many years the director of Florida Fish and Wildlife Research Institute, a premier state science and management laboratory. Finally, she was a mentor and role model to many young and not-so-young scientists, including me.

She would have been all for a National HAB Observing Network. In thinking about how I came to know her, I remembered the early days of something called GMNET, a rudimentary HAB observing network for the Gulf of Mexico, when I was a researcher in Louisiana. We thought that we had hit the event response jack pot when the development

of the internet allowed instantaneous, Gulf-wide communications and discussion about HABs and fish kill events. How things have changed as we talk about Event Response today. But she would also say, that all of these automated methods of observing HABs are wonderful and have their place, but we still need expert taxonomists to definitively ID HABs, and all HAB scientists need some basic taxonomic knowledge. When I became a NOAA HAB Program Manager, this spurred me to push for more opportunities for taxonomic training. Her expertise and gentle guidance will be missed.

Recording available on NHABON web site under webinars at the beginning of June 16, 2023 webinar. *https://ioosassociation.org/nhabon/*



Fig. 7. Karen with her early mentor, Professor Enrique Balech at the Sherkin Island, Eire, Taxonomy and Biology of Toxic Dinoflagellates & Other Harmful Plankton Workshop, 13–19 June 1989. Photo credit: Jan Landsberg.



Fig. 8. Karen during the 1983 Drøbak Phytoplankton Taxonomy course (inset); superimposed upon Karenia papilionacea previously known as the "butterfly type" of Karenia brevis. Photo credit: Miguel de Salas.

Gustaaf Hallegraeff, Tasmania

My first encounter with Karen was in 1983 in Drøbak, Norway, where she taught me dinoflagellate taxonomy at the Advanced Phytoplankton Course (APC 3). Several years later in 1989, I had the honour to spend quality time with her and her mentor Prof Enrique Balech at the 2nd Sherkin Island Toxic Dinoflagellates workshop (Fig. 7), followed by a personal visit to her Florida lab in 1991. I witnessed dead fish on local beaches and elderly tourists wearing face masks. Karen then was Chief of Florida's DNR Marine Science and Technology. Her office phone ran red hot but she still succeeded to make time to show me around and entertain. Omnipresent throughout our world oceans, the graceful swimming behaviour of *Karenia* "resembling a falling leaf" (her words) will forever remind us of Karen's hallmark kindness, respect and understanding in dealing with colleagues, industry and politicians in our at times challenging field of HAB science. May we all share the same love and grace for our science as she did.

Karen's focus on ever deeper understanding of blooms of the Florida red-tide dinoflagellate, first reported by Spanish explorers in the 1500s and initially called *Gymnodinium breve* Davis 1948, is legendary. Her 1979 PhD on its cellular ultrastructure (exploring 200 fixation schedules) first recognised that this species did not fit into *Gymnodinium,* and during the 2nd Conference on Toxic Dinoflagellate Blooms she thus proposed transfer to *Ptychodiscus*. This name is still reflected in the chemical nomenclature of brevetoxin analogues (*P. brevis*, PbTxs) investigated by her colleague Dan Baden. It was Terje Bjørnland who first presented evidence at an IUPAC Carotenoid Symposium in München 1984 that this dinoflagellate contained fucoxanthin derivative chloroplast pigments instead of peridinin, thus grouping it with other fish-killing dinoflagellates such as *Gymnodinium galatheanum* (now *Karlodinium veneficum*), (then called) *Gyrodinium aureolum* and *Gymnodinium nagasakiense* (both now *Karenia mikimotoi*). By 2000 sufficient molecular LSU rDNA sequences had become available to justify the discrimination of the genera *Karenia* G.Hansen & Moestrup, *Karlodinium* J. Larsen and in 2003 *Takayama* de Salas, Bolch, Botes & Hallegraeff, all comprised within their own family Kareniaceae. This opened the door for the description of many more species from multispecies *Karenia* bloom events in New Zealand, Australia, South Africa, Chile, China, Japan and the Philippines. My favourite publication by Karen is her Nova Hedwigia Beihefte 2008 account (with Jennifer Wolny and Allison Haywood) where she confirmed that Gulf of Mexico waters contain not just *K. brevis* but also *K. papilionacea, K. mikimotoi, K. selliformis, Karlodinium veneficum, Takayama pulchella, T. helix, T. tasmanica,* and at least three still undescribed morphotypes such as "Mexican hat" (Fig. 8). This at least partially explains why not all Gulf of Mexico, Florida, Delaware, Maryland, and Virginia *Karenia* blooms are equally harmful. But why the real brevetoxin producing *K. brevis* so far has only been confirmed from Florida remains unexplained. Similarly, the high fish-killing potential by *K. brevis* cannot be accounted for based on the ichthyotoxicity of brevetoxins only.

Barrie Dale, Oslo, Norway

Karen was one of my dearest and oldest (since the 1960s) colleagues and friend (Fig. 9). As one of a diminishing generation of scientists in the field, I feel an enormous loss for our science; but I also mourn the loss of a wonderful person with whom I was privileged to have shared a part of life´s journey. The history of HAB-studies has two main periods: early, scattered global studies prior to 1972 and the increasingly more integrated international "modern" research field that developed after the now famous 1972 "red tide" along the US East Coast. Karen was one of the few pioneers spanning both periods. It was not easy for her in the old days, struggling to understand this threatening aspect of phytoplankton ecology in Florida with a thriving tourist industry built on its sunny pristine marine environment. There is still much to learn about the fundamental causes of HAB, even after all the intervening years of research, and she also had to contend with fluctuating cycles of funding that many have experienced since. Funds were readily available immediately when toxic events occurred (when it was too late to investigate the causes), and greatly



Fig. 9. Karen with Barrie Dale at the 2008 International Society for Harmful Algae biennial meeting in Hong Kong where Karen was honored with the Society's Yasumoto Lifetime Achievement Award. Photo credit: Pat Tester.

reduced in years without events (important for documenting responses to possible causative environmental factors when the next event occurred). But Karen was very much her own person – confident in who she was and what she stood for, and she helped to pioneer not only the science, but also the development of important public awareness that allows the sensible management of "red tides".

We were soulmates who shared the unique excitement of an emerging "new" field of research together with a philosophy of life that greatly appreciated the decent honest fellowship we helped develop around the microscopic bit of natural history we began to understand. Karen from her studies of living dinoflagellates and "red tides" and I from our studies of living representatives of fossil dinoflagellate cysts. Karen was the first to appreciate the importance for HAB studies of our discoveries of dinoflagellate resting cysts (Wall and Dale at Woods Hole in the 60s and 70s). We had already identified the cysts of what is now called *Alexandrium catenatum* from the US East Coast bloom in 1972 before I left Woods Hole in 1974, and I subsequently described similar cysts for *Alexandrium* from the Oslofjord while working in Norway. Karen was the first to formulate the importance of such cysts as "seed beds" in sediment with the potential to excyst and initiate harmful blooms. There was a lot of exciting science to share as the modern phase of HAB studies began to emerge in the early 70s.

Bill Richardson, St. Petersburg, Florida

I first met Karen in 1994 when she interviewed and then hired me for a phytoplankton position in what was then the Florida Department of Environmental Protection, now the Harmful Algal Bloom (HAB) group of FWC, As the years have gone by it has become increasingly clear to me, how very fortunate I was. Although excited to now have a full-time job in my field of interest, I was not fully aware of the privilege it would be to work at FWC and especially to work for Karen. In truth, I had won the lottery.

Twenty-one years of having Karen as my supervisor, teacher, mentor and friend was as good as it could get. She filled those 21 years with a perennial smile and daily conversations about our work, what was known and needed to be known about Florida red tide and other harmful algal bloom species, while continually energizing our curiosity and excitement for our work. Her daily guidance and assistance to all of us in the HAB group was inspiring. She was a seemingly endless source of energy and goodwill, and fun to be with (Fig. 10).

Always leading by example, she demonstrated daily for us the path to becoming an accomplished scientist. Although it didn't take long to realize that professionally we wouldn't approach Karen's accomplishments or stature, we still benefited greatly by the personal interest she took in nurturing the necessary drive in each and every one of us. Walking down the hallway it was common for me to see Karen in the lab teaching techniques in microscopy or taxonomy to other fortunate and eager students or staff.

Several things will always keep me in awe of Karen. First, was her genuine interest and pleasure in helping those around her and her willingness to give so very much of her time. Time given, while the work on her desk grew ever larger



Fig. 10. Bill Richardson and Karen taken at the FWC/FWRI dedication of the KAS auditorium, 11 December 2002, St. Petersburg, Florida. Photo credit: Fish & Wildlife Research Institute, Florida Fish & Wildlife Conservation Commission.

and necessitated that she consequently work longer that day or night. For 21 years I saw her manage what looked to me to be an extraordinary workload, while at the same time always keeping herself available for the steady stream of questions and requests for guidance from me and other members of the HAB group, giving her full attention to each person, as if that were the only thing that she had to do that day. How she did that with such composure and patience - continues to amaze me. Additionally, in those 21 years I do not remember her ever speaking an unkind word about another person, again helping us all to be our better selves.

As we celebrate Karen's very significant contributions to marine science, FWC, and the positive impact she had on so many of us, whether we are still working or retired, we can remember how she lived her life and aspire to be more like her by generously giving our time and helping those around us.

Consuelo Carbonell-Moore, Corvallis, Oregon

Karen was an amazing human being whose mark will forever be embossed on those she touched. I first met Karen on a Monday March 1977. I had especially flown from Bogotá, Colombia to discuss my BA thesis, "Diatoms and Dinoflagellates of Cartagena Bay, Colombia" with her. I was familiar with her reports on the dinoflagellates of the Hourglass Cruises, while there was no one in my country who could help me with my thesis. I showed up unannounced at the Florida Fish Wildlife Institute in St Petersburg, Florida, not having confirmed whether she worked there or that at least she was in town. Traveling then was a lot easier than it is now. Airports were smaller, there was hardly any security, and my Dad was an executive at the Colombian Airlines and I could fly for free. Karen was at the time working on her PhD! and at the same time working at the institute. That morning I waited for her arrival with the certainty that all my questions were going to be answered. I introduced myself. Years later she mentioned that she had never forgotten the image of this young woman sitting down, waiting for her asking for help with her thesis. That was the beginning of a very long relationship. That week she kindly answered what she could and let me photocopy all the main references which I knew about, but didn't have access to in my country. In 1991 I ran into her at the Conference of Toxic Dinoflagellates in Newport, Rhode Island. By then she was already the director of the institute. When she learned that I had nice material to study, but I couldn't afford the high cost of a scanning electron microscope, she offered me the opportunity to use the Institute's SEM for free. I would have to pay for the polaroid film was before the digital era. I was then working at Oregon State University, I had come with a Fulbright scholarship to get a master's in Biological Oceanography even though my passion was dinoflagellates. I had contacted Max Taylor expressing my interest in learning about dinoflagellates with him after working with his monograph on the Dinoflagellates of the International Indian Ocean Expedition. Fate is strange. Max had responded my letter which I received nine months later, when I was already in Oregon. I still went to work with Max for a semester at University of British Columbia in Vancouver. I learned there how to use the SEM and later on I published two papers with that material. The last two years of my studies, I dated whom a few years later became my husband. After getting my degree I had to go back for two years to Colombia (USA government requirement). Once those two long years ended, I came back to Oregon to marry my fiancée and I also got a job at Oregon State University. And now, let's go back to Rhode Island. Without Karen I would have never been able to afford the use of an SEM. So, in early 1992 taking advantage of Karen's generous invitation, I went to St Petersburg to work with the SEM for two full weeks, from 6 am until midnight. Karen even provided where to stay at her mom's house. She even bought me groceries that first trip. I was after a specific taxon, *Blepharocysta striata* Schütt. And I looked and I looked, tried so many different samples, to no avail. The standard net for phytoplankton had a mesh of 64 µm and occasionally cells smaller were caught. But I was isolating cells with a micropipette and washing them four times before placing them on the stub for SEM observations. That was

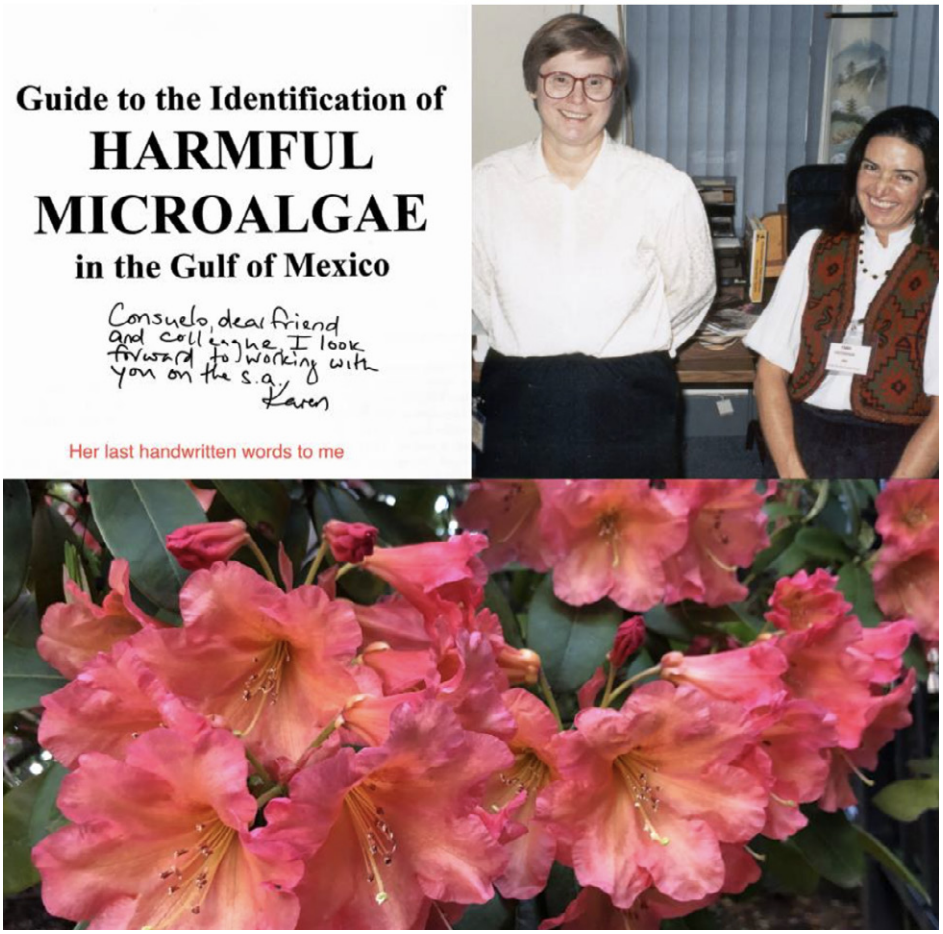

Fig 11. Karen and Consuelo Carbonell-Moore and Karen's last note to her. Photo credit: Consuelo Carbonell-Moore.

nervous wracking. In October of 1992, my late husband, Sandy Moore, who also worked in Oceanography at OSU was participating in the JGOFS cruises in the Equatorial Pacific. He brought me material that was discarded from the phytoplankton collections. These collections use the first phytoplankton MOCNESS nets, with a mesh of a very small opening, 25 µm. Besides, these nets as they open and close on demand, have perfect control for horizontal tows at a determined depth. I already had the hunch that *B. striata* was going to be a deep species as most of their reports came from deep samples. As I am looking at the microscope one of the deep samples, I couldn't believe my eyes. So, another trip to St. Pete confirmed my suspicions and eventually I described a new genus for *B. striata*. The SEMs that I took under Karen's generosity, allowed me to describe 17 new species of another podolampadacean genus *Lissodinium* and three new genera. My life after 1996 took a different turn and dinoflagellates were set aside until again, in 2002 I ran into Karen at another Toxic Algae meeting hosted by her at St Petersburg and then later in 2008, when I saw her in Montreal at the Dino 8 meeting. This time I had in my possession material that I had collected in the Indian Ocean during one of my cruises in the 1990's which had never been looked at as I had left oceanography. Karen, though retired, offered to help me have access to the SEM. This time the images acquired were digital! These images showed amazing small oceanic dinoflagellates, and the second part of my work on dinoflagellates started. So, once again, Karen had given me her support which let me advance further and further in the study of not only the Podolampadaceans, but other families of thecate dinoflagellates. After 2009 Karen and I were in continuous communication, I visited her every time I went to see my relatives who live two hours away from St Pete., exchanged Christmas cards and our last project was going to be a paper on the connection between the anterior sulcal plate and the apical pore. Unfortunately, my husband passed away unexpectedly in 2019 and slowed down my dinoflagellate work. Karen had met him many times, and I found some relief in her kind words about him. When I learned about Karen's departure, I was devastated as I had not said goodbye to that person who was pivotal in my development as a dinoflagellate taxonomist. I am now more devastated as I only learned about the service yesterday, which didn't give me enough time to join those people who not only respected her as a scientist but also loved her. Karen will forever be remembered as the sweet, cordial, understanding, tolerant, generous woman that she was (Fig. 11). Farewell my friend, thank you for having been in my life!

Vera Trainer, Seattle, Washington

Karen was my hero. Her 1981 paper with Haddad inspired me to learn more about the world of harmful algae. I met her in the early 1980s when I was Dan Baden's graduate student, invited to speak at a small, local symposium organized by Matt Murphy on Sherkin Island, Ireland (Fig. 12). During that conference, Karen got laryngitis and asked me to give her review talk on harmful algal blooms. She complemented and encouraged me. Then at night, we walked to the pub for a rousing evening of limericks and singing. We slept in the drafty cabins, listening to the horses chew on grass in the fields. Matt's daughter cooked corned beef and cabbage for us. It was an amazing, inspirational experience, especially because Karen took the time to make me feel welcome and included by introducing me to giants in the field.

Daniel G. Baden, Wilmington, North Carolina

I write today about Karen with mixed emotions, with sadness at her passing but with happiness at having known her for my entire academic research career.

I was a young graduate student at the University of Miami in the fall of 1973. My PhD supervisor Thomas Mende and I were reviewing possible subject for my PhD dissertation research proposal. We agreed that marine toxins were an interesting subject area. I thought Blue Ring Octopus venoms were exciting, Tom said Florida Red Tide was an interesting Florida problem.

I did some research about Florida Red Tide, and a name that kept coming up was Karen Steidinger at the Florida Marine Research Institute in St. Petersburg FL. I wrote a letter to Ms. Steidinger (pre-PhD days for her) and was surprised to get a letter response the next week. This began a back-and-forth about getting a starter culture of *Gymnodinium breve*. We agreed that I would drive to St. Pete to retrieve a culture to bring back to Miami. The timing was based on when she would return from "Tally", whatever that was. I arrived for my visit and found her office in a large old wooden building that looked like it was built in World War 2. Karen met me at her office door and said "Dan Baden, how are you?" It became the familiar greeting I would receive from her anytime we would meet.

Karen provided me a 10 mL culture of the classic W53 collected by Bill Wilson, a copy of their NH-15 media recipe, a promise not to provide the culture



Fig. 12. Matt Murphy, Vera Trainer, Enrique Balech, and Karen at Sherkin Island, Eire (13-19 June 1989, Taxonomy and Biology of Toxic Dinoflagellates and other Harmful Plankton). Photo credit: Sherkin Island Marine Station.



Fig. 13A. Enrique Balech, Karen Steidinger, Edward Schantz, Dan Baden, Carl Medcof, Alan White (3rd International Conference on Toxic Dinoflagellates, St. Andrews, New Brunswick, Canada, 8–12 June 1985). Photo credit: unknown.

to any third party, and the telephone number of Karen and of Beverly Roberts at the lab. I called that number and used their knowledge of Jim Breve many times that first year. The reason I mention this story is because Karen's generosity and friendliness helped form the foundation of a collaborative spirit that included Karen but expanded to include my developing ideas about what collaborative research meant.

Over the past 50 years, we have collaborated in joint publications. We have met at several of the International Red Tide/Toxic Marine Algae/Harmful Algal Bloom Conferences world-wide, characteristically by greeting me with "Dan Baden, How are you?!" (Note the exclamation point, it was always genuine). A special meeting was the Third International Conference on Toxic Dinoflagellates, where three pioneers in the field linking plankton research and toxin production were honored: Enrique Balech (Argentina, taxonomy and systematics), Edward Schantz (USA, saxitoxin and food biology), and Carl Medcof (Canada, Saxitoxin and molluscan biology) I believe the honoring tradition has continued for many if not all of the International HAB Conferences since (Fig. 13A).

I have followed her work on the taxonomy of *Gymnodinium breve* progress to *Ptychodiscus brevis* and finally resting at *Karenia brevis.* This monumental work appropriately honors Karen in the genera designation. Her work is foundational in nature. She was awarded the PhD in Biology in 1979.

She was always fascinated by the "unique architecture" of single celled algae and especially in *K. brevis*. I was always interested in the toxins from *Karenia brevis.* We were each interested in the environmental and biological harm that Florida Red Tides caused, and often over the years we spoke about tools and tests, controls and antidotes to Florida Red Tide toxins. I think this is the most unique part about Karen Steidinger's scientific curiosity; it was broadly based yet expertly focused.

Our communications had been reduced these past years, especially since my move to North Carolina. But we always exchanged Christmas cards, with her writing a short note in the card and signing it "Karen", and we responded with our always late Christmas card and a "missive" on what we were doing.



Fig. 13B. Karen and Dan Baden at the dinner banquet, Sixth Toxic Dinoflagellate Conference, 18–22 October 1993, Nantes, France. Photo credit: Don Anderson

Karen, I'll miss the collaboration, the many times we talked about science and research, the meetings (Fig 13B), and the research on the beaches in St. Pete. But mostly I'll miss the Christmas cards simply signed "Karen", telling me that you are still with us.

My condolences to your family and friends, each of whom have lost a dear friend and colleague.

Don Anderson, Woods Hole, Massachusetts

I was extraordinarily fortunate to have Karen Steidinger as a mentor, a friend, and a colleague. Those interactions began in 1974 when I was a graduate student searching for a thesis topic. A massive red tide of what we then called *Gonyaulax tamarensis* (now *Alexandrium catenella*) had occurred for the first time along the coast of New England, throwing the entire region into turmoil. My advisor and I felt there would be a solid topic for a dissertation within that event, and so I dutifully began growing cultures and doing experiments but lacked a clear direction. In my cultures, I kept seeing non-motile cells that collected at the bottom of the flasks, but I had no idea what they were and tended to ignore them. In one of those twists of fate that happen only too rarely, the first in our series of international HAB conferences was held in nearby Boston, with the focus being the Florida and New England red tides. I participated in that meeting and was captivated by Karen's presentation. She was among the first to recognize the importance of dinoflagellate resting stages or cysts in the dynamics of red tides. At that time, paleontologists had just discovered that the fossilized remains of a group of extinct organisms called hystrichospheres were actually the cyst walls of dinoflagellates, many of which were still living. Karen was quick to emphasize the importance of these life history stages, writing "*The recent investigations of Dr. David Wall ……lend credence to the speculation that pelagic, toxic dinoflagellate blooms might originate from dormant stages and that these stages might be associated with certain bottom sediments. This then brings up the question, if benthic resting*



Fig. 14. Karen and Don Anderson at the 13th ICHA Conference, 2008 in Hong Kong. Photo credit: Pat Tester

stages of certain dinoflagellates actually "seed" coastal red tides, are there localized areas of accumulation, or what we could call "seedbeds"?. It would seem that this avenue of research should have a high priority among phytoplankton systematists and ecologists.

I spoke to Karen at that meeting, telling her about the cells in my cultures, and as I hear from so many others, she graciously gave her time to this naïve graduate student, recommending that I travel down to Woods Hole Oceanographic Institution (WHOI) to seek guidance from Dave Wall, who was actively working on the linkages between fossilized and living dinoflagellate cysts. Thankfully, Dave also gave me his time and guidance, and we ultimately found the true resting cyst of *Gonyaulax tamarensis* (not the cells in my cultures, which were "temporary" or pellicle" cysts). This led to several important papers, a complete redirection of my thesis, and a postdoctoral position at WHOI, where I still run a research program that includes considerable work on resting cysts and their "seedbeds".

Throughout the years, Karen and I talked and corresponded frequently about the work we were doing and the progress in our field, punctuated every year with the exchange of birthday cards since we have the same birthday. We also met up at conferences and other meetings, where her calm and consistent scientific leadership was always evident. My career eventually advanced to the stage where I could reciprocate by nominating her for distinguished awards such as the *Yasumoto Lifetime Achievement Award* of ISSHA (Fig. 14), and the Phycological Society of America's *Award of Excellence*. It's fascinating to read through those letters now – there were so many accomplishments to highlight - the superlatives were many and the awards richly deserved.

I was deeply saddened by her prolonged illness and eventual passing. Karen had a profound influence on my scientific career and thus my life, and I miss her deeply.

Thank you, Karen.

Clinton Dawes, Tampa, Florida

Thank you for this opportunity to say a few words about an outstanding scientist and friend, Karen Steidinger, who I have known since the early 1970's. Karen joined my lab at USF after completing her BA in Zoology in 1968 and her MS in Marine Science in 1971. She was one of my earliest graduate students and was awarded her Ph. D in 1979 at the University of South Florida. Her dissertation was an ultrastructural study of the dinoflagellate *Ptychodiscus brevis*, the name was changed to *Gymnodinium breve* and more recently to *Karina brevis*. Part of her study was published as an abstract in the Journal of Phycology in 1977 two years before she completed her doctorate degree and her dissertation was published in the same journal in 1978, one year before she had her Ph. D.

The late Dr. Ernest Truby was a coauthor on those ultrastructural studies, a fellow doctoral student in my lab with her, and an outstanding research technician. Ernest worked with her at the Florida Department of Natural Resources and was co-author of several research papers with Karen. As noted, the species *Gymnodinium breve* was then transferred to the new genus, *Karina,* named in honor of her pioneering studies on dinoflagellates and other harmful Red Tide species.

Please understand, when Karen



Fig. 15. Karen (middle row, first left) with fellow USF graduate students (bottom row, first left, the late Dr. Ernest Truby) and friends at the home of Clinton (bottom row, far right) and Kathy Dawes, December 1977. Photo credit: Clinton Dawes.



Fig. 16. Kate Hubbard, Leanne Flewelling, Jan Landsberg, Pat Tester, and Barb Kirkpatrick at the memorial for Karen, 9 September 2023, St. Petersburg, Florida. Photo credit: Pat Tester.

joined our lab in 1974, techniques for the preparation of biological specimens for ultrastructural study were still developing and certainly not much was known regarding dealing with unicellular organisms such as dinoflagellates. The types of embedding plastics, chemical fixation, and ultrathin sectioning with glass and diamond knives were being developed. Thus, many of her early efforts were involved in enhancing these techniques, yet after three years in 1977, she published her first results at the International Seaweed Symposium in Santa Barbara California that resulted in national interest in her work.

Karen was one of the most gifted, focused, and friendly people that I have ever worked with and a model for her fellow graduate students as noted by other lab persons. While she carried out her studies at USF, she was also fully employed from 1963 onward at the Florida Department of Natural Resources in St. Petersburg, first as a technician (1963-1974), then as the Botany and Technical Sciences Supervisor (1974-1978). I remember her coming over on Saturdays to do ultrathin sectioning, fixing, and embedding of *G. brevis*, and working on the TEM with me.

In 1980 she became Chief of the Florida Marine Research Institute in the Florida Department of Natural Resources and continued as Chief until 1993. Even while directing a major state-wide facility for thirteen years, Karen continued to be involved in her studies of red tides and harmful algae and was both nationally and internationally recognized.

As shown in the photo in the Kudoboard (Fig. 15), she came to our student gathering; both Kathy and I remember those early years at USF with great pleasure. Karen led an intellectual life of great achievements and a social life of warm friendships. So, I am happy and honored to share a few of my thoughts about Karen with you today. Karen Steidinger will be remembered.

Endnote

On 9 September 2023, friends and colleagues attended (or listened in virtually) to a memorial service for Karen held at the Florida Fish and Wildlife Conservation Commission's, Fish and Wildlife Research Institute, St. Petersburg, Florida. In the afternoon, accompanied by bagpipe music, Karen's ashes were scattered by friends (Fig. 16) into the waters of Boca Ciega Bay, Gulf of Mexico, an area where she conducted so much of her red tide research.

Jan Landsberg, Parrish, Florida
kuatzin92@gmail.com

Pat Tester, Ocean Tester, LLC, Beaufort, North Carolina
Ocean.Tester@yahoo.com

New Chair and partial renewal of the GlobalHAB Scientific Steering Committee

On September 2023, the Scientific Steering Committee (SSC) of the IOC-SCOR programme, GlobalHAB (*www.globalhab.info*) was partially renewed. The composition of the new SSC includes four new members replacing previous ones:

Current SSC members

Renewed, incorporations in 2020

- *Clarissa Anderson* (Executive Director, Southern California Coastal Ocean Observing System -SCCOOS-, Scripps Institution of Oceanography, California, USA). New Chair
- *Hae Jin Jeong* (Seoul National University, Korea)
- *Malin Olofsson* (Swedish University of Agricultural Sciences, Sweden)
- *Heather A. Raymond* (The Ohio State University, USA)
- *Raffaele Siano* (Ifremer, France)
- *Aletta Yñiguez* (Marine Science Institute, Philippines).

New incorporations in 2023

- *Aifeng Li* (Ocean University of China, Qingdao City, China)
- *Luisa Mangialajo* (Université Côte d'Azur, France)
- *Jorge Mardones* (Instituto de Fomento Pesquero -IFOP-, Puerto Montt, Chile)
- *Silvia Nascimento* (Federal University of the Rio de Janeiro State, Brazil)

SSC members stepping down

- *Elisa Berdalet* (former Chair, 2016-2023, Institute of Marine Sciences, CSIC, Spain)
- *Po Teen Lim* (former Vice-Chair, 2020-2023, University of Malaya, Malaysia)
- *Neil Banas* (University of Strathclyde, Glasgow, UK)
- *Bengt Karlson* (Swedish Meteorological and Hydrological Institute, Sweden)

Liaisons with entities that have HABs in their terms of reference

- *Dave Clarke* (Marine Institute, Ireland), ICES-IOC-WGHABD
- *Raphael Kudela* (University of California Santa Cruz, US), GOOS and IPCC
- *Philipp Hess* (Ifremer, France), IPHAB
- *Maggie Broadwater* (NOAA, USA), IPHAB
- *Vera Trainer* (University of Washington, USA), PICES
- *Marc Suddleson* (USA), NOAA.



Members of the SSC at the ICHA 2023, Hiroshima, Japan. From left to right: Bengt Karlson, Dave Clarke, Raffaele Siano, Raphael Kudela, Marc Suddleson, Clarissa Anderson, Elisa Berdalet, Luisa Mangialajo, Philipp Hess and Aifeng Li.

Sponsors representatives

- *Henrik Enevoldsen*, IOC UNESCO/ IOC Science and Communication Centre on Harmful Algae at the University of Copenhagen, Denmark
- *Emily Twigg*, Scientific Committee on Oceanic Research, USA
- *Sun Yun*, Junior Professional Officer, IOC UNESCO/ IOC Secretariat, Paris, France

GlobalHAB is happy to announce that the new Chair of the SSC is Dr Clarissa Anderson. GlobalHAB thanks Clarissa and the current SSC members for accepting the commitment to serve GlobalHAB in the new term of the program. GlobalHAB also acknowledges the dedication of all past SSC members engaged with the programme since it was launched in 2016 (see *GlobalHAB webpage*).

Some SSC members attended the International Conference on Harmful Algae in Hiroshima last November 2023, and used the opportunity to hold a meeting to initiate this new period of GlobalHAB.

To conclude this note, GlobalHAB reminds ***the scientific community that it is invited to participate in the GlobalHAB programme, through seeking Endorsement*** of relevant research, monitoring, and modelling activities related to HAB projects that also contribute to foster international collaborations. Get the GlobalHAB endorsement application form, available on the webpage (*get involved*).

New GlobalHAB Chair, Clarissa Anderson



Clarissa Anderson is a biological oceanographer with expertise in ecological forecasting and remote sensing. After receiving a B.A. in Integrative Biology and Art History at UC Berkeley and a Marine Science Ph.D. at UC Santa Barbara, she completed several postdoctoral appointments, including a National Research Council fellowship and National Science Foundation postdoctoral award, before transitioning into a professional research position at UC Santa Cruz. Her research has focused on the prediction of harmful algal blooms and toxins in estuarine and coastal ecosystems as well as the fate and transport of harmful toxins to deeper waters and sediments. During her time as research faculty at UC Santa Cruz, she worked to establish the California Harmful Algae Risk Mapping (C-HARM) system with NASA Applied Science support. Clarissa is now at Scripps Institution of Oceanography serving as the Executive Director of the Southern California Coastal Ocean Observing System (SCCOOS) and the Director of the NOAA Cooperative Institute for Marine, Earth, and Atmospheric Sciences (CIMEAS). She continues to conduct research on phytoplankton ecology in coastal California. She is a member of the SCOR Working Group on Observing Air-Sea Interaction Strategy (OASIS), a U.S. CLIVAR Working Group on coastal climate solutions, the Science Advisory Team for the CA Ocean Protection Council (OPC), and the U.S. National HAB Committee (NHC).

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Director, Southern California Coastal Ocean Observing System (SCCOO
(CIMEAS)

New manual and guides



Barcelona to host 2024 UN Ocean Decade Conference

Three years after the start of the UN Decade of Ocean Science for Sustainable Development (2021-2030), a global conference will bring together the Ocean Decade community and partners to celebrate achievements and set joint priorities for the future of the Decade.

Hosted by Spain and co-organized with UNESCO's Intergovernmental Oceanographic Commission (IOC/UNESCO), the 2024 UN Ocean Decade Conference will take place on 10-12 April 2024 in the coastal city of Barcelona.

It will be a 3-day, in-person event co-led with a range of partners: Government of Catalonia and the Barcelona City Council through the Barcelona Capital Náutica Foundation, and the Spanish National Ocean Decade Committee, which is led by the Ministry of Science and Innovation through the Spanish Research Council (CSIC).

The conference will be a key moment for governments, leaders, maritime sectors, philanthropy, universities, private sector, NGOs and more, to take stock of the achievements of the first three years of the Ocean Decade and define a collective vision for the coming years. Participants will benefit from concrete examples and best practices in ocean science to deliver "the science we need for the ocean we want".

A key outcome of the 2024 UN Ocean Decade Conference will be the publication of a set of white papers related to the 10 Decade Challenges, that will identify future priorities for the Ocean Decade to generate the knowledge needed for science-based solutions related to global challenges, such as climate change, food security, biodiversity conservation, sustainable ocean economy, pollution and natural hazards.

A number of related high-level national and international events will take place before and after the main conference and there will also be scope for partners to propose and lead side events, exhibitions and networking events relevant to the conference themes on the days before the conference and in the sidelines of the conference itself.

"We are very pleased to be working hand-in-hand with Spain to bring this important milestone of the Ocean Decade to life," said Vladimir Ryabinin, Executive Secretary of IOC/UNESCO. "The conference will be an incredible opportunity for actors from around the world to collectively reflect on the use of science and knowledge for the sustainable management of the ocean, and in turn pave the way for the future of the Decade.

More details about the programme, registration process and calls for proposals for side events will be available soon. If you would like to receive updates, please *sign up here*.

Communication contacts:

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Spanish Institute of Oceanography-National Research Council (IEO-CSIC)
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About the Ocean Decade:

Proclaimed in 2017 by the United Nations General Assembly, the *UN Decade of Ocean Science for Sustainable Development (2021-2030) ('the Ocean Decade')* seeks to stimulate ocean science and knowledge generation to reverse the decline of the state of the ocean system and catalyse new opportunities for sustainable development of this massive marine ecosystem. The vision of the Ocean Decade is 'the science we need for the ocean we want'. The Ocean Decade provides a convening framework for scientists and stakeholders from diverse sectors to develop the scientific knowledge and the partnerships needed to accelerate and harness advances in ocean science to achieve a better understanding of the ocean system, and deliver science-based solutions to achieve the 2030 Agenda. The UN General Assembly mandated UNESCO's Intergovernmental Oceanographic Commission (IOC/UNESCO) to coordinate the preparations and implementation of the Decade.

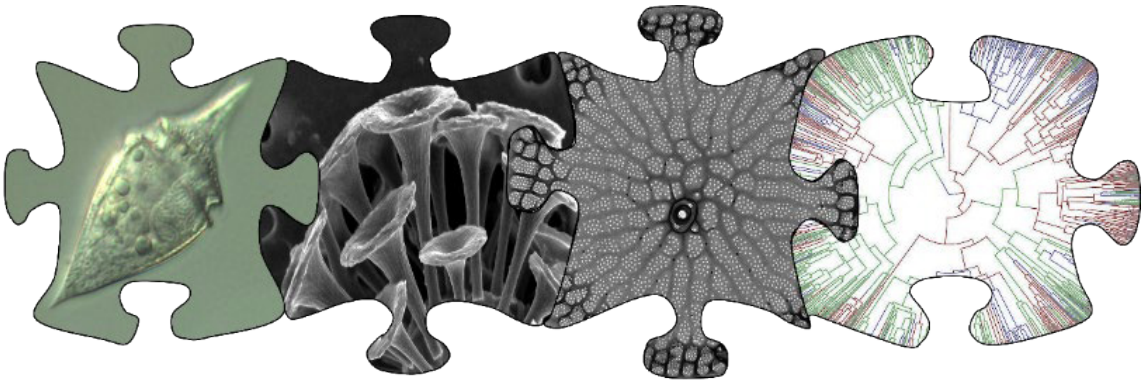
HAB-Solutions Programme Endorsed for UN Ocean Decade

A new initiative called "Harmful Algal Bloom Solutions (HAB-S)" has been endorsed as an UN Ocean Decade on Ocean Science for Sustainable Development programme at the meeting of the Decade Advisory Board on the 27th and 28th of November 2023.

HAB-S is led by the Intergovernmental Oceanographic Commission (IOC) and Food and Agriculture Organization (FAO) Intergovernmental Panel on Harmful Algae Blooms (IPHAB), in partnership with NOAA/NOS National Centers for Coastal Ocean Science (NCCOS), GlobalHAB, International Atomic Energy Agency (IAEA) and other key collaborators.

HAB-S aims to accelerate knowledge and understanding of the causes and effects of HABs at the level of scientists, societal stakeholders and public at large, and find solutions that reduce the frequency and severity of HAB events and mitigate their effects. It will provide novel and transformational science-based solutions for sustainable management and use of marine resources and ecosystem services affected by HABs in a changing world. We will keep you updated on developments here in HAN!

Contact for information: Sun, Yun *yu.sun@unesco.org*



13th Advanced Phytoplankton Course - APC13 Identification, Taxonomy, Systematics

Stazione Zoologica Anton Dohrn, Naples, Italy, 6-26 October 2024

The Advanced Phytoplankton Course series (APC) was initiated in the 1970s at the University of Oslo with the support of UNESCO and its Intergovernmental Oceanographic Commission (IOC). Subsequent Courses were held from 1985 to 2015 at the Stazione Zoologica Anton Dohrn (SZN), in 2012 at the University of Copenhagen and in 2019 at the Station Biologique de Roscoff (SBR). Over the last four decades, APC has contributed to the dissemination of the vast and ever-expanding body of knowledge on phytoplankton taxonomy, which has recently gained renewed interest because of its importance for interpreting advanced high-throughput imaging and molecular data. Many of the current experts in marine phytoplankton research have been trained at previous APC editions.

The 13th Advanced Phytoplankton Course (APC13) is organized by SZN together with SBR, the IOC Science and Communication Centre on Harmful Algae (IOC UNESCO / SCCHA) and Ocean Teacher Global Academy (IOC UNESCO / OTGA).

Course content

APC13 aims to provide participants with up-to-date expert knowledge on the identification and taxonomy of marine diatoms, dinoflagellates, coccolithophores and other phytoflagellates. The intensive 3-week program consists of lectures, demonstrations and practical sessions during which participants will be trained in classical techniques integrated by new approaches of automated imaging and molecular taxonomy. Participants will examine a diverse collection of preserved and live material, which will be identified with the aid of up-to-date taxonomic literature, and learn how sequence data resources can be used for identification purpose. Tutors will provide demonstrations of strain isolation and maintenance, preparation and observation of material in electron microscopy, and automated imaging tools.

In addition to criteria and methods for species identification in the different algal groups with various tools (light and electron microscopy, automated imaging tools, sequence data), course topics will include aspects of phytoplankton classification, cell ultrastructure, molecular phylogeny, biodiversity, ecology, biogeography, and harmful algal blooms.

Advise on taxonomic knowledge needed for all microalgal groups will be provided before the Course. An evaluation test will be conducted at the end of the Course.

Faculty

The APC13 tutors are renown phytoplankton experts:

- Nicolas Chomerat, IFREMER, Concarneau, France
- Bente Edvardsen, University of Oslo, Norway
- Wiebe H.C.F. Kooistra, Stazione Zoologica Anton Dohrn, Naples, Italy
- Carina B. Lange, University of Concepción, Chile
- Jacob Larsen, UNESCO IOC SCCHA, Copenhagen, Denmark
- Nina Lundholm, Natural History Museum, University of Copenhagen, Denmark
- Øjvind Moestrup, Department of Biology, University of Copenhagen, Denmark
- Marina Montresor, Stazione Zoologica Anton Dohrn, Naples, Italy
- Ian Probert, Station Biologique, Roscoff, France
- Diana Sarno, Stazione Zoologica Anton Dohrn, Naples, Italy
- Urban Tillmann, Alfred Wegener Institute, Bremerhaven, Germany
- Daniel Vaulot, Station Biologique, Roscoff, France & University of Oslo, Norway
- Adriana Zingone, Stazione Zoologica Anton Dohrn, Naples, Italy

How to participate

The application form with instructions is found at the *IOC UNESCO / OTGA site.*

In addition to filling the form, applicants will have to upload a motivation letter, their curriculum with the publication list and two recommendation letters.

Applications should be received not later than 5th January 2024, 23:59 CET.

The results of the participants' selection will be communicated in a month from the deadline.

Attendance is limited to 20 participants with PhD, MSc degree or equivalent, and with professional working knowledge of phytoplankton identification. The Course is held in English.

Participant selection will consider previous experience in phytoplankton,

relevance of APC13 to the present position and future involvement in monitoring and scientific projects, as well as chances for participants to share the information acquired during the course in their respective countries.
The registration fee of € 800 includes lunches (also vegetarian options), coffee breaks and social activities; it does not include travel and living expenses.

Information will be updated on this Stazione Zoologica *website*.

Mail can be addressed to *apc13@szn.it*

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Sponsors

APC13 is organised thanks to the logistic and/or financial support of the Stazione Zoologica Anton Dohrn, the Station Biologique de Roscoff, France and the IOC UNESCO / HAB and IOC UNESCO /OTGA. Additional resources have been provided by the Italian National Biodiversity Future Centre (NBFC), McLane Research Laboratory inc. and Carl Zeiss S.p.A. The list of sponsors will be updated as required.

Practical information

Naples is reachable through its *international airport* and the *HS-Rail network*. A choice of reasonable apartments, B&B accommodations and restaurants can be found in the immediate vicinity of the SZN or within a few stops of the metro. The cost of B&B accommodation in the SZN area starts from ca. 70 € per day close to the SZN, but may be cheaper in other districts of Naples.



Forthcoming

The next HAN issue will cover the 20th International Conference on Harmful Algae (ICHA) held on 5-10 November 2023 in Hiroshima, Japan. It will include highlights from the meeting, present the new ISSHA Council, provide information on the next ICHA meetings, and more.

With warmest wishes,
Dedmer van de Waal, ISSHA President

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Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

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Sato-Umi or Harmony with Nature

20th ICHA, Hiroshima 2023



Torii gate that welcomes visitors to the Itsukushima Shrine Island (Miyajima), UNESCO World Heritage at Hiroshima, Seto Inland Sea. Photo E. Bresnan

As the global outbreak of the novel coronavirus (COVID-19) had finally calmed down, the 20th International Conference on Harmful Algae (ICHA 2023) was held in Hiroshima, Japan, bringing together more than 500 participants from 40 countries around the world for the first time in five years. The Local Organizing Committee (LOC), chaired by Prof Ichiro Imai and the Scientific Committee (SC) of ICHA 2023 would like to express their gratitude to the ISSHA Council executives and participants for their various forms of cooperation.

During the preparation of ICHA 2023, the LOC has been facing difficulties derived from the COVID19 pandemic and rising prices in travelling costs. In this exceptional situation, we appreciate ISSHA members understanding about our giving up the original plan of an online and in person hybrid meeting



Local Organizing Committee for the 20th ICHA. From left to right: Satoshi Nagai (Vicechair), Katzuiko Koike (Vicechair), Masao Adachi (chair of program committee), Toshiyuki Suzuki (Vicechair), Ichiro Imai (Chair), Natsu Nakayama (secretariat) and Shigeru Itakura. Photo LOC ICHA 2023.

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Participants in the 20th ICHA, Hiroshima 2023. Photo LOC ICHA 2023.

that was initially announced by the LOC, and holding a conventional in-person meeting.

Japanese people have a long history of intimate ties with the coastal environment. This is defined by the "Sato-Umi" concept whereby biological productivity and biodiversity have been nourished through the intimate interactions of humans with their coastal ecosystems.

The theme of the 20th ICHA conference was "HAB Science and Human Well-being". The goal was to exchange relevant scientific information towards a greater understanding of HAB mechanisms, better and timely predictions of HAB occurrences and mitigation of their negative impacts.

In addition to the Opening Lecture and 9 Plenary Lectures, there were 216 oral communications, 45 ignite talks and 199 posters on 16 different topics: Ecology; Biology and Biogeography; Community/Species Interactions; Taxonomy; Microbiomes and Omics; Prediction and Modelling; Monitoring and Mitigation; Ciguatera and Benthic-, Ichthyotoxic- and Cyanobacterial HABs; Toxins, Biosynthesis and detection methods; Toxicology; Surveillance and Management; HABs in a changing world; Socio-economic impacts and Emerging issues.

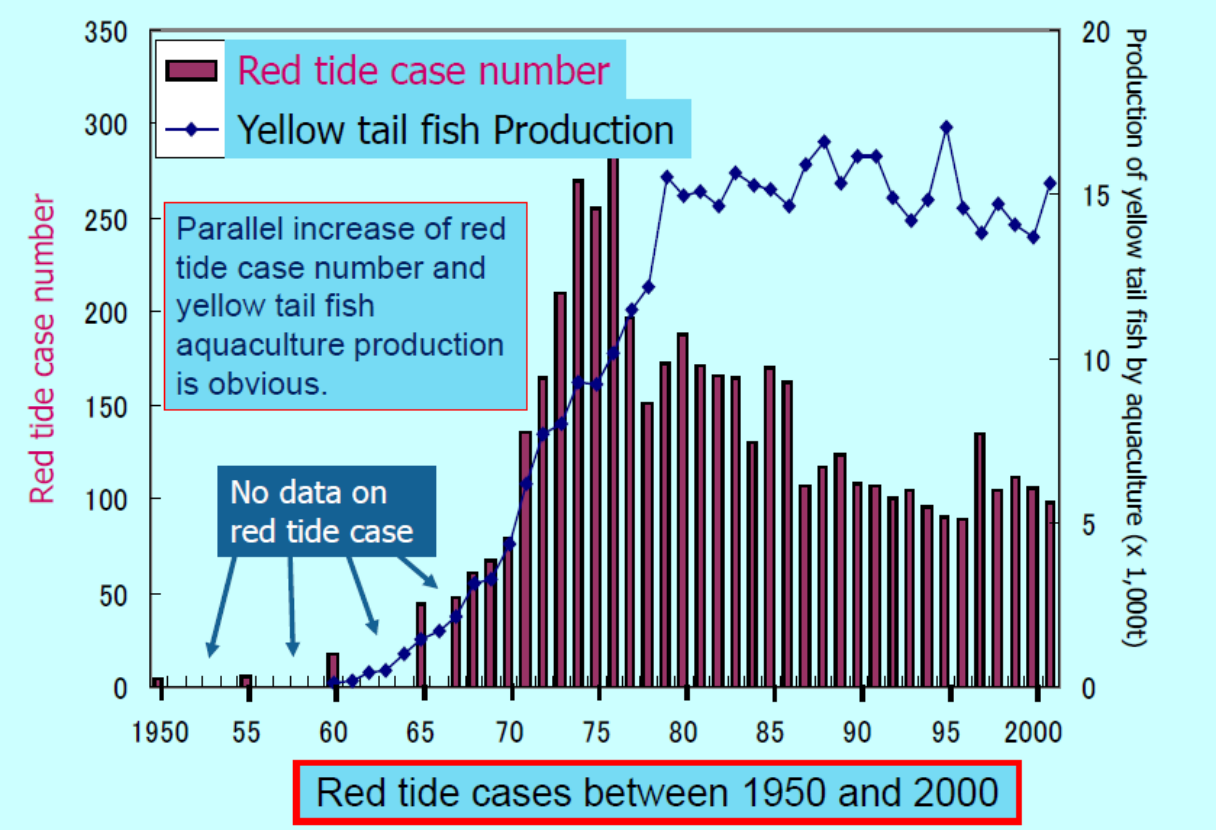
We are convinced that the ICHA 2023 has made a significant contribution to the scientific knowledge on harmful algae, and we sincerely hope that all participants thoroughly enjoyed their experiences in Hiroshima, the International City of Peace, along with Japanese cuisine and Sake.

Finally, we extend our deep appreciation to Dr. Wayne Litaker, our leaving ISSHA president, for the tremendous support provided over the past year to the preparation of ICHA 2023.

Prof. Ichiro Imai
Chair of the LOC of ICHA 2023

What conditions caused naughty algal blooms to turn into harmful ones?

Fukuyo-sensei opened the conference with a colourful review of red tide events and increasing fish cultivation in the Seto Inland Sea. *"Microalgae are kinds of friends struggling in the sea, in the same way as I do on land. This way of thinking may be quite specific for Japanese, or only me? In Japan we have various stone monuments for those we used to utilize as food or tools in order to express our gratitude and to pray for repose of souls of living things and spirits staying in instruments. In Japanese way, I feel naughty microalgae are friends working together, although I do not think to establish their monuments"* (Yasuwo Fukuyo, ICHA 2023, Hiroshima).



Long term series of red tides events and yellow-tail aquaculture; control measures applied in the late 70's. From Fukuyo-sensei ICHA 2023 opening address with his kind permission.



Aerial view of fish cages at the Seto Inland Sea (left) and the same area with a red tide aggregated around the cultures site. Images from the Seto Inland Sea Fisheries Agency taken from Fukuyo-sensei ICHA 2023 opening address with his kind permission.

Scientific Highlights of the 20th International Conference on Harmful Algae

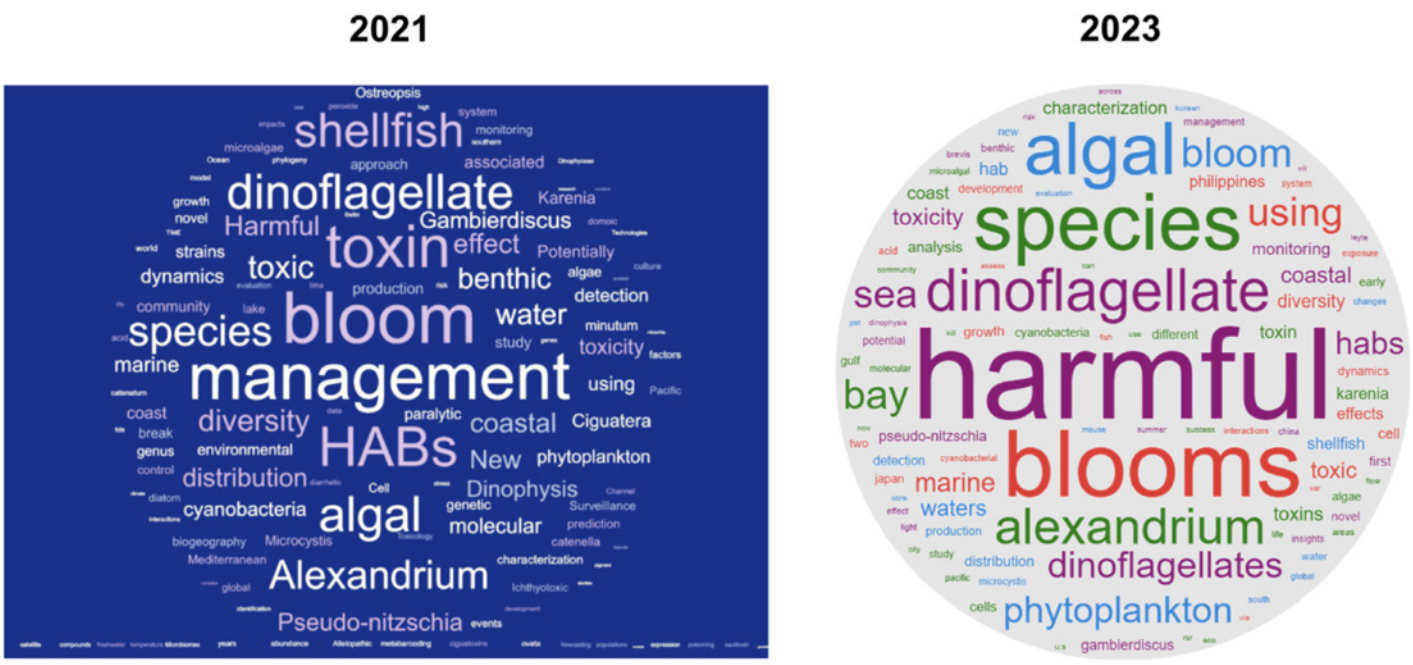


Fig. 1. Word clouds generated from all presentation titles in ICHA 2021 (left, from Fig. 1 in Van de Waal and Garcés 2022 [1]) and ICHA 2023 (right).

A summary of all the scientific highlights from a conference is a huge task and inevitably will miss important points. However, we hope these highlights will provide a flavour of the intense week experienced by the attendants to the 20th International Conference on Harmful Algae (ICHA 2023), Hiroshima, Japan, which can be shared with those who, unfortunately, could not join. It also aims to share the discoveries presented at the ICHA. This document is based on the summary of the opening and plenary lectures and the highlights selected by the session chairs of the conference. We sincerely thank all of them for their generous engagement and contribution during the busy week of the event. Finally, we hope this document will encourage the readers to continue the collaborative research on harmful algal blooms (HABs).

Word cloud as a summary

The 'Word cloud' method visualises words in the text by assigning sizes according to their occurrence frequency: the more frequent words will be shown larger. To find out the popular words and topics in the ICHA 2023, we generated a word cloud from all presentation titles registered at the conference. The same approach was conducted in the previous ICHA conference (19th International Conference on Harmful Algae, ICHA 2021) and both word clouds are shown here for comparison (Fig. 1). In the ICHA 2023, the most popular words were 'harmful, blooms, algal, species, dinoflagellate, phytoplankton and bloom', while those in the ICHA 2021 were 'bloom, management, HABs, toxin, dinoflagellate, shellfish and algal'. Although the most popular word in the ICHA 2021 was 'management', it was much less frequent in the ICHA 2023. The word cloud of the ICHA 2023 and ICHA 2021 captured several harmful dinoflagellate and diatom genera such as 'Alexandrium, Karenia, Gambierdiscus, Pseudo-nitzschia, and Dinophysis'. This suggests that these genera are well studied, possibly as they continue to cause problems and attract the interest of HAB researchers.

Opening lecture and plenary lectures

The opening and eight plenary lectures covered a diversity of topics related to harmful algae in marine and freshwater habitats around the world.

In the ICHA 2023 opening lecture, **Yasuwo Fukuyo** recalled ancestral cultural knowledge about HABs: '*Do not eat shellfish when snowmelt water runs into coastal seas*' is an old saying in Japan. If we aim to mitigate the negative impacts of HABs on humans and the environment, socio-cultural backgrounds from all over the world must be merged with scientific progress. Recently, this perspective has been taken in Aotearoa New Zealand and across the Pacific **(Kirsty Smith)**. She presented a collaborative activity with indigenous communities to establish a new rapid-test kit, developed with molecular and analytical chemistry techniques, to monitor HAB species and their toxins (with a focus on ciguatoxin-producers), and help food-safety in the area. **Vera L. Trainer** also emphasized that communities participating in science provide unique perspectives of the impacts and dimensions of the extreme events of *Pseudo-nitzschia* blooms on the Pacific coast of America. In her revision, Vera noticed that domoic acid production is tightly coupled to Fe and Cu acquisition by *Pseudo-nitzschia*. Understanding impacts on society is fundamental also concerning the freshwater lakes and drinking reservoirs threatened by cyanobacterial blooms **(Dedmer Van de Waal)**. In his talk, Dedmer showed how online searches for cyanobacterial blooms increase in warmer and drier summers, indicating an increased public awareness. **Po Teen Lim** presented the lessons learned about HABs which occurred in Malaysia during the past

two decades of monitoring and study using molecular tools: metabarcoding looks promising as an early warning tool and degradation of coral reefs may favour conditions for the proliferation of benthic harmful algal blooms (BHABs) caused by *Ostreopsis* and *Gambierdiscus* species. Concerning ciguatera poisoning (CP), **Masao Adachi** highlighted that different taxa could be the potential causative organisms of CP outbreaks in Japan: i) *Gambierdiscus silvae* that may produce Caribbean ciguatoxins (C-CTXs) or CTX-like toxins; ii) new undescribed *Gambierdiscus* phylotypes detected by metabarcoding analysis or iii) unknown organisms including genera other than *Gambierdiscus* may produce P-CTXs. **Luiz L. Mafra Jr** presented laboratory and field surveys indicating that plastic pollution might increasingly exacerbate the negative impacts of BHABs on marine organisms and human health through the food web in certain coastal areas.

Esther Garcés and **Mari Yotsu-Yamashita** addressed parasitic interactions and toxin pathways, respectively. **Esther** highlighted the conspicuous presence, in high-density mono-specific blooms of different dinoflagellate species, of numerous parasitoid species, some of them recurrently detected and sometimes coexisting in the same host species, and clearly some of them representing new diversity yet to be described. These parasitoids appear to have adapted to their hosts, thereby facilitating the maintenance, spread and recurrence of the parasite populations. **Mari** presented recent findings in the biosynthesis and metabolic pathways for tetrodotoxin and paralytic shellfish toxins using putative biosynthetic intermediates of saxitoxin (STX) and stable isotope-labelled compounds.



Fukuyo-sensei (centre) and WESTPAC colleagues. Photo CP Leaw.

Oral presentations

There were 36 parallel oral sessions which included 216 presentations in 16 categories. Some highlights are summarised below.

1. Ecology

This topic covered ecological studies of a wide range of organisms: cyanobacteria, haptophytes, diatoms, dinoflagellates and their related grazers, parasites and viruses. In these studies, data were collected using different methods, including morphology-based automatic imaging instruments [e.g., Imaging FlowCytobot (IFCB)], molecular tools [e.g., metabarcoding and quantitative PCR (qPCR)], chemotaxonomy (based on pigment analysis by HPLC), multi-omics approaches, or a combination of them.

Molecular tools are increasingly applied to monitor HAB species. Examples included metabarcoding alone in Japan **(Sirje Sildever)** or in combination with other methods for cyanobacteria in the USA **(Christopher J. Gobler)**; and a new single-cell multiplex PCR assay developed to assess the genotype frequency and toxin dynamics of *Pyrodinium bahamense* **(Kathleen Cusick)**. Imaging FlowCytobot (IFCB) used to identify the mechanisms driving the development and decline of *Dinophysis acuminata* blooms in the Nauset Marsh (USA) imaged *in situ* for the first time thin layer dynamics and phased mating processes in this species **(Serena Sung-Clarke)** and protozooplanktonic grazers later used in laboratory estimates of grazing losses **(Megan Ladds)**.

Analysis of long-term data series supported the development of a conceptual model illustrating atmosphere-ocean interactions in the Magellan fjords region (Chile); the unprecedented retreat of ice fields is impacting the fjords circulation dynamics and the subsequent changes in plankton community structure, including HAB events **(Máximo Frangopulos)**. An 18-years-record of toxic dinoflagellate cysts in NW Africa, obtained with sediment traps was related to atmospheric and hydrographic changes to identify the drivers of cyst production and explore future trends **(Surya Eldo Virma Roza)**.

Some emerging issues included the description of a great diversity of HAB species and abundance of *Alexandrium* cells found on drifting plastics in the Philippines **(Deo Florence L. Onda)** and new findings on the biological interactions between the high biomass bloom forming *Lepidodinium chlorophorum*, potentially harmful to oysters, and bacteria. The dinoflagellate production of Transparent Exopolymer Particles (TEPs) may facilitate positive bacterial interactions and nutrients availability **(Raffaele Siano)**.

Last but not least, 'Objectif Plancton', a citizen science program to assess phytoplankton communities' changes in France, was presented **(Laura Schweibold)**.

2. Biology and Biogeography

Extraordinary progress was presented concerning genetic trends that can help to explain evolutionary processes as well as the current ecological niches and biogeographic distributions of some HAB species.

For instance, transcriptomes of 17 dinoflagellate strains and phylogenetic analyses based on large multi-gene alignments of proteins and nucleotides

indicated that the sxtA gene may not have been laterally transferred between *Alexandrium* species and the related genus *Centrodinium punctatum* **(Shauna A. Murray)**. Laboratory culture-based observations demonstrated sexual mating, formation of planozygotes and diploid cysts, and cyst germination in *Karenia mikimotoi*, *Karlodinium veneficum*, *Prorocentrum donghaiense* and *Akashiwo sanguinea*. The presence of resting cysts of these species was confirmed and their distribution in sediments from Chinese seas determined using a combination of fluorescence in situ hybridization; light microscopy; single cyst sequencing for an ~1400 bp LSU rRNA gene (FISH-LM-SCS) and qPCR **(Ying Zhong Tang)**. A comprehensive genomic study revealed that the mitochondrial genes are relatively unstable compared to plastidial genes in nine *Skeletonema* species **(Satoshi Nagai)**. Results from laboratory experiments on the response of *K. mikimotoi* to light intensity (Fv/Fm curves) suggested that migration of field population from surface to sub-surface layers was to avoid inhibition of their PSII and get the optimal light irradiance **(Shota Higo)**.

3. Community/Species Interactions

Very interesting studies were presented on this topic combining cutting-edge experimental and analytical methods. This is an area that will certainly offer new progress in the near future to our understanding of small scale biological interactions involved in the development of HABs.

As an example, transcriptome and proteome analysis revealed that grazing by the protist *Paramecium* induced changes in multiple life cycle and metabolic processes in the cyanobacteria *Dolichospermum flos-aquae* including heterocyst differentiation and enhancement of nitrogen fixation **(Yuan Huang)**. Different laboratory experiments explored the allelochemical compounds and mechanisms of action among different microalgae. Free Fatty Acids (FFA) and Reactive Oxygen Species (ROS) can affect *Gymnodinium catenatum* **(Martin Fernando Encinas-Yanez)**, while the mucilage produced by benthic microalgae contributes to transport of allelochemical compounds **(Nadia V. Herrera-Herrera)**. Interestingly, seaweed cultivation could enhance the biodiversity of the co-occurring planktonic community **(Zhaoyang Chai)**.

4. Taxonomy

This topic included new progress on the taxonomy of several harmful taxa in coastal areas. The studies combined light microscopy, scanning electron microscopy and molecular sequencing applied to field samples and isolates of *Amphidoma*, *Coolia*, *Heterocapsa*, *Karenia*, *Karlodinium*, *Noctiluca*, *Protoceratium*, *Pseudo-nitzschia*, *Pyrodinium bahamense*, certain raphidophytes, nanoflagellates and cyanobacteria.

Information on new descriptions in taxonomy, combined with the toxinological characterization of the species is regularly updated in the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae, **(Nina Lundholm)** and drives the re-evaluation of certain groups of toxins, such as the goniodomins produced by some *Alexandrium* species **(Urban Tillmann)**. On the evolutionary side, a study on plastid molecular phylogeny suggested that the Kareniaceae started from an ancestral species with transient haptophyte symbionts; subsequently, 13 derivative lineages independently established the permanent plastids **(Kazuya Takahashi)**.

5. Microbiomes and Omics

This topic focused on several cutting-edge molecular approaches (e.g., metabarcoding, metagenome analysis, metatranscriptomics and metabolomics) used to investigate microbiome diversity or relationships within the microbial communities. Four communications addressing different microalgal groups were highlighted.

The combination of traditional culture-based methods with high throughput 16S rRNA amplicon sequencing was applied to analyse the microbiome composition associated with four *Gambierdiscus* species (*G. carpenteri, G. lewisii, G. lapillus* and a second *G. lapillus*-related genotype **(Shikder Saiful Islam)**. The study revealed a high diversity and species-specific bacterial composition with relevant biogeochemical and functional roles affecting growth dynamics of these dinoflagellates. A full-length 18S rDNA metabarcoding analysis of samples from the Texas Observatory for Algal Succession Time (TOAST) revealed that HABs diversity increased after an extreme event **(Lisa Campbell)**.

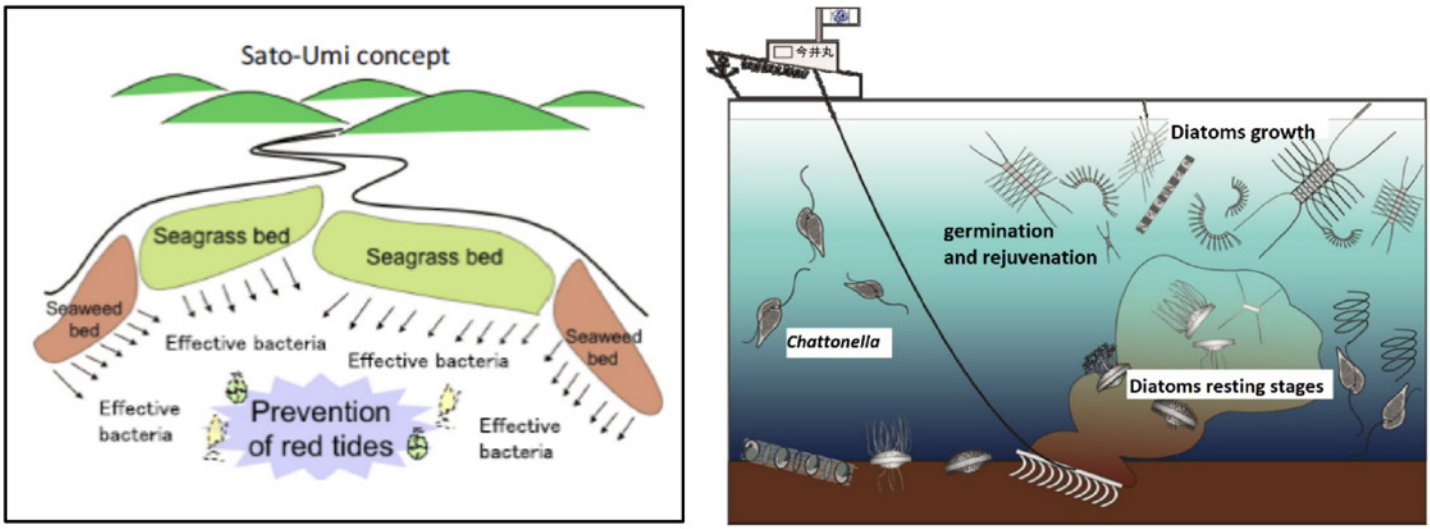
Metatranscriptomics and metabolomics aligned with ecological metadata revealed that the ichthyotoxic mixotroph *Chrysochromulina leadbeateri* exploits its toxicity to dominate the community and increase its prey availability for nutritional supplementation **(Uwe John)**. The success of the spreading and massive bloom formation of the microbenthic diatom *Cymbella janischii* may be favoured by bacteria in the mucous mat able to synthesize B-vitamins and with genes encoding the hydrolysis of diphosphates. Furthermore, a 3D reconstruction of the mats using microscopy and nano-focus X-ray CT supports the idea that the interior of the mat is filled with a dense soup of organic substances, which could be utilized by co-existing organisms **(Shinya Soto)**.

6. Prediction and Modeling

Several communications presented modelling or machine and deep learning approaches aimed to predict HAB events and manage their effects.

Trophic-transfer models throughout a critical marine food chain are constructed to predict the exposure to STXs of walruses in the Alaskan Arctic **(Patrick Charapata)**.

In the Philippines, the feasibility of establishing an automated HAB monitoring pipeline utilizing IFCB is investigated by training and evaluating a series of tree-based and convolutional neural network models in the identification and quantification of local HAB species **(Paul Samuel Ignacio)**. In Malaysia, machine learning models are developed to predict the occurrence of *Pyrodinium bahamense* and *Margalefidinium polykrikoides* **(Teruaki Yoshida)**. Microalgal abundance measurements from the Sabah's Department of Fisheries are combined with oceanic data (sea surface temperature and salinity, wind velocity, solar irradiance and chlorophyll a) provided by Copernicus Marine Services and NASA POWER databases. In Alfacs Bay (Spain, Western Mediterranean), forecast models based on neural networks and built on phytoplankton times series, showed that five years of observations of *Karlodinium* provided optimal results, but a longer series was needed for *Pseudo-nitzschia* **(Margarita Fernández-Tejedor)**. Abrupt changes in trends may diminish forecast capabilities



Left: The Sato-umi concept for HABs control applies the preservation of macroalgae and aquatic plants (Zostera marina) meadows as a basic stratrategy to prevent harmful proliferations exceeding alert levels. Right: If they exceed this threshold, the first prevention and mitigation strategy is to rake the sediments so that diatoms resting forms are resuspended and germinate, and their blooms outcompete noxious flagellates (e.g, C)hattonella). Source: Imai et al [2]

and therefore a continuous improvement of the models is needed.

7. Monitoring and Mitigation

Many studies were presented exploring the opportunities and required research concerning a variety of methods aimed at monitoring HABs and mitigating their impacts.

A meta-analysis was conducted on the published and unpublished studies that used one of 28 chemical, bacterial, physical and/or plant-based algal bloom treatments in field experiments on various water quality measurements, including phytoplankton pigments and cell density, microcystin and off-flavours **(Alan Wilson)**. The analysis concluded that most treatments failed to improve water quality in the field and highlighted the need for more research on alternative treatments. Similarly, experiments showed that hydrogen peroxide was not fully successful to control proliferations of the fish-killing *Prymnesium parvum* **(Iwona Jasser)**. DinoSHIELD is a bio-control technology using alginate hydrogel to immobilize *Shewanella* sp. IRI-160. DinoSHIELDs continuously release bacteria-derived algicides targeting dinoflagellates while limiting the dispersion of bacteria. Promising results were obtained in the application of DinoSHIELDs tested in mesocosms studies in the Broadkill River, USA **(Yanfei Wang)** and in laboratory experiments with *Karenia brevis* **(Kathryn J. Coyne)**. Detailed studies were presented characterizing the bioflocculation agent obtained from the fruits of terrestrial plants, in particular from *Diospyros mespiliformis*, which can be used to efficiently remove and lyse cyanobacterial cells **(Jabunali R. Gumbo)**.

Exciting new nanopore field technology will soon be ready for commercialization **(Robert George Hatfield)**. The assay presented uses recombinase polymerase amplification, an isothermal amplification technology that avoids some of the drawbacks of common PCR methods. Chilean HAB monitoring spans many scales and has recently incorporated metabarcoding and metagenomics technology to increase knowledge of the physiology and metabolism of phytoplankton communities and local HAB blooms **(Gonzalo Fuenzalida)**. PhytO-ARM is a new and exciting open-source design toolkit to aid in the deployment and edge computing for IFCBs and other imagers on fixed and Lagrangian platforms **(Michael Brosnahan)**. Concerning toxins, experiments to investigate the accumulation of dissolved domoic acid in mussels showed that these bivalves are not an adequate sentinel for that toxin, and alternate monitoring methods such as SPATT (solid phase adsorption toxin tracking) are more advantageous for understanding toxin concentration in the absence of harmful algal bloom **(Aubrey Trapp)**.

8. Ciguatera and Benthic HABs

This topic covered chemistry, data science, ecology and physiology of BHABs such as *Gambierdiscus* and *Ostreopsis*. Particularly, bioactive compound screening and discovery, modelling and biosensor development were advanced and noteworthy points.

The production of newly identified maitotoxins (MTXs, MTX-6 and -7) and gambierone analogues by two *Gambierdiscus* species, *G. cheloniae* and *G. honu*, were reported **(J. Sam Murray)**. Production of C-CTX-5 from *G. silvae* was documented and the detailed stereochemical diversity of C-CTX analogues was discussed **(Elizabeth M. Mudge)**. Experiments with a *G. polynesiensis* strain revealed the effects of temperature and salinity on growth and CTX production **(Tomohiro Nishimura);** modelling using these data sets predicted potential hotspots of this species' distribution and related ciguatera poisoning outbreaks in the future **(Dana K. Briscoe)**. A promising biosensor for the detection of P-CTXs in fish and seawater (detection limit 10-fold lower than FDA guidance level) was developed **(Mònica Campàs)**. A long term (2007–2021) study revealed *Ostreopsis* bloom trends along the Adriatic coast: densities increased until 2012 and declined from 2013 to date **(Stefano Accoroni)**.

9. Ichthyotoxic HABs

The GlobalHAB "white paper" on fish-killing algae, recently published by IOC/UNESCO [3] was presented **(Gustaaf M. Hallegraeff)**. This was the result of a workshop conducted in Puerto Varas, Chile, in October 2019, under the sponsorship of IOC, SCOR and the Government of Chile. It summarises current knowledge and gaps for future research about taxonomy, toxicity, bloom dynamics and impacts of fish-killing algal blooms. It provides



a roadmap for scientists, aquaculturists and insurance brokers to improve the management of these events. A comprehensive study of the massive and unprecedented bloom of the ichthyotoxic haptophyte *Prymnesium parvum* in the River Oder in Poland/Germany was presented **(Elisabeth Varga)**. Multiple drivers were identified and a combined effect of prymnesins and organic micropollutants was noticed. Another study revealed that *Karenia mikimotoi* significantly increased its ichthyotoxicity under axenic conditions, while different bacterial isolates could have different specific modulating effects on the algal ichthyotoxicity **(Fred Wang-Fat Lee)**.

10. Cyanobacterial HABs

This topic covered chemistry, genomics, mitigation, monitoring by IFCB or molecular approaches including metabarcoding, and taxonomy of cyanobacteria from freshwater to marine benthic environments, with the following highlights.

The first toxic *Microcystis* blooms and several new genera were reported from Malaysia **(Yin Sing Ong)**. A new toxic cyanobacterial genus and species, *Sphaerothrix gracilis*, with potential for industrial applications was reported from microplastic particles. **(Emily Curren)**. New imaging and pulse shape cytometry techniques provided more precise and species-specific information on filamentous cyanobacteria **(Kaisa Kraft)**. A novel early warning system using cyanotoxin-encoding genes predicted cyanotoxin production more accurately **(Jingrang Lu)**. A new ELISA test detected most of the microcystin variants **(Ingunn A. Samdal)**. Metabarcoding and LC-MS/MS analyses revealed benthic cyanobacterial mat diversity and their toxin production on Florida coasts **(David Erwin Berthold)**. Potential recycling of food waste and cyanobacteria biomass by salt-tolerant mushroom strains was reported **(S. K. Sim)**.

11. Toxins, Biosynthesis and Detection Methods

During the ICHA2023, we found out how the most novel methods are used to progress in the comprehension of toxin biosynthetic and metabolic pathways as well as in their detection. Thus, based on the colchicine mode of action, experiments suggest that STXs are biosynthesized *de novo* and also through salvage pathways (i.e, from intermediates in the degradative pathway). Colchicine inhibited nitrate assimilation upstream of glutamate biosynthesis in the *de novo* pathway **(Yuko Cho)**. Using LC-HRMS analysis and culturing techniques, several uncommon okadaic acid and pectenotoxin analogues were found in *Dinophysis norvegica* from northeastern US coasts **(Christopher O. Miles)**. A combination of short- and long-read amplicon sequencing, metatranscriptomics and metabolomics were used, with encouraging results, to characterize the oceanographic conditions associated with species-specific expression of domoic acid biosynthesis genes, in connection with elevated particulate domoic acid concentrations in Santa Barbara Channel, USA in 2019–2022 **(Steffaney M. Wood)**. Combining LC-MS/HRMS and LC-MS/MS, novel toxins, cyclic amines and products of their metabolism from *Vulcanodinium rugosum* were identified and quantified in contaminated shellfish in the Ingril lagoon hotspot in France **(Vincent Hort)**. HPLC coupled with ion-mobility QTOF mass spectrometry, showed that the structural diversity of the prymnesins produced by *Chrysochromulina leadbeateri* was larger than previously reported. Additional bioassays using RT-gill-W1 gill cells are run to identify the ichthyotoxins produced by this fish-killing microalga **(Thomas O. Larsen)**.

12. Toxicology

An in-depth revision is required on the toxicity of toxin groups, toxicity equivalency factors, and how all this is being conditioned by the accelerating change in the toxin profiles worldwide (but especially in Europe) due to global warming. Regulatory limits and toxicological endpoints for many toxin groups, including CTX, OA and STX, were analysed **(Luis M. Botana)**.

Microalgal toxins are part of still poorly understood chemical ecology. The effect of dinophysistoxin-1 (DTX-1) was studied on the growth, photosynthesis, antioxidant responses and cell cycle of the haptophyte *Isochrysis galbana*. DTX-1 was found to provoke cell cycle arrest in the G2/M phase, and the cell size and intracellular complexity were also increased in the 24-h batch culture **(Jiangbing Qiu)**.

13. Surveillance and Management

This topic covered chemistry, developing monitoring tools using molecular approaches including high-throughput microfluidic real-time qPCR technology, and developing monitoring frameworks, with the following highlights.

An effective analysis method using a high-throughput PCR system, Biomark HD real-time qPCR System, for the six *Alexandrium* species was reported **(Francesca Cucchi)**. From researchers to volunteers, from qPCR to drones and satellites including the unintentional dogs, exciting and effective approaches to HAB monitoring in the USA were presented **(Hannah Bonner)**. A duplex PCR assay targeting STX synthesis genes, sxtA4 and sxtG genes, was described as a promising tool for the early monitoring strategies of STX in aquaculture sites **(Greta Gaiani)**. *Alexandrium catenella* blooms may originate in Los Lagos region, Chile, and be dispersed to other aquaculture areas by hydrodynamics **(Óscar Espinoza-González)**.

14. HABs in a Changing World

This topic reported a wide range of studies to investigate bloom mechanisms of HABs influenced by climate change. The studies investigated HABs by various methods of chemistry, data science (modelling or statistical analysis), dynamics, ecology including mixotrophy, palynology combined with eDNA analysis, and physiology (growth experiments coupled with toxin production and molecular changes). Focused organisms were mainly dinoflagellates (*Alexandrium*, *Gambierdiscus*, *Karenia* and *Ostreopsis*), diatom

(*Pseudo-nitzschia*) and microcystin-producing cyanobacteria.

There is new evidence of unexpected variability due to climate change which hinders prediction of when and where HAB events will occur in a future ocean. These uncertainties affected studies on *Karenia brevis* and benthic microalgal species such as *Gambierdiscus* in the Gulf of Mexico **(Patricia M. Glibert**, **Sabrina Heiser)**, *Karenia selliformis* in Hokkaido, Japan **(Tomonori Isada)**, HAB species in the Bohai Sea and Yellow Sea, China **(Rencheng Yu)**, *Gambierdiscus* and *Ostreopsis* on Jeju coasts, Korea **(Wonho Yih)**, and *Alexandrium minutum* in the northwestern Iberian Peninsula, Spain **(Francisco Rodríguez)**. Conceptual models were raised: for the toxic *Alexandrium catenella* blooms in the Alaskan Arctic **(Donald M. Anderson)** and for predicting future changes in *Alexandrium* bloom dynamics and toxicity of shellfish in Southeast Alaska **(John R. Harley)**. Warmer conditions may increase dissolved DA production by *Pseudo-nitzschia multiseries* **(Raphael M. Kudela)** while rising pCO2 does not affect either its growth or toxin production **(William P. Cochlan)**. A nature-based solution was established to eliminate cyanotoxins in freshwater using biologically enhanced biocarbon (biochar) **(Jane Moore)**.

15. Socio-economic Impacts

Revealing the socio-economic impacts can influence policy formulations to establish better HAB monitoring strategies **(Mark Leonard S. Silaras)**. The studies in this category, therefore, investigated and summarised the current status and ongoing projects on social and economic impacts of HABs. The analysis of newspapers published in Japan between 1872 and 2023 revealed a social 'alarmist' perception of HABs and shellfish poisonings **(Kazumi Wakita)**. Natural sciences cannot be isolated from society: in the Philippines, HABs cause major economic losses to small-scale fishing communities **(Mark Leonard S. Silaras, Leni G. Yap-Dejeto)**.

16. Emerging Issues

This topic was mainly focused on toxin bioaccumulation and related physiological mechanisms in shellfish. In addition, symptoms of severe acute dermatitis caused by toxins were reported. Investigation into the adsorption of toxins on virgin and aged microplastics and an international qPCR workshop promoted by GlobalHAB were also reported.

Significant progress in understanding the action mechanisms of some toxins was reported: microcystin inhaled in mice **(Jiyoung Lee)** and portimine A causing dermatitis in fishermen associated with a bloom of *Vulcanodinium rugosum* in Senegal **(Philipp Hess)**. New insights into cellular processes underlying the slow depuration of DA in King scallops were reported **(José Luis García-Corona)**.

Ignite talks

There were two poster sessions including 244 presentations in 16 categories. Highlights of three Ignite Talk sessions covering45 presentations, follow.

A large-scale *Alexandrium catenella* bloom was first detected in the northern Bering Sea using an IFCB **(Evangeline Fachon)**. New Phagomyxa-like parasitoids were detected and isolated from Korean coastal waters **(Ye Seul Jeong)**. Accumulation of CTXs in herbivorous fish from Okinawa was confirmed and their dietary preferences elucidated using metabarcoding analysis **(Goshi Araki)**. Progress in the understanding of the biosynthesis of 5-hydroxymethyl uracil, the unique nucleotide replacing about forty percent of thymidine in dinoflagellates, may provide some keys to design specific strategies for HAB mitigation (**Emily Jolly**). Various topics from freshwater lakes to tropical oceans with specific focus on viruses, bacteria, silicate skeletons and even plastic waste were reported (**Juri Nazareth Mapa Ochotorena, Masaki Fujita, Kazumasa Yamada, Denise Ching Yi Yu)**. Two mitigation techniques used carbon nanostructures to remove HAB toxins and modified clay spreading to remove HAB species **(Amparo Alfonso, Jennifer H. Toyoda)**. Other topics included detection perspectives, new red tides, environmental quality control, parasites, ichthyotoxins, life cycles, effect of toxins on marine organisms and on model organisms, environmental cycles, and shellfish toxin profiles..

Acknowledgements

It would have been impossible to write up this highlight article without the help of the oral speakers and session chairs who summarised the highlight information to us. We greatly appreciate their support. We are grateful to Dedmer Van de Waal and Beatriz Reguera for their helpful comments. We are also grateful to all the organisers, including ICHA 2023 chair professor Ichiro Imai and local organizing and scientific committees, ISSHA executives and participants who contributed to the success of the 20th International Conference on Harmful Algae in Hiroshima, Japan. ***Arigatou gozaimashita, mata aimashou*** **(thank you very much and see you next time)!**

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The authors, Elisa Berdalet (left) and Tomohiro Nishimura (right). Photo at the Gala Dinner, ICHA2023.

ISSHA Societal Activities during the 20th ICHA Conference

The International Society for the Study of Harmful Algae (ISSHA) convened the 20th International Conference on Harmful Algae, held in Hiroshima, Japan, from 5-10 November 2023. The ISSHA Council had various meetings between May and November 2023 to organize the Conference activities, Council elections, travel awards, achievement awards and the ISSHA General Assembly.

General Assembly

The ISSHA General Assembly was held on Monday 6 November from 19:00 to 20:30. The General Assembly was opened by President Wayne Litaker and minutes from the previous assembly were approved. Various updates were provided including proposed by-law changes (Ian Jenkinson; see below), achievement awards (Dedmer van de Waal; see below), update on the Young Investigator Network Meeting (Misty Peacock, see below), ISSHA Treasurer's report (Nina Lundholm), Travel Awards (Po Teen Lim; see below), updates about the ICHA 2023 proceedings (Esther Garces and Ichiro Imai), details regarding ICHA2023 (Ichiro Imai), Council election results (Wayne Litaker; see below), and presentation of the ICHA 2027 in Aberdeen, Scotland (Christine Edwards). The General Assembly was followed by a presentation of the ICHA 2025 that will be held in Punta Arenas, Chile (Leanardo Guzman).



By-law changes

The by-law changes that were proposed and approved included the addition of one or two student representatives to the ISSHA Council, the inclusion of a new clause in the ISSHA statutes with a 'code of conduct' to guard against harassment and discrimination, and the agreement that the approval of minutes from the General Assembly should be done within two months.

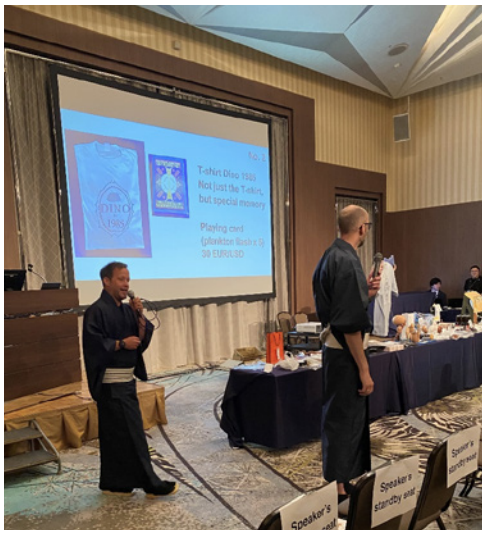
Young Investigators Network Meeting

This networking event was organized on Sunday 5 November by Misty Peacock, Natsuko Nakayama and Tomohiro Nishimura. It consisted of different activities, including games to illustrate effective communication, and listening, and participants discussed how to present oneself confidently. A total of 19 early career scientists attended the event.

Travel Awards

Travel awards have been given to a total of 36 young researchers and members of the International Society for the study of Harmful Algae (ISSHA) to participate the International Conference on Harmful Algae (ICHA2023) in Hiroshima city, Japan, from 5-10 November 2023. This biennial event is the biggest event for harmful algae researchers. The call for travel award application was announced through the website of ISSHA six months before the event, and applicants submitted their applications through the link of a google form available on the website. The call was also disseminated through emails to reach out to wider audiences of the HAB research community. All applications were evaluated by the ISSHA Travel Award Committee. With the funding from the Scientific Committee on Ocean Research (SCOR), five young scientists from the developing countries (China, Malaysia, Chile, and Thailand) were







Left: Frenzied activity at the ISSHA auction. Right: ISSHA Executive members and the new ISSHA President (right end) dressed for the occasion. Photos LOC ICHA 2023.

selected. ISSHA also provided ten additional travel awards with the funding from the society; this has benefited members from Australia, Austria, China, Ireland, Italy, New Zealand and United Kingdom. The funding support was obtained with the effort from the society to raise funds through the auction event held during each conference. We should not forget the generous support from all ISSHA members by donating items for the auction. In addition, nineteen postgraduate students and three HAB managers from the United States institutions were supported by the funding from NOAA, USA to attend the event in Hiroshima this year.

ISSHA Council members

ISSHA members voted on-line for the Executive Council, Council members and student representative. Some Council members stepped down after having (actively) served on previous Councils, including Vera Trainer (Past-president), Nina Lundholm (Treasurer), Elin Lindehoff (Secretary), Christine Band-Schmidt (chair of LOC ICHA 2021), Rosa Figueroa, Aifeng Li, Satoshi Nagai. Wayne Litaker thanked all past Council members for their services to ISSHA. Special thanks were made to Nina Lundholm for her long-standing service to the ISSHA Council, particularly as Treasurer.

The new ISSHA Executive and Council for the 2023-2025 period (new members and old ones occupying new executive positions are in italics):

Executive Council

PRESIDENT: *Dedmer van de Waal* (The Netherlands)
1ST VICE PRESIDENT: Po Teen Lim (Malaysia)
2ND VICE PRESIDENT: *Lorena María Durán Riveroll* (Mexico)
SECRETARY: *Ingrid Sassenhagen* (Germany)
TREASURER: *Marie-Yasmine Dechraoui Bottein* (France)
PAST-PRESIDENT: *Wayne Litaker* (United States)

Council members

Leila Basti (United Arab Emirates)
Laura Biessy (New Zealand)
Christine Edwards (United Kingdom)
Henrik Enevoldsen (Denmark)
Esther Garcés (Spain)
Tim Harwood (New Zealand)
Philipp Hess (France)
Ichiro Imai (Japan)
Mitsunori Iwataki (Japan)
Ian Jenkinson (France)
Luiz Mafra, Jr. (Brazil)
Shauna Murray (Australia)
Antonella Penna (Italy)
Mary Carmen Ruiz De La Torre (Mexico)
Toshiyuki Suzuki (Japan)

STUDENT REPRESENTATIVE:
Carolin Peter (Sweden)

The profiles of the ISSHA Council members can be found on the ISSHA website at *ISSHA Council*.

ISSHA 2023 Achievement Awards

The ISSHA 2023 Yasumoto Lifetime Achievement Award was won by **Ichiro Imai** and **Mireille Chinain** for their groundbreaking work on harmful algae, their contribution to the research field, as well as to society, and their mentorship for many students. We were honored this year by our special guest Prof. Yasumoto, who personally handed over the awards to this year's winners, and Dr. Takayama, who made the wood carvings.

The nomination of **Ichiro Imai** was led by Satoshi Nagai and presented during the award ceremony by Donald Anderson. It was supported by 18 colleagues from around the world, in-



Carvings of Dr Takayama ready for the awardees. Photo LOC ICHA 2023.

cluding Japan (7), Malaysia (2), United States (3), Chile (2), Mexico (2), Germany, Estonia.

Ichiro Imai illuminated the overwintering process of *Chatonella* through his discovery of resting cysts in the bottom sediments of the Seto Inland Sea, Japan and succeeded in artificially producing and germinating cysts under laboratory culture conditions after years of difficult and sustained effort. He identified encystment and excystment mechanisms and elucidated the life cycle of *Chattonella* from the analysis of the nuclear phase with Dr. Mineo Yamaguchi using cultured strains. From the ecophysiological properties of cysts, Professor Imai has shown that monitoring bottom water temperatures in early summer is effective in predicting the cyst-driven initiation of red tide outbreaks. Prof. Imai also discovered the resting cysts of the raphidophyte *Heterosigma akashiwo*, a very important HB species that occurs in temperate waters worldwide, and once again, elucidated the mechanisms of bloom development, including overwintering and bloom initiation.

Prof. Imai expanded his studies to the distribution and ecophysiology of diatom resting stage cells, documented their abundance in the surface sediments, provided spatial maps of their distribution in Japanese coastal waters, and confirmed the importance of light for germination. These results are also a significant breakthrough in the study of community variation and population dynamics of microalgae. Prof. Imai furthermore demonstrated that diatoms can be induced to germination/rejuvenation and rapid growth by resuspension to the euphotic layer using sediment perturbation. Application of these results has led to successful control of toxic blooms of *Alexandrium catenella* (formerly *A. tamarense*) in Osaka Bay (1400 km^2), where bottom sediment perturbations over 5 km^2 were carried out by the Osaka Prefectural and Fisheries Cooperatives in the springs of 2020 to 2023.

Prof. Imai identified and isolated a large number of growth-inhibiting/algicidal bacteria from the Seto Inland Sea, such as *Cytophaga* and *Alteromonas*. He examined the effects of various algicidal bacteria on the growth of HAB species in co-culture experiments and revealed



Prof. Ichiro Imai with Donald M. Anderson (left) after receiving the Yasumoto Award. Photo credit: Don Anderson.

the dynamics of those interactions. In the field, he found that algicidal bacteria increased during the final stage of red tides, such as with *H. akashiwo* in Hiroshima Bay, Japan. Furthermore, in lakes and ponds, Prof. Imai discovered that cyanobactericidal bacteria and growth-inhibiting bacteria exist on the surface of aquatic plants with high densities. Based on these findings, practical measures to control nuisance cyanobacterial blooms were provided to secure safe water resources by artificially creating aquatic plant zones. The research on algicidal bacteria in the control of HABs has opened up a new research field in aquatic ecology, and research in this area is currently growing.

Prof. Imai contributed significantly to the instruction of many young scientists working in the field of aquatic ecology. For over a decade, he has been involved in reviewing applications for funding within the US HAB research program "The Ecology and Oceanography of Harmful Algal Blooms (ECOHAB)." He has also contributed significantly to developing research societies such as the Plankton Society of Japan, the Japanese Society of Fisheries Science, and the Japanese Society of Microbial Ecology by being frequently invited or by helping to organize national and international conferences, symposia, and workshops. Of particular note is the honor of giving an invited lecture in June 2011 at the "Mycotoxins and Phycotoxins" session at the Gordon Research Conference in the United States, famous for its cutting-edge research presentations.

The nomination of **Mireille Chinain** was led by Patricia Tester and presented during the award ceremony by Marie-Yasmine Bottein. It was supported by nine colleagues from around the world, including China, French Polynesia (2), Australia, New Zealand, Mexico, and France (3). The following achievements were highlighted in the nomination letter, with the note that this is first time an ISSHA Yasumoto Award goes to a tropical Pacific scientist who is working at ground zero in ciguatera poisoning:

Mireille Chinain has played an integral part in all major breakthroughs in ciguatera research, such as chemical characterisation of ciguatoxin (with Dr. A. Legrand and Prof. T. Yasumoto), the taxonomic description of multiple *Gambierdiscus* species first based on morphology (with Dr. Faust) and later with help of genetics (with Dr. W. Litaker), the consistent production of ciguatoxin in culture by *G. polynesiensis*, the discovery of the new phenomenon of ciguatera poisoning from the gastropod *Tectus niloticus*, instigation in French Polynesia of an epidemiological surveillance program of human poisoning cases (with Dr. C. Gatti), and most importantly driving the elevation of ciguatera research as a priority topic for international forums such as IOC-IPHAB, FAO-WHO, and IAEA.

Since 2013, she has worked as an expert with the National Agency for Food, Environmental and Occupational Health Safety (ANSES), in the "Ciguatoxins" working group, and since 2014 as an expert with the International Atomic Energy Agency (IAEA). Since 2015 she served as one of the drivers for the Task Team on Global Inter-Agency Ciguatera Strategy for Improved Research and Management of IOC-UNESCO aimed at standardizing and strengthening research on ciguatera. Further Dr. Chinain has helped manage Ciguatera Poisoning (CP) risks at the international level serving as member of the FAO-WHO expert's meeting on CP in November 2018. She and her team hosted the IAEA Workshop on CP Field Monitoring and the Second Coordination Meeting of RAS 7/026 project in 2015. Being a highly valued and reliable collaborator, she has reached out over the years to many other others, e.g. in the US (Drs. M. Faust and W. Litaker), in Australia (Drs. R. Lewis, M. Holmes, S. Murry), the Pacific Island countries, China, and



Mireille Chinain, introduced by Marie Yasmine Dechraoui Bottein (right), accepts the Lifetime Achievement Award, bearing the name of Prof Yasumoto (left), in his esteemed presence. Photo credit: Clémence Gatti

mainland France, e.g., Dr. P. Hess to underpin the chemical understanding of the distribution of ciguatoxin analogues across tropical reef ecosystems. Materials Transfer Agreements were signed between ILM and the Cawthron Institute (Drs. P. Holland and L. Rhodes) New Zealand and NOAA (Dr. P. Tester) in the US as well. Mireille and her staff travelled from French Polynesia to Nantes to help support France's efforts to host ICHA2018.

The CIGUAPROD initiative coordinated and championed by Dr. Chinain, completed in 2019, aimed to generate a stand-alone facility for *Gambierdiscus* mass culture and toxin purification to provide her program, colleagues and eventually the HAB community with a reliable source of purified CTX standards. Even with the responsibilities of a laboratory, staff and innovative research and monitoring program, Dr Chinain has mentored numerous doctoral candidates working on various aspects of ciguatera.

Dr. Chinain's work has been acknowledged by national and international awards including the Prix Trégouboff Award 2005 in Marine Biology (Academy of Sciences of Paris, France), the Albert Sézary Award 2006 (National Academy of Medicine, Paris, France), and Tyge Christensen Prize 2010 (International Phycological Society).

There are few, if any, HAB scientists who have made breakthrough contributions in so many areas. But Dr. Chinain has made significant contributions in taxonomy, toxicology, ecology, biogeography and public health of HABs during her career. She has been a mentor and inspiration to many of us. Her leadership and persistence to produce repeated breakthroughs in helping mitigate a much-neglected tropical disease as evidenced by her contribution to the Experts Report on Ciguatera (2020), the Global HAB Status Report (2021) and Chapter 3 on Benthic HABs in the Joint FAO-IOC-IAEA Technical Guidance for the Implementation of Early Warning Systems for Harmful Algal Blooms (2023).

ISSHA 2023 Patrick Gentien Young Scientist Award

Tomohiro Nishimura received the ISSHA 2023 Patrick Gentien Award for his outstanding contribution to our understanding of the physiology, ecology and toxicity of harmful algae. He has proven to be a productive, collaborative, and impactful young scientist. His nomination was presented by Prof. Masao Adachi: Tomohiro Nishimura's research has focused on toxic dinoflagellates and diatoms in Japan and Oceania, with particular emphasis on benthic harmful algal bloom (BHAB) genera, including the macroalgae-associated *Alexandrium* (his original discovery), *Gambierdiscus*, and *Prorocentrum*, and harmful algal bloom (HAB) diatom genus *Pseudo-nitzschia*. His work included extensive studies of cell density and dynamics, the establishment of over 2,000 isolates, morphological and molecular phylogenetic classifications, toxicity and toxin production assessments, growth experiments under different environmental conditions, and the development of a sensitive and species-specific molecular biological detection and quantification method (qPCR assay). His research has elucidated species diversity, relations between species/clades and toxicity/toxin production, and the physiology and ecology of BHAB dinoflagellates and HAB diatoms.



Tomoshiro Nishimura, ISSHA Patrick Gentien Award 2023

His pioneering studies have significantly advanced the field of BHAB dinoflagellates and *Pseudo-nitzschia* research, and provided essential information for risk assessment of these organisms. His discovery of a *Prorocentrum* strain with high productivity of dinophysistoxin-1 has contributed to the industrial production of this compound as a standard material in Japan. In addition, his work on *Pseudo-nitzschia* has contributed to refining risk profiles and improving cell density trigger levels in the New Zealand Marine Phytoplankton Monitoring Programme, reducing unnecessary testing and bivalve harvest closures in New Zealand in the future. He has also contributed to research on other HAB/BHAB genera, such as *Azadinium, Coolia, Fukuyoa, Heterocapsa, Ostreopsis*, and toxic cyanobacteria genera with collaborators from various countries.

Maureen Keller Awards to best student presentations

Serena Sung-Clarke *(best oral presentation)*. Serena is a Ph.D. candidate with the Massachusetts Institute of Technology/Woods Hole Oceanographic Institution (MIT/WHOI) Joint Program in Applied Ocean Sciences. She is advised by WHOI's Dr. Michael Brosnahan. She



Serena Sung-Clarke



Kristof Möller



Loïc Plessis



Lucia Beatrice Cal

studies the ecology of the dinoflagellate *Dinophysis* using a mix of field observations, culture experiments, and molecular methods. Her presentation at ICHA2023 described her work investigating subsurface accumulation and phased sexual reproduction in a *Dinophysis* bloom population in the absence of their obligate prey. Serena earned her Bachelor of Arts with high honors from Swarthmore College in 2019, with majors in Biology and Political Science. As an undergraduate, she worked in Dr. Elizabeth Vallen's lab, studying cnidarian immune protein interactions. Her interest in HABs started during the summer of 2018, when she had the opportunity to work with Dr. Jacques Oliver and Dr. Lester Yuan at the U.S. Environmental Protection Agency (USEPA) analyzing freshwater and estuarine nutrient impairment and HAB occurrence. Before starting her Ph.D., Serena spent two years working for Cadmus on USEPA contracts, developing watershed health assessments, leading regulatory workshops for water utilities, and compiling research on chemical contamination. Now, as a student in the Brosnahan Lab, she pries into the mysterious lives of *Dinophysis* dinoflagellates and hopes to shed light on their response to starvation and life history processes.

Kristof Möller *(oral presentation honorable mention)*. Kristof Möller, from the Alfred-Wegener-Institute Helmholtz Centre for Polar and Marine Research (Bremerhaven, Germany), received the Maureen Keller Award for his presentation about: "Ichthyotoxicity of the harmful dinoflagellate *Alexandrium pseudogonyaulax* and predator-prey interactions with the copepod *Acartia tonsa*" co-authored by Urban Tillmann, Magdalena Pöchhacker, Bernd Krock, Elisabeth Varga, Florian Koch, Thomas Harris and Cédric L. Meunier.

Kristof Möller is a Ph.D. student in the Ecological Chemistry section at the Alfred-Wegener-Institute. His doctoral thesis focuses on the toxic dinoflagellate *Alexandrium pseudogonyaulax* in Northern European waters, particularly considering its recent increase in abundance. His research involves investigating bottom-up and top-down population controls of *A. pseudogonyaulax*, as well as assessing its toxicological effects on other trophic levels including zooplankton and fish. Additionally, he analyses how A. pseudogonyaulax has been introduced and what factors have contributed to its proliferation by conducting a time series analysis of long-term monitoring programs in Sweden, Norway, Denmark, and Germany. Kristof earned his Master of Science degree in biochemistry and Bachelor of Science degree in chemistry and biochemistry from the Ludwig-Maximilians-University in Munich. His master's thesis, which focused on establishing a method for extracting and quantifying vitamin B_{12} from seawater, sparked his interest in marine sciences. His research interests now encompass biotoxins, harmful algae blooms, and marine carbon dioxide removal techniques.

Loïc Plessis *(best poster presentation)*. Loïc Plessis, PhD student from the French Research Institute for Exploitation of the Sea (Ifremer) and Nantes University, was awarded the Maureen Keller Student Award for the best poster presentation titled "Portimine from *Vulcanodinium rugosum* induces severe pyroptosis in primary skin cells through ribotoxic stress response (RSR)" co-authored by Léana Gorse, Korian Lhaute, Fabienne Hervé, Damien Réveillon, Stéphanie Brédif, Patrice Brehmer, Étienne Meunier, and Philipp Hess.

Loïc's scientific journey began with a two-year diploma in applied chemistry at the Institute of Technology of Orléans University, followed by an internship in organic chemistry synthesis at Rothamsted Research, United Kingdom. He then pursued a double course in chemistry and biotechnology at two engineering schools at Strasbourg University, in European School of Chemistry, Polymers and Materials Science (ECPM) and European School of Biotechnology (ESBS). To complete his engineering diploma, he interned at the phycotoxin laboratory of Ifremer Nantes, working for the first time on toxic microalgae. Specifically, Loïc studied the chemical diversity of two toxic microalgae belonging to the genus *Azadinium*.

Currently, Loïc is conducting his

PhD research under the supervision of Dr. Philipp Hess between two laboratories: the METALG laboratory at Ifremer Nantes, Phytox unit, and the Groupe Rocher skin lab in Paris. His research focuses on investigating the impact of various microalgae and their metabolites on skin cells, with an emphasis on inflammatory effects. This includes studying both toxic microalgae for Ifremer, such as *Ostreopsis* and *Vulcanodinium*, and microalgae of cosmetic interest for Groupe Rocher. During his PhD, Loïc had the opportunity to collaborate with the Institute of Pharmacology and Structural Biology (IPBS) of Toulouse, which helped clarifying the mechanism of action of the emerging toxin Portimine from *V. rugosum*.

Lucia Beatrice Cal *(poster presentation honorable mention)*. Lucia Beatrice Cal received the Maureen Keller Student Award for her poster entitled: "Morphological and phylogenetic characterization of toxic *Coolia malayensis* from the West Philippine Sea", co-authored by Robert Jay Ramos, Juri Nazareth Ochotorena, Gian Carlo Gaetos, and Dr. Deo Florence Onda.

Lucia is a MS Marine Science student affiliated with the Microbial Oceanography Laboratory of the Marine Science Institute, University of the Philippines. She earned her Bachelor of Science degree, majoring in Biology, from the same university and took an interest in microalgae while taking a phycology elective as an undergrad.

She works as a research associate while pursuing her Master's degree under the "Real-time Monitoring and Early Warning for HABs Using High Throughput Imaging and Molecular Methods (the HABs Watch Project)" led by her adviser Dr. Onda, which aims to use a combination of machine-learning and the Imaging Flow Cytobot/IFCB (McLane Laboratories) to develop a real-time detection system for harmful phytoplankton. She is tasked with establishing and maintaining diatom and dinoflagellate monocultures, but is also being trained in using the IFCB. Her research is currently focused on benthic dinoflagellates, whose distribution patterns are not well-studied in the Philippines, and marine microbial ecology.









Top left, Local Organizers recognition at the closing ceremony (photo LOC ICHA 2023); Bottom left, Hiroshima Gokoku Shrine, location of cocktails before Gala dinner (photo E Bresnan); ISSHA most senior Council members relax at the Kagura performance: top right, Ichiro Imai-san and Ian Jenkinson (photo I. Jenkinson) and bottom right, ISSHA (past) President, Wayne Litaker (Photo E Bresnan).

First report of a *Pyrodinium bahamense* bloom off Djibouti, western Gulf of Aden

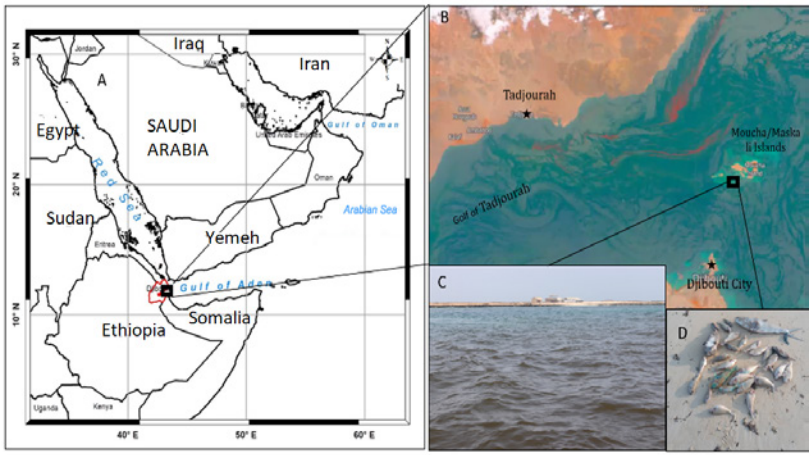


Fig. 1. A. Map of the study area and location of the dinoflagellate bloom off Djibouti, western Gulf of Aden. B. Satellite image from Sentinel-2 on September 22, 2023 showing high chlorophyll concentrations in coastal waters of Tadjoura, Djibouti. (C) Red water discoloration related to the bloom. (D). Fish mortality on the beach of the Moucha/Maskali Islands.

During summer, southwest Monsoon winds in the Gulf of Aden (GoA) induce an eastward outflow of surface waters. This outflow is compensated by an upwelling of cold, nutrient-rich deep water at the extreme west, in the Gulf of Tadjoura and the southern part of the Strait of Bab-el-Mandeb strait [1]. To the west of the GoA, these nutrient-rich waters are characterized by high chlorophyll concentrations [2]. In late summer, September 2023, a water discoloration event (red tide) accompanied by significant fish mortalities was observed in the Gulf of Tadjoura (Fig. 1). To understand the origin of this phenomenon, phytoplankton samples were collected using a 20-µm mesh size plankton net (Hydro-Bios) and environmental conditions were assessed at the sampling site off the Moucha/Maskali Islands on September 21, 2023 using a multiparameter probe (CastAway-CTD). Cells were identified by light microscopy [3] and enumerated using an Olympus inverted microscope (IX70-S8F2, Japan) [3]. The plankton samples revealed that the seawater discoloration was caused by a potentially toxic dinoflagellate, *Pyrodinium bahamense* [4]. The bloom, located near the coast, between the Marine Protected Areas of the Moucha/Maskali Islands and the city of Tadjourah, was dense enough to be detected by satellite imagery (Fig. 1. A). To the best of our knowledge, this is the first time that an analysis has been conducted in the Gulf of Tadjoura during a phytoplankton bloom. *Pyrodinium bahamense* developed a nearly monospecific bloom, reaching a density of 5.3 × 10^6 cells. L^{-1}. Other potentially toxic dinoflagellates such as *Dinophysis miles*, *Dinophysis caudata*, and *Lingulodinium polyedra* were detected but at very low cellular densities. Environmental parameters measured during the event were: salinity 36.6; temperature 32.9°C; pH 8.67, conductivity 64009 µS/cm, and seawater density 1021.9 kg/m^3.

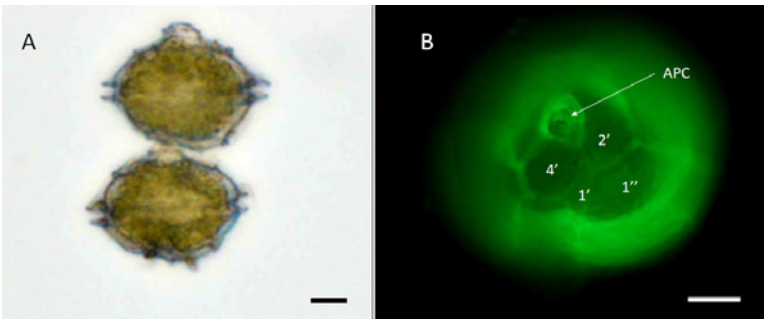


Fig. 2. A. Light micrographs of Pyrodinium bahamense cells from a sample collected on September 20, 2023, off the coast of Moucha/Maskali Islands. B. Epifluorescence micrograph showing epithecal plates and the Apical Pore Complex (APC) (scale bar = 10 µm).

Previous observations of *P. bahamense* blooms in Yemeni and Saudi Arabian coastal waters suggest that such blooms typically occur in late summer, although previous studies recorded lower cell densities of 10^4–10^5 cells. L^{-1} [5,6]. Our observations align with these findings, indicating that this species is present in coastal waters of the Red Sea in significant quantities in late summer through early autumn (June to October) [5,6]. In addition, toxicological analyses conducted on cultures of this species as well as water samples from the southern Red Sea region confirmed the toxicity of this species [6], which could explain the fish mortalities. Studies are needed to confirm the toxicity of the GoA strains of *P. bahamense* and their ability to produce toxins similar to those of the Red Sea strains. This is crucial for enhancing our understanding of associated risks and implementing preventive measures when necessary.

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Green tides of *Eutreptiella marina* (Euglenophyta) in Yelapa, Bahía de Banderas Jalisco-Nayarit México

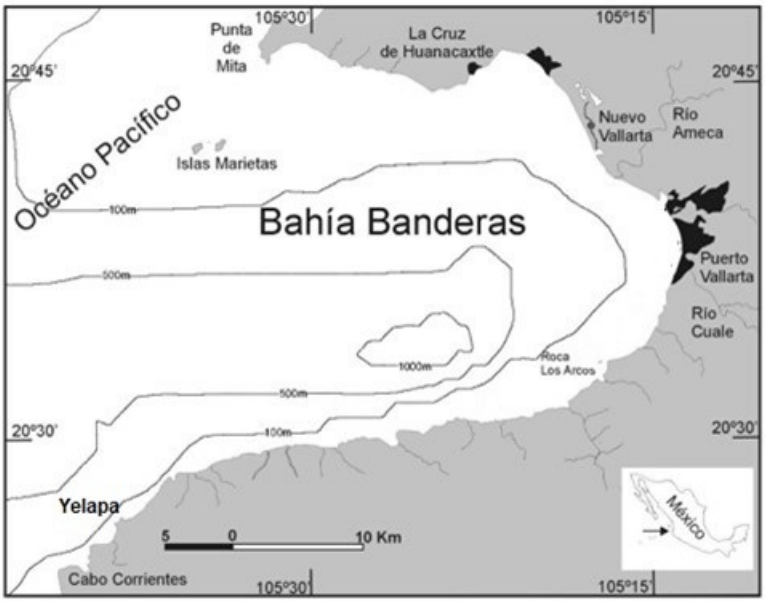

Fig. 1. Map of Yelapa area, municipality of Cabo Corrientes, in Banderas Bay Jalisco-Nayarit México.

In May 2022 a massive algal bloom caused greenish discolorations along the beaches of Yelapa Jalisco México (Fig. 1) (-105° 44´N; 20°48´W). Microscopical observations of bloom samples showed that the dominant microalgae agreed with the description of *Eutreptiella marina* by Norris [1] (Fig. 2). Single cells were about 15-80 µm long and 3-15 µm wide with a variable shape ranging from spherical to very elongated. A vertical profile of physical properties measured at the time of the bloom showed marked gradients of temperature, with 25.71°C at the surface, ~ 20°C at 10m and 14.56°C at the bottom, and a slight salinity gradient (34.08-34.24) (Fig. 3). Chlorophyll *a* reached values of 32.28 and 16.73 mg m^{-3} at the surface and 70 m respectively. It is well known that the genus *Eutreptiella* includes several species whose toxicity has been related to the cellular content of reactive oxygen species (ROS). Other *Eutreptiella* spp are used as pollution indicators [2, 3, 4]. These high biomass blooms (up to 7 million cells.l^{-1}) (Fig. 2) represent a latent risk for fish and other organisms in the bay. Likewise, its notable abundance can create severe anoxia causing mortality of marine fauna [5]. The bloom described here was one of the most extensive HAB of a euglenophyte microalgae ever reported in this area. The causes of this exceptional *E. marina* event are not very clear yet. It is well known that the occurrence of this species in Yelapa is favored by water circulation during the cold season. Upwelling of cold and nutrient-rich waters on the coast creates optimal conditions for proliferation of microalgae [3, 6]. An alternative explanation is that during that HAB event, Yelapa coasts were under the influence of El Niño Southern Oscillation (ENSO) in its cold La Niña phase (2021-2022). This atmospheric mode probably affected the oceanographic conditions that promoted the bloom of *E. marina*. Runoff of terrigenous material in rivers and local wastewater discharges may have contributed to promote these HAB events [7]. *Eutreptiella marina* is said to prefer waters with high concentrations of organic matter, ferrous compounds, and ammonium salts [1, 8]. Complementary studies should focus on the impacts of ROS produced by *E. marina* on the marine fauna, because these compounds cause erratic behavior and considerable mucus secretion by fish gills (e.g., *Xenistius californiensis*) in Bahía de Banderas [5]. During the bloom, no negative impacts were detected on the local marine fauna. There was no health impact from recreational activities either. Nevertheless, it is important to continue monitoring this area to identify spatial and temporal trends in *E. marina* populations and their impact.

Acknowledgements

This work was supported by the Project "Implementation of a Marine Environmental Monitoring Programme for Islas Marietas and Isla Isabel National Parks,



Fig. 2. Yelapa green tide of Eutreptiella marina during May 14-26, 2022. Micrograph of single cells showing different shapes (2A-E) taken under an inverted microscope with phase contrast. Aggregates at 200x (2F) and live cells filaments (2H) during the event. Photograph 2G courtesy of Océan. Rafael García de Quevedo.

Nayarit, México". Our thanks to Ocean. Rafael García de Quevedo for providing photos of the discolorations

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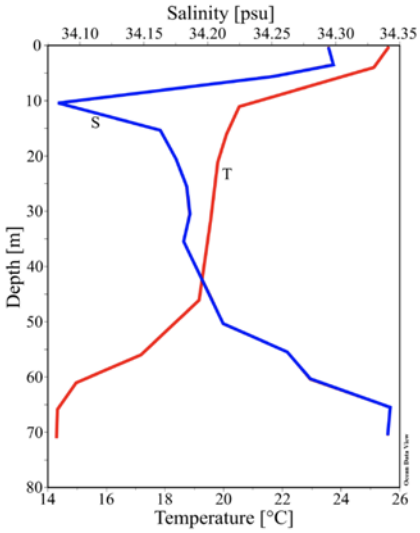

Fig. 3. Vertical distribution of temperature, salinity and depth in Yelapa, Jalisco during HAB events of Eutreptiella marina on May 26, 2022. Data obtained with a Sontek CastAway® CTD from Xylem and graphed with Ocean Data View software.

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NEW BOOK!

Harmful Australian Marine Microalgae

by Gustaaf Hallegraeff



This handy, pocket-sized guide arose from 30 years of notes for HAB training courses targeting Australian fish and shellfish farmers, public health officials, and environmental water quality managers.

Three previous editions (1991, 2002, 2015) have been updated to cover 114 dinoflagellates, 36 diatoms, 10 haptophytes, 5 raphidophytes, 4 dictyochophytes, 1 ciliate and 20 cyanobacteria, all illustrated by line drawings, light and electron micrographs.

Notes on harmful impacts, toxicology, global distribution and, where available, alert levels and counter measures are included.

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March 2024 | 192 pp |

Cook Islands benthic and epiphytic dinoflagellates

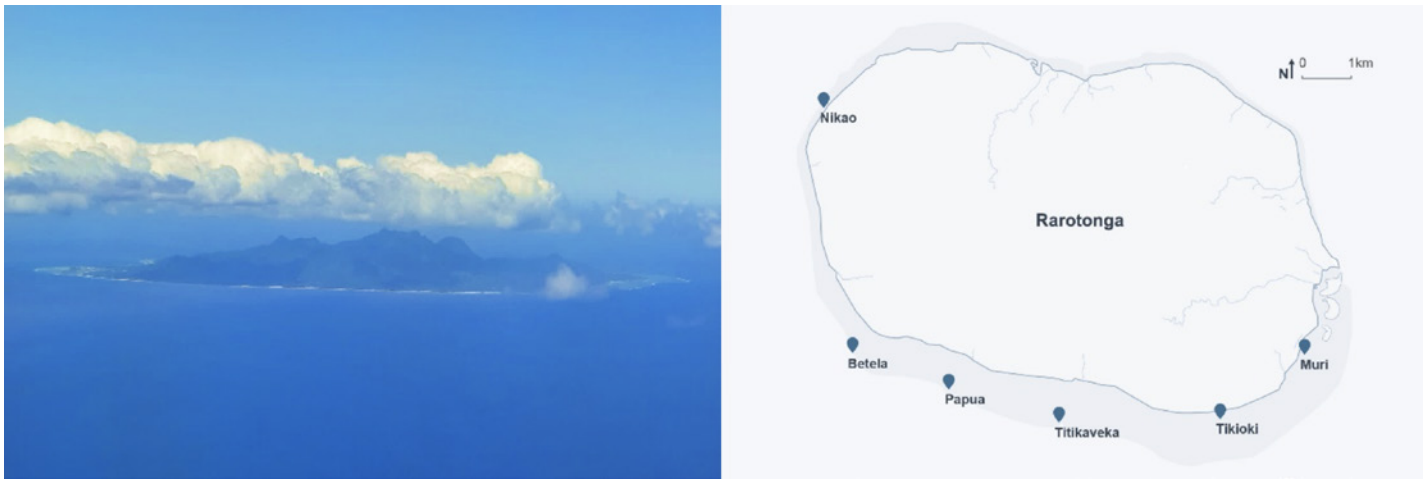

Fig. 1. (Left) Photograph of Rarotonga, Cook Islands from the air and (right) map of sites sampled in the Cook Islands, November 2022

In 1979, the genus *Gambierdiscus* Adachi & Fukuyo was described, with the type species being *G. toxicus* [1]. In 2017, in an article in HANews [2], it was noted that 'fourteen species of *Gambierdiscus* had been described with three of these being described in 2016, an increase of nearly 30% in one year'. Since then, the number has increased to nineteen species being recorded in AlgaeBase to date [1]. Seventeen of those species have been recorded in the Pacific region. The Cook Islands has been of interest because of the high rates of ciguatera poisoning in past years, due to the presence of *Gambierdiscus polynesiensis*, a ciguatoxin (CTX) producer [3]. A concern is that, with warmer seas being recorded in coastal waters of Aotearoa New Zealand, species found in the Cook Islands and other Pacific Islands may establish or, if already present, proliferate in the subtropical northern waters. It is therefore critical for risk assessments to be carried out to know what toxin producing genera are likely to be of future concern.

In November 2022, a research trip was carried out in Rarotonga, Cook Islands (Fig. 1) and samples were collected (Fig. 2) for metabarcoding (in preparation) and for isolations of living cells. The dominant *Gambierdiscus* species, as determined by DNA sequencing of cultured isolates, was *G. australes*. This species was isolated from macroalgal samples (*Turbinaria* sp. and *Halimeda* sp.) collected from the sites Papua and Titikaveka, and isolates tested were all maitotoxin (MTX-1) producers (Table 1). No CTX was detected. One of the many strains of *G. australes* isolated is now maintained in the Cawthron Institute Culture Collection of Microalgae (CICCM) as CAWD445.

At Muri Lagoon (Fig. 1), *G. honu* was successfully isolated from samples collected from *Padina* sp. and another isolate (CAWD446) was isolated from *Turbinaria* sp. at the Papua site. *G. honu* is a known producer of MTX-7 and gambierone analogues [4], but interestingly isolate CAWD446 did not produce MTX-7 above the limit of detection for this method (Table 1).

Gambierdiscus pacificus (CAWD449) was also isolated from *Padina* sp. in



Fig. 2. (Top left) Sampling in deeper water, Rarotonga, Cook Islands, November 2022; (top right) sampling at Tikioki with Ministry of Marine Resources staff; (lower left) passive sampling devices; (lower right) typical shallow coastal lagoon site.

Table 1. Biotoxins produced by Gambierdiscus species isolated from the Cook Islands, 2022.

		Toxins pg/cell							
Species	**CICCM code**	**G**	**44-MG**	**MTX-1**	**MTX-5**	**MTX-6**	**MTX-7**	**P-CTX-3B, 3C, 4A, 4B**	**Iso-CTX-3B /C, 4A/B**
G. australes	CAWD445	<0.01	**311**	**14**	**0.8**	<0.01	<0.01	<0.01	<0.01
G. honu	Muri16*	**4.4**	**159**	<0.01	<0.01	<0.01	**11.2**	<0.01	<0.01
G. honu	CAWD446	**42.5**	**138**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
G. lapillus	CAWD451	**0.6**	**108**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
G. pacificus	CAWD449	**5.7**	**210**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

*Not in Cawthron Institute Culture Collection of Microalgae (CICCM); G; gambierone; 44-MG: 44-methylgambierone; MTX: maitotoxin; P-CTX: Pacific ciguatoxin.

Muri Lagoon and produced 44 methylgambierone and gambierone, but no CTXs (Table 1).

An extremely slow growing *Gambierdiscus* isolate (yet to be tested for toxin production) was isolated from a sample collected at the island of Aitutaki, north of Rarotonga. This proved to be *G. lapillus* (CAWD451) as determined by DNA sequencing. It was isolated from the macroalga *Turbinaria ornata.* No CTXs were detected (Table 1). *Gambierdiscus* cells were also isolated from *Padina* sp. but these did not survive in culture.

Gambierdiscus species not observed in live samples during the current study, but which have been reported in the Cook Islands previously, include *G. lapillus* [5], *G. cheloniae* (a producer of MTX-6) [6] and *G. polynesiensis* (CTX producer) [7]. It was noted by Smith et al. [8], during a 2014 field trip, that although *Gambierdiscus* was present in high numbers at most sites (with five species being isolated and species determined by DNA sequencing and eight *Gambierdiscus* species being recorded using metabarcoding).

At Tikioki (Fig. 1) in November 2022, *Alexandrium* sp. 2 [9] was isolated from an artificial substrate which had been deployed for 48 hours. Cells were also isolated from samples deployed at Betela but did not survive. The cells were identified by DNA sequencing, as they were only present in their non-motile stage and were difficult to identify by microscopic observation (CAWD450). Despite being in culture for 10 months no motile cells have been observed to date. The isolate was tested for paralytic shellfish toxins by liquid chromatography tandem mass spectrometry but none were detected.

Ostreopsis lenticularis (CAWD447) and *Coolia malyensis* were isolated from samples collected from a submerged palm frond at the outer reef and were also identified by DNA sequencing. Production of palytoxin-like compounds by *Ostreopsis lenticularis* was assessed using a recently developed method that monitors the intact analogues (unpublished) and the oxidative cleavage method [10], but none was detected. Not isolated, but observed in most samples, were the dinoflagellate genera *Amphidinium* and *Prorocentrum.*

There did not appear to be a preferred habitat for *Gambierdiscus* or *Ostreopsis,* with species isolated from a wide range of macroalgal genera, including *Halimeda, Padina* and *Turbinaria.* The artificial substrate, *Asparagopsis,* and even a floating palm frond also gave rise to successful unialgal cultures. Species recorded by establishment of cell cutlures from the Cook Islands so far include *G. australes, G. caribaeus, G. cheloniae, G. honu, G. lapillus, G. pacificus,* and *G. polynesiensis.* This supports the hypothesis that toxic species of *Gambierdiscus* could establish on the macroalgae occurring in northern Aotearoa New Zealand, with habitat unlikely to be a barrier. It is not yet known whether the diversity of *Gambierdiscus* species is particularly high for the Cook Islands, or whether the species remain underestimated globally. As *Gambierdiscus* has been recorded on one occasion, and the related genus *Fukuyoa* on many occasions, in Aotearoa New Zealand it is only a matter of time before favourable conditions result in a proliferation of these dinoflagellates resulting in blooms.

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The Next International Conference on Harmful Algae (21st ICHA) is coming

Time flies, and it feels like just yesterday that the 20th edition of the International Conference on Harmful Algae (ICHA) unfolded. Held biennially, the most recent gathering took place from November 5 to 10, 2023, at the Grand Prince Hotel in Hiroshima, Japan. Now, preparations are underway for the upcoming conference, slated to take place in Chile, specifically in Punta Arenas on the northern coast of the Strait of Magellan at the southern tip of South America. The 21st edition of ICHA is scheduled from October 19 to 24, 2025, at the Dreams Hotel in Punta Arenas (Fig. 1).

These conferences are made possible through the tireless work, dedication, and collaboration of the International Scientific Society of Harmful Algae (ISSHA). Their support and guidance are instrumental in their success. The decision to host the 21st ICHA in Chile stems from a request made during the development of the 19th edition of the conference, held in La Paz, Mexico, in 2021. The request was presented to the Assembly of ISSHA, then chaired by Dr. Vera Trainer from the USA. Currently, Dr. Dedmer van de Waal from the Netherlands holds this position, who receives the post chaired by Dr. Wayne Litaker from the USA (2021-2023).

Harmful algal blooms (HABs) represent a pressing global issue marked by a rise in frequency, scope, and impact. The increase in these events is attributed to several factors, including extreme oceanographic climate fluctuations, such as El Niño and La Niña, the Antarctic Oscillation, and the broader effects of climate change. Additionally, nutrient overabundance from domestic and industrial effluent discharge into water bodies contributes to HAB proliferation. Furthermore, certain microalgae morphologies pose a threat to fish gills, particularly in the aquaculture industry. HABs, stemming from diverse microalgae species, can produce potent phycotoxins that accumulate in shellfish and pose a great threat to public health and aquatic ecosystems, including aquaculture systems (Fig. 2). Their severe impacts extend to social and economic sectors, exerting a significant toll on affected communities. In the last decades, HAB events have been escalating in frequency and expanding geographically, exacerbated by extreme oceanographic climatic events and global climate change. These blooms also affect freshwater ecosystems like lakes and rivers, prompting scientists worldwide to intensify efforts to understand their dynamics and impacts. Each country or region has created capabilities and inverted resources for monitoring and managing HAB occurrences. Effective policy decisions and management strategies are imperative in averting and mitigating the adverse effects of these events.



Fig. 1. Dreams Hotel in Punta Arenas, conference venue for the 21st ICHA conference venue in Punta Arenas, Magallanes, Chile.

With eighteen months remaining until the next conference, now is an opportune moment to underscore the significance of such scientific gatherings and the responsibility they entail for Chile as hosts. It's crucial to motivate attendance at the upcoming conference to be held in Chile. Emphasizing the importance of convening in the Southern Hemisphere, specifically at the southernmost tip of the Americas, in Chile, will be paramount. Additionally, the importance of Southern Chile as a natural laboratory and a global "hot spot" of HAB events, as well as the historical, geographical, and diverse natural attractions of Chile's southernmost region —the Magellan region— as the gateway to the Antarctic continent, will serve to entice participation further (Fig. 3).

These conferences typically convene between 400 and 600 attendees from no fewer than 50 countries, presenting a significant undertaking for the host country, organizing institution, and local organizing committee. Since December 2023, the Local Organizing Committee has convened several meetings, establishing various subcommittees tasked with orchestrating the logistics and commitments associated with this scientific gathering. A substantial contingent from the national and Latin American scientific communities is expected, and the conference will prominently address HABs and marine phycotoxins. Nevertheless, the participation and support of the entire global community, spanning diverse institutions and individuals engaged with HABs, are encouraged to contribute to the planning and execution of this crucial scientific event through the International Scientific Committee.

The upcoming 21st edition of ICHA, scheduled to take place in Punta Arenas, Chile, marks the third occasion the conference will be hosted in a Latin American country. Previous editions were held in Brazil (Florianópolis) in 2016 and Mexico (La Paz) in 2021. Notably, three-quarters of these conferences have historically been hosted in countries located in the northern hemisphere. The exceptions to this trend include meetings held in South Africa, Australia, New Zealand, and Brazil.

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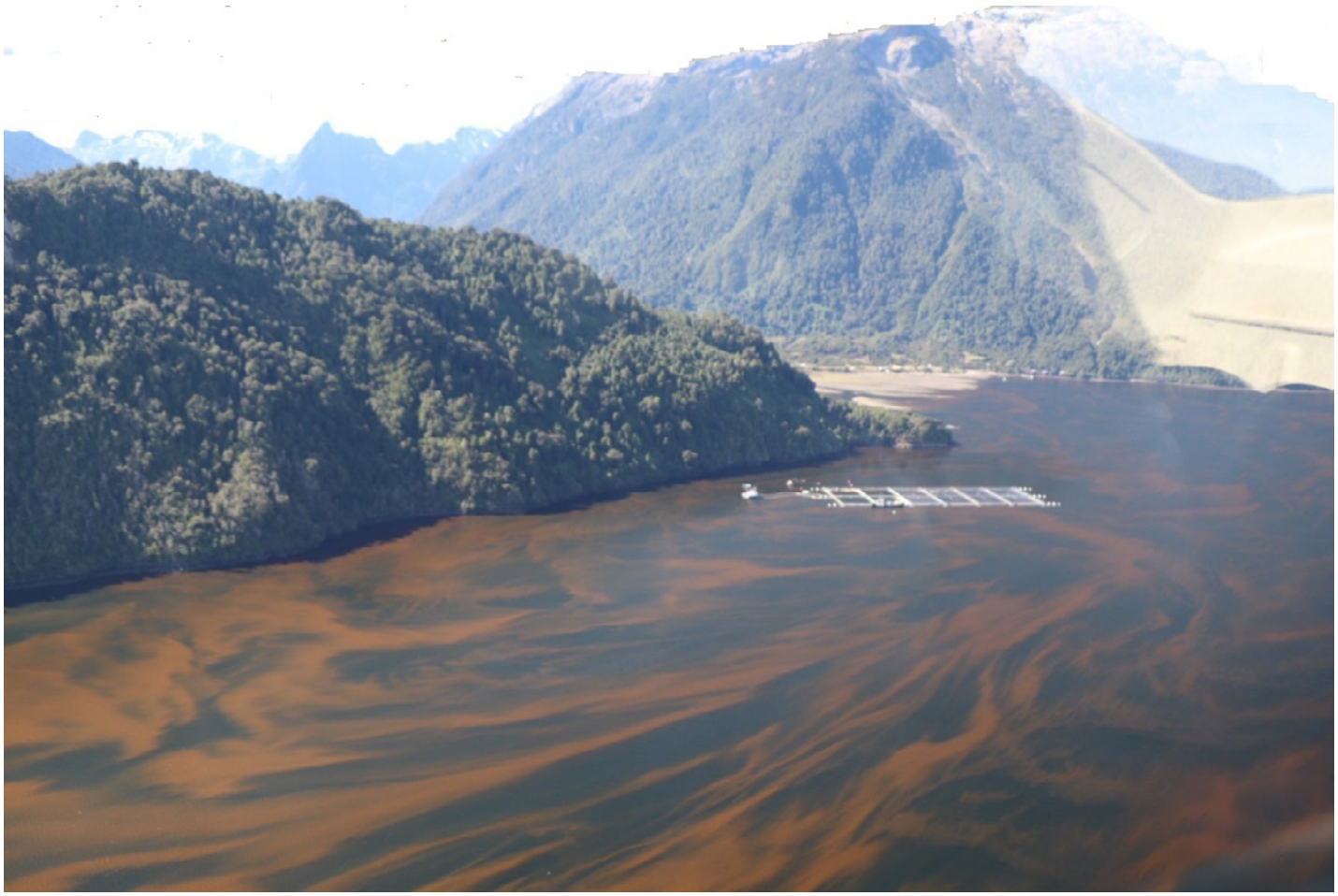

Fig. 2. "Marea café" (brown tide) of Heterosigma akashiwo in Comau Fjord, Los Lagos. Photo by Pamela Urrutia



Fig. 3. Towers of Paine National Park, Chilean Patagonia. Photo University of Magallanes



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Macroalgal blooms, dystrophy, discolourations and fish kills in a Tyrrhenian lagoon



Fig. 1. Collection of dead fish from an extensive dystrophic event in Orbetello Lagoon, Italy, July 2024

Some coastal lagoons have undergone strong processes of anthropic change with the result that they have been transformed from mesotrophic to eutrophic and, eventually, hypertrophic environments. This, by altering their typically resilient euryhaline and eurythermic biocoenoses, has favoured the dominance of *facies* of opportunistic species, microphytes and macroalgae, with impressive vegetative development [1].

Some of these species decay rapidly with rising summer temperatures, mainly as a result of increased bacterial activity [2].

The high organic load on the sediments triggers anaerobic bacterial processes that first lead to the formation of ammonia and rapidly to sulphate reduction and production of hydrogen sulphide. This last process produces dystrophy, kills the sediment fauna and even the vagile fauna if it does not be-

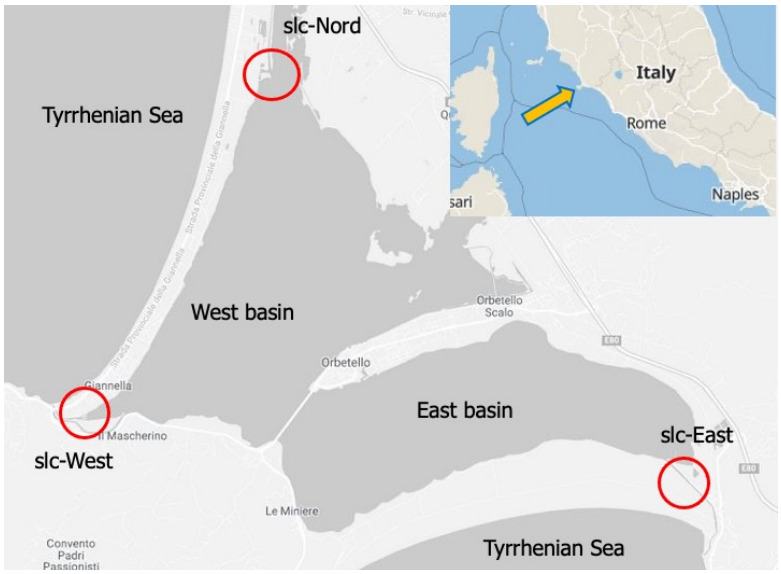

Fig. 2. Orbetello lagoon with its three sea-lagoon canals (slc), two in the West basin and one in the East basin

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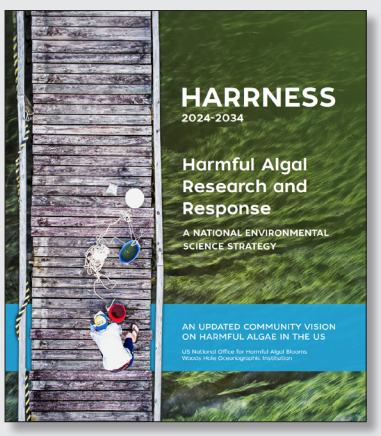



Fig. 3. Extensive mat of Valonia aegagropila in March in the West basin. The mats consisted of large 'balls' floating on the bottom (benthopleustophytic)

come aware of the toxic gas release on time (Fig. 1). Dystrophy is a degradation phenomenon through which the hypertrophic ecosystem reduces its enormous amount of accumulated energy (organic matter). Afterwards, endemic populations may be re-established, but hypertrophy quickly leads to the sedimentation of new organic deposits, and the cycle restarts [3]. The more eutrophic an ecosystem is, the higher the frequency of dystrophic episodes.

The Orbetello lagoon is a shallow, non-tidal, hypertrophic environment with very low flushing rates. It is located on the Tyrrhenian coast of Italy, (42° 25' – 42° 29' N; 11° 10' – 11° 17' E) and consists of two connected basins, West and East, of 15.25 km^2 and 10.00 km^2, respectively. The lagoon has three canals which communicate with the sea, two in the West basin (SLC1; SLC2) and one in the East basin (SLC3) (Fig. 2). To get an idea of the degree of hypertrophy, in May 2024, nutrient concentrations in the water column of the West basin were: 238 µM of N-NO_3, 27 µM of N-NH_4 and 3,23 µM of P-PO_4 [4]. This strong eutrophication supports an impressive growth of Chlorophyta *Chaetomorpha linum* and *Valonia aegagropila*. *C. linum* is a cosmopolitan opportunistic species that produces vegetative blooms in many parts of the world, favoured by nutrient availability, particularly P [5, 6]. This species is very resilient to extreme conditions, being able to survive even dystrophies, although large mats of the alga decay close to the bottom. *V. aegagropila* (Fig. 3) is a typical Mediterranean lagoon species and thrives in mesotrophic environments. It is among the first macroalgal species in the lagoon to decay under critical summer conditions [2]. This fragility is probably due to poor defense against bacterial activity, which increases with temperature rise, since in laboratory culture, in the absence of sediment, the species tolerates temperatures up to 36°C (Lenzi, unpublished data).

In February 2024, the macroalgal biomass estimates were about 30,000 tonnes in the West basin and 23,000 tonnes in the East Basin (Fig. 4). *C linum* represented 50% of the total biomass, *V. aegagropila* 30%; the remaining percentage was largely made up of Rhodophyta and 5-7% by the Ochrophyta *Gongolaria barbata.* With the exception of *C. linum*, all these species decay under summer conditions.

In July 2024, following a sudden rise in temperature, much of the macroalgal biomass decayed and sulphate-reducing bacteria gained the upper hand. Extensive areas of milky colouration developed in both the West and the East basins, as a result of the release of H_2S from the sediments and its oxidation to colloidal sulphur as it rose in the water column (Fig. 5). Subsequently, secondary bacterial growth occurred, probably anaerobic sulphur-oxidising purple bacteria, and the process affected most areas of the two lagoons (Fig. 5). Indeed, the waters everywhere remained strongly anoxic with very low redox values.

Figure 6 shows the trends in dissolved oxygen (DO, mg/L), redox (Eh, mV) and temperature (T, °C) values obtained on 24 July from multi-parameter probes. The values slowly evolved for the better in the West basin, with a slight rise during the daytime phases (DO = 3 mg/L; Eh = 160 mV) as a result of photosynthetic activity, and then returned to very low levels during nighttime respiration (DO = 0 mg/L; Eh = -400 mV), while in the East basin the values remained consistently very low

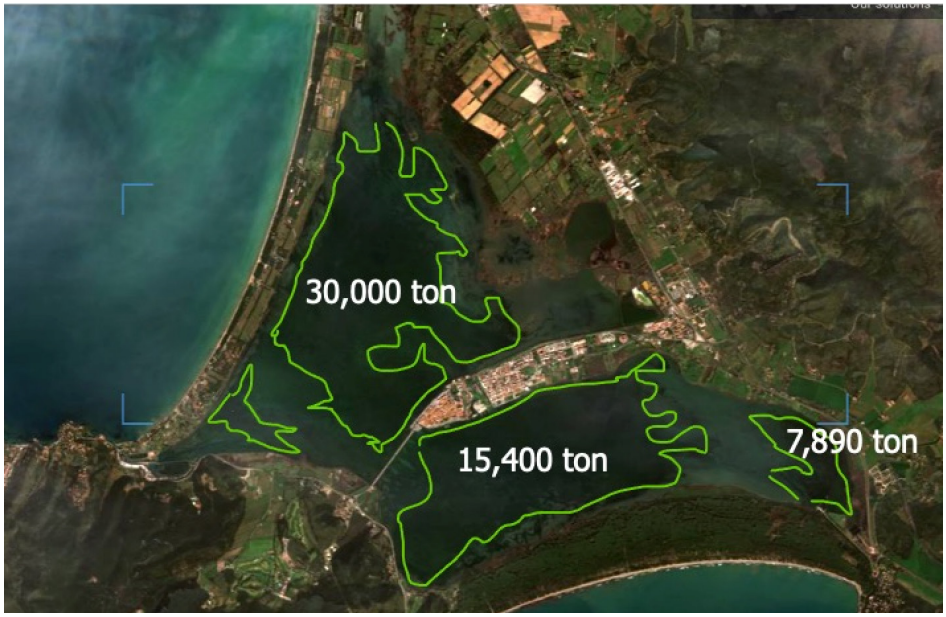

Fig. 4. Distribution of high-density macroalgal mats and estimates of their biomass (C. linum 50%, V. aegagropila 30%, others 20%) in the Orbetello Lagoon in February 2024

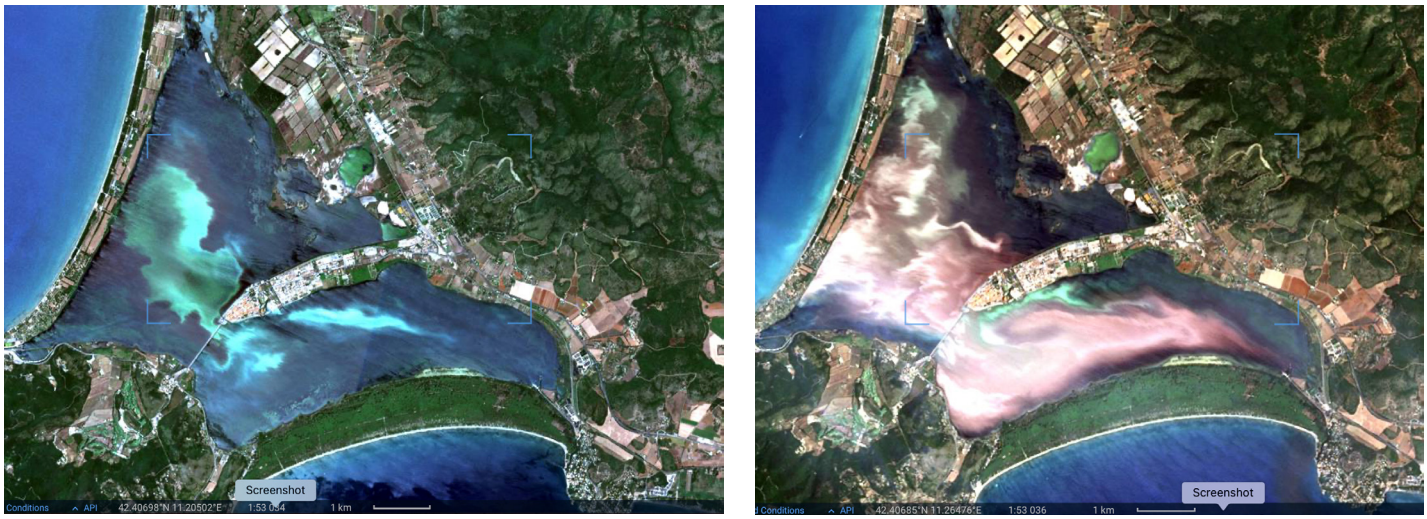

Fig. 5. Sentinel-2 images. Left, July 16, 2024: H2S development and whitewater formation (colloidal sulphur); Right, July 24, 2024, evolution and spread of the phenomenon, with brown debris masses in suspension, and likely development of anaerobic sulphur-oxidising purple bacteria, on the right.

over 24 hours (DO = 0 mg/L; Eh = -400 mV). Temperatures varied between 29°C and 32°C.

The die-off affected all bottom populations including vagile species, and more than 70 tonnes of fish (above all grey mullet, sea-bream and sea-bass) were collected (Fig. 1). Smaller fish were not harvested as at 32°C, they decomposed quickly and sank. This dystrophy has resulted in severe damage to the lagoon fishery and the economy of Orbetello city. Significant damage was caused to the tourist industry due to the discoloration of the lagoon waters that flowed into the sea, preventing bathing along the beaches, and because of a putrid smell, caused first by bacterial growth and then by the decomposition of fish. The high and above all persistent temperature rise, combined with the high load of labile organic matter, is an important cofactor triggering dystrophy.

The abundance of suspended particulate organic matter was estimated at 3.23±0.70 g/L. Considering that for the West Basin alone (avg depth 1 m), the extent of the phenomenon was approximately 930 ha, the suspended organic

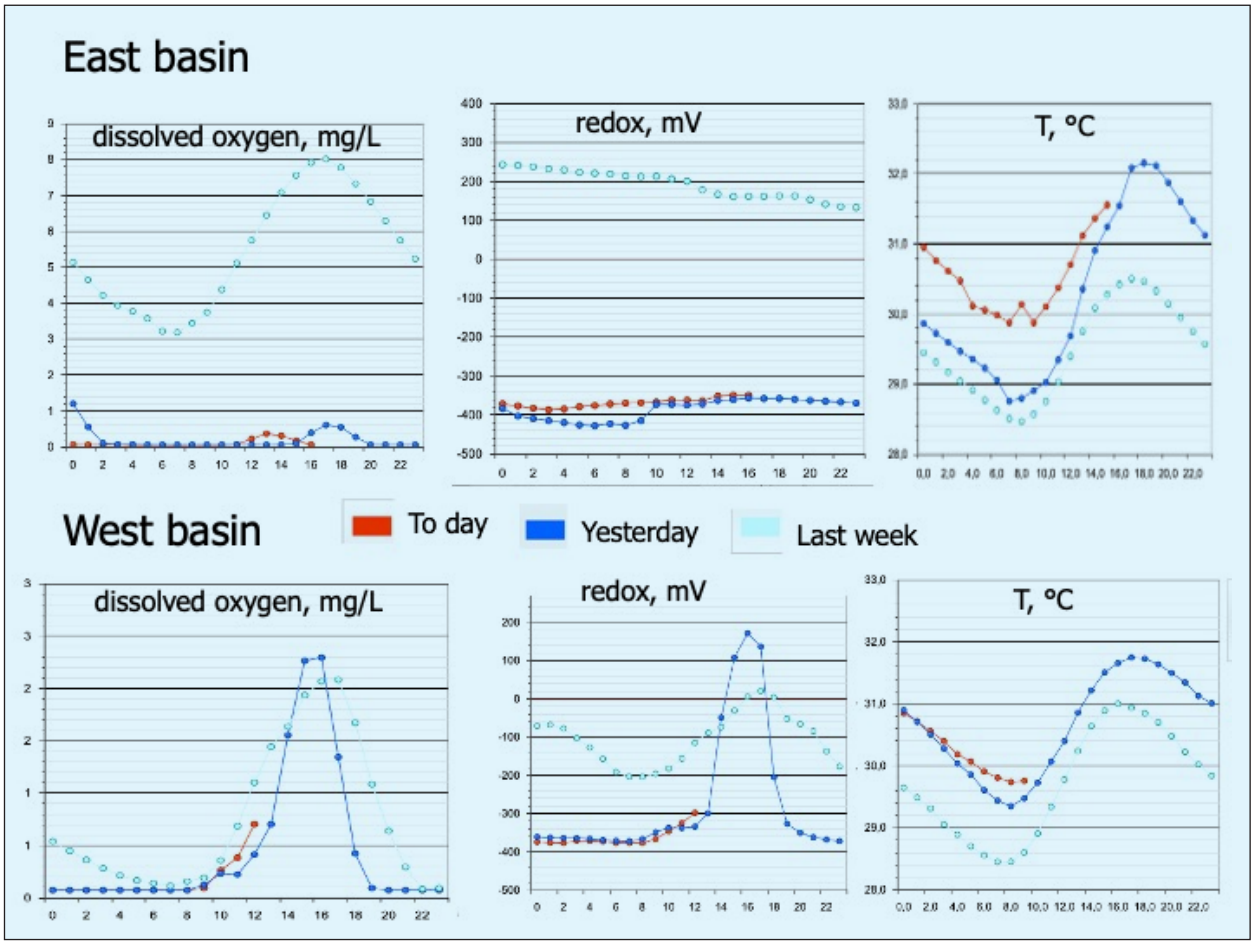

Fig. 6. Dissolved oxygen, redox and temperature values obtained from the multi-parameter probes located in the central areas of the two basins. In light blue, the average values for the week preceding the date of 24 July 2024; in blue, the values for the day preceding that date; in red, the values for 24 July. On abscissas, the hours 0 am to 11 pm; on ordinates, mg/L, mV and °C, for dissolved oxygen, redox and temperature, respectively.



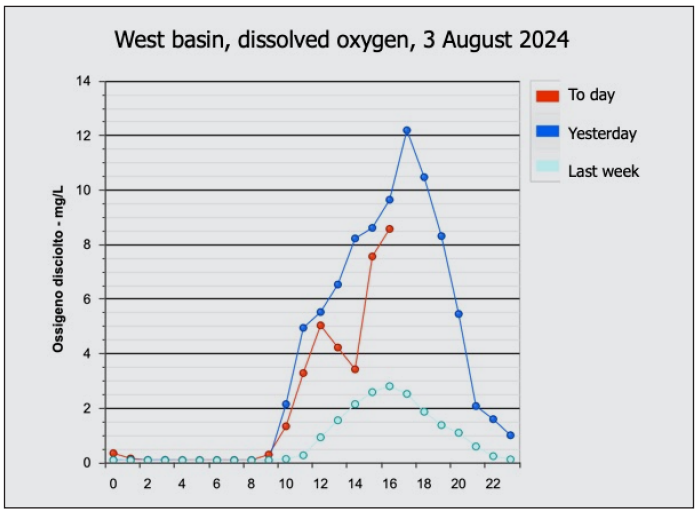

Fig. 7. Left, Sentinel-2 image of 5 August 2024. Evolution of the dystrophic phenomenon that occurred in the Orbetello lagoon, with development of Cyanobacteria in the West basin (green water); Right, dissolved oxygen in the central area of the West basin: high values during the daytime, but reaching zero due to intense respiration during night and early morning hours.

matter could have totalled over 30,000 tonnes. This suggests that a significant quantity of debris, able to trigger new dystrophic phenomena, was still available.

The evolution of dystrophic phenomena can take several pathways, much depending on weather and climate conditions:

Pathway 1: Dry winds from the western quadrant favour faster recovery, as they cool the water more rapidly at night, which reduces bacterial activity and favours oxygen uptake. In this case, the water tends to become very dark due to suspended detritus. which is attacked by aerobic bacteria.

Pathway 2: On the contrary, a humid, sea breeze does not promote night-time heat exchange but encourages intense bacterial activity, prolonging anoxia and promoting new dystrophic phenomena.

Pathway 3: Positive evolution of the phenomenon is provided by growth of Cyanobacteria, which produce oxygen and counteract the consequences of dystrophy; this case developed in the West basin in August 2024 (Fig. 7).

The probable secondary development of purple bacteria that occurred in July 2024 in the Orbetello lagoon has yet to be confirmed; it would be a record for the area on the basis of its extent.

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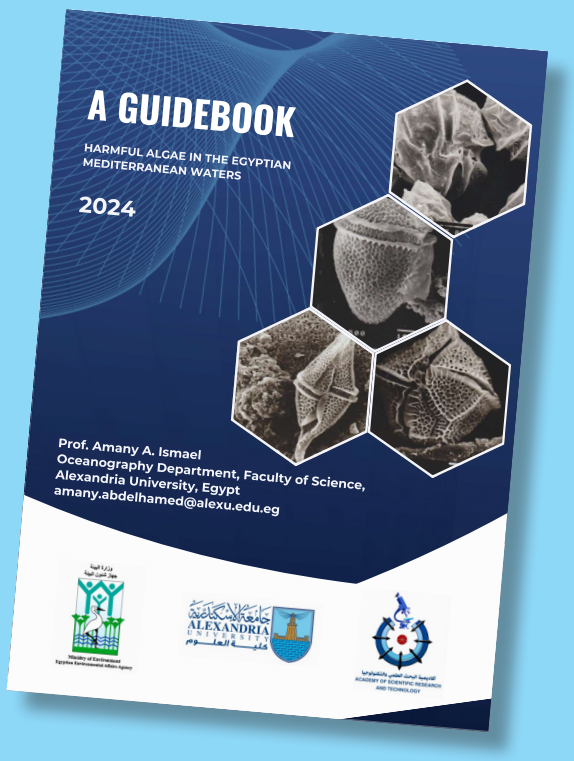

Harmful Algae in the Egyptian Waters

The author of this guidebook, Prof Amany Ismael, is the current Chair of the IOC Regional Group HANA (Harmful Algae in North Africa).

The book can be freely downloaded from the IOC website:
https://oceanexpert.org/document/34750

HARRNESS: An Updated Roadmap for HABs in the US

"*HARRNESS 2024–2034: Harmful Algal Research and Response, A National Environmental Science Strategy*" [1] has been released through the US National Office for Harmful Algal Blooms at the Woods Hole Oceanographic Institution (Woods Hole, Massachusetts, USA). This national strategy builds on major accomplishments from past efforts, provides a state of the science update since the previous decadal HARRNESS plan (2005-2015) [2], identifies key information gaps, and presents forward-thinking solutions to advance research, monitoring, and management of HABs in the US.

Major achievements on many fronts since the last HARRNESS are detailed in this report. They include improved understanding of bloom dynamics of large-scale regional HABs such as those of *Pseudo-nitzschia* on the west coast, *Alexandrium* on the east coast, *Karenia brevis* on the west Florida shelf, and *Microcystis* in Lake Erie. The report highlights advances in HAB sensor technology, allowing deployment on fixed and mobile platforms for long-term, continuous, remote HAB cell and toxin observations. Emerging HABs or impacts are also described.

Many other issues are also highlighted. For example, freshwater HABs now occur in many inland waterways and their public health impacts through drinking and recreational water contamination have been characterized and new monitoring efforts initiated. Furthermore, freshwater HAB toxins are finding their way into marine environments (sometimes termed the "freshwater-to-marine continuum) and contaminating seafood with unknown consequences. After many years with little or no issues with *Dinophysis* and diarrhetic shellfish poisoning (DSP events), blooms have been documented at multiple locations along the US coast, but the causes of this apparent expansion are not understood. Similarly, blooms of fish- and shellfish-killing HABs are occurring in many regions and are especially threatening to aquaculture.

The science, management, and decision-making necessary to manage the threat of US HABs continue to involve a multidisciplinary group of scientists, managers, and agencies at multiple levels. The initial HARRNESS framework (2005-2015) and the National HAB Committee (NHC) that was formed at that time have proven effective means to coordinate the academic, management, and stakeholder communities interested in national HAB issues and provide these entities a collective voice through this updated HARRNESS national strategy.

The US Congress and Executive Branch have supported most of the advances achieved under HARRNESS (2005-2015) and continue to make HABs a national priority. Congress has reauthorized the Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA) multiple times and continues to authorize the National Oceanic and Atmospheric Administration (NOAA) to fund and conduct HAB research and response, has given new roles to the US Environmental Protection Agency (EPA), and required an Interagency Working Group on HABHRCA (IWG HABHRCA). These efforts have been instrumental in coordinating HAB responses by federal and state agencies.

The update process leading to HARRNESS 2024-2034 began with discussions via a webinar and follow-on survey for the US HAB community. A 26-member Scientific Steering Committee (diverse in geographical location, expertise and career stage) was assembled and divided into core focus groups to concentrate writing efforts within major HAB programmatic areas: 1) **Observing systems, modeling, and forecasting;** 2) **Detection and ecological impacts**, including genetics and bloom ecology; 3) **HAB management** including prevention, control, and mitigation, and 4) **Human dimensions**, including public health, socioeconomics, outreach, and education. Focus groups were tasked with addressing: a) our current understanding based on advances since HARRNESS 2005-2015; b) identification of critical information gaps and opportunities; and c) proposed recommendations for the future. Committee members sought input from an additional 35 contributors and reviewers to ensure production of a comprehensive document. Affiliations spanned academia, federal and state agencies, institutions, industry, the nonprofit sector, private industry, and tribal organizations.

The aim of this interdisciplinary effort was to provide summary information that will guide federal, state, local and tribal agencies and nations, researchers, industry, and other organizations over the next decade to collectively work to address HAB problems in the United States.

The vision statement for HARRNESS 2024-2034 has been updated, as follows: ***"Over the next decade, in the context of global climate change projections, HARRNESS will define the magnitude, scope, and diversity of the HAB problem in US marine, brackish and freshwaters; strengthen coordination among agencies, stakeholders, and partners; advance the development of effective research and management solutions; and build resilience to address the broad range of US HAB problems impacting vulnerable communities and ecosystems."***

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Biotic control of harmful algal bloom formation and toxin production (BIOTOX)

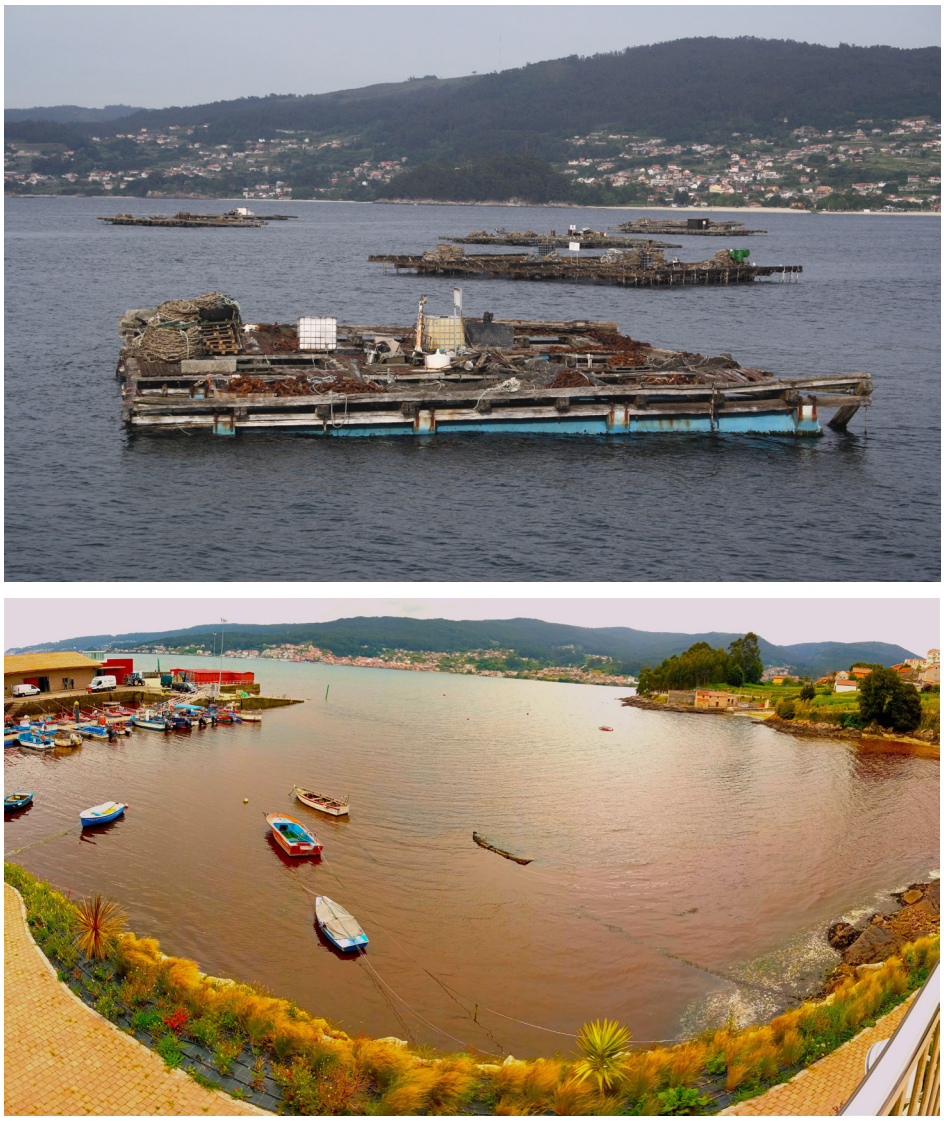

Fig. 1. Top, Mussel rafts in Bueu, the hot spot of DSP events in Galicia; Bottom, Red tide of Alexandrium minutum in Campelo, at the head of the ría. Ría de Pontevedra, nW Spain (Photos by Fran Rodríguez) 2018.

BIOTOX (2022-2026) is a new coordinated project funded by the Spanish Ministry of Science and Innovation. BIOTOX combines expertise in Harmful Algal Blooms and the dinoflagellate culturing capacities of the VGOHAB group (*https://vgohab.com/*) of the Spanish Institute of Oceanography in Vigo with the scientific capacity in microbial plankton interactions and the mesocosm facility of the Biological Oceanography Group (GOB) at the University of Vigo (*http://gobio.webs.uvigo.es/index.php?lang=es*) to explore the role of biotic factors on the development of Harmful Algal Blooms (HABs) in the Galician Rías (NW Spain), using *Alexandrium minutum* as a model system.

HABs may cause significant socioeconomic impacts in the Galician Rías, which support intensive aquaculture and fisheries favoured by the prevalence of coastal upwelling conditions from spring to autumn (Figure 1). As a result, high primary productivity and periodical occurrence of algal blooms, including harmful species, are observed in the Rías. The increasing occurrence of harmful microalgae (and associated shellfish harvesting closures) in the Rías Baixas has been linked with the decreasing extension and intensity of the upwelling season in the NW Iberian Peninsula in recent decades [1]. The initiation, duration and intensity of HABs have been related to different biological, chemical and physical factors, but the relative impact of abiotic and biotic factors is still poorly understood.

BIOTOX aims to assess how biotic interactions of *Alexandrium minutum* with its bacterial microbiome, parasites and angiosperms affect bloom formation and toxin production, and to tackle outstanding problems in marine microbial ecology. Quantification of the impact of biotic interactions on HAB formation and toxicity, and their regulation by environmental conditions, are essential to improve our understanding of HAB dynamics and toxicity. These ambitious goals require an ample range of biological knowledge and advanced methodological approaches.

BIOTOX formulates four hypotheses: 1) Temporal variations in the composition of the bacterial microbiome influence growth and toxin production of *A. minutum*, 2) The impact of the bacterial microbiome on *A. minutum* bloom development and toxin production is mediated by chemical substances promoting algal growth and/or toxin production, 3) Parasitic infection plays an important role during *A. minutum* bloom development, influencing growth, toxicity, life cycle and genetic variability of the host, and as consequence, the size and duration of the bloom and 4) Bioactive substances derived from *Zostera marina* meadows inhibit growth and toxin production of *A. minutum* and are released into the water column, impacting the microbial community.

To test these hypotheses, a working plan combining field sampling and different experimental approaches was defined.

Intensive sampling at a station in the Galician Rías over a two-year period allows characterization of the microbiomes (bacteria and parasites) associated with blooms of *A. minutum.* Combined 16S and 18S rRNA amplicon sequencing and the use of advanced imaging flow cytometry (IFC) (CytoSense®) characterize the pico-, nano- and micro-phytoplankton components of the community.

Exclusion experiments, where bacterial activity is blocked by adding antibiotics, and transplant experiments, where quasi-axenic *A. minutum* is inoculated on natural bacterial communities collected from these proliferations

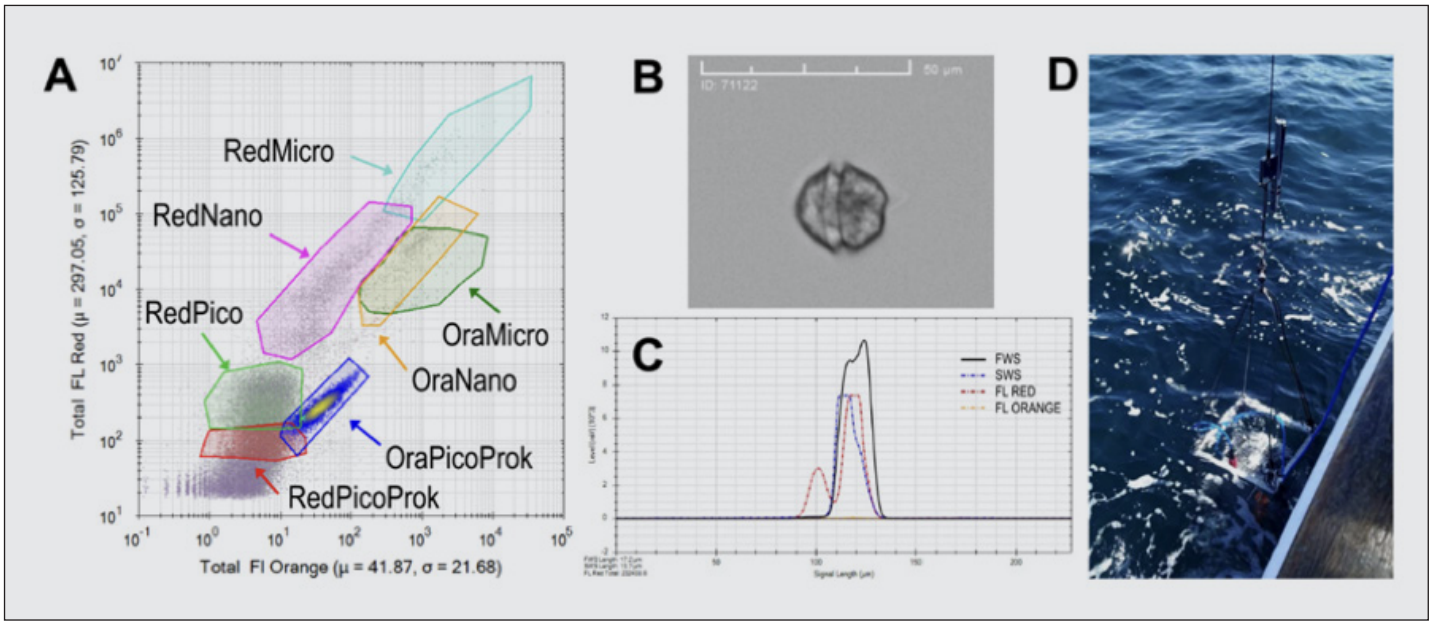

Fig. 2. Several products obtained from imaging flow cytometry (CytoSense®). (A) Cytogram of pico- and nanophytoplankton. Cell concentration data are obtained for RedPicoProk (Prochlorococcus), OraPicoProk (Synecochoccus), RedPico (pico-eukaryotes), RedNano (nano-eukaryotes) and OraNano (nano-cyanobacteria and Cryptomonas) plankton components (as defined in Thyssen et al. 2022); (B) Image of Alexandrium minutum and (C) associated pulse shape; (D) Submersible mode for in situ sampling of the water column.

provide information about the effect of bacterial microbiomes on the dynamics and toxin production of these blooms. As a complementary tool, the analysis of the bacterial metatranscriptomes derived from field and transplant experiments provides insightful information to unveil molecules potentially driving allelopathic interactions; these will be corroborated using simple bioassay experiments.

The effects of parasites on *A. minutum* growth, life cycle transitions and cellular toxin content are being studied by exposure of *A. minutum* cells to different host-parasite ratios; parasitic exudates from these treatments are tested to evaluate their allelopathic effects. The potential link between life cycle and toxin content will be studied using clonal cultures with no capacity of zygote formation and in sexual populations.

Finally, to evaluate if bioactive substances derived from *Z. marina* meadows released into the water column inhibit *A. minutum* growth and toxin production, microcosm experiments with natural exudates of *Zostera marina* and mesocosm experiments including natural *Zostera marina* beds are ongoing.

Preliminary results

- Samples for imaging flow cytometry (IFC) are analyzed a few hours after collection, avoiding the use of fixatives that usually alter the morphological and physiological properties of the cells (Fig. 2). Micro-phytoplankton cell densities at genus or species level are estimated from the supplementary information provided by pulse shapes of each particle and the collection of acquired images (Figure 2B-C). In addition, the equipment can be submerged at different depths to obtain in situ information on the vertical distribution of phytoplankton assemblages and species related to water column structure (Figure 2D).

- Analyses of the bacterial microbiome of *A. minutum* strains isolated from Ría de Vigo showed a dominance of Flavobacteriales and Rhodobacterales. These groups were previously associated with natural dinoflagellate blooms [2] (Fig. 3). Ongoing DNA metabarcoding of samples collected during the progression of a natural bloom in 2023 will soon provide further insights into the microbiome of *A minutum* in the field.

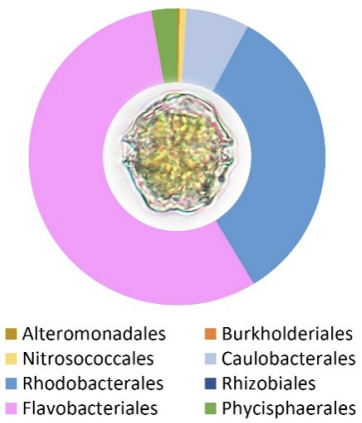

Fig. 3. The composition of the bacterial microbiome of A. minutum strain V1, isolated from Ría de Vigo is dominated by Flavobacteriales (mostly Winogradskyella) and an uncultured Rodhobacteraceae.

- Monitoring data from the fixed sampling location showed that *A. minutum* cell density was directly related to the prevalence of *Parvilucifera sinerae* infections (Fig. 4 A-B), the only dinoflagellate parasite identified at this site. Thirty four clones of *P. sinerae* were isolated to perform infection studies. Infection can be monitored by flow cytometry (Fig. 3C), since it is characterized by a DNA peak to the left of the peak corresponding to the basic DNA content of *A. minutum* (control), whereas peaks to the right of the control indicate dinoflagellate growth. Our preliminary results suggest that at certain parasite:host ratios, *Alexandrium* toxin quota (GTX3 content per cell) may increase in some strains (Fig. 4 D-E) and that this infectious process depends on the host susceptibility.

- Increasing exudate concentrations from unaltered *Z. marina* leaves during 24 h significantly reduced *A. minutum* growth rate even at the

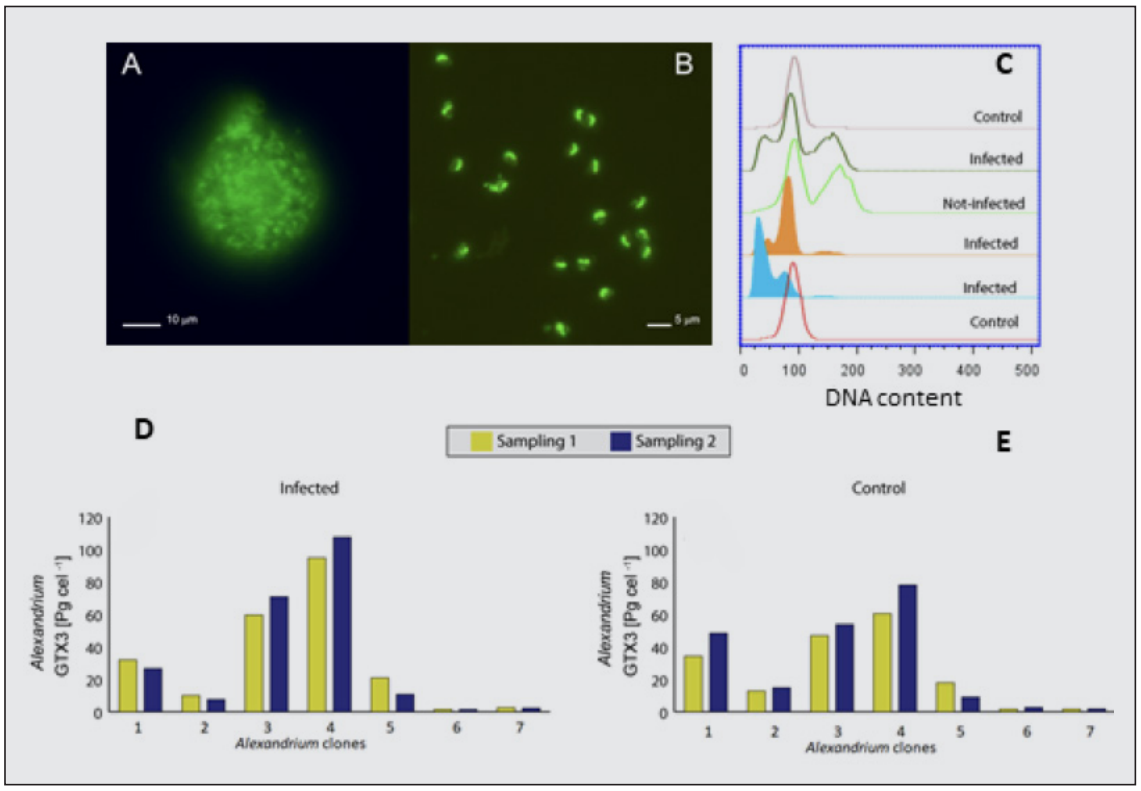

Fig. 4. Parvilucifera infectans Infection stages (A-B) after DNA staining with sytox green. Fully infected cell of A. minutum (A) and its parasite free stage (B). (C) Flow cytometry histogram showing DNA content peaks for controls (uninfected and synchronized dinoflagellate cultures) and for infected and uninfected growing cultures. Toxin content per cell (GTX3) of Alexandrium cells from different clones (1:1, host:parasite ratio) in infected (D) and uninfected (control) cultures (E). Sampling 1 and 2 indicate replicate experiments.

lowest concentration in preliminary microcosm experiments (Fig. 5). The highest concentration tested prevented any increase in dinoflagellate numbers throughout the experiment. Parallel cultures treated with antibiotics led to the conclusion that the growth rate decline was a direct effect of substances released by *Z. marina* or its attached microbiome and not an indirect effect mediated by the stimulation of coexisting bacteria [3]. New experiments have been designed: i) to assess whether the observed inhibitory effect varies seasonally and with the tide level , and ii) to elucidate whether *Zostera marina*'s microbiome has a role on the negative interactions between seagrass meadows and dinoflagellates. Further stage investigation plans include: 1) identification of the bioactive molecules potentially responsible for the dinoflagellate response and 2) In depth assessment of the morphological and metabolic changes on *A. minutum* cells induced by the allelopathic compounds

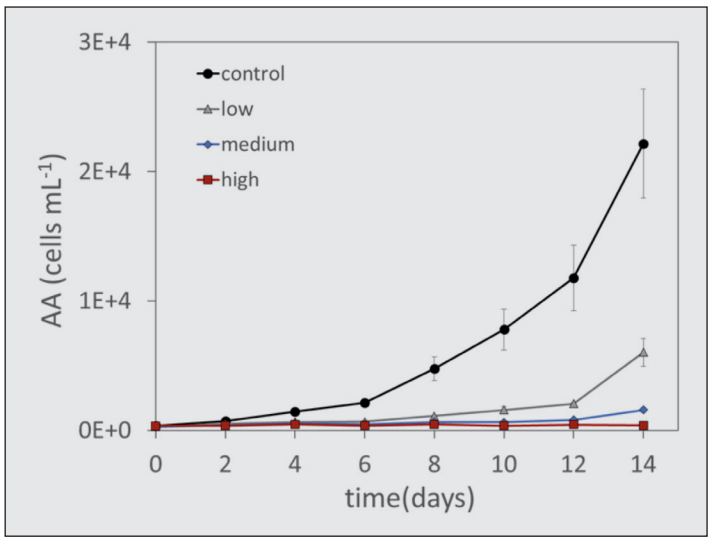

Fig. 5. Changes in abundance of A. minutum exposed to different concentrations (0, low, medium and high) of the substances released by unaltered Z. marina leaves during a 24 h incubation.

Acknowledgements

We thank all the people involved in the project BIOTOX for help with sampling and analytical work. We thank the crew of R/V Mytilus and R/V Kraken for their assistance during the work at sea. This research is supported by a grant from the Spanish Ministry Science and Innovation through the PID2021-125643OB-C21 and PID2021-125643OB-C22 contracts.

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Introducing Citizens of the Sea!

We are officially launching this new charity in May this year to harness the range and capability of offshore cruisers and ocean racers to collect ocean biodiversity data at scale. Citizens of the Sea (Fig. 1) will be equipping fleets of vessels to collect eDNA, photogrammetric and environmental data as they race across oceans and cruise between Pacific Islands in the coming months.

One key innovation is the ***TorpeDNA*** (Fig. 2), a ground-breaking plankton net device designed to collect environmental DNA (eDNA) from sea surface water samples while towing the device behind a boat for only 5 minutes. Its innovative features include affordability, compact size, and enhanced usability, facilitated by a streamlined torpedo design that enables operation at up to 12 knots speed. The simple protocol avoids contamination and allows users to isolate eDNA samples rapidly and with minimal hands-on time. The samples are then sent to the Cawthron Institute for swift metabarcoding analyses of bacteria, plants and animals detected in the samples. With enough spatio-temporal coverage, this approach can help measure ocean health, detect rare or endangered species, and more effectively monitor HABs and other pathogens.

With climate change threatening marine ecosystems, monitoring biodiversity and the spread of harmful algae is an urgent and important task. In collaboration with sailors, scientists, and local communities we can revolutionise how data is collected in the ocean, to benefit marine management, biosecurity, and climate science.

Learn more at: *https://www.citizensofthesea.org*

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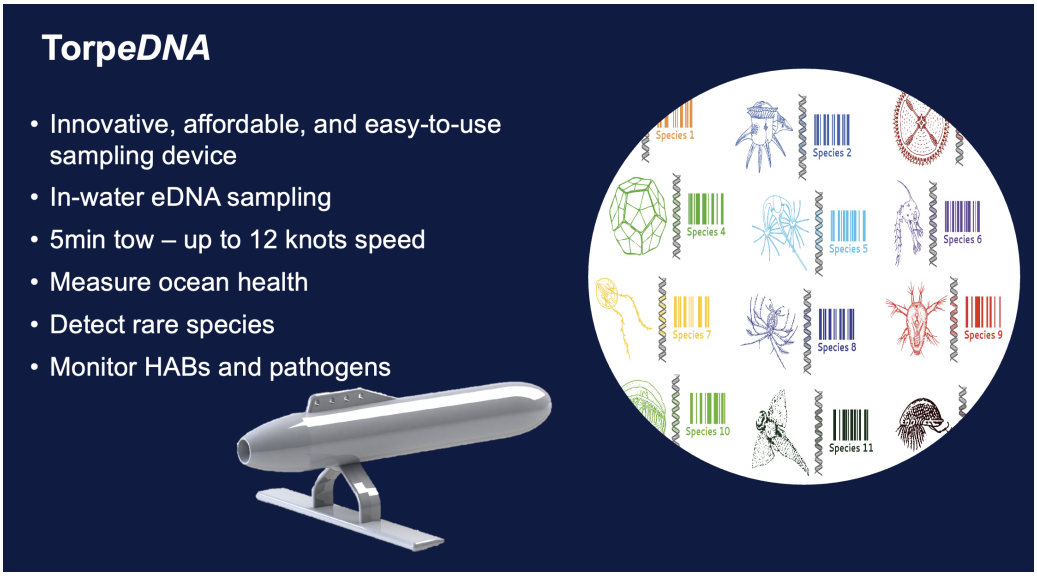
Fig. 1. Citizens of the Sea, launch May 2024.



Insights from an ad hoc International Workshop on Dinoflagellate Cysts in Vigo (Spain)

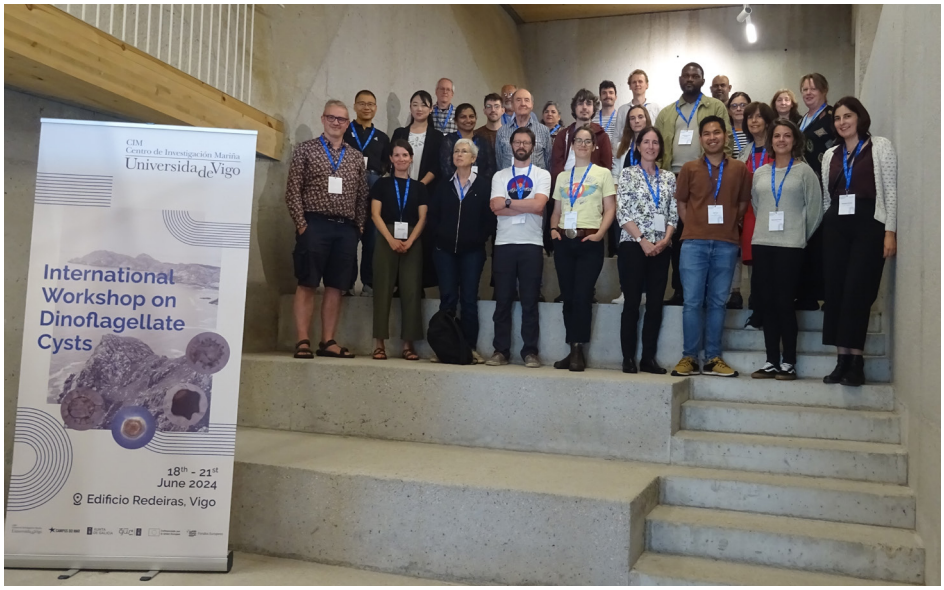


Fig. 1. Participants in the International WS on Dinoflagellate cysts by the hall of the Redeiras building in Vigo, Spain

Between June 18th and 21st, 2024, an International Workshop on Dinoflagellate Cysts was held in Vigo (Galicia, Spain). This event, hosted by the Marine Research Centre (University of Vigo) brought together 27 scientists from 13 countries, including Belgium, Cameroons, Canada, China, France, Germany, Greece, Mexico, Norway, Portugal, Spain, UK, and USA (Fig. 1).

Participants were primarily biologists and geologists with specialized training in various fields such as microscopy, genetics, biogeochemistry, computer science, taxonomy, and more, but they all shared a common denominator on using **dinoflagellate cysts as a research tool**. The major goals of the workshop were to facilitate discussions on cutting-edge research, improve methodological approaches, and explore new collaborative opportunities related to the field of dinoflagellate cyst research.

Cysts are resistant stages in the life-cycles of many dinoflagellates which among other functions are involved in the survival and dispersal of the species. Some taxa are potentially toxic harmful algal bloom (HAB) forming species, such as *Alexandrium* spp., *Gymnodinium catenatum*, *Gonyaulax spinifera* complex, *Lingulaulax polyedra* (syn. *Lingulodinium polyedra*), *Pyrodinium bahamense* and *Protoceratium reticulatum* (Fig. 2). After being produced in the upper water column, their cysts sink to the ocean floor where some species can remain viable for more than a hundred years.

Dinoflagellate cysts have great geological preservation potential and have left fossil records from earlier times. This gives us the opportunity to study this group of organisms from both perspectives: biology and geology. In the workshop, the participants shared their latest findings and techniques and discussed cyst applications in various disciplines, such as modern and past (late Quaternary) biology and ecology. **Barrie Dale**, Emeritus Professor at the University of Oslo (Norway) highlighted the importance of integrating both perspectives (living and geological) to obtain a more comprehensive understanding of dinoflagellate ecology and their response to environmental changes.

The four-day workshop was held in the Redeiras building, a traditional fishermen's house recently restored for scientific and cultural events. It is located in O Berbés, an emblematic neighbourhood in the historic centre of Vigo , and offers beautiful views of the ría. The setting chosen for this event was very pertinent, since Ría de Vigo is known for its high primary productivity and marine biodiversity. This coastal inlet, site of the largest fishing port in Europe, has been a major source of food s for the local population and, today, along with the other Galician rias, is a leading area of world importance for aquaculture and seafood production. Red tides

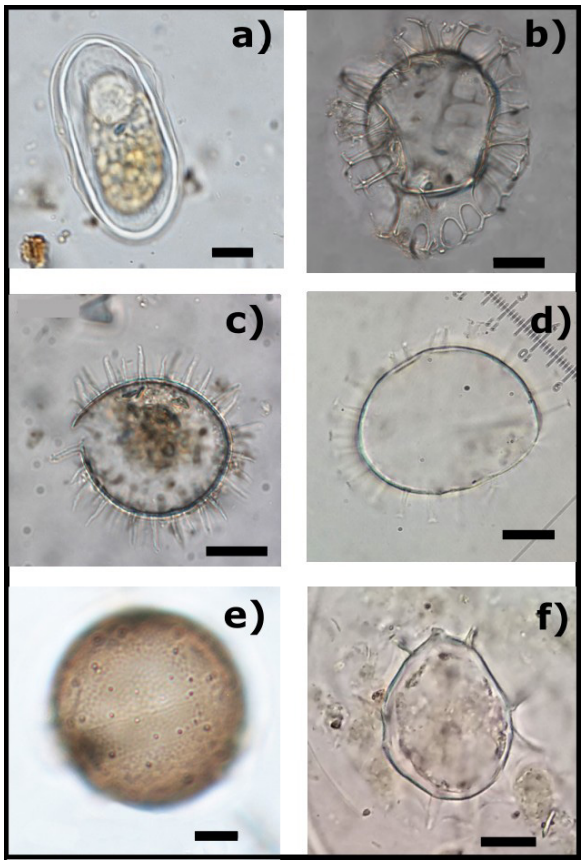


Fig. 2. Cysts of potentially toxic HAB-forming dinoflagellates a) Cyst of Alexandrium sp. from the Chukchi Sea (taken by Vera Pospelova); b) Cyst of Gonyaulax spinifera complex (cyst equivalent Spiniferites mirabilis) from sediments off Portugal [1]; c) Cyst of Lingulaulax polyedra (cyst equivalent Lingulodinium machaerophorum) from sediment off Portugal; d) Cyst of Protoceratium reticulatum (cyst equivalent Operculodinium centrocarpum sensu [2] from sediments off Cape Blanc, NW Africa ([3]; e) Cyst of Gymnodinium catenatum from sediments of the Ría de Vigo, NW Iberia [4]; f) Cyst of Gonyaulax sp. (cyst equivalent Impagidinium aculeatum) from water samples off Cape Blanc NW Africa [3] Scale bars = 10 µm.



Fig. 3. Daniel Rey García, Director of the host institution (CIM), greets the participants.

and HABs caused by dinoflagellates and other microalgae have been a recurrent phenomenon in the rias for many years, and are the focus of extensive research, including studies conducted by **Iria García-Moreiras** (CIM–University of Vigo, Spain), and by María Portela and Beatriz Reguera (VGOHAB Group, IEO-CSIC), who actively participated in the workshop discussions.

Daniel Rey García, director of the hosting institution (CIM), welcomed the participants and stated that the event provided an excellent platform for scientists to share their knowledge, experiences, and perspectives (Fig. 3). He highlighted the importance of continued research on dinoflagellate cysts to better understand marine ecosystems and climate change. The workshop started with an icebreaking session where participants introduced themselves and their research directions. The program included interactive oral presentations, hands-on laboratory sessions and a field trip. A total of 18 oral presentations were followed by enthusiastic discussions on various aspects of dinoflagellate cysts. These included the use of AI in palynology, palaeoecological signals and preservation of cyst assemblages, cyst taxonomy and extraction techniques, genetic and organic-geochemical approaches, and their application in studies of past cyst distributions and ecology (late Quaternary studies), as well as in modern biology and ecology (including bloom dynamics and the detection of potentially toxic species).

Robert Williams (Norwegian Offshore Directorate, Norway) underlined that high-resolution scanners and image processing techniques, which are already commonly used in pathology for the diagnosis of diseases, have the potential to be used for digitalizing microscope slides and automating the analysis of dinoflagellate cyst assemblages. Moreover, he explained how machine learning and AI techniques can assist in interpreting dinoflagellate cyst assemblages and speed up the identification and counting process. Whilst these techniques currently require a high financial investment and are still under development for marine palynology, the participants agreed on their promising future applications in the field of cyst analysis.

Highlights of interest to the HAB community included the talk by **Vincy Winifred** from the University of Minnesota (US) who, together with her collaborators, observed high numbers of *Alexandrium catenella* cysts in palynologically processed surface samples from the Bering Sea. **Vincent Iratçabal**, from the University of Southern Brittany (France), presented the first results of his research team on dinoflagellate cyst mapping in a coastal area commonly affected by toxic events produced by cyst-forming dinoflagellates, which leave a record in the sediments. **Ana Amorim** (University of Lisbon, Portugal) and **Beatriz Reguera** (IEO-CSIC, Spain) promoted a line of discussion on the need for more dinoflagellate cyst studies to broaden our understanding of their distributions, abundances and diversity and improve our understanding of the HAB dynamics produced by cyst-forming species.

Haifeng Gu, from the Third Institute of Oceanography-MNR (China), presented their latest results on diversity estimates based on metabarcoding data from cysts extracted from surface sediments, and showed that genetics can help to study the biogeography of toxic species, particularly those whose cysts are difficult to identify microscopically (Fig. 4). **Girish Beedessee**, from Northumbria University (UK), showed how high-throughput omics technologies are bringing new insights into the understanding of the functioning of dinoflagellate genomes, and presented a



Fig. 4. Haifeng Gu presented the latest research of his team on metabarcoding in dinoflagellate cysts to study dinoflagellate diversity and harmful algal bloom (HAB) dynamics.



Fig. 5. Pjotr Meyvisch explained what fossilizable biomolecules are and their relevance in the interpretation of fossil dinoflagellate cyst records.

new technique being developed which may help understand the genome biology and secondary metabolism of dinoflagellates and their response to environmental stresses. **Jan Janouskovec**, from the University of Southampton (UK), remarked that interdisciplinary collaboration is important to resolve the phylogenetic tree of dinoflagellates (Fig. 5). The use of multigene phylogenies is helping resolve dinoflagellate relationships but there is still the need for more data on groups well represented in the fossil records and from extant families of uncertain affinity. **Pjotr Meyvisch** (Ghent University, Belgium) gave an insightful talk on the chemical composition of the cyst wall (studied by infrared spectroscopy) and how it supports the assessment of the ecology, preservation and fossil history of dinoflagellate cysts (Fig. 5).

During the session on palaeoceanography, we learnt more about the detection of (palaeo)environmental signals from dinoflagellate cyst assemblages. For instance, **Anne de Vernal** (Geotop–University of Quebec in Montreal, Canada) proposed *Nematosphaeropsis labyrinthus* as a new tracer of the upper mixed layer depth. **Amy Dale** (GeoResearch Consulting, Norway) and **Eldo Roza** (MARUM–University of Bremen, Germany), showed their sediment trap research and explained how such studies provide valuable insights into the production, transport, preservation, and accumulation of cysts in offshore marine environments, which can help to interpret the sediment cyst signals and obtain more accurate palaeoenvironmental reconstructions from sedimentary records. Several researchers presented their latest reconstructions of past marine environments from down-core cyst records. These included **Fang Gu**, affiliated to the University of Göttingen (Germany) and the University of Geoscience (China), who presented a reconstruction of the last 16 thousand years in the Brazil-Malvinas Confluence, and **Eugenia Fatourou**, from the National and Kapodistrian University of Athens (Greece), of the last 1.1 million years in the Gulf of Corinth (Northeast Mediterranean). Furthermore, we learned from the presentation by **Karin Zonneveld** (MARUM–University of Bremen, Germany) how dinoflagellate cyst assemblages from coastal sediments enable reconstructions of past climate changes and their relationship to the early pandemics in Europe during Roman times. Finally, **Javier Helenes**, from the CICESE Institute in Mexico, remarked that comparing older fossil cyst records with more recent ones is very useful for the interpretation of past environmental signals from sediment cyst assemblages and can help evaluate future scenarios.

During the microscope session at the Botany Laboratory on the University de Vigo Campus (Fig. 6), participants compared dinoflagellate cyst morphotypes from different regions of the world. Various aspects related to sample storage, laboratory processing, identification, and cyst nomenclature were also covered in the laboratory and discussion sessions. **Vera Pospelova** (University of Minnesota, US) and **Kenneth Mertens** (IFREMER, Concarneau, France) led the discussions and made detailed presentations on cyst extraction techniques and new developments regarding cyst taxonomy and nomenclature.

A field trip was organised to the Cies Islands, a unique natural area and part of the Atlantic Islands National Park (UNESCO natural heritage candidate). During a tguided tour of the central is-



Fig. 6. The workshop participants during the laboratory session in the Botany Laboratory on the University of Vigo campus.



Fig. 7. Participants in the tour pose by the "Pedra da Campá" (Bell Stone) in the Cíes Islands National Park.

land (Isla del Faro) (Fig. 7), participants learned about the biodiversity and ecological importance of the National Park, the geomorphology of the area, the dynamics of seabirds and other local fauna, and ongoing projects to remove invasive species. Participants were impressed by the stunning views, unique geological formations, and the high number of breeding yellow-legged gulls with their newborn chicks.

The final round table provided a summary of the key topics discussed during the previous days. It also aimed to identify needs and challenges, and to outline future strategies. B. Reguera pointed to the fact that palynologists are applying a dichotomous classification of cyst formers into autotrophs and heterotrophs, often ignoring the capacity of mixotrophic behaviour in most dinoflagellates [5]. The word "mixotrophy" appeared in the title of only one presentation. Several scientists emphasized the importance of merging data from various fields to enhance biogeographic studies and gain a better understanding of the variability in past and present dinoflagellate communities.

Regarding future directions, it was suggested that standard protocols for sample collection, processing and data analysis should be established to ensure data comparability across different regions. Additionally, there is a need to reach a consensus on cyst taxonomy and nomenclature. It was also highlighted that the development of molecular techniques, such as metabarcoding, is a priority for future research to complement traditional morphology-based identification methods in present-day and past studies on dinoflagellate distribution and ecology. An effort should also be made to implement the use of sediment traps in cyst dynamics' studies, which will immensely help interpret paleoecological signals. Given that cyst-forming dinoflagellate are extremely sensitive to environmental changes, excystment and encystment dynamics provide valuable tools to monitor the effects of climate and anthropogenically induced changes in marine ecosystems.

Workshop organisers (Fig. 8) and participants wrote a "white paper" that summarizes the current state of research in dinoflagellate cysts and outlines future directions. Hopefully, this workshop and its resulting publications will demonstrate the importance of dinoflagellate cysts as tools in the study of marine biodiversity, biogeography, HABs, and palaeoceanography, in addition to their use for evolutionary studies and phylogenetics.



Fig. 8. Members of the international scientific committee. From left to right: Vera Pospelova, Ana Amorim, Iria García-Moreiras (local organiser), Kenneth Mertens and Karin Zonneveld.

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Harmful Algal Bloom Impacts on Sustainable Ocean Foods Highlighted at United Nations Event



Fig. 1. The UN Division for Ocean Affairs and the Law of the Sea supports the implementation of UN Sustainable Development Goal 14 to conserve and sustainably use the oceans, seas and marine resources for sustainable development

Harmful algal blooms were a key discussion topic at the 24th meeting of the United Nations Informal Consultative Process on Oceans and the Law of the Sea (*ICP-24*) in New York, 17-19 June 2024. The three-day event, led by the *UN Division for Ocean Affairs and the Law of the Sea* (Fig. 1) featured individual presentations and panel discussions with UN delegates addressing the ocean as a source of sustainable food. ICP-24 emphasized sustainable ocean foods as an area where coordination and cooperation at the intergovernmental and inter-agency levels should be enhanced, and recognized the impacts of harmful algal blooms (HABs) on the safety and security of sustainable ocean foods.

During this event, Philipp Hess (PHYTOX Director, *IFREMER*) discussed the biological and chemical diversity of HABs in a changing climate and related risks to food safety and security; Maggie Broadwater (*ECOHAB* Program Manager, NOAA/NCCOS) discussed the impacts of HABs on the environment, societies and economies; and Don Anderson (Senior Scientist, *WHOI*) shared technologies and approaches to HAB early warning, mitigation, and control, with a focus on NCCOS-funded research on *Alexandrium catenella* blooms in Alaska and control technologies for *Karenia brevis* in Florida (Fig. 2).

The *HAB Solutions Programme*, recently endorsed by the UN Decade of Ocean Science for Sustainable Development, was highlighted during the presentations. This program is led by the Intergovernmental Panel on HABs (*IPHAB*), and will augment existing international coordination efforts through the IPHAB, GlobalHAB and IOC Regional Working Groups, and bolster cooperative efforts to reduce the adverse impacts of HABs on sustainable ocean foods. *HAB Solutions* aims to enhance knowledge and understanding of the causes and effects of HABs at the level of scientists, societal stakeholders, and the public at large, through comprehensive public awareness campaigns, education initiatives, and interdisciplinary solutions that reduce the frequency and severity of HAB events and minimize their adverse effects on the social, economic and natural environments.

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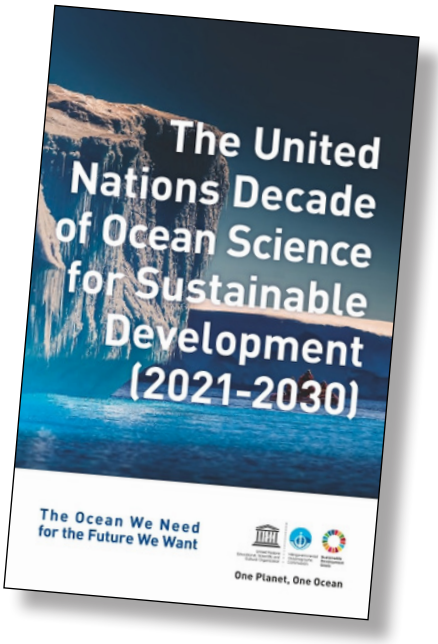



Fig. 2. Philipp Hess, Maggie Broadwater, and Don Anderson at the UN ICP-24 meeting. Philipp and Maggie currently serve as the Chair and Vice Chair, respectively, of the IOC Intergovernmental Panel on HABs (IPHAB), and are working to advance the IPHAB's HAB Solutions Programme

Upcoming events

HAB Session at the International Aquaculture Society Meeting – Aquaculture 2025

A Harmful Algal Blooms sesión will be organized at the **Aquaculture 2025** meeting, International Aquaculture Society, in New Orleans from March 6-10, 2025 – *https://www.was.org/Meeting/code/AQ2025*

The abstract submission deadline is September 30.

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7th XMAS

2025 Xiamen Symposium on Marine Environmental Sciences

January 14-17, 2025

HAB session at Xiamen Simposium on Marine Environmental Sciences (XMAS) 2025

The Xiamen Symposium on Marine Environmental Sciences (XMAS) was first initiated by State Key Laboratory of Marine Environmental Science, Xiamen University (MEL) in 2014 to promote interdisciplinary studies in marine environmental science. Since then, it has grown to become one of the largest conferences in marine science in Asia.

The upcoming XMAS 2025 will take place in Xiamen, China, from January 14-17, 2025. During this event, a session on Harmful Algal Blooms will be hosted: ***Alleviating the Impact of Emerging Harmful Algal Blooms (HABs) to Coastal Ecosystems and Seafood Safety for a Sustainable and Healthy Ocean***

Conveners: Po Teen Lim, Haifeng Gu, Xiaolong Yu, Chui Pin Leaw

This session welcomes studies on emerging HAB events, including research on microalgae and macrophytes harmful events in the Asian Pacific region and beyond. Topics of interest include:

1. Dynamics of HABs, from molecular to ecological aspects, including transboundary events.
2. Knowledge gaps on the physiological and molecular responses of HAB species from tropical to temperate regions.
3. Early warning systems of HABs using novel technologies.
4. Development of monitoring (automated and in-situ) and mitigation (modified clays and other) technologies to minimize the impacts of HABs to mariculture.
5. Trends and emerging HAB events (species, occurrence, and frequency) of regional importance under changing climate conditions.

Presentations related to international collaborations and joint research efforts in addressing the expanding HAB issues in the region are also welcomed.

Details of the session can be found on the XMAS 2025 website: *https://xmas.aconf.org/all_session_proposal*

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ISSHA 21st International Conference on Harmful Algae (ICHA)

The 21st edition of ICHA will be held in Punta Arenas (Magallanes, Chile) from October 19 to 24, 2025

More information
https://issha.org/icha-conference/ and *https://icha2025.org/*

Important dates
Registration Opens: January 13th 2025
Early Bird ends: May 16th 2025
Late Registration ends: Sept. 19th 2025

Abstract submission
Opens January 20th 2025
Deadline: June 1st 2025

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November 1, 2024

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