

# Guidelines for the study of climate change effects on HABs



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# INTRODUCTION

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## **Best Practice Guidelines for the Study of HABs and Climate Change**

Our planet Earth is changing. Marine and freshwater ecosystems are experiencing intense natural and anthropogenic pressures that will generate unforeseen changes in their structure and functioning. The drivers of climate change have already altered the dynamics and interactions of the biotic and abiotic components in these ecosystems, and these changes are anticipated to accelerate in the future. Embedded within natural aquatic ecosystems are Harmful Algal Blooms (HABs) that are noxious to aquatic organisms as well as human health and wellbeing.

There is concern that climate-driven changes will exacerbate HABs at a time when humans are increasingly reliant on aquatic systems for food and drinking water, livelihoods, mariculture and recreational resources. But there are many unknowns. What trends are evident in HAB distribution, frequency and severity? Might the drivers of climate change alter ecological outcomes to promote HABs? How might HABs and other planktonic species adapt to a changing environment? And, how can we prevent or mitigate future HABs impacts? These are only some of the important questions for which the scientific community should seek answers.

The need to support this process forms the basis of the GlobalHAB Programme, launched by IOC UNESCO and SCOR, with the aim of promoting international and multidisciplinary coordination of the research on HABs ([www.globalhab.info](http://www.globalhab.info)).

HAB science today is founded on studies dealing with a great diversity of topics and harmful organisms, using a variety of continuously evolving experimental methods and approaches. The rich insights obtained to date have been key to supporting research on the potential impacts of climate change on HABs. But more quantitative intercomparisons are now needed amongst studies as well as global comparisons of generated data, which is hampered by the diversity of methods and approaches that have brought us so far.

The challenge to achieving greater harmonization of our experimental and observational practices is substantial, although it is acknowledged that this is not necessarily the case in all situations. The major aim of these guidelines is to communicate standardized strategies, tools, and protocols to assist researchers studying how climate change drivers may increase or decrease future HAB prevalence in aquatic ecosystems.

These guidelines represent a first step that will help inform HAB scientists, students, and researchers entering the field, as well as scientists seeking to incorporate HAB studies into existing and developing ocean and freshwater observing systems.

The idea of a guide towards more uniform research practices and strategies for the study of how climate change drivers may affect HABs in aquatic systems stemmed from a March, 2013 workshop in Friday Harbor, USA and was reaffirmed at the Harmful Algal Blooms and Climate

Change Scientific Symposium, May, 2015, in Göteborg, Sweden. The proposal then was encouraged by the broader international community at the International Conference on Harmful Algae held in Florianopolis, Brazil in October 2016, and GlobalHAB subsequently endorsed the initiative. GlobalHAB thanks the commitment of the Editorial Board in driving this initiative since it started in 2018. We also thank all participants in the development of the diverse chapters and all the reviewers whose suggestions improved the final version of the document.

Being aware that science on HABs is a continuously evolving process, we hope that this document will be enriched with new contributions in the future.

December 2021

*The GlobalHAB Scientific Steering Committee*

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# CHAPTER 1

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## Guidelines for the study of climate change effects on HABs: Introduction and Rationale

**Mark L. Wells**<sup>1</sup>, **Michele Burford**<sup>2</sup>, **Anke Kremp**<sup>3</sup>, **Marina Montresor**<sup>4</sup>, **Grant C. Pitcher**<sup>5</sup>

<sup>1</sup> School of Marine Sciences, University of Maine, Orono, Maine USA; State Key Laboratory of Satellite Ocean Environment Dynamics (Second Institute of Oceanography, SOA), Hangzhou, China

<sup>2</sup> Australian Rivers Institute, Griffith University, Nathan, Queensland, Australia

<sup>3</sup> Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany

<sup>4</sup> Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Napoli, Italy

<sup>5</sup> Department of Forestry, Fisheries and the Environment, Cape Town, South Africa

Harmful Algal Blooms (HABs) span tropical to polar environments in both marine and freshwaters, with contemporary records suggesting that HAB impacts have become more prevalent over the past few decades (Hallegraeff 1993, Smayda 2002, Glibert et al. 2018). HABs generally fall into two broad categories. One category comprises a comparatively small set of phytoplankton species that produce potent toxins or chemical compounds that negatively affect humans or other species (e.g., Berdalet et al. 2017a, b, Pitcher et al. 2017, Glibert et al. 2018). The second category encompasses high biomass production by a broad range of mostly non-toxic phytoplankton species that lead to ecological disruption in surface waters, hypoxia and anoxia in subsurface waters, or both (e.g., Glibert and Burford 2017, Pitcher and Jacinto 2019). These high-biomass blooms most often, but not always, can be attributed to anthropogenically-enhanced nutrient input. High biomass HABs are a very active and important area of research, and forecasting these events requires understanding the nutrient flux into coastal and freshwaters relative to dilution by circulation and mixing. Toxic HABs, on the other hand, not only can generate major ecological disruption, but also can severely impact human health and aquaculture activities, leading to a heightened interest in understanding their drivers. However, despite many decades of research, our forecasting skills for toxic algal blooms still are rudimentary. Even so, evidence-derived conjecture about “HAB favourable” conditions combined with the accelerated progression of climate drivers has generated concern that HAB frequencies and distribution may increase in the future. But direct, systematic evidence of a HAB-climate relationship so far is lacking (e.g., see Hallegraeff et al. 2021). A more coherent research strategy is needed to obtain the mechanistic understanding of potential HAB/climate linkages (Hallegraeff 2010, Wells et al. 2015, 2020). This knowledge will support more realistic forecasting for HABs in the future ocean, and help to foster mitigation strategies that minimize these potentially increasing impacts.

The central challenge to improving our practical knowledge of climate effects on marine and freshwater HABs is understanding the mechanisms of species’ proliferation and interaction with their biotic and abiotic environment. What physiological or ecological traits enable toxic bloom forming phytoplankton species to sporadically flourish in waters that appear indistinguishable from other times when they are largely absent? Moreover, how might climate change encroach upon and alter these interactions in ways that might favour, or suppress, toxic HAB species within the planktonic and benthic assemblages? It is the complexity of this matrix of environmental, physiological, and ecologi-

cal factors that HAB research has focused on over the past decades. But progress has been frustratingly slow. In the face of accelerating climate change, it is time that we re-evaluate the approach to HAB research.

Of the many important questions, it can be argued that three are central:

### **1. What changes are occurring in the geographic distribution, character, frequency and intensity of HABs in the changing aquatic environments?**

The linkages between climate and/or eutrophication and the perceived increase in marine HABs have recently been studied quantitatively (Hallegraeff et al. 2021). While observational datasets in some regions indicate temporal trends of increasing HABs, other regions show HABs to be decreasing. However, these findings are limited by several factors that constrain interpretations, including variations in sampling efforts and limitations of existing long-term observational programs (Hallegraeff et al. 2021). Intensified monitoring efforts associated with increased aquaculture production, and the regional emergence of new HAB syndromes or impacts are considered responsible for the global increase in the number of HAB events (Hallegraeff et al. 2021). The study of freshwater HABs faces similar problems in isolating climatic effects from those attributable to the expansion of local stressors. Datasets are too few, are not spatially distributed widely enough, and HAB species reports often occur only when freshwater or seafood becomes sufficiently toxic to generate human health hazards or cause economic damage. More problematic, the environmental conditions before and during bloom initiation are almost never recorded, and few observation sites collect information on the plankton communities from which toxic species emerge. Most researchers then are left to only speculate about the causes and timing of HAB outbreaks. To capture the nuances that regulate HAB development we must move beyond the societal-based definition and detection strategy of “HABs”— identifying blooms that are sufficiently toxic to negatively affect human or ecosystem health — to one that recognizes them as the special subset of ecological episodes within diverse communities, comprising pre-bloom selection, bloom initiation, cell accumulation, and bloom termination.

### **2. How do HAB species respond to climate drivers through acclimation or adaptation?**

The ability of HAB species, relative to other phytoplankton, to acclimate or adapt to accelerating environmental and ecological changes will shape their future trajectories of enhancement or suppression. The study of acclimation and adaptation has long relied on manipulating conditions in culture studies (e.g., Kremp et al. 2012, Tatters et al. 2018), but changes may take prolonged periods to occur, limiting the number and depth of these investigations. Acclimation, namely those responses arising from nuanced changes in expression of the existing genome (West-Eberhard 2003), is more easily investigated than adaptation given the comparative species response times (weeks vs. years to decades). But new genomic approaches are beginning to reveal greater insights (e.g., Schaum et al. 2018). Although many current studies are moving beyond considering only single drivers, the interplay of strengths and weaknesses in experimental strategies has resulted in few collective answers to some basic questions. For instance, what are the optimal and tolerance windows of HAB species to multiple drivers relative to other members of the phytoplankton community, and how does the rate of environmental/ecological change foster or limit the capacity for selection of specific phenotypes/genotypes?

### **3. How may climate drivers alter future toxic HAB effects on human health, ecosystems, economics, and food and water security?**

There are numerous studies of HAB effects on human health, on ecosystems, and on food and water security, but far fewer on economic effects (see Trainer 2020) and only recently has there been a shift



towards considering how the combination of climate drivers may modulate the collective imprint that future HABs leave on human and environmental wellness. Beyond the need for better insight to climate/HAB interactions, “traditional” HAB research also must expand to embrace sociologists and economists if we are to obtain the knowledge base necessary to forecast this collective future HAB imprint.

So how best to proceed? One of the primary impediments limiting the ability of HAB science to address these questions stems from one of its strengths: the blend of experimental methodologies and observations on the diversity of toxic and other harmful species. Studies to date have given us both broad and detailed insights but so far only a fragmented understanding of toxic HAB species and their ecologies. The wide range of methodological approaches also hamper attempts to synthesize across studies, whether on the same organism or toxins, or across field observational programs and time series. Key methodological differences include, but are not limited to, basal seawater and media enrichments, culture techniques and conditions, experimental design, field techniques, analysis methods, and parameters measured, all of which have the potential to influence study outcomes. These diverse approaches have limited our ability to quantitatively pool these findings among HAB genera and species to develop a more comprehensive understanding of what sets HAB organisms apart, how they respond to natural variability in coastal/freshwater conditions, to anthropogenic pressures, and importantly, how might climate drivers affect their bloom prevalence.

The guidelines here are a step towards overcoming this weakness through recommendations from the international HAB research community on approaches to standardize laboratory and field practices where possible. Clearly, it will be challenging to accomplish this goal given the “biological” differences among HAB species (physiology, growth requirements, life histories, trophic interactions, etc.), their habitats, and the unavoidable variability among laboratory experiments. Similarly, HAB field observations and monitoring programs often face unique limitations, which in turn can affect HAB modeling strategies. Nevertheless, some degree of collective agreement on unifying research principles and standards will help move the field of HAB research to better meet societal needs.

The chapters in this document emphasize the need to study the effects of climate drivers on HABs at local to regional to global scales. Each of these scales impart different requirements but include:

- observational studies to record changes in community structure,
- mechanistic studies with single to multiple drivers to understand responses at an organismal level,
- process studies to understand competitive outcomes within plankton communities,
- adaptation studies to inform on plasticity and changes in genotypes,
- and data analysis and modelling studies to integrate findings to predict how future HABs may differ from contemporary HABs.

As we move forward, the improved understanding must be integrated with sociological and human wellness studies to fully address the health, cultural, and economic issues important to society.

Four philosophies underpinned the writing of these guidelines. The first is that although the central purpose is to lead researchers of climate change/HAB interactions towards more uniform practices and strategies, guidelines must not be so restrictive as to preclude the flexibility that leads to discovery of unexpected relationships. This is a critical point; while research proposal designs and outcomes can benefit from these guidelines, these guidelines should not be used as inflexible rules.

Second, active and interested HAB researchers have a wide diversity of available resources and expertise. It is important that these guidelines also provide avenues for those at less well-equipped sites to still provide valuable contributions to research, observation and modelling efforts, particularly given the need for a more global assessment of climate/HAB interactions.

The third is to guide researchers towards more comparable/compatible approaches and strategies for field and laboratory studies that facilitate quantitative and statistical intercomparisons among independent studies across regions. That is, to help strengthen the collective assessment of how climate change may influence HABs.

Finally, there is a recognition that a new balance of mechanistic and scenario-based studies, including scientific community scale strategies (e.g., common garden experiments; Boyd et al. 2018), are needed to better characterize and forecast generalized HAB responses to climate-driven changes in coastal and fresh waters.

The chapters cover experimental practices for studying climate driver effects on the prevalence of HAB species within diverse phytoplankton communities, how these responses are influenced by acclimation and adaptation, the design, justification, and management of long-term field observation programs, how to improve present HAB database design and operation, and future steps to improve modelling practices for HAB study and forecasting. The specific recommendations presented in each chapter include both existing practices or directions as well as new concepts, all intended to help guide new researchers entering the field and experts alike. Mentioning here only a few, experimental studies should include gradients of multistressors for HAB species and key competing non-HAB species. Studies addressing acclimation and adaptation should be designed in collaboration with modelers and exploit relevant omic techniques. Long-term HAB observation programs need to incorporate environmental and ecological observations, perhaps best done through alignment with on-going networks. Current HAB databases need to be remodelled to increase their interlinkages and connections to other environmental/biological databases, and expand their spatial coverage, particularly in the coastal seas of Africa, south Asia, the Arctic and Antarctic. Finally, modelling studies should evolve to be an umbrella linking all of the above, emphasizing multistressor responses and adaptation of HAB species and strains, put into the context of regional forecasts of progressive changes in coastal and freshwater systems.

The ultimate goal for HABs and climate change research is to understand the potential changes climate drivers will have on the prevalence of HABs; a goal that will require appraisal across many regions. Achieving this level of insight requires expanding HAB research beyond its current geographical footprint to include nations and locales not currently engaged. So while this document is targeted to be informative for experts, it also is designed to guide new researchers with limited resources on how to best enter the field and provide valuable data and findings. Only by expanding the research and observational base can we can hope to gain a truly global insight on how climate change may be affecting HABs.

These Guidelines for the Study of HABs and Climate Change are intended as foundation on which to build, and we encourage the research community to consider contributing chapters on other aspects of HAB/climate change research. The guidelines are designed and published as an on-line resource to increase its availability, and it is planned that there will be periodic reassessment, revision, and augmentation as new methods and understanding shift the key research targets.

HAB research lies at a juncture between continued reliance on past, somewhat fragmented research approaches and practices, and the adoption of new strategies that better address the complex interactions among climate drivers, environmental change, physiology, ecology, sociology and economies that control the realized impacts of HABs. The guidelines towards harmonizing approaches presented here will help to foster more quantitative intercomparisons among studies, and thereby accelerate development of the knowledge base for improving HAB forecasting skills in the contemporary and future coastal oceans.

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# CHAPTER 2

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## Observing changes in harmful algal blooms over time: long-term observations for studying impacts of climate change

**Anthony J. Richardson**<sup>1,2</sup>, **Ruth Eriksen**<sup>3</sup>, **Gustaaf M. Hallegraeff**<sup>4</sup>, **Wayne Rochester**<sup>2</sup>, **Grant C. Pitcher**<sup>5</sup>, **Michele Burford**<sup>6,7</sup>

<sup>1</sup> School of Mathematics and Physics, University of Queensland, Brisbane, QLD, 4072, Australia

<sup>2</sup> CSIRO Oceans and Atmosphere, Queensland Biosciences Precinct, St Lucia, QLD, 4067, Australia

<sup>3</sup> CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart, TAS, 7001, Australia.

<sup>4</sup> Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, Tasmania 7001, Australia

<sup>5</sup> Department of Forestry, Fisheries and the Environment, Private Bag X2, Rogge Bay 8012, Cape Town, South Africa

<sup>6</sup> Australian Rivers Institute, Griffith University, 170 Kessels Road, Nathan, Queensland 4111, Australia

<sup>7</sup> School of Environment, Griffith University, 170 Kessels Road, Nathan, Queensland 4111, Australia

*“Improved information on the linkages between HABs and climate will emerge only through the establishment and maintenance of long-term phytoplankton monitoring programs adequately supported by environmental monitoring. At present our understanding and ability to predict how climate may select for HABs are severely limited by the scarcity of long-term records”*

Pitcher et al. (2018)

### 2.1 The need for time series in a changing world

Most marine science involves process studies, such as short-term field programs, experimental manipulations and modelling, whereby researchers study a part of a system in depth to identify and understand underlying processes. Such process understanding is a fundamental aspect of research. But process studies alone are not sufficient to unravel many aspects of a system.

Time series provide a vital long-term perspective, delivering critical information on *the state and trend* of an ecosystem unavailable through process studies. The long-term perspective afforded by time series provides our best opportunity to separate human-made from natural variation, a key requirement in attributing human impacts and managing ecosystems in the Anthropocene. Consequently, much of the focus of HAB research today is conducted within the context of a changing global environment and the need to continuously monitor the state of the Earth (Pitcher 2012). Because of the socio-economic impact of HABs on marine and freshwater ecology, the aquaculture industry, human health and water quality, time series of HABs play a prominent role in ecosystem assessments (e.g. Brett et al. 2020, Farrell et al. 2020, Barua et al. 2020). Time series data underpin our global and regional ecosystem assessments, such as those by the Intergovernmental Panel on Climate Change (IPCC 2014), the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES 2018), the United Nations World Ocean Assessment (United Nations [UN] 2016), and the State of Environment reporting in many countries (e.g. Evans et al. 2017).

Time series also play an essential role in ecosystem management. The biogeochemical and ecosystem models that we use to inform management decisions require testing and continued refinement (Franks 2018). The adaptive management cycle for complex systems involves the development of management strategies, their implementation and evaluation, from which we then can learn and inform revised management strategies, thereby completing the cycle (Medema et al. 2008). No model of HABs in a region would be considered sufficiently robust to inform management decisions if it had not undergone rigorous assessment against time series data. For example, management strategies to reduce eutrophication are continually tested against time series data of phytoplankton and HABs, ensuring management strategies are working, such as in the Chesapeake Bay and watershed report card (Anon 2019).

From a research perspective, time series describe how a system is changing, providing insights into how it operates, and thus generating hypotheses for future process studies to test. For example, following analysis of 30 years of time series data for *Dinophysis acuta* and *Dinophysis acuminata* from the Galician Rías Baixas, Díaz et al. (2016) deduced that these species exhibited different seasonal abundance patterns depending on climate conditions. During cool summers only *D. acuminata* was present, but in warm summers *D. acuta* replaced *D. acuminata* in late summer. Díaz et al. (2016) highlighted that the inoculum of *D. acuta* cells off the Portuguese coast was critical for predicting blooms and that alongshore currents might be responsible. This finding provides fertile ground for more process-based modelling (Chapter 6), experimental work, and field work to test the validity of the inoculum hypothesis (Chapter 2).

Networks of time series provide the opportunity for comparative analyses across regions to learn about a system, a key feature of the GEOHAB program and its successor GlobalHAB (Berdalet et al. 2018). Comparative analysis of ecological time series across similar systems that differ in terms of their initial conditions, drivers, species present or management, can provide fundamental insights into system behaviour (Wells et al. 2015). Considering that HAB events result from a complex interplay of physical, chemical and biological drivers (Glibert et al. 2018a), the ability to apply sophisticated statistical and modelling techniques to networks of time series to disentangle multiple human and natural drivers and stressors is powerful (Franks 2018). Networks of time series can also help to assess whether a system is driven by large-scale drivers or organising principles, and thereby test macro-ecological theory (Horne et al. 2016, Campbell et al. 2021). Analysing global trends has been the focus of SCOR Working Group 137 on Global Patterns of Phytoplankton Dynamics in Coastal Ecosystems (Paerl et al. 2015), now continued as IOCWG TrendsPO.

Despite the demonstrated value of time series observation of HABs, Wells et al. (2015) stated “there is an absence of high quality time-series data in most regions currently experiencing HAB outbreaks, and little if any data from regions expected to develop HAB events in the future”. This is especially concerning considering that climate change is changing marine ecosystems (Poloczanska et al. 2013), including HABs (Wells et al. 2015, Wells and Karlson 2018, Xiao et al. 2019, Nohe et al. 2020, Trainer et al. 2020). The lack of time series data hampers HAB research, especially in attributing changes to climate change and in prediction. Consequently, an increasing number of reports of synchronous trends in HABs and the environment are not adequately supported by appropriate time series data, analyses and underlying mechanisms (Pitcher 2012).

Here we start with a brief synthesis of potential and observed impacts of climate change on HABs to highlight the key drivers. We then detail the types of data needed to be collected in a time series, including ways to measure HABs and the key drivers. We document statistical considerations that can be used to inform the design of time series. Based on our experience, we then bring together practical information for operating a time series program. Finally, we provide some recommendations for designing a global network. We hope that this review will provide momentum for the development of a global HAB time series program.

## 2.2 How might climate change impact HABs?

Although there is growing evidence that climate change is impacting all types of marine life, including phytoplankton (Poloczanska et al. 2013), there is still considerable uncertainty in understanding the breadth of impacts of climate change on HABs. Our growing understanding of how climate change impacts HABs comes from observations, experiments, models and logical expectations. Expanding our time series observations will be invaluable for identifying how climate change is impacting HABs and are needed to generate hypotheses to test with experiments and models (Hallegraeff et al. 2021).

There is concern that climate change may increase the incidence and severity of HABs. The potential linkages between climate change and HABs have been well reviewed by Hallegraeff (2010), Wells et al. (2015), and Wells and Karlson (2018) and will only be summarised here to provide context for discussing variables that need to be measured (Figure 1). The key variables we will discuss here – based on Wells et al. (2015) – are temperature, stratification, ocean acidification, eutrophication and biological interactions such as grazing. Many of these are related to ocean hydrodynamics – including currents, temperature, stratification, turbulence and nutrient enrichment – and they have a large effect on distribution, abundance and composition of HABs.

		Environmental Factor				
		↑ T°C	↑ Stratification	↑ OA	↑ Cultural Eutroph.	Grazing
<b>HAB Type</b>	Diatoms (e.g., <i>Pseudo-nitzschia</i> spp.)	↕ +	↓ ++	↕	↓	↕
	Toxic Flagellates (e.g., <i>Alexandrium</i> , <i>Pyrodinium</i> , <i>Gymnodinium</i> )	↑	↑ ++	↕	↑	↕
	Benthic (e.g., <i>Gambierdiscus</i> spp.)	↕ ++	↑ ++	?	↑	↕
	Fish Killing (e.g., <i>Heterosigma</i> spp.)	↑	↑ ++	?	↑ +	↑ +
	High Biomass (e.g., mixed spp.)	↕	↕	↕	↑ ++	↕
	Cyanobacteria (e.g., <i>Nodularia</i> spp.)	↑ +	↑ ++	↕	↑ ++	?
	Cell Toxicity	?	?	⋮	↕	↕

Figure 1. Different HAB types and the direction and magnitude of environmental drivers (from Wells et al. 2015).

Warming is the most obvious effect of climate and there is evidence that it is impacting HABs. Although there are fewer data available on phytoplankton than many other marine groups, their response to climate change in terms of distribution and phenology is faster than most other groups. For example, in a global meta-analysis, Poloczanska et al. (2013) found that the timing of phytoplankton blooms was advancing by ~7 days per decade and by ~400 km per decade poleward. Temperature affects many aspects of cell physiology including photosynthesis, motility, growth and cyst germination (see references in Wells et al. (2015), Brandenburg et al. 2019, Seto et al. 2019). Many marine species are expected to adjust their range, with poleward expansions (Poloczanska et al. 2013), and the expectation is that HABs will do the same, although there is limited current evidence (but see Hajdu et al. 2000, Llewellyn 2010, McLeod et al. 2012). The phenology of many marine species is moving earlier (Poloczanska et al. 2013), and there is evidence that some HAB species are doing the same in some areas, including *Dinophysis* spp., *Prorocentrum* spp., *Pseudo-nitzschia delicatissima*, *Pseudo-nitzschia* spp. in the North Sea (Edwards and Richardson 2004, Chivers et al. 2020, Nohe et al. 2020). Given that

many HAB species prefer warmer temperatures, including most cyanobacteria and many dinoflagellates, there is an expectation that HAB species will increase with warming, although other species and groups may respond differently (Wells et al. 2015).

Global warming heats up the surface ocean and melts ice sheets, leading to enhanced stratification, reduced turbulence and altered nutrient and light regimes, all of which will have profound effects on phytoplankton (Finkel et al. 2010). On a global scale, phytoplankton are predicted to decline in tropical regions and increase in polar regions (Richardson and Schoeman 2004, Woodworth-Jefcoats et al. 2017) as stratification increases, which could lead to large-scale changes in where blooms occur and what species might dominate. With most HAB species being dinoflagellates, and this group preferring stratified conditions, there could be an increased tendency toward HABs in warmer, more stratified regions. As dinoflagellates dominate the phytoplankton late in the successional sequence during summer when stratification is most intense, the expectation is that HABs could have a longer seasonal window. There are some examples suggesting that changes in stratification are already influencing HABs on a regional and more local scale. In the California Current System there has been an increase in events of red tide colouration with increased stratification (Ryan et al. 2014); and the emergence of *Dinophysis* spp. off the coasts of the US (Campbell et al. 2010) and Canada (Taylor et al. 2013) has also been attributed to changes in stratification.

More CO<sub>2</sub> in the atmosphere is leading to more CO<sub>2</sub> dissolved in the ocean, altering carbon chemistry and leading to ocean acidification and lower pH. Altering the carbonate chemistry affects carbon acquisition, metabolism and photosynthesis, but responses vary among species (see Wells et al. (2015) and references therein). Ocean acidification can benefit dinoflagellates (Brandenburg et al. 2019) more than other phytoplankton groups (Beardall et al. 2009) because they will require less energy to capture CO<sub>2</sub>. Evidence is accumulating that disparate HAB species such as the dinoflagellate *Alexandrium* producing paralytic shellfish toxins, the fish-killing dinoflagellate *Karlodinium*, and diatoms of the genus *Pseudo-nitzschia* producing the toxin domoic acid may increase their cellular toxin content under ocean acidification scenarios (Fu et al. 2010, Sun et al. 2011, Tatters et al. 2013). A satisfactory working hypothesis as to why this should be so is still lacking.

There is general agreement that cultural eutrophication, which increases nutrient concentrations and changes their molar ratios, increases phytoplankton abundance, including HAB species (Heisler et al. 2008). However, climate change is also changing nutrient concentrations in the ocean, as climate drives oceanographic conditions at a variety of scales. Climate change has led to the strengthening of upwelling in Eastern Boundary Current regions (Sydeman et al. 2014); how nutrient enrichment processes at a local and regional scale will alter with climate change will have profound repercussions for HABs, but is a major gap in our understanding (McKinnon et al. 2007).

The primary grazers on HABs are microzooplankton, although copepods, invertebrate filter feeders and fish all ingest HABs (Turner 2006). HABs could produce allelopathic toxins to deter grazers through lethal or sublethal effects. For example, zooplankton grazing could stimulate domoic acid production by *Pseudo-nitzschia* diatoms (Lundholm et al. 2018), although other studies have shown no effect (Olson and Lessard 2010). The copepod *Acartia tonsa* stimulates production of saxitoxins in the dinoflagellate *Alexandrium minutum* (Selander et al. 2006). Zooplankton grazing rate is likely to increase with temperature, although how this might affect HABs and their toxin production is unknown. The magnitude of zooplankton grazing pressure can also exacerbate or temper blooms, and spreads carbon export over larger spatial scales (through diel vertical migration) and so minimises the potential for hypoxia (Wells et al. 2015).

In summary, there is growing observational evidence and mechanistic understanding that climate change will alter the frequency, severity, timing and spatial patterns of some HAB species but not others, but exactly which species will be affected, whether there will be increases or decreases, and where problems will worsen or ameliorate remain open questions. More time series will provide the



evidence required. There is no *a priori* reason why climate change should affect all HAB species in all geographic regions in similar ways. However, if dinoflagellates generally increase in importance under climate change, then this could have important implications for the biological pump (Guidi et al. 2015).

## 2.3 Types of Data Needed

With the growing realisation that more HAB time series are needed, and recognition of the need for a unified global effort in terms of HAB monitoring, the GEOHAB program helped identify the types of data required. Using this, and building on the review by Wells et al. (2015), we provide a suite of environmental and biological parameters that could form the basis of time series and complementary field programs to support climate-related research and modelling efforts, and provide a rationale for each (Table 1). It is proposed that a core set of measurements be maintained at each site of monitoring (in bold in Table 1), and those sites with the capacity could collect auxiliary variables that could provide a deeper understanding of HABs (in plain text in Table 1).

### 2.3.1 ENVIRONMENTAL VARIABLES

To measure the key environmental drivers of HABs – viz. temperature, stratification, ocean acidification, cultural eutrophication and grazing – there is a suite of physical, chemical and biological variables that could be collected (Table 1). Many of these variables are already routinely collected in state or national time series programs for HABs, but others might need to be added. Further, it is important that observations are highly resolved and sustained to address the range of scales that influence coastal ecosystems and HABs (Pitcher 2012). With the development of new sensor technology and autonomous systems, continuous environmental monitoring is often possible (Glibert et al. 2018b).

### 2.3.2 THE HAB COMMUNITY

There is a suite of different methods for observing HABs, including species and abundance functional types based on pigments from HPLC, abundance based on flow cytometry of cells, species information based on molecular approaches, HAB groups based on toxin profiles, and species information based on cysts from sediment cores (Cullen 2008; Sekula-Wood et al. 2011; Barron et al. 2013; Glibert et al. 2018b). As each method has advantages and disadvantages for measuring HABs, using multiple approaches will ensure a more complete picture and provide greater confidence. The explicit representation of the biological complexity of HAB species needs to be matched with appropriate complexity in the representation of environmental and ecological parameters to allow either short-term regional forecasts or long-term predictions of their response to global climate change (Pitcher 2012).

The traditional way to sample HABs has been to use light microscopy, supplemented by electron microscopy for difficult to identify species, although this can be time consuming and expensive. Using instruments that can autonomously collect high-frequency HAB and environmental data can minimise the need for expensive survey vessels and human sampling and analysis, but can also currently be prohibitively expensive when considering technician time and instrument costs. One state-of-the-art HAB sampler is the Imaging FlowCytoBot. This instrument, described as an automated underwater microscope, includes a flow cytometer and an imaging unit. Fluorescence or scattering of individual cells is used to get cell abundance. Each cell is imaged and identified, mostly to species or genus, using automated image analyses and supervised machine learning (Olson and Sosik, 2007). Such instruments can work continuously over a period of six months, relaying images back to the laboratory to produce real-time observations. Another innovative instrument is the environmental sampler processor that can be fixed on a mooring. Using automated molecular probe technologies has successfully demonstrated near-real-time in situ detection of HAB species and their toxins (Scholin et al. 2009; Seltenrich 2014; Herfort et al. 2016).

Table 1. List of measurements that could be considered at time series sites in a HAB monitoring program. Includes environmental drivers and HAB measurements. Core variables are in bold.

Variable type	Type	Variables	Rationale
Environmental drivers	Physical	<b>Temperature (profiles)</b>	Many HABs have specific temperature preferences, often warm, stratified conditions (Wells et al. 2015)
		<b>Salinity (profiles)</b>	Lower salinity waters, especially in estuarine environments, lead to greater stratification and can benefit HABs. Greater stratification can lead to altered nutrient availability (Marinov et al. 2010).
		<b>Precipitation</b>	Estimate of riverine runoff. Many HABs are stimulated by micronutrients associated with riverine runoff (Doblin et al. 1999). Runoff also changes stratification.
		Winds and Wave heights	Obtained from marine forecasts. HABs often accumulate during gentle winds.
		Light attenuation (profiles)	Changes in atmospheric conditions (cloud cover, particulate loads) and changes in coastal inputs (sediment, dissolved organic matter) affect light intensity, which along with temperature, influences the germination of dinoflagellate cysts (Anderson et al. 2005). Cyanobacterial species and bloom-forming raphidophytes and dinoflagellates may have photoprotective compounds that provide an advantage (Carreto and Carignan 2011).
	Chemical	<b>Macronutrients (N, P, Si), reduced N (NH<sub>4</sub>, urea, DON, DOP) (at discrete depths)</b>	HAB species implicated in high biomass events can be related to increases in macronutrient concentrations through eutrophication (Paerl et al. 2014) or to natural enrichment events. The form of N influences the cellular toxin content for a range of dinoflagellate species (Kudela et al. 2010) and for the diatom <i>Pseudo-nitzschia</i> (Auro and Cochlan 2013).
		Dissolved oxygen (at discrete depths, profiles)	An index of the oxygen available for respiration and of bacterial degradation.
		Carbonate system (pH, pCO <sub>2</sub> , alkalinity, total CO <sub>2</sub> ) (at discrete depths)	Effects of projected decreases in surface ocean pH and increased pCO <sub>2</sub> are not uniform across HAB species, and in many instances experimental results are equivocal (Wells et al. 2015). Effects on phytoplankton may include changes to growth/biomass, production of toxins (Van de Waal et al. 2011), changes in community composition (Feng 2009) and impacts on higher trophic level grazers (Caron & Hutchins 2013).
		Sediment characteristics, sediment resuspension	Important for cysts.
	Biological	<b>Chlorophyll a (at surface and discrete depths or profiles)</b>	An index of total phytoplankton biomass.
		<b>Phytoplankton community composition and abundance (including benthic species where needed)</b>	Quantitative where possible, otherwise qualitative. Information on the broader phytoplankton community helps with the interpretation of changes in HAB species.
		Microzooplankton and macrozooplankton biomass and community composition	Zooplankton are important grazers of HABs and can influence bloom formation (Turner 2006). HAB species may protect against grazing through production of allelochemical phycotoxins, while ingestion of toxic species can affect zooplankton grazing rates, fecundity, growth, survival (Schulze and Kjørboe 2009; Wells et al. 2015).

Table 1. cont.

HAB		<b>HAB species abundance and composition</b>	Time series of HAB species.
		<b>Toxins</b>	Determine the toxicity of HAB species in your area and in the particular event. Can be collected dissolved in water (<0.4 µm, collected in SPATT (Solid Phase Adsorption Toxin Testing) bags), intracellular (particulate) concentrations, or in shellfish.
		Phycocyanin probes (where appropriate)	To estimate cyanobacterial abundance.
		Cysts	Abundance and composition of cysts in the sediment. Helps elucidate seeding events and contributes to understanding bloom formation and lifecycles
		Markers for detecting presence of toxin genes (e.g. microcystins, saxitoxins)	Provides background conditions and early warning (Murray et al. 2011),
		Markers for quantifying cell numbers	Particularly useful at low abundances,
		qPCR	Can be used to estimate the amount of DNA and thus the abundance of particular species.
		Markers for gene expression	To assess physiological state of HAB species.
		IFCB (Imaging FlowCytoBot)	Useful for high resolution time series for selected HAB species.
	Cysts	Abundance and composition of cysts in the sediment	Helps elucidate seeding events and contributes to understanding bloom formation and lifecycles.

Nowadays, collecting molecular data in ecological time series is critical and leads to enhanced collaborations, insights and research output. In particular, DNA barcoding has been used to reveal patterns in biodiversity and ecosystem responses to anomalous climatic events including heatwaves (Berry et al. 2019). High-throughput sequencing (metabarcoding) of targeted genetic markers has revolutionised research into the diversity of protists (Amaral-Zettler et al. 2009, Santoferrara 2019). It can yield millions of sequences, focusing on the 18S and 28S ribosomal RNA gene in eukaryotes. The obtained sequences can be used to identify the taxa present in a sample. Metabarcoding methods are becoming more robust (Santoferrara 2019). The quantitative Polymerase Chain Reaction (qPCR) is also available to estimate the amount of DNA (related to cell numbers) of organisms that are difficult to identify using light or fluorescence microscopy (Penna and Galluzzi 2013, Hatfield et al. 2019). qPCR is an extremely sensitive, fast, molecular method useful for the detection and quantification of cryptic

species (*Pseudo-nitzschia* spp.) or those with difficult morphotypes (*Alexandrium* spp., *Chattonella* spp., *Ostreopsis* spp.) (Penna and Galluzzi 2013).

Many time series, particularly those derived from aquaculture monitoring programs, record only HABs, but this severely limits the ability to glean insights into underlying ecosystem dynamics. Harmful species should be treated as intrinsic components of phytoplankton communities that have complex interactions with the environment, each other, and grazers (Chapter 2). Without placing HABs in the broader context of the phytoplankton community, we will not be able to adequately understand the response of HABs to climate change in the future.

## 2.4 Statistical considerations for the design of time series

There are a number of recommendations for the design of HAB time series programs that can be provided by considering statistical aspects of the data and potential analyses (Table 2).

### 2.4.1 FREQUENT SAMPLING

It is desirable to match the frequency of sampling to the time scale of temporal variation of the organism of interest to avoid aliasing. Aliasing happens if you sample too infrequently for the phenomenon of interest and can lead to artificial cycles appearing. An obvious example of aliasing is the classic wagon-wheel effect, where wagon wheels on moving stage coaches appear stationary or moved backwards in Western movies because the sampling frequency – the number of frames recorded per second on the film – is too slow to render the phenomenon accurately. The short generation times (days to a week) of HABs and other phytoplankton suggest frequent sampling is needed, and that sampling longer time scales can lead to aliasing. Presently many HAB time series undertake weekly sampling, and even at this frequency there could be some aliasing of the signal. Automated sampling methods based on cytometry, images or molecular probes might help alleviate aliasing problems and makes it much more practical to sample frequently.

There is often a debate about whether it is better to take more samples spatially when sampling or more samples through time. Because of the rapid growth of HABs and other phytoplankton, time series are probably best sampled more frequently in time rather than in space. However, a definitive answer would require a pilot study comparing the variance associated with HABs through time with that in space.

### 2.4.2 ANALYSING TIME SERIES IN THE FREQUENCY DOMAIN REQUIRE LONG TIME SERIES

To identify the dominant frequencies in long time series data of HABs, valuable insights might be provided by time series techniques common in electrical engineering. Time series can be envisaged as the superposition of cycles with different frequencies – from short-term, tidal and event-scale variation to longer-term, climate variability such as that associated with the El Niño Southern Oscillation. Spectral analysis decomposes a time series into its characteristic cycles. The simplest spectral analysis uses periodograms, which identify the periods (i.e., frequencies) of the dominant cycles in a time series assuming these are constant over time. By contrast, wavelet analysis allows the dominant frequencies to change over time by estimating the frequencies over a local neighbourhood. Wavelet analysis, and to a lesser extent periodograms, require long time series. Spectral techniques are probably best applied to automated techniques for monitoring HABs such as the Imaging FlowCytoBot, which provide high frequency, regular sampling, and to high frequency time series of physical and chemical parameters.

### 2.4.3 EQUALLY-SPACED SAMPLING THROUGH TIME

Having approximately equal time steps makes it much easier to use periodograms and wavelet ana-

Table 2. Statistical considerations and rationale to consider when designing a time series program.

Recommendation	Rationale
Conduct frequent sampling	Match your frequency of sampling to the time scale of temporal variation of the organism of interest to avoid aliasing
Time series approaches in the frequency domain require long time series	Wavelet analysis periodograms require long time series
Maintain equally-spaced sampling through time	When considering temporal autocorrelation in time series data, it is easier if samples are approximately equally spaced in time, allowing easier application of periodograms and wavelet analysis
Sample sufficient volume for rare species	Ensure you collect sufficient water volume to capture adequately the abundance of rarer species of interest
Record numbers counted for each species and the volumes sampled	In GLMs and GLMMs, it is better to model counts as a response and use volume as an offset, rather than model numbers per unit volume
Minimise pseudoreplication	Only collect multiple samples from the same site at the same time if sampling error is high
Be aware of taxonomic changes	Analyses need to consider changes in taxonomy; a change log is essential
Establish the length of time series needed to identify significant trends	A power analysis to ascertain the length of time series needed should consider seasonality
Include other anthropogenic stressors in the sampling	To assess a climate change signal, other anthropogenic stressors should be included in analyses
Conduct comparative analyses	Use GLMs and GLMMs to bring different datasets together to make time series. Choose time series for the same species so they come from a range of environmental conditions. Comparative analysis across sites minimises the chances of spurious local correlations
Combine multiple HAB data types	A robust estimate from a time series can be derived by combining different data types into a single statistical model

lysis to identify the dominant frequencies of variation in time series. Regular sampling also makes it much easier to estimate temporal autocorrelation in time series data (the degree that one sample in time is related to subsequent samples in time). This is because the degree of autocorrelation is typically assessed by sliding the time series backward in time one step at a time, and it is only valid to do this if the time interval is similar.

#### 2.4.4 SAMPLE SUFFICIENT VOLUME FOR RARE SPECIES

Rare species – those which have many zero counts – are more difficult to model statistically than more abundant species. It is worthwhile considering the rarest species you are interested in and ensure you collect sufficient water volume to sample this species adequately. For some larger, rarer species, sometimes a towed fine mesh net can provide more robust abundance estimates than bottle samples. For example, using 10 years of monthly data at a time series station on the Great Barrier Reef, Australia, the large filamentous cyanobacterium *Trichodesmium* was present in 62% of samples using a 12-L bottle, but present in 94% of samples collected with a 100- $\mu$ m mesh net that collected ~20 m<sup>3</sup> of water (AJR unpublished data).

#### 2.4.5 RECORD NUMBERS COUNTED FOR EACH SPECIES AND THE VOLUMES SAMPLED

Although species abundance in time series programs has traditionally been analysed as numbers per unit volume (per litre or m<sup>3</sup>), it is not the most appropriate way to analyse such data. Regression models such as generalised linear models (GLMs, with fixed factors) and generalised linear mixed models (GLMM, with fixed factors, and random factors often related to sampling) are frequently used to analyse ecological data. Accessible introductions to these models can be found in Crawley (2013) and Zuur et al. (2009). Both GLMs and GLMMs can have a non-normal error structure, which is valuable with count data. When building a statistical model, it is more appropriate to use untransformed or raw data as the response (dependent variable) when possible, and model characteristics of the data by choosing the appropriate error structure (e.g. normal, binomial, Poisson, negative binomial) and link function (the transformation that links the mean of the response (dependent variable) to the predictors (known as the linear predictor)). In GLMs and GLMMs, it is therefore better to model the response of species abundance as counts and use the volume of sample counted as an offset (to standardise counts by), rather than model numbers per unit volume as the response. However, an inherent problem with modelling HABs is that they are often absent (i.e., lots of zeros). Depending on the number of zero abundances for the response variable (known as zero inflation), one can model count data as Poisson (moderate number of zeros), negative binomial (more zeros), or use a zero-inflated negative binomial or a hurdle model (for even more zeros, Zuur et al. 2009). Both the Poisson and negative binomial error structures use a log link function by default. If the volume counted is unknown for each sample and counts cannot be modelled explicitly, then there is little choice but to use the numbers per m<sup>3</sup> as the response, with a log-transformation of the response and a normal error structure (when there are zeros) or with a gamma error structure that has a log link function (if there are no zero values). See Franks (2018) for examples of using these techniques with HAB time series. The main point is it is best to store the volume counted and the raw count in your database (Chapter 5). A final approach that might be useful for understanding the spatial and temporal variation in HABs given the large number of zeros are maximum entropy methods (Harte and Newman 2014).

#### 2.4.6 MINIMISE PSEUDOREPLICATION

Pseudoreplication is the phenomenon of artificially inflating the number of samples in a study because samples are not independent, leading to larger type I error rates. An example could be collecting multiple samples from the same site at the same time. This is generally not worthwhile, unless sampling variability is high at the site (e.g. due to high local spatial variation) relative to the temporal variation. Pseudoreplication can be considered explicitly with a GLMM.

#### 2.4.7 BE AWARE OF TAXONOMIC CHANGES

Changes in taxonomy can present challenges for statistical analyses. A change in name for a particular species should be dealt with in database curation and should thus not pose a problem. However, more of a dilemma is the distinction in how you treat a species that is found for the first time (and could always have been identified if present) versus a species that you are only able to identify now for the first time (i.e., it might have been present before but it could not be identified). When a species is found for the first time and it could always be identified, true zeros can be assumed before this date because it would have been seen previously if it was there. When in a position to identify a species for the first time, following taxonomic training for instance, the length of time series for this species is from that date onwards. This situation is fairly common, as para-taxonomists commonly learn to identify more species as they gain experience. Other times, a particular species might be split into multiple species (e.g. the *Alexandrium tamarense* species complex; John et al. 2014), and only after that date someone is able to separate them. This is where a change log in a database is essential – a record of the first date that the taxon was able to be identified. There are other situations with HAB time series data that are worth noting also. For example, toxin monitoring programs often only monitor once

a toxin has been detected (or a human poisoning has occurred) so do not provide true zeros. Also, cryptic species are often difficult to identify with light microscopy but could be recorded as species complexes (e.g., *Pseudo-nitzschia delicatissima* complex, or *Alexandrium tamarense* complex) and can still provide valuable information.

#### 2.4.8 LENGTH OF TIME SERIES NEEDED TO IDENTIFY SIGNIFICANT TRENDS

A key question asked before starting a time series, particularly by funders, is how long do you have to sample before you can detect a significant trend. To answer this question one can use a power analysis (Gerrodette 1987). If you have preliminary data from a pilot time series, a power analysis can be used to estimate the number of samples required to detect trends in abundance data using regression analysis, given a type I error rate (e.g. 0.05), estimates of sample variability, and the rate of change (effect size). An important consideration is that HAB time series are highly seasonal, and this semi-predictable source of variation should be separated from other variation so significant trends can be detected with shorter time series. Thus sampling at a sufficiently high frequency to describe the seasonal cycle and event-scale variation will enable resolution of seasonality, event-scale variation and trends.

#### 2.4.9 COMPARATIVE ANALYSES

A key feature of the GEOHAB program and its successor GlobalHAB is the ability to learn about HABs by using comparative analyses across regions (GEOHAB 2003, Kudela et al. 2018). By comparing ecological time series from systems that differ in terms of their species, environmental conditions, and levels of human impact or management, comparative analyses can provide new insights into how a system behaves, its large-scale forcing, and the ecological consequences. Comparative analyses across diverse sites (e.g. tropical and temperate, estuarine and shelf) minimises chances of spurious local correlations. A challenge is that sites may vary in their sampling gear, sample volume counted, sampling depth or time series duration. Comparative analyses may be as simple as adding a term as to how the sites vary (e.g. sampling gear) to the regression model. A valuable comparative analysis could focus on the proposed nine 'model' HAB taxa (Wells et al. 2015) that have a variety of different impacts on people and ecosystems – *Alexandrium catenella* (paralytic shellfish poisoning), *Pseudo-nitzschia multiseriata* (diatom, domoic acid poisoning), *Heterosigma akashiwo* (fish killing), *Cochlodinium polykrikoides* (fish killing), *Karenia brevis* (fish killing), *Dinophysis* spp. (diarrhetic shellfish poisoning), *Aureococcus anophagefferens* (high biomass), *Nodularia spumigena* (cyanobacteria), *Gambierdiscus* spp. (ciguatera fish poisoning).

#### 2.4.10 INCLUDE OTHER ANTHROPOGENIC DRIVERS

To attribute changes in the abundance, timing, composition, behavior or toxicity of HABs to climate change, multiple anthropogenic stressors should be considered (Brown et al. 2011). The impact of particular stressors can intensify, weaken or have no effect in the presence of other stressors (Griffith et al. 2012).

#### 2.4.11 COMBINING MULTIPLE HAB DATA TYPES TOGETHER

Although microscopy, molecular techniques, cytometry, pigments and toxins all measure some aspect of HABs in time series, each has its own strengths and weaknesses. Multiple time series such as these can be analysed separately, but a more robust approach might be to combine these different data types into a single statistical model. For example, two noisy time series might be combined to provide a single series with smaller standard errors. Alternatively, a combined analysis may have increased power for hypothesis testing or statistical inference concerning relationships with environmental variables. However, analyses that require uncommon model structures may require custom formulation and fitting using maximum likelihood methods or Bayesian statistical modelling.

## 2.5 Operating a time series program

Initiating and operating a time series program is time consuming and often under-appreciated compared with more traditional outputs such as publications. However, publications have a limited shelf life, but high quality, long-term, time series programs are likely to make a greater lasting contribution to science through their impact on society and different research fields.

Operating a time series program presents many challenges and below we discuss some of these and their solutions. A guiding principle that can help making operational decisions concerning a time series program is: "Would I make the same decision if the program lasts for 50 years?". This contrasts with the more common short-term expediency in science and it allows you to focus on getting the decision right in the long term.

### 2.5.1 PUBLISHING OPPORTUNITIES

There is a concern amongst scientists that initiating a time series program might lead to reduced research output and that you might have to wait many years before you can publish. Although the establishment phase of a time series program can be time consuming, there are still many publishing opportunities along the way. For example, it is now possible to publish methodological descriptions of observing programs – this provides peer-review of your approaches and also provides information for others wanting to start up their own programs. For example, the journal *Frontiers in Marine Science* has a Research Topic on "Best Practices in Ocean Observing", which provides a forum for publishing methodological papers that underpin ocean observing programs, including those that monitor HABs (e.g. Eriksen et al. 2019). Another possibility is that early publications can focus on questions that can be answered from short time series. For example, what are the key species present, what is their spatial variation, how do they vary seasonally and how do they vary in response to climate variability (e.g. Thompson et al. 2015). It is even possible to publish climate change papers from short time series. Although little can be said about climate change from data shorter than 20 years in length (Poloczanska et al. 2013), by collating historical data from the region you can produce composite time series that span more than 20 years and then analyse long-term change (McLeod et al. 2012, Ajani et al. 2020). Any historical phytoplankton data that is compiled and not in existing open-access databases can also be published as data papers in scientific journals such as *Ecology* and *Scientific Data* (Davies et al. 2016, 2018). There is also the opportunity to produce outputs that are not typical publications, such as ecosystem assessments (Barua et al. 2020, Brett et al. 2020, Farrell et al. 2020). Finally, once a program is established, publication output can grow exponentially (see Edwards et al. 2010 for the North Atlantic Continuous Plankton Recorder program). So there are many opportunities for publications. Another consideration is a philosophical one; publications have a shelf life, so their citations decline over time. Quality time series data are different – every additional year makes the whole time series more valuable. Perhaps initiating and supporting collection of time series data and making them available to the broader community will eventually make a greater scientific contribution and be a longer-term legacy than publishing one's own scientific papers.

### 2.5.2 THE VALUE OF A RELATIONAL DATABASE

Databases in relation to HABs are covered in detail in Chapter 5 of this book, so we focus here on the need for databases in time series programs. In the past it was common to store ecological time series data in a spreadsheet. Nowadays, a relational database, where relationships between stored items of information are recognised, is essential. A database streamlines data entry through forms and improves quality control by restricting particular fields to certain values and putting flags on out-of-range values. A database helps to curate and archive data. A database simplifies data delivery to external websites, as data can be exported automatically to online web servers. Data for analysis are easily extracted from databases, and changing taxonomic capabilities of analysts through time can be accounted for using a change log. A relational database also allows the storage of auxiliary environmen-



tal variables, some of which might be on different time and space scales (for example, a fluorescence profile taken at the time of phytoplankton sample collection), and queries can be written to match the biological and environmental data. Designing and delivering such a relational database requires database experts.

Using a species table in a relational database ensures consistency in taxonomic names through updates to taxonomic tables. Each species name recorded should be consistent with the current accepted scientific name in WoRMS (World Register of Marine Species, <http://www.marinespecies.org>) and should have an AphiaID. Accepted name changes can be tracked through the scientific literature and communication with expert collaborators. Digital reference collections are valuable for maintaining taxonomic consistency of phytoplankton identification from light microscopy, and these can also be stored in a database.

### 2.5.3 ADVANTAGES OF FREELY-AVAILABLE DATA

Scientists often feel some understandable apprehension in making data freely available. Our experience is that rather than reducing your publication output, the collaborations that follow from making time series data freely available usually increases your publications. Making your data freely available generally makes the time series more secure financially, because there is greater data uptake (Stevens et al. 2006), often a key requirement in long-term funding. Further, funding agencies and journals are now increasingly requiring data be made freely available. We can only develop a global network by sharing data.

The HAB research community is making solid progress in making HAB data available (Chapter 5). OBIS [[jobis.org](http://jobis.org)] focuses on the global distribution of HAB species toxic to humans and fish as covered by the *IOC-UNESCO Taxonomic Reference list of Harmful MicroAlgae*, while HAEDAT [<http://haedat.iode.org>] focuses on recording HAB events that adversely impact human society whether by high biomass (leading to hypoxia/anoxia, clogging of fish gills, beach closures), fish kills, or seafood poisoning (<http://haedat.iode.org/>). HAEDAT makes data on HAB events freely available via the web from the ICES (North Atlantic) and PICES areas (North Pacific) and IOC Regional Networks in South America, South Pacific, Asia and North Africa. It is important to recognise that HAEDAT data are restricted to HAB events that had an impact on human society. Such event data are challenging to analyse because of different sampling approaches, counting methods and monitoring intensity, but still they can be used to form proxy time series through time. For example, Piontkovsky and Castellani (2009) used one-off cruise data to build a 50-year zooplankton time series in the tropical Atlantic Ocean. Such proxy time series could be useful for establishing the long-term seasonality, against which seasonality from new and standardised time series can be assessed. Taking the lead of the *International Panel for Climate Change* (IPCC) consensus reporting mechanism, and to complement the World Ocean Assessment, HAEDAT and OBIS data are being used to generate Global HAB Status Reports (Hallegraeff et al. 2017, 2021).

### 2.5.4 THE IMPORTANCE OF HUMAN CAPITAL

A time series program requires team members with a wide range of expertise, often including phytoplankton identification, field work, databases, instrumentation, statistical analysis, molecular methods, and pigment analysis, amongst others. Having access to such expertise is often difficult outside Government research organisations. Another important lesson when running a time series program is to build some redundancy in staffing expertise. Having multiple people with the ability to do field work, laboratory analyses, and database manipulations ensures that the survey is more resilient to vagaries of staff turnover, being on holidays, maternity leave or long-service leave.

### 2.5.5 PARTNERING WITH TAXONOMIC EXPERTS

Opportunities to formally train in taxonomy are rare and diminishing. Developing a HAB time series is easier if you partner with phytoplankton taxonomists. Taxonomists are remarkably willing to give of their time to help ongoing training of young (and sometimes older) scientists. Being able to capture digital images and sending these to a network of taxonomic collaborators means that one can simultaneously identify unknown specimens and improve your taxonomic skills. Such collaboration with taxonomic experts often leads to new records of species in a region or even new species to science.

### 2.5.6 STREAMLINING METHODS

Although the aim of time series programs is to have constant sampling methods and counting procedures over time, in reality this is not always possible or even desirable. Sampling programs usually evolve from their initial conception as methods are streamlined and improved. Having a perspective that the time series will last 50 years, enables methods to evolve in the early stages. For example, para-taxonomists often improve their ability to discriminate between taxonomic groups with experience and training, and setting-in-stone the taxonomic groups counted at the start of the time series can be a mistake. This highlights the importance of a change log that was mentioned earlier.

### 2.5.7 TIME SERIES AS A RESEARCH HUB

The infrastructure (moorings), regular sampling, and scientific momentum surrounding time series programs often results in them becoming a hub for research (e.g., Zingone et al. 2019). Opportunistic and supplementary programs often piggy-back on sampling trips and provide additional physical, chemical and biological measurements including extra sensors on moored infrastructure. Because of their valuable data, time series often become the focus of new, integrated, process-oriented field, experimental and monitoring programs (Anderson et al. 2014). This leads to a critical mass of researchers, richer collaborations, broader perspectives, new insights, and ultimately greater scientific output.

### 2.5.8 IMPACT BEYOND RESEARCH

Research impact through publications is often not the only metric by which long-term observing programs are judged. Increasingly, government funders expect observing systems to support marine management and HAB time series are well placed to deliver on that need. Increasing aquaculture production and the requirement to protect human health has resulted in many countries developing and implementing monitoring and management strategies to minimise the risk of placement of unsafe product on the market (Silke 2009). These programs usually comprise an integrated system of monitoring, from phytoplankton to end-product testing, and are governed by relevant legislative requirements that are in place to protect consumer safety and to safeguard the industry (Pitcher 2012). There is a growing awareness of the need for international harmonisation of monitoring food safety standards, and there is a global structure of regulation in place from CODEX through to national food safety authorities working towards this objective. These intensive monitoring programs will ultimately provide an excellent record of the trends and fluctuations of HABs. Many HAB researchers already work closely with the aquaculture industry and Government agencies to ensure safe seafood products.

Time series of HABs also have valuable information to provide about the state and trends of ecosystem health, on local, regional and global scales, and can inform policy. Time series data are valuable for the assessment of high spatial resolution coastal models used to support water quality management (Walsh et al. 2016, Franks 2018). Even water quality models not focused on HABs typically have functional groups for diatoms and flagellates or small and large phytoplankton (Skerratt et al. 2019), information typically provided by HAB time series programs. Becoming an indispensable part of ongoing management and policy decisions maximises the chances of continuation of time series.

## 2.6 A global network

Here we discuss three arguments to support and expand a global HAB monitoring network.

### 2.6.1 FUNDING

The foundation of any long-term observing system is sustained funding. One successful funding model has been for the relatively small biological observing community to team up with the much larger – and better organised – physical oceanographic observing community to push for a large and integrated observing system. Partnering with the broader observing system community will also help exert political influence and develop a world class biological observation system.

For example, in Australia the Integrated Marine Observing System (IMOS) is a \$US11 million per year program spanning physical to biological data streams (Moltmann 2011). All participants have benefited from being part of an integrated and coordinated system, allowing more direct lines of communication and influence with government. HABs are an ideal case study for GOOS programs because there is a need for physical, chemical and biological observations, there is a pressing societal need, and hydrodynamic modelling is an essential part for the design of a HAB early warning system. There is also a call to include HAB observations within the Global Ocean Acidification Observation network (GOA-ON). We recommend that HAB researchers make contact with these programs to initiate collaborations, which will help support existing HAB sampling programs and initiate new ones.

### 2.6.2 PARTNERING WITH INDUSTRY

The biggest collectors and customers of HAB data are the aquaculture industry. Some industries are keen to work with researchers to make their data available, but others are not, or only after a period of embargo of commercially sensitive data. For the purpose of quickly identifying developing problems, industry sometimes collect data themselves on the HAB species present, often sampling frequently and sometimes over decades, but usually lacking information on the broader phytoplankton community and environmental data. What researchers can bring to the industry table is the capacity to develop innovative solutions, especially when their time series are harnessed to build and assess process-based hydrodynamic models that predict HAB outbreaks.

We recommend that HAB researchers initiate closer collaborations with the aquaculture industry. Leveraging data from the aquaculture industry is a powerful and efficient way to develop enough sites to gain a regional picture of HABs. We also recommend that the global HAB database HAEDAT investigate collaborations with the aquaculture industry, as this would substantially improve the global database because it includes true zeros. This would ensure that HAEDAT becomes a shared international resource for time-series data.

### 2.6.3 DESIGNING A GLOBAL NETWORK

Ocean and climate processes know no national boundaries and the ubiquitous nature of HABs suggests that a cooperative and coordinated global network is needed. Such a network is timely, given the current UN Decade of Ocean Science for Sustainable Development (2021-2030). Existing long-term HAB time series, particularly those over a decade, could form the backbone of a global network, and new time series using consistent methodology for comparative analyses should be established. Developing a global network of time series will make each individual time series more secure, as termination of individual time series would weaken the whole. To develop a global network, there is also value in harnessing the large community of phytoplankton researchers, beyond those that focus on HABs. For example, SCOR WG 137 (now IOCWG TrendsPO) on Global Patterns of Phytoplankton Dynamics in Coastal Ecosystems (Paerl et al. 2015) has already brought together many existing phytoplankton time series, but there still lacks a unified vision of a global network.

There are three main objectives of a global HAB time series network. First, to promote the integration of the fragmented coastal environmental research community and its linkage to policy makers, environmental managers and the wider global observing system communities. Second, to develop and implement an internationally co-ordinated strategy for the gathering, acquisition, exchange and analysis of these data. Last, an ultimate goal could be to encourage and provide the essential datasets for the development and application of predictive process-based models to better manage HABs in the future, and the role that time series play in such modelling efforts.

To initiate such a global HAB time series network, the design principles of the initial design of GOOS should be customised (modified from IOC 1998).

1. Define objectives to meet needs of the research and user community
2. Assume contributions are long-term and systematic
3. Ensure consistency of methods as far as possible, but have flexibility where needed
4. Focus on ubiquitous problems benefiting from a global network
5. Cover a range of activities including sampling, quality control and data delivery
6. Follow a specified data policy for the management, processing and distribution of data
7. Consider systems outside the network that can contribute to and/or benefit from the network
8. Review the design regularly

How might we design the global network itself? HABs are generally coastal or shelf phenomena, so a truly global system spanning the vast expanse of the open ocean is neither practical nor needed. One possible design approach would be to ensure coverage based on bioregionalisation. The most common global bioregionalisation is that of Longhurst (1998), but its focus is on the open ocean. We recommend use of the Spalding et al. (2007) bioregionalisation. They defined 232 coastal and shelf ecoregions based on expert opinion. Having HAB time series data in the world at each of these ecoregions would cover a wide range of different systems and undoubtedly provide extensive opportunities for comparative analyses. Although there would be ecoregions that have few problems with HABs, these ecoregions would provide valuable contrasts. A gap analysis could prioritise marine ecoregions where HABs are common (using the HAEDAT database) and where there are no current time series locations. Climate change affects different parts of the world ocean in different ways (Bopp et al. 2013), and it should include monitoring stations that represent hotspots of warming, ocean acidification, stratification, nutrient change, and deoxygenation.

Beginning in 1997, the physical oceanographic community of GOOS released a blueprint for what the global observing system for ocean climate would look like, detailing the needed temporal and spatial coverage of its major platforms, and tracking its implementation through time (IPC1998a,b). Having a target for globalisation has the benefit of providing a target that can be tracked through time – motivating the research community and focusing the attention of funding bodies. For example, the ARGO network, which had only 544 floats in 2002, reached its design specification of 3,000 floats globally in 2007, and has maintained this coverage ever since. Successful examples of what can be achieved with long-term monitoring data include the North Atlantic Continuous Plankton Recorder program (1940 until present; Reid et al. 2003) and the Narragansett Bay 1950-1989 environmental monitoring program (Li and Smayda 2000, Smayda 2003). We recommend that the HAB research community design a prototype global HAB monitoring program so that it can be seen as a serious, viable and effective complement to global ocean observing programs.

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# CHAPTER 3

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## HABs under global change: Experimental conditions and approaches

**Dedmer B. Van de Waal**<sup>1</sup>, **Lennart Bach**<sup>2</sup>, **Elisa Berdalet**<sup>3</sup>, **Karen M. Brandenburg**<sup>4</sup>,  
**Sanna Suikkanen**<sup>5</sup>, **Sylke Wohlrab**<sup>6,7</sup>, **Per Juel Hansen**<sup>8</sup>, **Anke Kremp**<sup>9</sup>

<sup>1</sup> Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

<sup>2</sup> Institute for Marine and Antarctic Studies, Ecology & Biodiversity, University of Tasmania, Hobart, TAS, Australia

<sup>3</sup> Institute of Marine Sciences (ICM-CSIC), Passeig Marítim de la Barceloneta, 37-49, 08003 Barcelona, Catalonia, Spain

<sup>4</sup> Utrecht University (UU), Utrecht, The Netherlands

<sup>5</sup> Finnish Environment Institute, Marine Research Centre, Helsinki, Finland

<sup>6</sup> Alfred Wegener Institute (AWI), Helmholtz Centre for Polar and Marine Research, 27570 Bremerhaven, Germany

<sup>7</sup> Helmholtz-Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), 26129 Oldenburg, Germany

<sup>8</sup> Marine Biological section, University of Copenhagen, Helsingør, Denmark

<sup>9</sup> Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Warnemünde, Germany

### 3.1 Introduction

Global environmental change involves shifts in our climate (e.g. temperature and rainfall), climate drivers (e.g. atmospheric  $p\text{CO}_2$  and cloud cover), and associated environmental changes (e.g. nutrient concentrations and salinity). These global environmental change drivers are expected to affect the frequency, duration and magnitude of harmful algal blooms (HABs) (Wells et al. 2020). Experimental approaches can give insights in the responses of HAB species and populations, either in isolated experiments of single strains and populations, or within natural communities and ecosystems. Here, we discuss general experimental conditions and approaches as a step towards a more standardized approach, which may facilitate designing and setting up experiments to unravel the potential effect of global environmental change drivers on HABs. Our aim is not to provide an exhaustive overview on all experimental approaches in HAB research, but rather to touch on what we think are important considerations when performing these kinds of experiments. We hope that our chapter helps in finding the appropriate experimental conditions, experimental systems and species' traits to any given global environmental change related question, ranging from fundamental cellular processes to competitive interactions and community dynamics. A more unified experimental approach should improve inter-comparison between studies and, ultimately, to draw more consistent trends that support our predictions on the future of HABs.

We first provide a short overview of the scales covered by different experimental approaches (3.2), followed by a general overview on the isolation of HAB species and general practices regarding culturing conditions (3.3). We subsequently describe a number of experimental boundary conditions (3.4), and provide an overview of the main global environmental change drivers (3.5). Afterwards, we discuss a number of general experimental approaches, as well as the assumptions and applications associated with these different methods (3.6). Lastly, we discuss key traits that provide basic insights in the

responses of HAB species, populations and communities toward global environmental change drivers (3.7).

## 3.2 Scaling of experiments

Experiments that manipulate and control the environment of HAB species are used to understand their responses to environmental change drivers. These experiments can be performed at a range of scales, from small-scale bottle experiments in the laboratory to large-scale mesocosms in the field. Different experimental approaches may be preferred at different organizational levels, ranging from single to multiple strains at the population level, up to multiple species at the community level. Experiments thus scale along a gradient generating a high degree of understanding but lacking natural complexity on the one side, to a high level of natural complexity, but with a lower degree of understanding. For instance, small-scale batch experiments may aid our mechanistic understanding at the cellular level by revealing responses of certain species or strains to changes in specific environmental factors. However, they lack the natural complexity and therefore cannot be directly applied to make projections of how populations will respond to global environmental change drivers. Conversely, large-scale outdoor mesocosms incorporate natural complexity, thereby testing more realistic scenarios of global environmental change. Yet, such experiments generally reveal little understanding of the underlying mechanisms, due to the large number of interactions and confounding factors.

Combining different experimental scales will support our understanding of the impacts of global environmental change on HABs. Obviously, it is unrealistic to cover all scales within one experiment and capture a mechanistic understanding of a very complex system. By applying a comparable set of experimental conditions and analyzing key traits, an experiment at any scale may be coupled to any other experiment at an adjacent scale (from cells to populations to communities to ecosystems, and vice versa). Moreover, testing the same set of traits following similar methods allows comparison across experiments, laboratories, and habitats, thereby improving integration of results, for instance by meta-analytic approaches and modelling. Experiments are an essential source of data for the parametrization and calibration of mathematical models forecasting and projecting global environmental change effects on HABs and translating mechanistic processes at the cellular level to implications for population and community dynamics at the ecosystem level (Chapter 6). In addition, components of existing models can be more easily transferred from one HAB species to another, or from one region to another, with appropriate parameterization and calibration based on intercomparable experimental data.

## 3.3 Culture conditions

### 3.3.1 ISOLATIONS AND ESTABLISHMENT OF STOCK STRAIN CULTURES

Strains of HAB species that have been kept in culture for a long time will adapt to the conditions under which they have been maintained and a strong selection towards strains that best survive in the laboratory will take place (see Berge et al. 2012, Chapter 4). Therefore, we recommend to work on newly acquired cryopreserved strains from culture collections, or freshly isolated strains. New strains can generally be isolated using standard procedures for microalgae (*sensu lato*, including cyanobacteria; Andersen and Kawachi, 2005). Isolation of cells is usually done using a drawn-out Pasteur glass pipette or a microloader pipette tip that does not break as easily as glass pipettes. The best results are obtained using sterile filtered water (0.2 µm pore size filter) from the sampling location at temperatures comparable to the *in situ* temperature at the time of isolation. For this purpose, walk-in climate rooms or temperature-controlled incubators are very practical, but not essential. Nutrients can be added initially at relatively low concentrations (especially in the case of oligotrophic species),

and progressively increase the proportion of the culture media. However, the requirements are very species-specific and some newly isolated cells will need rapid transfer into full strength growth medium. Recipes of different culture media can be found in Andersen et al. (2005). Smaller species, such as some cyanobacteria, are difficult to select by pipette. In such cases, dilution series of field collected water samples can be done, and several automated methods exist such as flow cytometry with cell sorting (for details see Andersen, 2005).

While diatoms and cyanobacteria can be considered “photosynthetic, autotrophic phytoplankton” and grown as such, an increasing number of HAB species within the groups of dinoflagellates, haptophytes, chrysophytes, chlorophytes, and raphidophytes are in fact mixotrophs, combining photosynthesis and heterotrophy through phagotrophy and/or osmotrophy (Jeong et al. 2010, Mitra et al. 2016, Stoecker et al. 2017). Some mixotrophs include organisms that have their own chloroplasts, now generally referred to as “Constitutive Mixotrophs” (CMs; Mitra et al. 2016). Some of these species can easily be grown without prey (facultative mixotrophs), while others (e.g. *Karlodinium armiger*) are dependent on prey for growth using standard nitrate-based media (Berge et al. 2008). Some CMs are quite selective in which prey they ingest, while others are quite omnivorous (e.g. Jeong et al. 2010). Although most of the CM species can be grown without food in monocultures, they may lose the ability to feed in monoculture after some time through acclimation or adaptation (Hansen and Tillmann 2020, see also Chapter 4). Other HAB species, like the ciliate *Mesodinium rubrum/major* complex, and the dinoflagellate *Dinophysis* spp., do not have their own chloroplasts and are currently referred to as Non-Constitutive Mixotrophs (NCMs). They are dependent on the supply of chloroplasts from their prey for growth, although they have considerable control of the chloroplasts (Hansen et al. 2013, Rusterholz et al. 2017). Many of the HAB-NCM species depend on certain prey species; they are specialist NCMs. Culturing these species thus also requires isolation and co-culturing of the associated prey species, which will have their own optimal growth and culturing conditions as well.

A particular group of HAB taxa have a predominant benthic phase (e.g. *Gambierdiscus* spp., *Ostreopsis* spp., *Prorocentrum lima*). They produce mucilaginous substances by which they attach to surfaces such as corals, macroalgae, rocks and sand. In general, they can grow in culture flasks without the natural substrate. Many benthic dinoflagellates also seem to be mixotrophic, likely ingesting bacteria or benefiting from organic compounds supplied by the bacterial metabolism. For some research purposes it may be necessary to work with axenic cultures (e.g. to elucidate the direct and indirect role of bacteria on dinoflagellates growth and toxin production, allelopathy, etc.). However, eliminating bacteria from benthic dinoflagellate cultures has proven very difficult or even ineffective (e.g. Tarazona-Janampa et al. 2020).

### 3.3.2 STOCK CULTURE CONDITIONS

For laboratory experiments with HAB species (and phytoplankton in general), a number of culturing conditions are important to consider for ensuring optimal growth, which usually are the conditions used to establish stock cultures. These include the type of growth medium and nutrient concentrations, light intensity and duration of the light:dark (LD) cycle, temperature, provision of sufficient inorganic carbon, and the need for mixing. Here, we highlight general aspects regarding the culturing of algae and cyanobacteria, and for further details we refer to the excellent overview provided by Andersen (2005).

For marine autotrophic HAB species, the most commonly used growth media are nutrient enriched natural seawater F/2 (Guillard 1975, Guillard and Ryther 1962) and K (Keller et al. 1987). For freshwater and brackish water HAB species (notably cyanobacteria) the typical growth media are WC (Guillard and Lorenzen 1972), BG-11 (Allen 1968) and Z8 (Kotai 1972). The freshwater growth media are typically artificial and prepared using deionized water, enriched with macronutrients, salts, trace elements and vitamins, and are usually sterilized by autoclave (>20 mins at 121°C), but can also be filter-steri-

lized (0.2  $\mu\text{m}$  pore size). The pH often needs to be adjusted, which is done prior to sterilization. When the medium is autoclaved, vitamins are added aseptically afterwards (filtered through 0.2  $\mu\text{m}$  pore size). When investigating isolates from a specific lake, it is recommended to use water from this lake as a base for culture media, with modified nutrient concentrations according to a media recipe, and subsequent filter-sterilization.

Marine growth media often use natural seawater enriched with macronutrients, such as nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ) and silicate (for diatoms), trace elements and vitamins using the same sterilization procedures as for freshwater media. The culture media composition can also be adjusted to represent more natural conditions with often relatively lower nutrient concentrations and adjusted salinities. Usually, marine growth medium may be diluted to e.g. K/10 (i.e. 10 times dilution of K medium). Moreover, marine growth media may be diluted with sterilized deionized water, whereas salts may be added to freshwater growth media to adjust salinities, for instance when growing brackish water species. Seawater sourced from the location where HAB species were isolated is ideally used, though this is not always feasible (e.g. as may be the case of isolates from culture collections, or from contaminated areas).

As an alternative to filtered natural seawater, there are various recipes for artificial seawater. These may be used for instance when seawater is not readily available, or when the exact chemical composition needs to be controlled. Examples of artificial seawater growth media are ASW (Goldman and McCarthy 1978), ESAW (Berges et al. 2001, Harrison et al. 1980), and AQUIL (Morel et al. 1979). In addition, YBCII (Chen et al. 1996) has been developed for  $\text{N}_2$  fixing cyanobacteria and lacks dissolved inorganic nitrogen. However, not all HAB species grow well on artificial media. For better success, species can be stepwise acclimated, with incremental increases of artificial medium mixed with natural seawater. To test whether the medium is suitable, growth rates achieved with artificial medium should be comparable with the growth rates obtained when grown in enriched natural seawater media, and species should show similar morphological and functional characteristics.

Light conditions that typically are used in culture experiments aim to represent the saturating part of the light response curves, and generally range from 50 to 250  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Brandenburg et al. 2018a, 2019b). However, it is advisable to start with lower light intensities, with subsequent stepwise acclimatization to higher light levels if required to reach light saturation. The LD cycle that is selected will depend on the latitude and growing season of the HAB species used for the experiment, generally being 12:12 for species isolated in tropical waters and 16:8 or 14:10 for species isolated in temperate latitudes. Similar to the LD cycles, the temperature used will strongly depend on the latitude of isolation, and may be in the range of 20-28°C for species isolated in the tropics, and 15-25°C for species isolated in temperate regions (Brandenburg et al. 2018a, 2019b). Lastly, aeration is sometimes applied to allow both mixing and a continuous supply of  $\text{CO}_2$ . Yet, various HAB species, particularly dinoflagellates, are known to be fragile to small-scale turbulence (e.g. Berdalet et al. 2007), and potential adverse effects of aeration should be tested by microscopy for morphological aberration, and by comparing overall growth rates. When aeration is not an option, experiments are ideally kept dilute to prevent a drift in carbonate chemistry toward lower  $\text{CO}_2$  concentrations and higher pH values (for further discussion see section 3.6.2). For heterotrophic and some mixotrophic HAB species, culture medium should include a prey source, such as bacteria, phytoplankton, and/or other protists (Mitra et al. 2016, see also section 3.3.1).

## 3.4 Experimental conditions

### 3.4.1 EXPERIMENTAL CONDITIONS AND ACCLIMATIZATION

Before starting an experiment, one has to determine the experimental conditions, which is not trivial. Using the experimental conditions resembling the location from which the cells were isolated may

allow better extrapolation to the local field conditions, yet it complicates comparison across studies and may add confounding factors. Conversely, while growing HAB species from different locations under the same conditions may allow direct comparison among studies, such an experimental design would test for adaptation (i.e., strain variability), and responses would depend on the differences between experimental and local conditions. Both ways of choosing experimental conditions have their advantages and pitfalls, which are important to consider prior to setting up experiments and, importantly, when interpreting the experimental results.

Once the experimental conditions have been established, cells need to be acclimated to these conditions. Acclimatization reduces potential carryover effects from stock culturing conditions, which may differ between laboratories, and acclimation of cells thus allow better comparison across studies. Moreover, species or strains may differ in their ability to adjust their physiology to new conditions. The periods for acclimatization vary (see also Andersen 2005), but we recommend at least ten generations, as this allows a near complete replacement (>99%) of the population by cells grown under the experimental conditions. Please note that when allowing much longer acclimatization periods, i.e. beyond 50, it is important to consider the potential for genetic adaptation (see Chapter 4), which will be species dependent and differ between environmental and climate change drivers (Collins et al. 2020, Reusch and Boyd 2013).

### 3.4.2 CHOOSING CLIMATE AND ENVIRONMENTAL DRIVERS

Experiments addressing the impacts of global environmental change drivers and HABs should obviously target key climate change drivers, such as elevated CO<sub>2</sub> levels, as well as the shifts in climate by itself, such as warmer temperatures. Moreover, they can target indirect environmental changes as a consequence of shifts in the climate and its drivers, including, but not limited to, increased upper mixed layer stratification, and increases and decreases in pH, salinity, nutrient availability, and exposure to PAR and UV radiation (e.g. by shifts in stratification or cloud cover; Boyce et al. 2010, Hutchins and Fu 2017, Moore et al. 2018). To accommodate the increasing complexity of changing conditions, experiments can be scaled from single factor manipulations in single strain batch cultures, to multiple drivers on natural communities in microcosm or mesocosm experiments. These extremes represent a gradient between a reductionist approach, providing information on mechanisms and processes of climate change responses, and a holistic approach targeting the broad impacts of multi-driver scenarios in natural communities (Boyd et al. 2018, Wells 2019). Generally, the projections of the IPCC assessment reports (IPCC 2014 or newer versions) should be considered when testing for impacts of projected environmental and climatic changes. We note that changes do not occur instantaneously, and the response of a species to the expected change might involve long-term evolutionary processes, which may or may not differ from observed plasticity responses (Boyd et al. 2018, Collins et al. 2020, see also Chapter 4).

Responses of HAB species to global environmental change drivers should ideally be tested using environmental gradients. These gradients are most informative when they fall within the range of the observed variability in the natural habitat of the respective species, but also within the range of future projections for these variables in the region of origin of the tested HAB species. Gradients may also extend to more extreme conditions as this may be required to establish the shape of the response curve. Gradients are tested using so-called response curves, which theoretically follow a typical shape based on fundamental physiological or metabolic principles affected by the investigated driver (Fig. 3.1). Between and within species, the response curves may shift in slope and location of the optima, reflecting specific differences in plasticity, and the potential for adaptation (Fig. 3.1, Brandenburg et al. 2018, Litchman et al. 2007). Testing response curves is essential to determine optimal growth conditions for a given species and its maximum (or minimum) tolerable limits. Furthermore, response curves provide fundamental insights in the sensitivity of species' response variable to any climate driver. For example, at certain ranges growth or toxin content may strongly increase, while at other



ranges, these traits may not change or even decline (Fig. 3.1). Revealing the tolerable limits and the fundamental responses along environmental gradients will help understanding the potential effects of natural variability and global environmental change drivers.

Natural variation in temperature and salinity affects HAB species' growth and physiology, with growth responses to temperature gradients often following a unimodal, left-skewed function (thermal reaction norm, Fig. 3.1.A), and response curves to salinity gradients often following a bell-shaped curve (Fig. 3.1.C). For each response curve, its shape determines how strongly a species or strain is affected by a relative change in the environmental driver (see also section 3.5). Natural variations in nutrient concentrations can affect a number of species traits (see also section 3.7), including the toxicity of HABs, and change in growth rates and carrying capacities (i.e. potential bloom magnitude). Growth response curves to nutrient concentrations, as well as nutrient uptake kinetics, reflect a saturation function from nutrient limitation to saturation (Fig. 3.1.B). Growth response curves to nutrient gradients are not only used to assess optimal growth conditions, but are also important to evaluate the competitive ability of a species (for details see section 3.6.2.2). The assessment of response curves by single-driver experiments is also an important prerequisite for the design of multiple-driver experiments by identifying environmental parameters with a strong and potentially superior influence on the overall performance of a species (for details see section 3.5.5, Boyd et al. 2018, Brennan and Collins 2015).

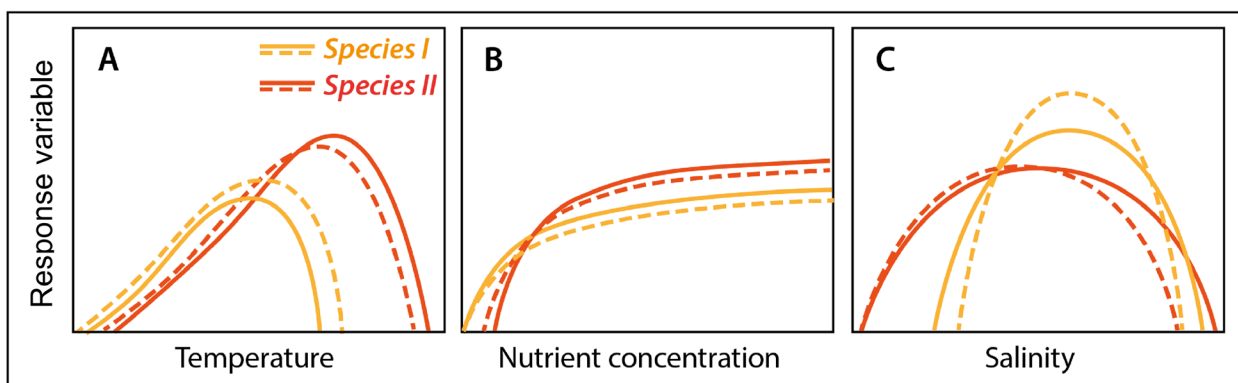


Fig. 3.1. Response curves along gradients of A) temperature, B) nutrient concentrations and C) salinity, where the different lines for the same species represent intraspecific variation of the response.

## 3.5 Global environmental change related drivers

### 3.5.1 CARBONATE CHEMISTRY

Increasing CO<sub>2</sub> emissions have led to enhanced atmospheric pCO<sub>2</sub>, from 280 μatm before the industrial revolution to approximately 410 μatm in 2018 (IPCC 2014, Tans and Keeling 2021). Atmospheric pCO<sub>2</sub> is expected to reach >500 μatm by the end of the century (Rogelj et al. 2016). Roughly 30% of the anthropogenically emitted CO<sub>2</sub> is absorbed by the oceans (Gruber et al. 2019), which leads to substantial changes in the marine carbonate chemistry, most notably a rapid decline in seawater pH (i.e. Ocean Acidification, OA). In more productive coastal and freshwater systems, the impact of elevated atmospheric CO<sub>2</sub> levels on the dissolved CO<sub>2</sub> concentrations remains elusive, as this will depend on numerous factors, such as trophic state, organic carbon loads from the catchment, and microorganisms' respiration in the water (e.g. see Hasler et al. 2016, Sobek et al. 2005).

For testing the effects of changes in dissolved CO<sub>2</sub> concentrations on HABs, earlier guidelines have suggested the use of fixed carbonate chemistry treatment values based on future atmospheric pCO<sub>2</sub> projections for 2100 ranging between 430 and 1,000 μatm (Barry et al. 2011). However, adopting these atmospheric target levels neglects the high variability of carbonate chemistry in the water compared

to the atmosphere. For example, HABs often form in highly productive waters where photosynthetic and respiratory processes can drive dissolved  $p\text{CO}_2$  far out of the range found in the atmosphere from supersaturating to limiting conditions (Hansen 2002, Verspagen et al. 2014). Given the potential for  $\text{CO}_2$  limitation, and also because responses are often non-linear, we recommend that the impact of shifts in  $p\text{CO}_2$  is examined by exposing a HAB species of interest to a gradient of  $\text{CO}_2$  concentrations, ranging from limiting concentrations (e.g.  $<100 \mu\text{atm}$ ) to a number of projected future concentrations, and possibly beyond, in standard growth experiments (see also section 3.4.2.).

Manipulations of  $\text{CO}_2$  levels can be achieved in various ways and there are pros and cons to each approach (for details see Gattuso et al. 2011). The most intuitive approach is to aerate the growth medium with premixed gases that contain the target  $p\text{CO}_2$  levels. Such an air- $\text{CO}_2$  mixture can be obtained commercially or by mixing pressurized air with  $\text{CO}_2$ . When experiments are aerated, the water is directly enriched during growth of the investigated HAB species but this bears the risk that cells get damaged by the bubble stream and may cause profound experimental artefacts (Rost et al. 2008, Van de Waal et al. 2014a). Alternatively,  $p\text{CO}_2$  can be adjusted with the additions of dissolved sodium carbonate (or sodium bicarbonate) solutions in combination with acid or base (Gattuso et al. 2011). Manipulations restricted to the time before inoculation have the disadvantage that carbonate chemistry conditions may change profoundly (and even unrealistically) during growth and photosynthetic  $\text{CO}_2$  acquisition of the HAB species, which could constitute another type of experimental artefact. A third, less common, approach is to aerate a small amount of seawater with pure  $\text{CO}_2$  gas and then use small amounts of this highly  $\text{CO}_2$ -oversaturated water to establish the treatments (Riebesell et al. 2013). Especially for dinoflagellates, which are often sensitive to aeration-induced mixing, this approach is a good alternative for maintaining stable pH conditions during experiments.

Ocean acidification experiments require close control of the carbonate chemistry conditions in experimental systems. This can be achieved in closed systems with no or only a small headspace volume and an airtight seal to minimize changes in carbonate chemistry through air-sea  $\text{CO}_2$  exchange. Furthermore, carbonate chemistry can change substantially during biomass build-up due to  $\text{CO}_2$  fixation, which may be prevented by running dilute batch approaches (see also section 3.6.2.1). Alternatively, when interested in the impacts on population biomass and system carrying capacity, carbonate chemistry can be allowed to drift towards low (and even limiting)  $\text{CO}_2$  concentrations (e.g. Berge et al. 2010, Hansen et al. 2007). Manipulation of  $\text{CO}_2$  concentrations in water by aeration with different levels of  $p\text{CO}_2$  will lead to changes in not only  $\text{CO}_2$ , but also pH, total DIC, as well as inorganic carbon speciation in the water. Thus, studies in which the  $p\text{CO}_2$  has been manipulated without correcting for changes in pH and DIC cannot differentiate the effects of changes in  $\text{CO}_2$  concentrations from the direct pH effects, nor changes in DIC. Many, if not all marine phytoplankton, as well as many freshwater species, can utilize bicarbonate ( $\text{HCO}_3^-$ ); in fact, they are often predominantly  $\text{HCO}_3^-$  users (e.g. Eberlein et al. 2014, Ji et al. 2020, Price et al. 2008, Rost et al. 2006, Trimborn et al. 2008). Thus, increases in  $\text{HCO}_3^-$  concentrations, for example through an increase in pH or DIC, may enhance inorganic carbon acquisition. Changes in pH may also directly affect growth of phytoplankton, where an increase or a decrease in pH can even become growth limiting (Berge et al. 2010, Hansen et al. 2007). Studies of two harmful dinoflagellates (*Amphidinium carterae* and *Heterocapsa oceanica*) have demonstrated that a reduction in external pH from 8 to 7 was associated with a lowering of intracellular pH, and may have been the cause of a decreased growth rate (Dason and Colman 2004, Giraldez-Ruiz et al. 1997, see also Kallas and Castenholz 1982). Thus, carbonate chemistry should be monitored in order to account for the sensitivities of the carbonate system and exposed species.

Carbonate chemistry conditions in water can be assessed by measuring two different parameters of the carbonate system and calculating the other parameters with open access software such as CO2SYS (Pierrot et al. 2006) or seacarb (Gattuso et al. 2020). Measurable carbonate chemistry parameters are pH, total alkalinity, dissolved inorganic carbon ( $\text{DIC} = \text{CO}_2 + \text{HCO}_3^- + \text{CO}_3^{2-}$ ),  $p\text{CO}_2$ , and  $\text{CO}_3^{2-}$  concentration. The analytical methods to determine these parameters differ widely and each method

has distinct advantages and disadvantages. Open access “Best practices guides” are available to help scientists entering this research field with setting up and using the best analytical techniques (Dickson et al. 2007), and designing meaningful ocean acidification experiments (Riebesell et al. 2011). We highly recommend making use of these guides to navigate around the numerous pitfalls associated with the diverse range of carbonate chemistry analyses and manipulations.

### 3.5.2. TEMPERATURE

Mean global air temperatures will continue to rise over the 21<sup>st</sup> century if CO<sub>2</sub> emissions are not reduced. In current scenarios, the projected temperature increase at the sea surface for the next century is 1 to 10°C, depending on the region. The most dramatic increases in sea surface temperatures are expected for high latitudes (Hutchins and Fu 2017, IPCC 2014). Temperature manipulations can be achieved by incubating experimental units (cultured isolates, experimental communities) in temperature-controlled incubators, water baths, and incubation chambers. It is important to consider that temperature responses are unimodal (Fig 3.1.A), and experiments with temperature are ideally performed with multiple (>3) temperature treatments to include the full reaction norm (see also section 3.4.2). Another way to account for this is to expose cultures to a gradient of temperatures, e.g. in a temperature gradient bar (or a temperature table; Hinnert et al. 2017, Watras et al. 1982). These are typically metal devices cooling on one end of the table/bar and heating on the other end. Such devices, allowing the simultaneous testing of multiple temperatures, are particularly suited to generate temperature response curves (i.e. reaction norms) of response variables, which describe temperature adaptations of an organism and respective trait responses.

### 3.5.3 SALINITY

Climate change will cause shifts in salinities of marine and freshwater systems. In some regions of the world, sea surface salinities will rise due to enhanced evaporation, while in other regions, particularly cold-temperate and arctic waters, more precipitation, river runoff and melting ice will cause freshening of coastal waters (Durack et al. 2012) where HABs predominantly occur. Determining salinity reaction norms will provide information on the capacity of species to deal with shifts in salinity conditions. Marine species with narrow salinity range preferences are expected to be affected to a greater degree than coastal and estuarine species that often have wide tolerance spans to salinity. The aim of salinity experiments can be to determine the range of salinities supporting growth and maintenance. For this purpose, cultures may be grown at different salinities, starting from prevailing salinities, then doing successive stepwise transfers into lower and higher salinities, after acclimatization periods of several generations at each new condition (e.g. Suikkanen et al. 2013).

Similar to temperature, salinity responses are unimodal (Fig. 3.1.C), and responses to salinity should be tested across a gradient of salinities in order to determine the full reaction norm. A salinity gradient can be created by diluting artificial seawater using deionized water. Dilution of natural seawater may risk reducing concentrations of distinct chemical compounds to which species might be adapted. This, in turn, may compromise the interpretation of such experiments as actual salinity responses might be affected by micro-nutrient concentration and composition. Note that prior to using artificial seawater, some acclimatization and testing is required (see also section 3.3). In freshwater salinity experiments, salinity should be increased with a representative combination of minerals. Otherwise, sodium chloride can be added to achieve a salinity gradient, with stepwise acclimatization to the increasing salinities (Tonk et al. 2007). When using water from natural freshwater environments, it is important to consider hardness and conductivity of the water as determined by presence and relative concentration of minerals and salts. When designing culture experiments, e.g. with toxic cyanobacteria, these conditions need to be examined prior to experiments, as they can substantially affect growth and toxin production (Carneiro et al. 2013).

### 3.5.4 NUTRIENTS

Climate change will also affect the availability of nutrients in open oceans, coastal areas and inland waters. Surface ocean warming leads to enhanced stratification, which can limit inorganic nutrient supply (from upwelling mechanisms) and affect its chemical speciation in coastal and open ocean regions. In contrast, human activities such as discharge of organic and inorganic nutrient-rich water from agriculture and sewage can lead to eutrophication of coastal zones and inland waters (Berman and Bronk 2003, Heisler et al. 2008, Smith et al. 1999) and alter nutrient ratios (N:P). N and/or P often limit phytoplankton growth in most coastal regions while large parts of the open ocean, and some coastal regions, can be limited by iron (e.g. the Southern Ocean or North Pacific, Moore et al. 2013; the Californian upwelling regions, Hutchins and Bruland 1998). Changes in the supply of nutrient concentrations and forms can strongly impact HAB development.

With phytoplankton growth, nutrient levels decrease and may shift from non-limiting, to limiting and ultimately causing starvation (see also Figs. 3.1.B and 3.4.A). Thus, it is difficult to control nutrients as a treatment, particularly in batch experiments (for further details see section 3.6.2.). This is due to the nature of the growth response to nutrient concentrations, which reflects a saturating function (as shown in Fig. 3.1.B). Such a saturation function is characterized by the maximum uptake or growth rate ( $V_{\max}$  or  $\mu_{\max}$ ), the half saturation constant ( $K_{1/2}$ ), and the uptake or growth affinity (Van de Waal and Litchman 2020, see also section 3.7.3).

When assessing the effect of nutrient limitation, other factors should be kept under optimal conditions. Also, when testing nutrient limitation, high biomass build-up should be prevented (e.g. by providing the limiting nutrient at sufficiently low concentrations), as that may lead to marked shifts in pH,  $\text{CO}_2$ , DIC, and light. When using batch cultures, sampling at least during the mid-exponential, late exponential and early stationary phase is recommended in order to allow the assessment of responses at different growth phases from non-limiting ( $\mu = \max$ ) and limitation ( $0 < \mu < \max$ ), to starvation ( $\mu = 0$ ). Nutrient concentrations at the time of sampling should be reported.

We note that many HAB species, particularly dinoflagellates, are able to utilize organic nutrient sources (e.g. Berg et al. 1997, Collos et al. 2014, Hattenrath-Lehmann and Gobler 2015, see also section 3.3). This will allow them to persist even when they have low growth rates and affinities for inorganic nutrients, provided that the cultures are not axenic (which is often the case).

### 3.5.5 MULTIPLE DRIVERS

Global environmental change in marine and freshwater environments involves a combination of changes, including elevated  $p\text{CO}_2$  and temperatures, as well as shifts in light availability, UV irradiance, and nutrient concentrations (Doney et al. 2012, IPCC 2014). Although an increasing number of HAB studies combine different global environmental change drivers, for instance elevated  $p\text{CO}_2$  or warming combined with nutrient limitation (Eberlein et al. 2016, Fu et al. 2008, Sun et al. 2011), our understanding of these putative interactive effects is still at its infancy (Van de Waal and Litchman 2020). We therefore encourage future experiments testing for multiple factors, from cells to communities, in order to better approach the intricate complexity of climate change impacts on HABs (Boyd et al. 2018, Wells et al. 2015).

Scaling up experiments from single to multiple drivers can become impractical, however, as adding more levels of each driver causes the number of experimental treatments to grow exponentially (Fig. 3.2.A). When investigating three or more drivers (or single strains compared to populations or communities), it might be considered to move from a mechanistic gradient approach (response curves) to a scenario-based approach (e.g. IPCC scenarios), or to combine both approaches to reduce the number of treatments (Fig. 3.3.B; Boyd et al. 2018). This, though, requires careful prior consideration regarding the choice of drivers and their combinations (see Boyd et al. 2018 for further details).

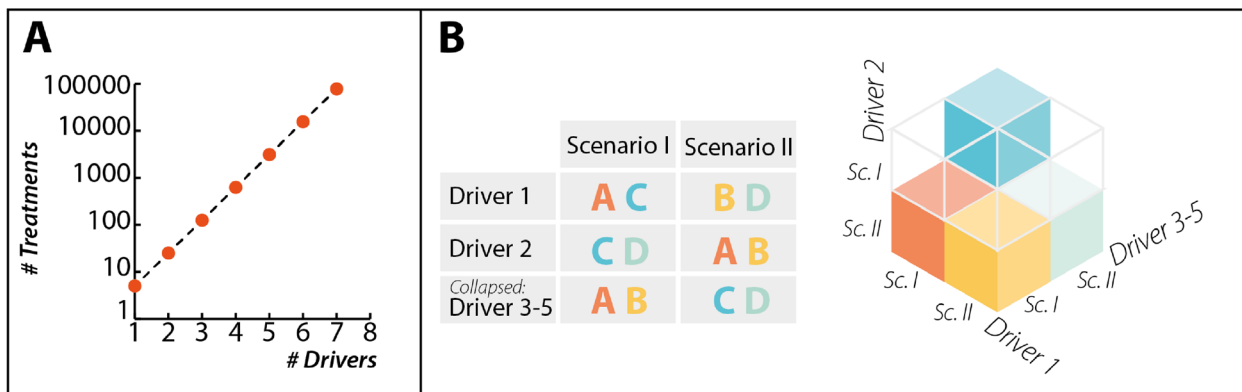


Fig. 3.2. A) Number of possible treatment combinations arising from the incorporation of multiple drivers in an experimental design with a gradient of 5 levels per driver. B) Example of a scenario-based experimental design that includes two main drivers (Driver 1 and 2), and three further collapsed drivers (Driver 3 - 5).

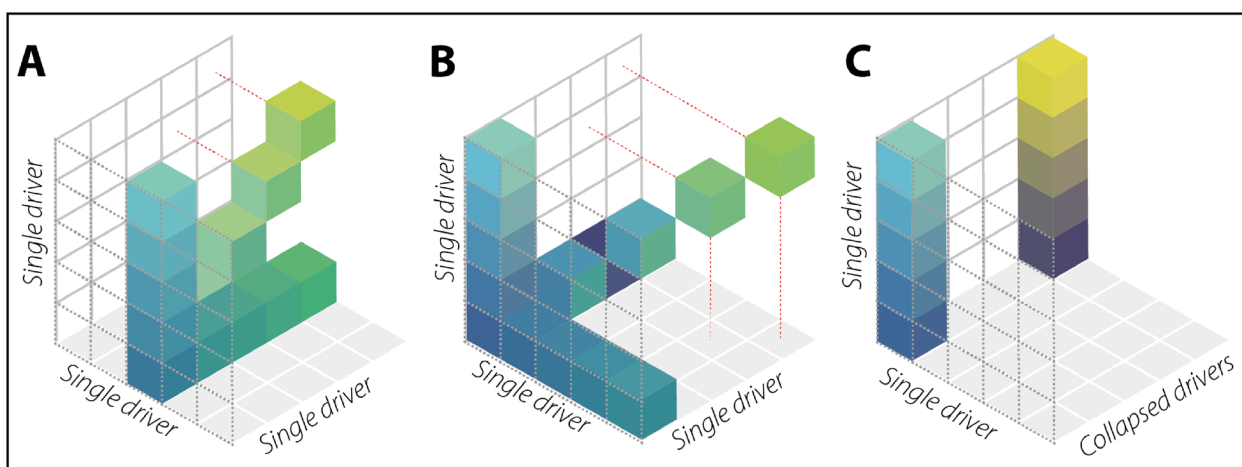


Fig 3.3.: Examples of experimental designs to investigate the interactions of multiple drivers. A) Example of a reduced design with two drivers (gradients) and the exclusion of lower-order interactions, B) example of a reduced design with three drivers (gradients) and the exclusion of lower-order and two-way interactions, C) example of a collapsed design with a single driver gradient tested against two levels of multiple drivers collapsed into one treatment. Modified after Boyd et al. (2018).

Experiments that investigate the interactions of multiple drivers can be designed as full-factorial, reduced or collapsed designs, or as hybrids (Fig. 3.3). A full-factorial design, i.e. the investigation of several levels of each driver and all their combinations to construct response curves, generally has a practical limit of three variables (Boyd et al. 2018). A reduction in the number of interactions (“reduced design”; Figs. 3.3.A,B) can be achieved by testing the effects of each driver independently and the combined interactive effects of all drivers together, by excluding lower order interactions (Fig. 3.3.A) and two-way interactions (Fig. 3.3.B; see also Gunst and Mason (2009) for further details). Another alternative is the “collapsed design”, where gradients of the effect of a main driver of interest, or a driver with a superior effect, are tested against all other drivers of interest that are “collapsed” into a second combined driver (Fig. 3.3.C). The “collapsed design” thus creates a matrix similar to the two-way full-factorial design, but with several variables of interest.

## 3.6 Experimental methods

### 3.6.1 LARGE SCALE EXPERIMENTAL SYSTEMS – MESOCOSMS

Mesocosms are artificial enclosures of (semi-)natural ecosystems which can be used to study HAB responses to climate change variables. There is little consistency in the literature at what point a “microcosm” becomes a “mesocosm”. In general, mesocosms vary considerably in size, from less than

0.1 m<sup>3</sup> to over 1,000 m<sup>3</sup>, and have been developed for both indoor and outdoor experiments (Stewart et al. 2013). Indoor mesocosms have the advantage of controlling climatic conditions such as irradiance, wind and rainfall, while outdoor experiments have the benefit of testing impacts of global environmental change drivers under natural climatic variability. The timing of operating mesocosms for HAB research should align with the natural occurrence of the respective HAB species of interest. Land-based mesocosms (both outdoor and indoor) ideally use natural water with associated plankton communities. Communities used to seed experiments are often sieved (e.g. <200 µm) to exclude larger-sized plankton, as well as vertebrates, such as amphibians and fish. While this procedure is common in mesocosm experimentation, it affects the composition of the food web and trophic cascades, and therefore it is arguably the degree of realism of the experiment. Indeed, we emphasize that confinement of communities, even in large mesocosms, will lead to artifacts (indicated below) that must be considered for the interpretation of the data (Bach and Taucher 2019, Carpenter 1996, Sarnelle 1997).

Mesocosms come closest to capturing the complexity of natural ecosystems, while maintaining some form of control on the applied experimental conditions and treatments (Schindler 1998, Stewart et al. 2013, Stibor et al. 2019). They have the advantage that a water mass containing natural communities can be monitored over a relatively long period of time without the problem of horizontal advection. This allows the investigator to observe species succession patterns, which helps to understand the emergence of HABs. Furthermore, mesocosms enable abiotic or biotic manipulations that may not be allowed in natural environments (e.g. enrichment by nutrients). Although mesocosms have been applied to HAB research (Brussaard et al. 2005, Kana et al. 2004), and are increasingly applied to climate change studies (Stewart et al. 2013), the number of experiments using mesocosms to test responses of HABs to climate change are limited (Hansson et al. 2013, Riebesell et al. 2018, Wohlrab et al. 2020).

Through their usually large size, the operation of mesocosms also comes with a number of challenges, including limitations on the number of systems for different treatments and sufficient replication, personnel and infrastructural demands, and experimental treatment control. Clearly, having more experimental units is desirable as it avoids choices regarding the number of treatments and/or replicates. When only a limited number of systems is available, experiments can be performed with sufficient replicates, but with a limited set of treatments, or, vice versa, along a treatment gradient lacking replication. Natural communities enclosed in mesocosms involve many species and trophic levels that can range from viruses and bacteria to heterotrophic protists, zooplankton, and in some cases fish and/or amphibians, in addition to the phytoplankton and HAB species' communities. To account for this complexity, it is advisable that all trophic levels and presumed key organisms are quantified in the sampling and analysis program, as well as the assessment of dissolved and particulate nutrients, and measurements of the distinct treatment parameters. In the case of time and budgetary limits to such a more exhaustive sampling program, it may be advisable to involve colleagues with complementary expertise, as this may greatly improve our understanding of the system and its response to global environmental change drivers.

Important methodological pitfalls to avoid in mesocosm experimentation include (1) biofouling, (2) accumulation of sinking materials, and (3) impacts of excessive sampling. Biofouling on the walls of mesocosms can lead to considerable nutrient consumption and to pronounced changes in the mesocosm light environment and should therefore be kept at a minimum. Wall growth can be reduced, either by regular physical removal to prevent development of epiphytes (Riebesell et al. 2013, Verschoor et al. 2003), or by the inclusion of polypropylene strips that can be used to assess and account for epiphyton densities (Kazanjian et al. 2018, Roberts et al. 2003). Dealing with biofouling is not trivial and should be considered early in the process of developing the experimental design as it is central for the data quality. Accumulation of sinking organic matter at the bottom of the mesocosms can create anoxic microhabitats when the material degrades. It is therefore advisable to remove this material before accumulation becomes significant. This can be done by collecting sinking material in conical sediment

traps at the bottom, with the additional advantage that this material can be used for investigations of organic export out of the euphotic zone (Boxhammer et al. 2016). Excessive sampling of certain components of the food web (e.g. copepods) can lead to selective removal of organisms and alter trophic cascades. This problem can be accounted for by approximating the growth rate of the sampled organisms and adjusting the sampling volume and frequency to reduce the sampling impact. Additionally, it is advisable to employ non-invasive sampling tools as much as possible to avoid this effect (e.g. use underwater cameras instead of plankton nets to quantify organisms).

### 3.6.2 SMALL-SCALE EXPERIMENTAL CULTURING – BATCH AND CONTINUOUS MODE

Small-scale culturing experiments can capture the responses of strains of single species, as well as pre-defined populations and communities. These systems may address intraspecific and interspecific interactions, as well as controlled grazing and parasitism. To maintain control, small-scale systems typically lack the more complex multi-interaction network of natural food-webs. Controlled small-scale culturing studies can provide mechanistic understanding of responses of specific cellular processes toward environmental variables, while experiments with multiple strains, both separate and together, can reveal how general these responses are for species and populations. Testing multiple phytoplankton species, including co-occurring non-HAB species, and/or effects of single grazer and parasite control informs about the impacts of global environmental change drivers on community and trophic interactions, as well as their feedback on HAB species. The strength of such small-scale culturing experiments is particularly exploited when kept simple, and allows testing many variables and strains, with targeted combinations of species and/or higher trophic levels. Importantly, small-scale culturing experiments can easily include a control treatment and sufficient replicates to gain insights in the statistical variation and thus the significance of the findings. In the following sections, we focus on experiments testing phytoplankton. We refer to Chapter 6 for further details on trophic interactions.

#### 3.6.2.1 BATCH CULTURING

With batch culturing, cells are grown without any ongoing dilution and it represents the most basic type of experimental system. Batch experiments can be performed in volumes as small as a micro-well plate to, technically, any large size. The choice of the experimental volume mainly depends on the number of desired treatments and replicates, as well as the amount of biomass required for measuring the various parameters that characterize the functional traits (see also section 3.7). Importantly, any form of biomass should be monitored through time to establish a growth curve, and assess the duration of exponential growth and timing of the stationary phase. It is recommended to include both biovolume and cell counts as biomass indicators, since cell sizes may change with treatments and along the growth phases, and it is especially relevant for diatoms like *Pseudo-nitzschia* (e.g. Montresor et al. 2016). We also note that chlorophyll-a and carbon can also be sensitive to experimental treatments, and that inferences based on these biomass indicators should be carefully considered.

When provided with saturating light conditions and non-limiting nutrients, at the optimal temperature and salinity, the exponential phase will closely represent the maximum growth rate. Allowing cultures to shift from exponential growth to non-exponential growth, and ultimately reach the stationary phase, implies a limitation. Ideally, any limitation is imposed as a treatment, for example with reduced nutrients in the culture medium. Under these conditions, cultures will reach limitation at the end of the exponential phase, and starvation during the stationary phase where nutrients are depleted and growth becomes zero (Fig. 3.4.A). Thus, conditions in traditional batch experiments are not stable, and vary with biomass build-up. To minimize these changes, systems can also be run as 'dilute batch', where cultures are harvested at the early exponential phase (Fig. 3.4.B). Under these conditions, shifts in nutrients (or any other resource) can be controlled within any pre-set limits (e.g. allowing <5% changes in a certain resource). In addition, harvesting cells in the early exponential phase minimizes the risks of light stress due to increased shading, thus avoiding unintended "multi-stressor

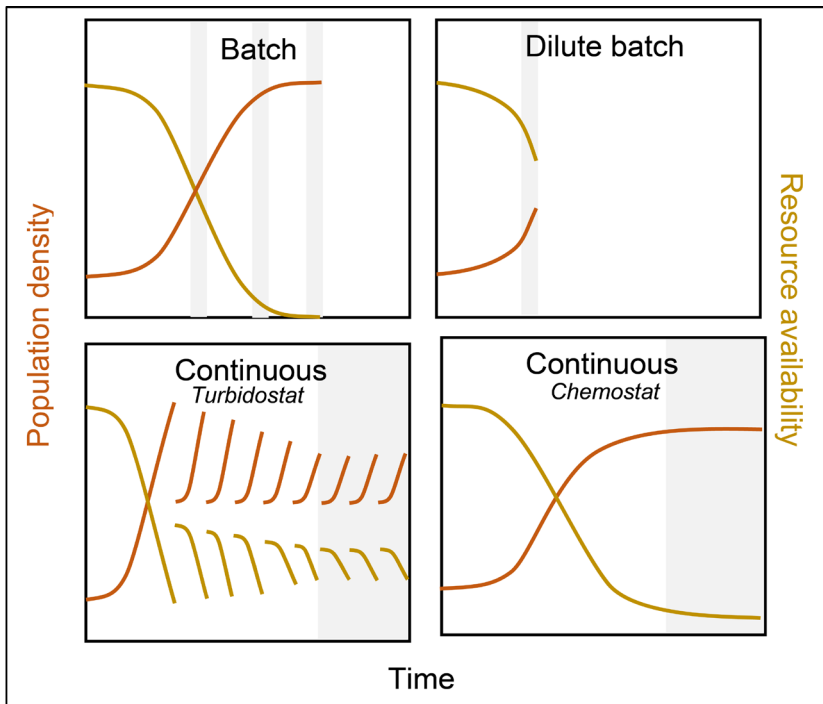


Fig. 3.4. Schematic overview of population density (orange solid lines) and resource availability (yellow dashed lines) dynamics in different experimental systems with (A) Batch, (B) Dilute batch, (C) Continuous with controlled biomass (i.e. turbidostat), and (D) Continuous with controlled dilution rate (i.e. chemostat). Grey areas in (A) denote distinct culture phases (assuming negligible lag-phase) with exponential, limited and starved, in (B) only exponential, in (C) exponential or limited, and in (D) limited.

experiments". For a dilute batch approach, sufficient acclimatization is a prerequisite, as the impact of any lag phase can have a considerable effect on estimated growth rates and estimates on physiological responses.

From the various global environmental change drivers, testing the impacts of light, nutrients, salinity and temperature are relatively straightforward. For example, effects of nutrients and salinity can be tested by adjusting the culture medium to the appropriate treatment levels, while for temperature the experiments are performed in temperature-controlled climate rooms, incubators, and water baths, or using cooling jackets or a cooling finger. As indicated earlier (in section 3.4.2), effects of these treatments are non-linear and thus temperature gradients should be tested in order to establish reaction norms and allow for trend analysis, especially for salinity, temperature and  $p\text{CO}_2$ , as responses to these drivers have been shown to be non-linear. For nutrients, growth responses are also non-linear, following Monod-type saturation curves (Monod 1950, 1949, see also section 3.4.2). For batch experiments, it is recommended to carefully estimate biomass over the duration of the experiment to assess the timing of growth phases, allowing sampling for trait analysis at least once for each growth phase. Because the carbonate chemistry is highly sensitive to biomass build-up, the impact of  $\text{CO}_2$  on growth is ideally tested in a dilute batch approach, or semi-continuous cultures with regular manual replacement of culture with fresh growth medium. Experiments running into the stationary phase may allow testing the impact of any driver on the carrying capacity (e.g. de Senerpont Domis et al. 2014, Riebesell and Tortell 2011).

An alternative to dilute batch cultures are exponential fed batch cultures (Fischer et al. 2014) in which fresh growth media is continuously added proportionally to the culture volume. Consequently, the culture volume and supply rate of the growth medium increase exponentially. Under such experimental conditions, exponential growth can be maintained over prolonged periods of time (Fischer et al. 2014).

### 3.6.2.2 CONTINUOUS CULTURING

Continuous cultures involve the ongoing dilution of an experimental system with growth medium. This can either occur in a turbidostat mode with a variable pumping rate to target a predefined biomass, or



in a chemostat mode with a fixed pumping rate to target a predefined net population growth rate. As such, continuous cultures have the great advantage of achieving near maximum growth (turbidostat) or nutrient limited growth (chemostat) for sustained periods of time and under well-defined conditions (Figs. 3.4.C-D). In the continuous mode, a fresh medium with nutrients is pumped in, while a spent medium with algal biomass is pumped out. Consequently, cultures experience the dilution as a 'mortality' factor, preventing full nutrient depletion, and allowing sustained growth where the extent of limitation can be controlled (e.g. see also Tilman 1982).

Continuous cultures are ideally used for testing impacts of global environmental change drivers on HAB-related traits that are closely linked to growth rate (e.g. toxin synthesis). At steady state growth conditions, net growth rates (determined by the nutrient inflow) are the same across treatments, and thus allow testing of the direct effect of any global environmental change driver on toxin quota (e.g. for  $p\text{CO}_2$ : Eberlein et al. 2016, Sun et al. 2011, Van de Waal et al. 2009). Moreover, they allow testing of the impacts of combined environmental drivers, for instance exposing nutrient limited chemostats to a gradient of  $p\text{CO}_2$  or temperature. Moreover, continuous cultures can be used to test synergistic and antagonistic interactions between HAB species, as well as between HAB and non-HAB species, and they have applied for studies on resource competition (Passarge et al. 2006, Tilman 1982, Van de Waal et al. 2011), evolution (Novick and Szilard 1950, Rosenzweig et al. 1994, see Chapter 4), and natural community dynamics (Harrison and Davis 1979, Hutchins et al. 2003, Sommer 1985). Note that classical continuous culture devices cannot be used for benthic HABs, because these microalgae attach strongly to the surfaces. Thus, most studies on benthic HABs are confined to using batch and semi-continuous systems. Continuous flow chambers designed for coral studies (e.g. Yates and Halley 2003) might be suitable for studying benthic HABs as well, though that remains to be tested.

### 3.7 Traits

Trait-based approaches are increasingly used to study the effects of changing environmental conditions on phytoplankton community composition and functioning (e.g. Kruk et al. 2017, Litchman and Klausmeier 2008, Weithoff and Beisner 2019). Knowledge of trait changes are key to the understanding of climate change effects on phytoplankton (Roselli and Litchman 2017, Van de Waal and Litchman 2020). Focusing the experimental design on measuring key traits in HAB experiments will greatly facilitate cross study comparisons, and furthermore support scaling from cellular functional traits to population and community dynamics.

Functional traits are properties of an organism that describe its life history, behavior, physiology and morphology and thereby define ecological functions such as reproduction, resource acquisition or defense against predation (Table 3.1, Litchman and Klausmeier 2008, McGill et al. 2006, Weithoff and Beisner 2019). Traits determine the success of species in communities under specific environmental conditions. Trait changes along gradients of environmental drivers not only describe the response of phytoplankton species, but can also identify functional consequences, and thereby allow the coupling of different organizational levels and experimental scales (Duan et al. 2021). Moreover, experimentally derived trait data, specifically of trait changes, can be used for the parameterization of mathematical models (see also Chapter 6).

Changes of traits along relevant gradients of the major global environmental change drivers yield reaction norms that describe the phenotypic plasticity of any trait of interest (for details see section 3.4.2; see also Chapter 4), defining plastic ranges of the examined isolate, and at the same time may indicate the potential for selection and adaptation under changing environmental conditions (Collins et al. 2020, 2014). Although measuring changes in traits across gradients of environmental drivers is laborious and time consuming, this approach will be important for defining the reaction norms needed to assess and model the impacts of global environmental change drivers on HABs (see also Chapter 6).

Table 3.1. Overview of HAB functional traits and a number of examples (for further details see Litchman and Klausmeier 2008, McGill et al. 2006, Weithoff and Beisner 2019).

Type	Trait	Examples	
Morphological	Cell volume/size		
	Growth form	Colonial Filamentous	
Physiological	Light acquisition	Pigment type Accessory pigments	
		Carbon acquisition	Carbon Concentrating Mechanisms Photosynthesis
	Nutrient acquisition		Nutrient uptake kinetics Nitrogen fixation Mixotrophy
		Biochemical	Toxin production Allelochemical production
			Resting stage formation
	Behavioral	Vertical migration	

In order to improve our overall understanding of HAB species' responses to global environmental change, it would be highly valuable to gain insights to the responses of a comparable set of traits that determine the growth, competitive success and toxicity of HAB species. These include cell size, toxin production, toxin quota, and allelopathy, as well as a number of more specific additional traits, such as inorganic carbon and nutrient acquisition, and resting stage formation. The regulation of traits may also be associated with elemental quotas and ratios, which can therefore potentially serve as 'currency' to unify HAB ecology across taxa, habitats, and scales (Brandenburg et al. 2020, Duan et al. 2021, Meunier et al. 2017).

### 3.7.1 GROWTH RATE AND CELL SIZE

Growth rates describe physiological responses at large, are highly plastic, and often respond directly to changes in environmental conditions. In experiments testing effects of global environmental change drivers, growth rates are typically used as a response parameter whereas in evolutionary studies they are often considered as a measure of fitness (see also Chapter 4). Growth rates along environmental gradients are most commonly determined in batch experiments on cultured isolates using standard methods (Wood et al. 2005), but can also be calculated for target species within natural communities, for instance in mesocosm experiments (see section 3.6.1.). Growth rates are estimated from measurements of a proxy of biomass (e.g. cell number, biovolume, chlorophyll-a content, carbon content, in-vivo fluorescence; see section 3.6) over several days (see also Wood et al. 2005).

Calculation of growth rate in batch cultures is based on the exponential growth phase, which should be identified from several (a minimum of 3) time points (see also Fig. 3.4). Identification of the exponential growth phase can be visually assessed by expressing the biomass parameter in logarithmic

scale against time. The exponential phase can also be numerically determined by calculating the changes in growth rate over the course of the experiment; the period where growth rates are constant and highest reflect the exponential phase with maximum growth (under the imposed conditions). These growth rates are typically based on the specific growth rate, which is calculated following:  $\mu = (\ln N_t - \ln N_{t-x}) / (t_t - t_{t-x})$ , where  $N_t$  and  $N_{t-x}$  refer to the biomass (as biomass unit per volume of water) at time point  $t$  and an earlier time point  $t-x$  (in days), respectively. The unit for specific growth,  $\mu$ , is the reciprocal  $d^{-1}$ . Further details can be found in Wood et al. (2005).

Cell size or volume is a morphological trait that is closely linked to growth, reproduction, cellular metabolism and resource acquisition, as well as various ecological interactions including predation, and as such has been considered a master trait (Brown et al. 2004, Litchman and Klausmeier 2008). Small cells generally grow faster and are more efficient in the acquisition of limiting nutrients compared to larger cells. Generally, cell size is less plastic as a trait than growth rate in single taxa, and will respond to changes in environmental parameters more at the level of community assembly than within a species (Litchman and Klausmeier 2008). Plasticity in size may occur as a result of accumulation of carbon under nutrient depletion, as well as the development of a food vacuole for heterotrophic or mixotrophic HAB species. Consequently, an increase in cell volume can be observed in batch cultures when entering into the stationary phase, if nutrients become limiting. Moreover, especially in the case of dinoflagellates, increases in cell size may also reflect the change from a vegetative phase to resting stages or sexual reproduction. Changes in cell sizes are important to determine how relevant biochemical parameters or traits change as a function of HAB species' biomass response to an environmental change driver. As an example, if toxin content per cell increases proportionally with cell size under any given driver, shifts in toxin content may not result from actual changes in toxin synthesis, but rather from intracellular accumulation or release. Expressing toxin contents by cell volume should avoid such limitations, and may reveal actual shifts in toxin synthesis. We note that while most toxins are measured as intracellular compounds, toxins could be released into the medium during the exponential or stationary phase, as shown in the case of domoic acid (e.g. Godinho et al. 2018). For these toxins, it is therefore important that the extracellular fraction of toxins is also assessed in order to gain insights in their overall synthesis.

### 3.7.2 TOXIN PRODUCTION AND ALLELOPATHY

Toxin production represents the most prominent harmful effect of HABs on human and animal health, and knowledge of responses of this trait is particularly relevant for the assessment of impacts of environmental change drivers on HAB toxicity. Toxins produced by HAB species represent a variety of chemical structures, including several unknown compounds, which can either be expressed constitutively along environmental gradients or whose production is enhanced or fully inducible based on the prevailing conditions (Lassus et al. 2016). The vast majority of these secondary metabolites are derived from basic biosynthetic routes through enzymatic transformations, which can be further modified into various structural analogues (Cembella 2003). As such, they have not evolved with a particular purpose but are either present because they confer a selective advantage or are biologically neutral. This hinders attempts to clearly assign a particular ecological function to this group of secondary metabolites, and increased toxin levels can therefore be associated with changes in other traits. For example, changes in nutrient availability can strongly affect cellular toxin content in HAB species. This effect is often attributed to an accumulation of toxins within the cells when growth is restricted but photosynthesis and assimilation of non-limiting elements persists, so that primary metabolite precursors and secondary metabolites become excessively present (Bates et al. 1991, Brandenburg et al. 2020, Van de Waal et al. 2013). Grazing by copepods seems for some species to represent a 'true' induction signal for toxin production, but also depends on nutrient availability (Harðardóttir et al. 2015, Selander et al. 2008, 2006, Tammilehto et al. 2015).

The toxicity of cells is not only determined by the total toxin cellular content, but also the toxin profile (contribution of different toxin analogues), which may also change in response to environmental change drivers (e.g. Fu et al. 2010, Liu et al. 2016, Tatters et al. 2013, Van de Waal et al. 2010, 2009). It is advisable to monitor changes in toxin production and composition along with other traits (e.g. growth rate and cell size), as this allows a more comprehensive view on the way toxins are modulated. Importantly, knowing not only growth and biomass responses towards global environmental change related drivers, but also those of toxin content and composition, will improve our overall understanding of mechanisms underlying HAB toxicity.

HAB toxins primarily comprise compounds that accumulate through the food web and are classified with reference to the human toxin syndrome they cause (Cembella 2003). Some HAB species produce toxins affecting fish specifically and causing massive mortalities on aquaculture and fisheries (e.g. Andersen et al. 2015, Mardones et al. 2015). Yet, many HAB species also produce so-called allelochemicals, with adverse effects on co-occurring competitors or grazers (Legrand et al. 2003, Ternon et al. 2018). Allelopathy therefore is an important trait that determines ecological interactions and can cause patterns of dominance or changes in the community structure independent of competitive or trophic interactions (John et al. 2015, Legrand et al. 2003). Chemical compounds underlying allelochemical interactions are largely of unknown structure, hence they can only be quantified indirectly, which is often labor- and time-intensive. Generally, toxicity or allelopathy tests include bioassays that assess the effect of the allelochemicals on other species, most notably the ability to lyse *Rhodomonas* cells (e.g. Ma et al. 2009, Tillmann et al. 2008), or the ability of unknown compounds to lyse blood cells (Eschbach et al. 2001). Although allelochemical production is generally considered as an important trait for the growth of many HAB species, quantification has often been neglected, in part due to technical limitations, in part because the nature of the chemical agent is unknown.

### 3.7.3 SPECIFIC ADDITIONAL TRAITS

Besides the key traits considered above, other more specific traits may also greatly support our understanding of the functional responses of HAB species toward changing environmental conditions. They provide further insight into the mechanisms of physiological responses, on the acclimation capacities of the studied organism, their competitive abilities, and may furthermore help to explain the success of certain species (also in relation to other non-HAB species) under changing environmental conditions, particularly when involving multiple drivers.

Photosynthetic performance is described by traits such as cellular chlorophyll concentration, maximum rates of photosynthesis ( $P_{max}$ ), light compensation point ( $I_k$ ) and photosynthetic efficiency (i.e. initial slope; alpha) that can be obtained from  $P-I$  curves based on photosynthesis rates determined along a light gradient. These can reflect different light use strategies among taxonomic groups and/or communities, as well as the acclimation potential to low light levels, relevant under, for instance, bloom conditions (Litchman and Klausmeier 2008). Moreover, these traits are used as photosynthesis parameters for models on primary production (Bouman et al. 2018). Fluorescence can be used as *in vivo* proxy to assess photosynthetic performance and primary productivity (for details see Suggett et al. 2011).

Nutrient uptake traits include the maximum nutrient uptake rate,  $V_{max}$ , the half saturation constant for nutrient uptake,  $K_{1/2}$ , and the nutrient uptake affinity  $\alpha$  ( $V_{max}/K_{1/2}$ ; see also Van de Waal and Litchman 2020). Together, these traits characterize the nutrient uptake strategy of a species, population or strain. Generally, nutrient uptake traits are highly plastic, and can differ considerably among taxonomic groups (Litchman et al. 2007), and between genotypes within a population (Brandenburg et al. 2018). Specific nutrient uptake strategies are typically associated with certain habitat conditions and lead to dominance of respective phytoplankton groups. However, several HAB species may also use alternative strategies to outcompete other phytoplankton. For instance, mixotrophic species with capacity to utilize organic nutrients or ingest preys may thus escape traditional resource competition.

Life cycle traits include the formation of resting stages and sexual reproduction, complex processes that determine the potential to survive unfavorable conditions, colonize new habitats and generate intraspecific diversity that promotes adaptation (Chapter 4). Transitions between actively growing cells and resting stages can be quantified in experimental studies (Hinnert et al. 2017). Yet, documenting and quantifying sexual reproduction it is not straightforward as it requires observation of living cultures (Figuerola et al. 2005), or population genetic analyses (Montresor et al. 2016). In the case of dinoflagellates, life cycles are particularly complex as they include the formation of asexual, ecdysal or temporary cysts (e.g. Bravo and Figuerola 2014) that can also be induced as a response to changing environmental conditions.

Some additional traits only apply to certain groups of phytoplankton, such as  $N_2$  fixation in cyanobacteria, allowing species to acquire inorganic nitrogen from atmospheric  $N_2$ , and mixotrophy, allowing species to acquire organic carbon and nutrient sources. Both traits may provide a competitive advantage under nutrient-limited conditions, that is common in both freshwater and marine systems (Elser et al. 2007). Nitrogen-fixing (diazotrophic) cyanobacteria use the nitrogenase enzyme to convert atmospheric  $N_2$  gas to ammonia. In environments with recurring cyanobacterial blooms,  $N_2$  fixation by diazotrophic cyanobacteria may play a significant role in shaping the entire plankton community (e.g. often in anthropogenically eutrophied lakes, Smith et al. 1999; in the Baltic Sea, Karlson et al. 2015). Mixotrophy is common in many HAB taxa, especially dinoflagellates, haptophytes, and raphidophytes. In constitutive mixotrophs, which have their own chloroplasts, mixotrophy allows some species to compensate for carbon limitation during light limitation and for nutrients under nutrient-limiting conditions (Hansen 2011, Smalley et al. 2003). However, food uptake may in some species be stimulated by light (e.g. *Karlodinium veneficum*, *Dinophysis* spp. and *Mesodinium rubrum*), and some species will feed irrespective of the availability of nutrients (e.g. *Fragilidium* spp., Hansen 2011, Hansen et al. 2013). Most of the studies on food uptake in mixotrophs have been done in the laboratory, where the prey is offered live. In field studies, small plastic beads (1-2  $\mu$ m), and radioactive or fluorescently labelled prey (e.g. bacteria) have been used in tracer studies in nano-sized mixotrophs (Beisner et al. 2019). In micro-sized mixotrophs, live prey with a different fluorescence profile (e.g. cryptophytes) or prey that have been labelled can be used. Quantification of the percentage of cells with vacuoles has typically been done using an epifluorescence microscope or a flow cytometer (see Hansen and Tillmann 2020).

Although not directly considered a trait, the cellular elemental composition (e.g. C, N and P) may reflect the regulation of physiological traits, including toxin production (Brandenburg et al. 2020, Meunier et al. 2017, Van de Waal et al. 2014b). Elemental composition of phytoplankton is particularly sensitive to changes in light, nutrients and  $CO_2$  levels (Geider and Roche 2002, Sterner and Elser 2002). Elemental ratios can be assessed both in single organisms, populations, and entire communities, and may thus serve as a common 'currency' connecting multiple organizational scales. For instance, ecosystem models require changes in C, N and P fluxes, as well as ratios therein, which are typically parameterized based on single strain studies to allow model projections for entire ecosystems (e.g. Hense et al. 2013). Thus, the inclusion of elemental analyses in HAB experiments will allow comparison across studies, taxa, and habitats, a more effective use of data in ecosystem models, and a common currency linking different organizational scales including detrital contribution as a source of nutrients.

### 3.8 Recommendations

1. Newly isolated strains should be taxonomically characterized using a combination of morphological and molecular data. Additional information concerning site, date, natural environmental conditions at the origin, and stock growth conditions should be provided in order to build up a

database on the strains used to investigate the effects of climate drivers on HAB taxa. For further details see Chapter 5;

2. Experimental studies aiming to investigate environmental effects on harmful algal or cyanobacterial species should ideally use regional climate and environmental change projections as treatment values;
3. Experimental studies should report general stock culture and experimental conditions, including species/strain names, temperature, pH, etc. to allow comparison across studies;
4. Experiments should prevent drifts in carbonate chemistry and/or studies should monitor and report fully constrained carbonate chemistry (pH, CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, alkalinity). For further details, see earlier guidelines (Dickson et al. 2007, Riebesell et al. 2011);
5. Use low levels of a limiting resource, while providing other resources in ample supply in order to avoid high biomass build-up and/or co-limitation by other resources;
6. In order to account for non-linear responses of HAB species to global environmental drivers, more studies should test gradients rather than single treatments;
7. More studies should incorporate multiple global environmental change drivers, as these factors do not act alone;
8. Include key competing non-HABs, as proliferation of HABs will also depend on responses of competing species;
9. Quantify key traits such as growth rate, cell size, nutrient uptake rates, cyst formation, and toxin production to assess responses to change in experiments;
10. Use standard analytical methods to establish trait values and to facilitate intercomparison.

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# CHAPTER 4

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## Studying the acclimation and adaptation of HAB species to changing environmental conditions

Gwenn M. M. Hennon<sup>1</sup>, Josefin Sefbom<sup>2</sup>, Elisa Schaum<sup>3</sup>, Sonya T. Dyhrman<sup>4,5</sup> and Anna Godhe<sup>2†</sup>

<sup>1</sup> College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks, Alaska, USA

<sup>2</sup> Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden

<sup>3</sup> Institute for Marine Ecosystems and Fisheries Science, University of Hamburg, Hamburg, Germany

<sup>4</sup> Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY, USA

<sup>5</sup> Department of Earth and Environmental Sciences, Columbia University, New York, NY, USA

† Deceased before final submission

### 4.1 Overview of acclimation and adaptation

Studying adaptation and acclimation in HAB species is at the very core of understanding their responses to predicted future climatic scenarios. For definitions of key terms such as acclimation and adaptation used in this chapter, please refer to Box 1. When organisms are exposed to changes in their environment, they will respond through a combination of migration, acclimation and adaptation, or become extinct. The relative contributions of acclimation, adaptation, or extinction will co-vary with the genetic architecture of the population or species and severity and direction of the change. Teasing apart the influences of acclimation vs adaptation on a phenotype is ultimately not always straightforward, as they are non-exclusive processes.

Acclimation involves changes in the phenotype of an individual organism without a corresponding change to the genetic code (West-Eberhard 2003). If and when environmental conditions later revert to the initial state, acclimation is reversible such that an acclimatized organism can then re-adjust

#### Box 1: Definitions of key terms

Acclimation – physiological habituation of an organism to a change in a particular environmental factor.\*

Adaptation – evolutionary process involving genetic change by which a population becomes fitted to its prevailing environment.\*

Climate driver – an environmental parameter expected to be influenced by climate change (e.g.: temp, CO<sub>2</sub>).

Response variable – a physiological or genetic parameter measured after altering a climate driver.

\* *Henderson's Dictionary of Biological Terms (12<sup>th</sup> Ed.)*



to the earlier phenotype. Acclimation allows for survival over multiple habitats because acclimation facilitates tolerance of sub-optimal conditions (Bradshaw 1965, Pelletier et al. 2009). The ability of an individual to acclimate to new conditions, also termed phenotypic plasticity, can occur over relatively short times (<1 – 10s of generations). Plasticity is thought to have costs such as a decreased overall growth rate or decreased fitness in other conditions, although these costs have proved difficult to quantify (Auld et al. 2010, Chevin et al. 2010). Plasticity should be favored when these costs are minimized (Pigliucci 2001). Theory predicts that unstable environmental conditions should select for enhanced phenotypic plasticity (Botero et al. 2015, Ashander et al. 2016, Haaland and Botero 2019), thus phenotypic plasticity can also be considered an adaptation to variable environments.

Adaptation to a new condition is an evolutionary process that is generally not reversible and is associated with a trade-off or cost of decreased fitness in the previous condition while increasing fitness in the novel environment (e.g., Collins and Bell 2004). In general terms, studying adaptation entails a comparison between contemporary/derived and ancestral populations or species, *i.e.* there is a historical aspect. Evolution alters the genetic make-up of populations, therefore the contemporary gene pool will be different from the ancestral gene pool and thereby ancestral and contemporary populations will differ in their trait distributions under the same conditions. For an evolutionary response to occur, at least one generation must pass, however in all but the most detrimental conditions evolution has been observed to occur over at least ~70-100 generations (Lenski et al. 1991, Schlüter et al. 2014, Schaum and Collins 2014). Poorly suited phenotypes are out-competed, and eventually the fitness of the population or species is tweaked towards a trait optimum, as favored genotypes and the involved alleles become more common. The relatively rapid generation time of many HAB species permits studying adaptation using experimental evolution within selection experiments. Selection experiments can be used to characterize the ability of individuals, populations, and communities to adapt to new conditions by subjecting a population to projected climate drivers over a course of many generations. At the end of the selection experiment, reciprocal transplant experiments can be used to compare trait optima between ancestral and derived (evolved) populations (Collins 2011).

In addition to the 'fast forward' approach of experimental evolution, the life cycles of some phytoplankton species allows for a 'rewind' approach using resurrected resting stages. Many microalgal species (including HAB species) form resting stages that can be revived up to 100 years after they formed (Lundholm et al. 2011, Härnström et al. 2011). This allows for a 'rewinding the tape' approach where physiology can be compared between present and past populations (Ellegaard et al. 2013). If a species forms resting stages, the genetic diversity harbored in the sediments can function as a seed-bank that buffers the population from crashing via continuous reseeded with viable cells with different physiological capabilities (Kremp et al. 2016, Sundqvist et al. 2018). Therefore, it has been hypothesized that seed-banks can circumvent some of the need for adaptive phenotypic plasticity in an unstable environment, by allowing the coexistence of differentially adapted genotypes. To disentangle the degree to which a population has acclimated to a specific condition or has specialized by adaptation, a good starting point is to run a set of experiments to establish a reaction norm of physiological responses to climate drivers and thereby assess the tolerance range and the phenotype of acclimation.

## 4.2 Acclimation-Tolerance Studies

Most studies on the impacts of climate change on harmful algae focus on short-term acclimation or tolerance experiments. Tolerance studies measure the limits of an organism's niche and where it can live (e.g.: what are the upper and lower bounds of temperature or salinity) while acclimation measures what an organism is doing (e.g.: how fast is it growing, changes to other physiological parameters) within the limits of its tolerance range. Acclimation and tolerance experiments are designed to be performed on short timescales (tens of generations) and can investigate the acclimation responses and tolerance range of whole communities or isolates from extant or seedbank populations. The major

benefits of acclimation experiments are their short duration, the ease at which multiple drivers can be tested, and the greater number of strains or replicates that are logistically possible to test (see Chapter 3).

Acclimation studies are useful for screening a large number of cultured strains or mixed microbial communities to study their responses to a single or multiple climate drivers. Acclimation studies assess the underlying physiological responses of the species/population/community on short time scales where responses are likely not due to heritable genetic change. Examples of previous studies that have tested the responses of HAB species and co-existing phytoplankton to CO<sub>2</sub> and temperature perturbation include Kremp et al. (2012) and Tatters et al. (2012b, 2018). Other examples include experiments that survey the acclimation responses from both HAB and non-HAB formers from the same broad functional groups (Hennon et al. 2017). Such studies can help constrain whether acclimation experiment results should be generalized to a wide or narrow range of marine microbial taxa, however studies with non-HAB phytoplankton groups suggest that intra-specific variability can be on par with or exceed inter-species variability (Schaum et al. 2012, Hattich et al. 2017), so more information on strain variation is needed. Most model projections of phytoplankton ecology are currently based on acclimation studies that generalize across broad functional groups of phytoplankton (e.g.: diatoms and dinoflagellates as in Follows et al. (2007) and Dutkiewicz et al. (2015). A greater library of acclimation studies with HAB species could improve the taxonomic resolution in such models to the point that they could become useful for HAB prediction (Hennon and Dyhrman 2020, Chapter 6). As model projections show, the success of organisms in future climate scenarios is dependent on both their functional response and the response of other organisms occupying a similar niche (Dutkiewicz et al. 2015). Therefore, it is important to constrain the response variables of harmful algae and their nearest competitors to future climate conditions (see Chapter 3).

Investigation of both single, and a combination of climate variables in multi-driver experiments (Boyd et al. 2015), are tractable in acclimation studies. Studies suggest that future climate conditions can have interactive effects on HAB species (Sun et al. 2011, Fu et al. 2012, Tatters et al. 2012a), meaning that the functional response to different climate drivers cannot be assumed to be additive. To constrain the choice of climate drivers and strains Boyd et al. (2018) recommend reducing the matrix to the most impactful climate drivers for your region and the organism that is most important from a policy perspective. The study of HAB species has obvious importance to regional policy and communities, so these should be prioritized in studies of phytoplankton responses to climate change. It is recommended to use several (>3) treatment levels for a climate driver to assess the reaction norm (response curves) of each species or strain even at the expense of replication (Boyd et al. 2018, Chapter 3). To guide the choice of experimental design Boyd et al. (2019) developed a tool to assist researchers in exploring the outcomes of several theoretical climate drivers (<https://meddle-scor149.org/>). Acclimation experiments should ideally explore the limits of an organism's tolerance to a climate change driver and find non-linear responses with respect to that driver. The results of these experiments can guide the choice of climate drivers for a multi-driver study and the levels of perturbation appropriate for longer term adaptation experiments.

Studies have shown that different timescales of acclimation to a perturbation in climate drivers like elevated CO<sub>2</sub> can also produce different physiological responses. For example, Kim et al. (2013) found that growth rates and swimming speeds of the raphidophyte *Heterosigma akashiwo* were significantly slower for cells after a short acclimation (1-5 generations) to elevated CO<sub>2</sub> than after an acclimation period of 11-12 generations, and furthermore, the highest CO<sub>2</sub> concentration required longer acclimation times to reach stable physiology. Hennon et al. (2014) found that the enhanced carbon uptake of the diatom *Thalassiosira pseudonana* at elevated CO<sub>2</sub> decreased after cells were acclimated for an additional 20-33 generations in a nitrate-limited chemostat. These results suggest that acclimation to multiple drivers (e.g., nutrient limitation and elevated CO<sub>2</sub>) or more extreme changes (e.g., very high CO<sub>2</sub> concentrations) may require longer acclimation times for cells to reach stable physiology.

Performing a pilot study to determine the period of acclimation after which a cell has reached a stable response is useful here and provides crucial knowledge about the duration required to acclimate strains under different culture conditions and for cells with different life history strategies, or evolutionary backgrounds. Once established, the duration of acclimation can also provide important constraints for future models that attempt to capture realistic seasonal or short-term variability in climate variables. In practice, studies comparing physiology after shorter versus longer acclimation periods in phytoplankton are rarely reported in the literature so if a pilot study is impractical, an acclimation period of at least 10 generations is recommended to ensure adequate acclimation.

Although they are of clear value, acclimation experiments can only measure short-term responses to climate drivers whereas most climate perturbations will occur gradually relative to phytoplankton generation times and potentially allow species to adapt. As some studies suggest however, it may be possible to infer how a species is likely to evolve based on how a species acclimates to a given condition (Collins et al. 2013). For example, Schaum and Collins (2014) showed that the plasticity of 16 phytoplankton strains correlated with the direction and magnitude of the evolutionary response (Schaum and Collins 2014).

#### 4.2.1 ACCLIMATION EXPERIMENT RECOMMENDATIONS

- Carry out a pilot study to estimate the timescales of acclimation and tolerance ranges for each species.
- If a pilot study is impractical, allow for at least 10 generations to reach stable acclimation and less than 50 generations to avoid adaptation.
- Take advantage of the relative ease of acclimation experiments to study single strains of HAB and non-HAB species in isolation and characterize their reaction norms with respect to one or two dominant climate change drivers.
- Once reaction norms to climate drivers are established for a single species or strain, use these results to guide the choice of perturbation level for longer term, multiple species, and multiple-driver experiments.

### 4.3 Adaptation Studies

It is vital to understand how HAB and non-HAB species (which compete for limiting resources) will evolve under future conditions to predict how HAB prevalence will be impacted by climate change. Two complementary techniques—resurrection ecology and experimental evolution—are potentially useful for estimating rates and extent of adaptation and evolution of HABs to climate drivers. Because HABs are single-celled protists or cyanobacteria with growth rates on the order of 0.5–2 day<sup>-1</sup>, it is possible to run experiments for hundreds of generations of asexual reproduction in the timespan of about one year to estimate adaptation rates to future climate conditions. In addition, the formation of resting stages is a known feature of several important groups of HABs (e.g., the dinoflagellate *Alexandrium* and the cyanobacterium *Nostoc*), allowing these groups to be resurrected from deep sediments to study adaptation. Resting stages of other non-HAB dinoflagellates were shown to be capable of surviving > 100 years buried in sediment (Hinnert et al. 2017) and can be revived to measure the rate of adaptation of these populations to environmental changes (climate drivers) that have occurred over the past century (Figure 1).

The field of resurrection ecology utilizes the resting stages of ancestral populations and thereby offers a promising method to investigate HAB responses to environmental change. Physiological and genetic/molecular exploration of past and present populations can reveal how species have historically coped with change. Often it is also possible to estimate the time-frame over which adaptation or evolution has occurred by dating the sediment core and back tracking to significant events (e.g., pre-industrial and post-industrial) (Figure 1). For example, Godhe et al. (unpublished) isolated resting stages of

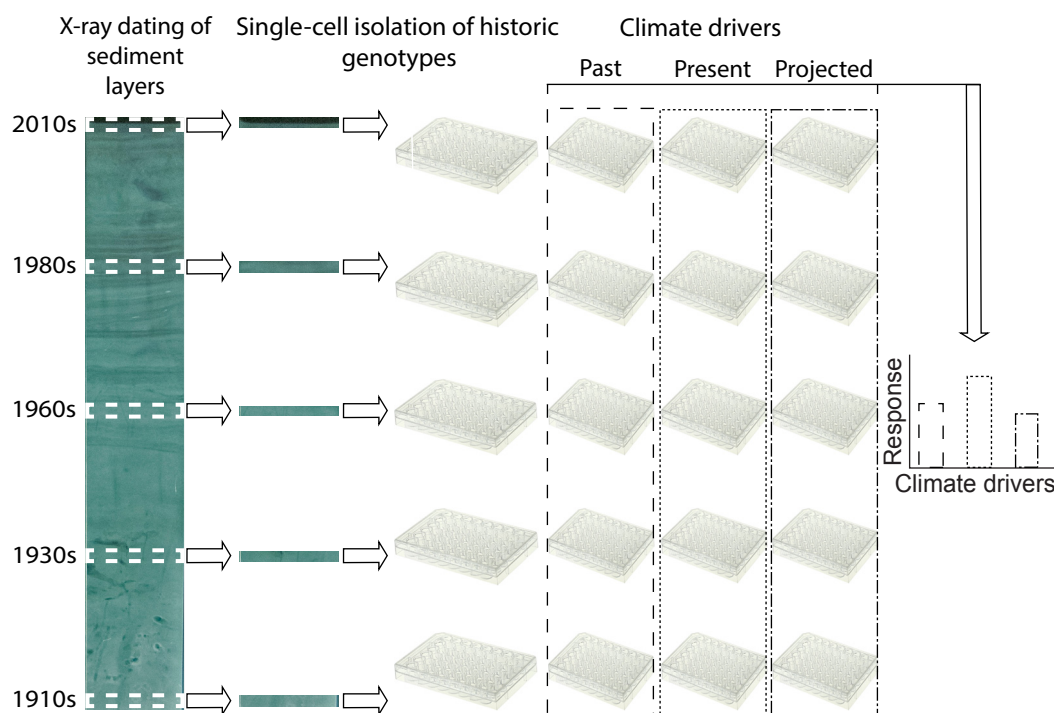


Figure 1. Workflow for resurrection ecology experiments. First, collect a sediment core from the field. The layers in the sediment core are then dated to estimate sedimentation rate. The core is then sliced (often ~1 cm thick), with the outer parts removed so as to prevent contamination between layers. Resting stages can then be isolated from each layer, and once revived, physiological and molecular examination of past and present populations can be carried out under various climatic drivers of interest.

the diatom *Skeletonema marinoi* from recent and historical populations and investigated their adaptive response to the increased eutrophication caused by agricultural intensification in the surrounding area of a Danish fjord. Resurrection ecology allows for comparisons of past and present strains both in terms of their physiology and genetics, it is also a valuable analog for climate change in that it integrates multiple drivers over thousands of generations including natural variability in the context of whole ecosystem interactions. The drawbacks of resurrection ecology are that only certain species form resting stages, there may be a degree of selection in which strains are able to be revived in the laboratory setting, and the environmental changes of the past may not be good analogs for changes in the future. To minimize the potential for laboratory resurrection bias, it is important to mimic historical conditions as closely as possible when attempting to resurrect resting stages. It is also important to perform physiological and molecular examination (e.g., genome, transcriptome sequencing) of the resurrected strains as soon as possible after germination to avoid experimental evolution.

In addition to resurrection ecology, another approach for studying evolution and adaptation is experimental evolution (i.e., selection experiments). Experimental evolution approaches have been used to examine many aspects of evolutionary biology, including the process of microbial adaptation (Lenski et al. 1991, Bell 1997, Cooper et al. 2003). Generally, representatives of a population or species are exposed to one or more drivers for sufficient generations to either generate mutations *de novo* or for evolution to occur through sorting of existing standing genetic variation (see review by Kawecki et al. 2012). Often, representatives of the same genotype(s) are kept under conditions without the driver to account for evolution in response to control or laboratory conditions. Some species can be cryopreserved and serve as ancestral representatives that allow researchers to replay the evolution experiment and test the reproducibility of the outcome (Blount et al. 2008). After a period of hundreds to thousands of generations, the evolved and ancestral (cryopreserved or 'lab-evolved') populations are examined for differences in fitness, phenotypic traits, and/or changes in the genetic code.

The choices of population size, initial genetic diversity and number of generations are key decisions in designing a selection experiment. In contrast to acclimation and tolerance experiments, selection experiments require a larger number of generations, usually >250 (Reusch and Boyd 2013). Although, depending on the system examined and the question posed, this number will vary. For example, in an experiment conducted on a genetically diverse population (e.g., natural population or artificially mixed set of genotypes), evolution may be largely driven by rapid sorting, and occur within less than 100 generations. For low-diversity asexually growing cultures, a minimum of 250 generations is probably required to observe adaptation (Lenski et al. 1991). When the initial genetic architecture is limited (as is the case when starting from a single clone), evolution is expected to occur largely by de novo mutation and/or substitution. In addition, the fitness advantage of a mutation will greatly influence the number of generations needed for it to become a majority or fixed in the population (Elena and Lenski 2003) and some multi-step beneficial mutations may require > 20,000 generations (Blount et al. 2008). In general, it is thought that mutations that convey small fitness advantages require more generations to become fixed (Kawecki et al. 2012).

Exponential growth rate is the most commonly used fitness response variable for asexually reproducing organisms such as bacteria (Elena and Lenski 2003). While this metric may be appropriate for bacteria evolving in a detrimental environment, HAB species have shown a mixture of responses to climate drivers such as increased CO<sub>2</sub> concentrations (Raven et al. 2019), including enhanced growth. Therefore, exponential growth rate may not be the most useful response variable for all studies, especially when organisms are evolving in what would have to be considered an ameliorated environment. Hence, it is advisable to also consider other attributes such as toxin production, swimming behavior, and the suitability of the organism as a food source in selection experiments with HAB species to determine the potential for evolution of a trait that is important to a species in its respective ecosystem (see Chapter 3).

The greater control that experimental evolution provides allows researchers to test species' responses to future conditions, replicate and replay evolution under single or a combination of climate drivers with the starting genetic diversity of their choice (ranging from clonal or multi-species). This method can be used with HAB species (Flores-Moya et al. 2012) and other phytoplankton that do not form resting stages. Some of the drawbacks of experimental evolution include that they require long time commitments that may not be tractable for slower growing species or species that are difficult to cultivate in the lab. Often in experimental evolution the conditions imposed in a laboratory setting are extremely simplified compared to an organism's natural habitat. Rarely are conditions as stable in nature, as they are during a typical selection experiment (Collins 2011), although experimental evolution is now increasingly carried out in multi-driver experiments (Flores-Moya et al. 2012) and fluctuating conditions (Schaum and Collins 2014, Schaum et al. 2018). The difficulty of long-term selection experiments will necessarily restrict the number of replicates, species, and conditions that are possible to test. Because experimental evolution is simplified, the experiments are suited to uncovering the mechanisms of adaptation to a single or few climate drivers, but may miss important interactions between species or be performed on too short a timescale to capture the mutations needed to make bigger evolutionary leaps. Ultimately, although there are several limitations to performing adaptation experiments (both resurrection ecology and experimental evolution), they are essential tools for studying the response of HAB species to climate change.

#### 4.3.1 ADAPTATION EXPERIMENT RECOMMENDATIONS

- Resurrection ecology experiments are best suited for species with resting stages from regions where historical environmental change is a good analog for future climate drivers and where species interactions or complex natural conditions create high diversity.
- When attempting to resurrect resting stages, try to mimic the historical conditions as closely as possible to avoid bias in which strains are revived.

- Test the physiology and genetics of resurrected strains as soon as possible (within 10s of generations) to avoid experimental evolution.
- Before beginning experimental evolution, perform a power analysis to determine the number of replicates needed to observe a small and large effect size between the control and selected populations.
- If possible, cryopreserve an ancestor of the starting genotype(s) to allow for 'replay' selection experiments and comparisons between the ancestor, the control, and adapted phenotypes.
- Unless pilot studies or previous experiments suggest otherwise, plan for a minimum of 250 generations of experimental evolution (longer for weaker selection pressure), test the effect size every 50 generations or so to determine if adaptation has occurred.
- Consider whether climate drivers are stable or fluctuating in the species' natural environment and whether mimicking the natural fluctuations is feasible for the experiment.
- Consider measuring more than one response variable (e.g., growth rate, toxin content, cell size, chemical composition, competitive fitness) that is appropriate to the ecology of your organism.

#### 4.4 Considerations for acclimation and adaptation experiments with HABs

In addition to the recommendations above, there are a number of special considerations for studying how climate change will impact harmful algal species. Previous guides exist for outlining the best practices in culturing phytoplankton for ocean acidification experiments (LaRoche et al. 2010), multi-driver experiments (Boyd et al. 2019), measuring carbonate chemistry parameters (Dickson et al. 2007), and Chapter 3 of this guide covers experimental considerations for climate drivers and response variables for HABs in greater detail. This section focuses on general considerations for experimental design for acclimation and adaptation experiments rather than the technical details of measurements and culturing. In designing an acclimation or adaptation experiment, the investigator will be faced with many choices such as: the choice of climate driver conditions (including their timescale of exposure and variability), and the choice of which response variables to measure, all guided by the choice of species and the geographical region of interest (Figure 2).

Choice of HAB species and geographical region are the key elements of experimental design in the study of the impacts of climate change on harmful algal blooms (Figure 2A). Ideally, the researcher should consider the most important HAB threats to their region of interest and also identify other non-HAB species that occupy a similar niche to study the relative fitness change with an acclimation or adaptation experiment. It may also be important to consider how rising temperatures in particular may allow for new HAB threats to emerge in higher latitudes *via* range expansion (Barton et al. 2016, Gobler et al. 2017). The choice of geographical region may also be influenced by the HAB species of interest, with the researcher using previous literature on the habitat range of a HAB species to define a region of interest. Once the species and region of interest have been identified, these choices can be used to inform the choice of climate and response variables to be measured.

For maximizing the usefulness of acclimation studies on HABs for modeling applications, it is important to use a range of realistic present and future climate conditions and measure a functional curve for each response variable for each species (as in Figure 2B). The choice of climate drivers (e.g., temperature, CO<sub>2</sub> combinations thereof), which is discussed in greater detail in Chapter 3, would ideally be based on environmental parameters thought to influence the fitness of HAB species relative to their closest competitors; however these relationships are often unknown. Literature from the geographical region of interest can inform the baseline environmental conditions and climate projections can be based on the representative concentration pathways (RCP) scenarios for CO<sub>2</sub> (Ciais and Sabine 2013) which project the climate outcomes under different greenhouse gas emissions scenarios. A gradient of five or more levels within individual treatments will help define a functional response curve ranging from baseline to different projected future conditions for each climate variable (see also Chapter 3).

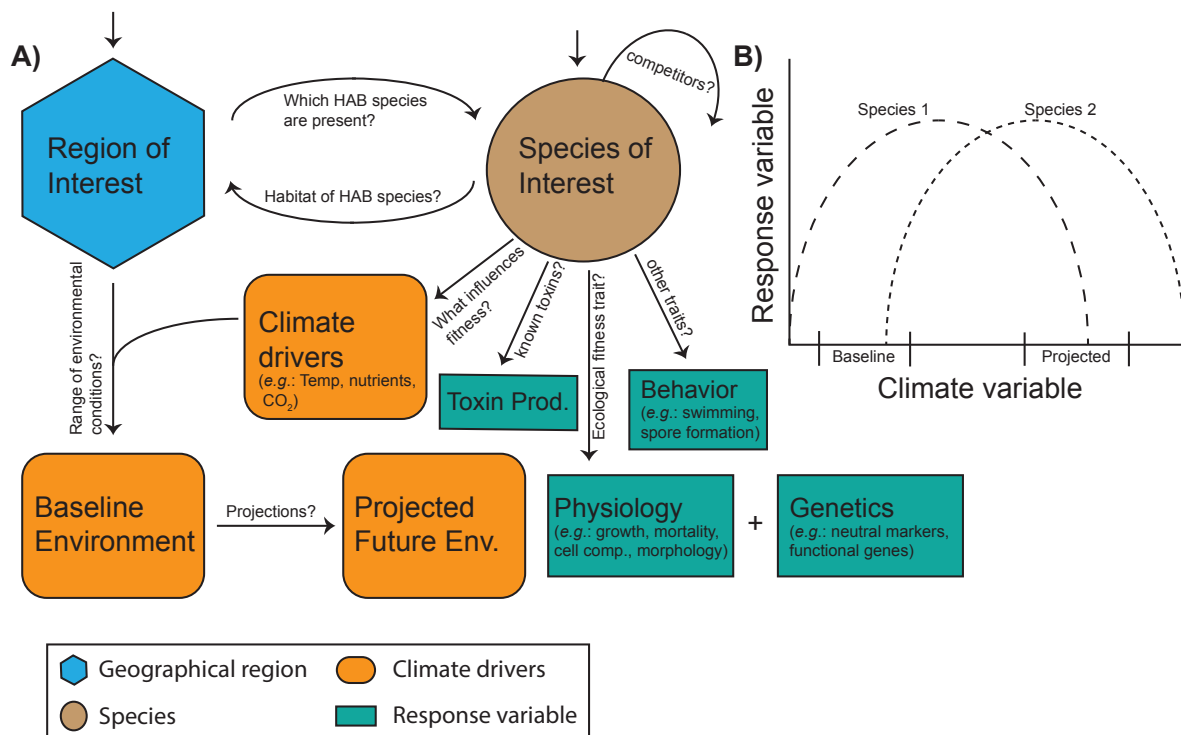


Figure 2: Experimental design considerations to determine the responses of HAB species to climate change. (A) Starting with a decision about either a geographical region of interest or a HAB species of interest, the diagram outlines a series of guiding questions that will aid the experimentalist in choosing climate drivers and response variables as well as the range of values that should be assayed. (B) The theoretical response outcomes of the experimental design are shown for two competing species under baseline and projected climate conditions.

Previous studies have shown that functional response curves of HAB species can be non-linear (Hennon et al. 2019). Extreme conditions will be necessary to establish the shape of the functional curve (e.g. very low or high temperatures or CO<sub>2</sub> concentrations that are at the limits of species tolerance). Such extremes in climate variables may be criticized in that they do not represent realistic future scenarios, however they will be appropriate for species that experience large environmental fluctuations such as in coastal and highly seasonal regions. Thus the choice of environmental perturbation should also consider the natural variation in the ecosystem as a guide in the experimental design process.

The choice of response variables to measure in each experiment must be guided by the choice of species as well as the variable's importance for guiding modeling forecasts (Figure 2, see also Chapter 3). For example, growth rates are the most common response variable needed as input to ecological models and should be reported if at all possible, but mortality, lag phase duration and biomass yield can also be informative as to the cell physiological state (Hennon et al. 2018). Toxicity is another key variable for forecasting the impact of harmful algal species, however not all the toxins are characterized, so the ability to measure toxicity is species-specific. Behavioral changes, such as in swimming, can also have important impacts on harmful bloom formation and have been shown to be altered by changes in pH (Kim et al. 2013). Formation of resting stages is another biological strategy common to many HAB species that may be influenced by climate variables such as temperature, nutrients, and pH (Agrawal 2009). Changes in algal cell morphology, such as cell size and chain formation could also influence the physical properties of bloom formation and predator avoidance (Pančić and Kjørboe 2018). Finally, changes in macromolecular cell composition (e.g., N:P ratio) could signal changes in nutrient requirements that alter the fitness of harmful algae relative to their nearest competitors (Toseland et al. 2013, Liefer et al. 2019).

Molecular measurements can also be powerful tools for corroborating physiological measurements and elucidating potential mechanisms of shifts in physiology or fitness. Markers of population genet-

ics such as microsatellites, amplified fragment length polymorphisms (AFLPs) and single nucleotide polymorphisms (SNPs) quantify gene flow and genetic drift. Microsatellites are non-coding repetitive regions in the DNA. Because they are non-coding they are also presumably neutral, meaning they are not affected by selection and are thus suitable for looking at patterns of gene flow among populations both spatially and temporally (e.g., Ruggiero et al. 2018). AFLPs resemble microsatellites in the sense that they are informative for investigating patterns of gene flow (e.g., Alpermann et al. 2010, Sassenhagen et al. 2015). AFLPs are however not necessarily always neutral. Whether or not specific AFLPs are neutral or not can be tested, and can thereby bias patterns of gene flow but may instead be informative in terms of patterns of selection. SNPs resemble AFLPs in that they may or may not be neutral. However, unlike AFLPs, with SNPs the location is usually known. It is for instance possible to sequence AFLP fragments, of interest, and locate the SNP(s) to see if it is located in a known gene. Targeted amplification of key functional genes with quantitative PCR (qPCR), such as toxin biosynthesis pathway genes, can inform harmful algae responses to climate drivers in cases where the genes have been elucidated (Stüken et al. 2011, Brunson et al. 2018). Using non-targeted approaches such as genomics, transcriptomics, and proteomics can also be informative by exploring the presence and regulation of functional genes (as reviewed by Hennon and Dyhrman 2020). Genomics is useful in exploring the gene complement of functional genes and how they can be gained and lost in a population (Harke et al. 2016). Transcriptomics is useful for uncovering mechanisms of acclimation to a climate driver by exploring changes in functional gene expression (Hennon et al. 2015) and also for assessing gene complement in organisms that are not well studied or have large genome sizes (Keeling et al. 2014). Proteomics is less sensitive, than other non-targeted approaches and typically requires a transcriptome or genome for analysis, but can be particularly useful for understanding the biological responses of algae such as dinoflagellates that largely regulate their cell metabolism at the post transcriptional level (Lin 2011).

#### **4.5 Future directions and outlooks**

Experiments with HAB species have revealed a variety of acclimation responses to climate change perturbations that should be integrated into a larger prediction framework. Similar to how weather forecasts have been vastly improved since the 1950's, HAB forecasting could be drastically improved with concerted efforts to develop mechanistic models that utilize phytoplankton acclimation and adaptation data (see Chapter 6). The near-term goal of HAB species experiments should be to improve accuracy of regional forecasting for HAB events. Regional HAB model frameworks already exist in some cases (McGillicuddy et al. 2011, Anderson et al. 2016) and could be augmented by integrating phytoplankton physiological and ecological mechanisms uncovered from acclimation studies (e.g., temperature reaction norms) to better forecast potential changes in frequency and intensity of HABs in response to events like anomalously warm years. While many species have significantly increased growth rates under elevated CO<sub>2</sub> and temperature predicted for future ecosystems (Hennon and Dyhrman 2020), their responses tend to be unimodal (Hennon et al. 2019), with a decline in growth rate as conditions exceed a certain threshold. Future studies should focus on characterizing the shape of the species acclimation response curves to find thresholds rather than finding significant changes between modern and a single predicted future level of CO<sub>2</sub>. Studies also suggest an important role for microbial interactions in shaping the fitness of HAB species (Amin et al. 2015). Future studies should seek to identify interactions between HABs and other marine microbes, including the impacts of competition, allelopathy, and parasitism, as well as synergistic and mutualistic interactions. Regional models would require data on local species' acclimation responses to environmental variables including light, temperature and nutrients, as well as a parameterization of these key microbial interactions and behaviors.



The iteration of regional model development, environmental monitoring, and isolation of new local strains for physiological experimentation will guide research efforts on the species and conditions that are most relevant to forecasting HAB events in each region. The long-term goal of HAB species experiments should be to predict the fitness of HAB species relative to their competitors under future ocean conditions. Building on regional model frameworks (see Chapter 6), long-term forecasts could be created by incorporating physiological data for HAB and competitor species responses to future temperatures and CO<sub>2</sub> concentrations. Such long-term forecast models would have to consider long-range species dispersal and adaptation rates. Ocean circulation models are now very advanced and have been coupled with ecological models to predict which phytoplankton functional type will dominate in each region under modern (Follows et al. 2007) and future conditions (Dutkiewicz et al. 2015, Barton et al. 2016). Similar models have also been used to understand shifts in distributions of HAB species (Gobler et al. 2017). Given the observations of increasing poleward range of many HAB species (Hallegraeff 2010), these modeling tools should be more widely used to guide preemptive monitoring in regions where HABs have not previously been observed, yet are likely to become prevalent due to rising temperatures. These models could be tested by comparing the outputs with regular HAB monitoring to refine and improve ecological model frameworks.

Constraining our estimates of adaptation rates of HAB species through experimental evolution should be another important goal. Model frameworks exist to estimate evolutionary drift of a population (Thomas et al. 2012), but rely on assumptions with little supporting data for real species. A few studies have done long-term evolution experiments with HAB species (Flores-Moya et al. 2012, Tatters et al. 2012b) and found significant increases in evolved growth rates. Researchers should invest their effort in long-term evolution experiments for prevalent HAB species such as *Karenia brevis*, *Aureococcus*, and *Pseudo-nitzschia* with a trove of supporting sequencing and ecological data (Brand et al. 2012, Frischkorn et al. 2014, Di Dato et al. 2015, Wurch et al. 2019). By comparing acclimated and evolved responses of these 'model' HABs and their co-occurring competitors, it may be possible to predict the evolved responses of other HAB species based on their acclimation responses (Collins et al. 2013, Schaum and Collins 2014), avoiding intensive long-term evolution experiments for most species.

Analysis of omics data promises to open another avenue for understanding the forces that shape HAB species success under modern and future ocean conditions (Hennon and Dyhrman 2020). *De novo* sequencing of HAB species with large genomes such as dinoflagellates is rapidly becoming possible, as DNA sequencing becomes more affordable and accessible. Meta-omics techniques can also be used by monitoring programs to characterize HAB species responses during the formation of harmful blooms and compare these responses with those of cultured species in the lab (Frischkorn et al. 2014, Wurch et al. 2019). Thus, omics techniques can provide a way to test assumptions about the mechanisms of environmental and ecological drivers of HAB events and could be used to improve forecasting of future events.

#### **4.6 Summary of recommendations for HAB experimentalists**

1. Develop close collaborations with modelers and monitoring programs to guide choice of experimental HAB organisms and conditions.
2. Improve near-term forecasting by measuring acclimated growth rate responses of HAB species to a range of environmental conditions and key microbial interactions.
3. Incorporate adaptation responses of representative species into modeling frameworks to improve long-term HAB forecasting.
4. Leverage omics to improve the prediction of HAB species responses to future/multifactorial conditions.

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# CHAPTER 5

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## Databases for the study of harmful algae, their global distribution and their trends

**Adriana Zingone**<sup>1</sup>, **Laura Escalera**<sup>1</sup>, **Eileen Bresnan**<sup>2</sup>, **Henrik Enevoldsen**<sup>3</sup>, **Pieter Provoost**<sup>4</sup>, **Anthony J. Richardson**<sup>5,6</sup>, **Gustaaf Hallegraef**<sup>7</sup>

<sup>1</sup> Stazione Zoologica Anton Dohrn, Naples, Italy, zingone@szn.it

<sup>2</sup> Marine Scotland Science, Aberdeen, Scotland, U.K.

<sup>3</sup> Intergovernmental Oceanographic Commission (IOC) of UNESCO, IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Denmark

<sup>4</sup> Intergovernmental Oceanographic Commission (IOC) of UNESCO, IOC Project Office for IODE, Oostende, Belgium

<sup>5</sup> Centre for Applications in Natural Resource Mathematics, School of Mathematics and Physics, University of Queensland, St Lucia, Queensland, Australia

<sup>6</sup> CSIRO Oceans and Atmosphere, Queensland Biosciences Precinct, St Lucia, Queensland, Australia

<sup>7</sup> Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia

### 5.1 Introduction

The scientific questions most frequently posed about HABs are whether their incidence is increasing, if their geographic range is expanding, and what are the mechanisms behind any observed trends. Indeed, a global expansion of HABs and its possible drivers have long been debated. Eutrophication, human-mediated introduction of non-indigenous harmful species, climate variability and change, and aquaculture development have all been suggested as possible causes for the expansion and increase of HABs (Smayda 1990, Hallegraef et al. 2010), although the evidence of such a global increase is weak (Zingone and Wyatt 2005; Hallegraef et al. 2021a,b). Capacity and monitoring efforts to detect harmful species and events have also increased over the last 40 years, increasing the reporting of harmful events across the world's seas. Concurrently, information on the absence of events and on decreasing trends, like all negative results, is rarely available.

Whether HABs are increasing or not, their impacts are a global and growing concern. As the human population continues to increase along with its need for resources, HABs will pose a growing risk to the safety and security of the seafood industry and to coastal ecosystems and resources, and will impede development of recreation and tourism industries. The variable effects of climate and anthropogenic pressure on the coastal environment suggest that harmful species and their impacts on human activities will also change over time and on different spatial scales. For these reasons, improved approaches to quantify changes in distribution patterns of harmful species and events at multiple spatial and temporal scales are urgently needed to facilitate forecasting of where and when HABs may occur, and changes in their frequency and intensity. These forecasts are fundamental to support effective management of HABs and to optimise the use of maritime space in coastal areas.

Two main questions identified in these guidelines concern changes in the geographic distribution, character, frequency and intensity of HABs, and the need to forecast how climate change will alter

future HAB effects on human health, ecosystems, economics, food and water security. Both questions require a global assessment of the current distribution of harmful species, toxins and HAB events, along with their links to environmental conditions, including climate drivers. However, data on the presence and density of harmful species and their impacts are scattered in the ever-growing scientific and grey literature, while resources within statutory monitoring programs are primarily focused on the protection of human health and not publication of data in peer-reviewed journals.

The lack of a global, evidence-based overview of the distribution and trends of HABs and their impacts has stimulated the development of a Global HAB Status Report (GHSR, Hallegraef et al. 2017, Zingone et al. 2017, Hallegraef et al. 2021a,b), delivered in 2021, which has been endorsed by several international agencies (IOC, ICES, PICES and IAEA) and supported by the Government of Flanders. The GHSR aims to provide the first quantitative assessment of the status and current changes of HAB occurrence across the world seas. The first efforts towards a GHSR have taken advantage of several public databases that have been populated with information on harmful algal species and harmful events over the last decades in an effort to overcome the fragmented and underexploited nature of the available information. A central finding of the GHSR is that improved datasets and their harmonization will be needed to show definitive trends between climate and changing HABs.

In this chapter we provide a summary of the three main databases containing information on harmful marine algal species and events that are available in the public domain. We highlight strengths and weaknesses of each database, as well as requirements for further development. We also describe some additional phytoplankton datasets that do not hold data about harmful algal blooms specifically, but contain relevant information that can be exploited to assess global HAB distribution and trends. The overarching goal of this chapter is to assess if these data are appropriate to address the key questions about HABs and climate drivers delineated above. We highlight the main data gaps and provide a series of recommendations to overcome the current limitations.

## 5.2 Databases Focusing on Harmful Algae

### 5.2.1 GENERAL FEATURES

Two broad categories of harmful microalgae are recognized. The first includes species that produce toxins, i.e., chemical substances that may impact human health or marine animals by poisoning, either directly or through vector organisms that accumulate the toxins. Microalgal toxins may accumulate in cultivated seafood above threshold concentrations, leading to managed closures of shellfish harvesting areas/fisheries to protect human health, which can have a negative economic impact on associated aquaculture industries and fisheries (Skjoldal and Dundas 1991, Fernández et al. 2003, Sakamoto et al. 2020). In some instances, toxic events may result in human illness/mortalities (Tagmouti-Talha et al. 1996, García et al. 2004) along with mortalities of fauna belonging to higher trophic levels e.g., sea birds or marine mammals (Coulson et al. 1968, Gible et al. 2021). It should be noted that toxin production within species is variable due to intraspecific and physiological differences (Flynn and Flynn 1995, Pizarro et al. 2009), which can be influenced by environmental conditions. In addition, it can be argued that toxicity (e.g. seafood toxicity) only occurs when vectors and target species are present during blooms of toxin-producing species. This is not always the case as in some instances the toxin-producing species can be present but shellfish are absent. To be precise, these species are therefore only 'potentially' toxic, but are often referred to as toxic in this chapter and elsewhere for brevity.

The second category of harmful species includes microalgae that do not produce any toxic compounds but may still have adverse effects on marine fauna, e.g., by mechanical damage to fish-gills (Bell 1961) or associated with anoxia (Pitcher and Probyn 2011) or high ammonia concentrations (Okaichi and Nishio 1976). In addition, many microalgal species may cause seawater discolorations (e.g., Satta et al.



Table 1. Characteristics of 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/information.

	Taxonomic Reference List of Harmful Micro Algae	HABMAP-OBIS	HAEDAT
<b>Field</b>	Taxonomy and toxins	Biogeography	Socio-economics, health, ecology
<b>Includes</b>	List of toxic species	Toxic species records with or without impact	Non-toxic or toxic harmful events with an impact
<b>Excludes</b>	Non-toxic HAB species	Records of non-toxic HAB species	Records of blooms or toxic species or toxin concentrations in shellfish without impacts
<b>Literature</b>	References on species taxonomy* and toxicity*	References of species records*	References of the event or reports by national representatives entered when appropriate
<b>Geographic coordinates</b>	No	Yes*	Yes*
<b>Time</b>	No	Yes	Yes
<b>Environmental data</b>	No	At times in the literature cited	At times in the reports
<b>Impacts</b>	No	Not needed	Yes*
<b>Source</b>	Editors of the list	Regional groups of Editors	National representatives of IOC-ICES-PICES groups
<b>Web site</b>	<a href="http://www.marinespecies.org/hab/">www.marinespecies.org/hab/</a>	<a href="http://ipt.iobis.org/hab">ipt.iobis.org/hab</a>	<a href="http://haedat.iode.org/">haedat.iode.org/</a>

2010) or form mucilage, scums or foams (e.g., Escalera et al. 2018), which have an adverse effect on the use of coastal waters for fisheries, recreation and tourism activities.

The distinction between toxic and non-toxic harmful events can be difficult. For example, water discolorations can be caused by toxin-producing species with no apparent toxic effects, while mass mortalities can be caused by toxins produced by microalgae (Skjoldal and Dundas 1991), or mechanical damage to the marine animals (Bell 1961), or anoxia (Pitcher and Probyn 2011). The boundary between harmful and non-harmful microalgae is also subtle, because any microalgal species may have a harmful impact when they reach high densities. A clear example is provided by many ecologically beneficial diatoms that produce polyunsaturated fatty acids (PUFA) which, in the case of intense blooms, can impair copepod reproduction and development (Ianora et al. 2004). The three HAB databases described in this section deal with different aspects of toxic and non-toxic harmful microalgae and their related impacts (Table 1). Specific characteristics of each database are described in the subsequent sections.

## 5.2.2 THE IOC UNESCO TAXONOMIC REFERENCE LIST OF HARMFUL MICRO ALGAE

### 5.2.2.1 Description

The database includes formally accepted names of planktonic and benthic microalgae that produce toxins (Moestrup et al. 2009, [www.marinespecies.org/hab/](http://www.marinespecies.org/hab/)). The number of species has nearly doubled since the first version of the list in 2002 (Fig. 1A) which included only four of the six algal groups present today (Fig 1B, C), to which cyanobacteria (blue-green algae) have recently been added. The increase in species results from several advances in knowledge: i) discovery of toxin production in additional species belonging to genera already known to include toxin-producing species, e.g., some

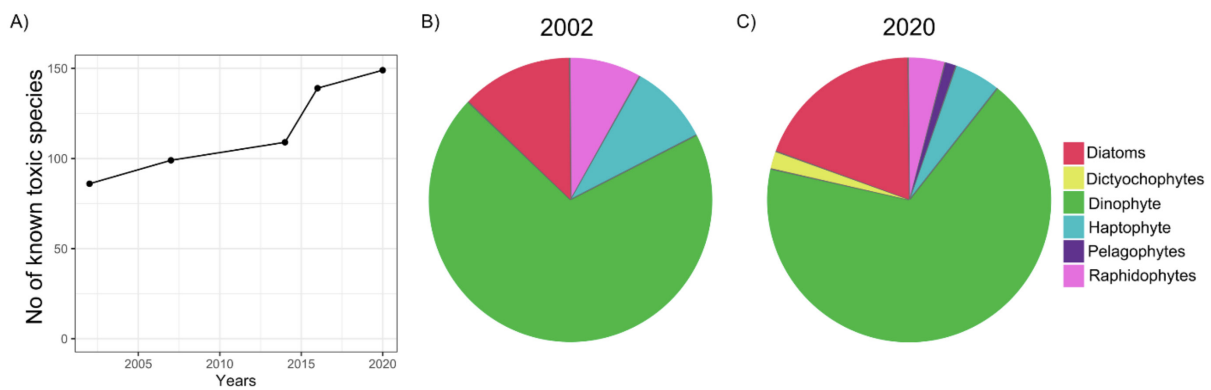


Fig. 1. A) Trend of the number of microalgal species known to produce toxins, as listed in the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Moestrup et al. 2009 and onwards, accessed in different years). B) Proportions of different taxonomic groups represented in the first version of the IOC-UNESCO reference list and C) as of October 2020. Cyanobacteria are not included.

*Pseudo-nitzschia* species have only recently been found to produce domoic acid, ii) finding of toxin production in species of genera previously not known to include toxic species, such as *Aureococcus anophagefferens* and *Centrodinium punctatum*, or iii) discovery of new toxins and new toxin-producing species such as *Vulcanodinium rugosum* and several *Azadinium* species.

#### 5.2.2.2 History

The need for an agreed, official list of toxin-producing species was formalized at the 4<sup>th</sup> IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB IV, Vigo, Spain, 1997). Consequently a 'Task Team on Algal Taxonomy' was established, comprising experts of different taxonomic groups. A first version of the list was completed in early 2002 and hosted on a server of the Department of Biology of the University of Copenhagen. The list has undergone continuous revision and additions. Since 2010 it has been hosted as part of the World Register of Marine Species (WoRMS).

#### 5.2.2.3 Content

For each species on the list, the information includes:

- Accepted scientific name according to the *International Code of Nomenclature for algae, fungi, and plants* (Turland et al. 2018), the basionym (the first name applied to the species) and synonym/s, with Author/s and years for each name.
- Type locality.
- Academic reference of the first description of the species as well as the publication(s) in which it was given its taxonomically accepted name, if different.

This information is often supported by illustrations. In addition, the type of toxins produced is described, along with up to (usually) three references with information on toxicity, toxins or toxic effects. Additional notes are present in some cases.

#### 5.2.2.4 Management

The list has a chief editor and an editorial board, with each of its members in charge of keeping the list updated for a particular group (family, order or phylum). The taxonomic information is automatically linked to WoRMS, which in turn relies on AlgaeBase (Guiry and Guiry 2020) for nomenclatural changes.

#### 5.2.2.5 Strengths and Weaknesses

The *Taxonomic Reference List of Harmful Micro Algae* is the starting point for an assessment of toxic microalgae from anywhere in the world. It provides the most up-to-date and accurate nomenclature, as well as historic taxonomic or nomenclatural changes.

The list deliberately does not cover species that have formed non-toxic HABs, such as diatoms harmful to finfish by mechanical damage (e.g., some *Chaetoceros* species), nor species associated with

non-toxic discolorations (e.g., *Noctiluca* spp.) or anoxia (e.g., *Triplos* spp. and *Cerataulina pelagica*). As noted previously, any microalgal species at high concentrations may have a negative or harmful impact. In addition, species causing anoxia in semi-enclosed systems may not do so in more exposed areas with higher flushing rates. Species that cause water discoloration in some areas are part of a local microalgal community that may have no specific impact in other areas. For these reasons, a global list of species that cause adverse effects without producing toxins would be impractical.

Although useful as a reference for correct nomenclature, the *Taxonomic Reference List of Harmful Micro Algae* does not contain literature on morphology and cannot be used to assist in the identification of individual species.

### 5.2.3 HABMAP-OBIS: DATABASE ON THE GEOGRAPHIC RANGE OF HARMFUL SPECIES

#### 5.2.3.1 Description

Based on the *IOC UNESCO Taxonomic Reference List of Harmful Micro Algae* (see Section 5.2.2), the database HABMAP-OBIS collects the information on the distribution of potentially toxic species regardless of their actual impacts. The result is a referenced biogeography database of potentially toxic species. The primary source of the information collected by HABMAP-OBIS is peer-reviewed scientific literature, but PhD theses, reports, and any additional online material are also used as far as they are retrievable and can be attributed to a reliable author. Source material also includes the IOC-Harmful Algae News newsletter and the proceedings of various HAB conferences, which hold many records of potentially toxic species that have not been published in peer-reviewed journals.

#### 5.2.3.2 History

An informal, collective effort to map the occurrence of toxic microalgae was initiated at early international HAB conferences (Lund 1989, Rhode Island 1991). The use of these maps in multiple contexts by the scientific, policy and management communities highlighted the need for records to be complete, traceable, and associated with a reliable reference. The idea of groups of editors handling regional records of HAB species ranges was developed within the International Society for the Study of Harmful Algae (ISSHA) at St. Petersburg (USA) in 2001, and the 6<sup>th</sup> Intergovernmental Panel on HABs (IPHAB VI, St. Petersburg, USA 2002). At the first ISSHA Council (Hornbæk, Denmark, 2004), the *ad hoc* Committee on Special Projects was established and given the task of promoting the project HABMAP. Since 2006, HABMAP and other IOC data initiatives have been incorporated into the Ocean Biogeographic Information System (OBIS).

#### 5.2.3.3 Content

The database is organized in worksheets arranged by regions, where lines correspond to individual records of potentially toxic species and include associated information (Table 2). Some data fields, such as the species name, the source literature and the coordinates, are mandatory and have an established format following the Darwin Core standards for biodiversity informatics, to be readily converted into entries for OBIS and properly mapped (for details of these standards, see <http://iobis.github.io/hab/>). When available, additional information on species maximum density, toxins detected and their concentration is also included in the database, along with supplementary notes.

Where the taxonomic status or name of a species has changed since the information was published, the current accepted name is reported whenever possible, using the *IOC UNESCO Taxonomic Reference List of Harmful Micro Algae* (see Section 5.2.2) and the relevant taxonomic literature. One key detail is the assignment of a quality flag (QF) to the identification of each species in the database (Zingone et al. 2015). QF scores are based on expert judgement and take into account the identification method used, the known distribution of the species, possible issues in its taxonomic history, and any additional information in the reference used as the source of the information. As each record for a single species is reported in one line of the worksheet, regional databases can consist of 1000s of entries, e.g., about 2,500 for the Mediterranean Sea (Zingone et al. 2021).

Table 2. Information collected in the HABMAP-OBIS file for each species from each site in a given published reference. Asterisks (\*) indicate mandatory information. Detailed information on the content and its format can be found at <http://iobis.github.io/hab/>.

<b>Identifiers</b>	Scientific name*	Valid name of the toxic species
	Reported name (if different)	Name of the species in the reference
	Identification status	Quality flag of the identification
	Reference*	Literature reference
	Additional references	For the same record, if any
	HAEDAT URL	Link to related events in HAEDAT
	Last modified	Date of data entry
<b>Date</b>	Date*	Date of the species collection
	Verbatim date	
<b>Location</b>	Latitude*	Coordinate of the sampling site
	Longitude*	Coordinate of the sampling site
	Coordinate uncertainty	(m)
	WKT	For data concerning transects or polygons
	Locality	Geographic name of the site
	Minimum depth	Sampling depth
	Maximum depth	Sampling depth
<b>Abundance</b>	Value	Cell density
	Unit	
<b>Toxicity</b>	Toxin	Toxin name from the reference
	Value	Toxin concentration from the reference
	Unit	
<b>Remarks</b>		Any additional comments

#### 5.2.3.4 Management

The HABMAP-OBIS database is compiled by a group of 12 lead editors, each assisted by an editorial group, responsible for occurrence data from one of the following regions: 1. East Coast USA/Canada, Greenland; 2. Florida, Caribbean, Central America, Venezuela; 3. South America; 4. West Coast USA/Canada, Alaska; 5. Australia/New Zealand; 6. Southeast Asia; 7. North Asia; 8. Indian Ocean; 9. Benguela (South Africa and Namibia); 10. Mediterranean, Black Sea; 11. West/North Europe, Scandinavia; and 12. Pacific/Oceania. Editors are experts in the field of harmful algae of the specific regions, and are charged with retrieving the relevant literature, reviewing the individual papers, checking the taxonomic status of the species, considering possible taxonomic and nomenclatural changes, assigning a QF to the identifications, and filling in the fields of the worksheet for each record of a potentially toxic species (Table 2). The process of data entry has received a great impetus from the GHSR project and the related initiative to publish a Special Issue of the journal *Harmful Algae* (Hallegraeff et al. 2021b) that includes invited regional overviews (see Section 5.4). In fact, data from the literature for some regions not covered so far are still being entered into the database.

### 5.2.3.5 Strengths and weaknesses

The database is a valuable tool for tracing species geographic ranges and identifying possible risk areas in regions of the world where data on harmful species are available. The compilation by regional experts who assess the quality of the data in the published literature ensures the information in the database is robust. The database can also be used in a dynamic way, e.g., by comparing maps obtained from HAB species records in different decades. HABMAP-OBIS data flow into the general OBIS dataset, where they can be merged with data from different inputs and be used with visualization tools to trace ranges and record trends of genera containing potentially toxic species (Fig. 2).

One of the main limitations of the HABMAP-OBIS dataset is the lack of data on absences (i.e., where a species was not found), although this information is available, at least for well-studied areas. Data related to incorrect identifications cannot be included either, not even with QFs or the criteria for being considered wrong. In addition, coverage is scant for many regions of the world where taxonomic studies are scarce.

## 5.2.4 HAEDAT: THE HARMFUL ALGAL EVENT DATABASE

### 5.2.4.1 Description

The Harmful Algal Event Database (HAEDAT) is a database that contains information about harmful algal events from across the globe. Data are summarized into 'events' associated with a management action or negative economic/ecological/social impact. In contrast to the other two databases already described, HAEDAT also contains reports of impacts not related to toxins, e.g., high biomass blooms, anoxia and mucilages. Within HAEDAT, a harmful algal event is defined as one of the following:

- (i) water discoloration, scum or foam with a socio-economic impact caused by potentially toxic or non-toxic microalgae;
- (ii) biotoxin accumulation in seafood above concentrations considered safe for human consumption;
- (iii) high density of toxin-producing algae, which results in a shellfish or other invertebrate harvesting ban, or a closure of a beach as a precautionary measure to protect human health; and
- (iv) any event where humans, animals or other organisms are negatively affected by algae (i.e., including non-toxic algal species).

HAEDAT includes toxic and non-toxic harmful events (non-toxic discolorations, mucilage, anoxia or mechanical damage to fish and shellfish) even in cases where there is no information about the causative organism. In contrast to HABMAP-OBIS, HAEDAT does not include records of toxin-producing species or blooms where no impacts have been recorded (Table 1). Events recorded in HAEDAT may or may not be associated with a published data source.

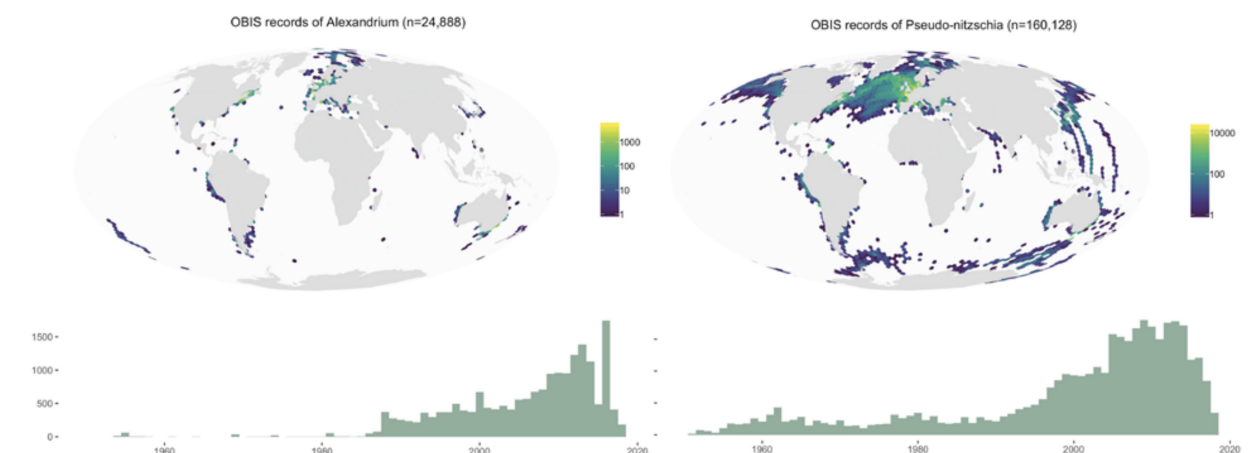


Fig. 2. OBIS maps and time series of records of the genera *Alexandrium* and *Pseudo-nitzschia* between 1950 and 2019, obtained by merging the datasets specifically established within HABMAP-OBIS with all data from other sources that are present in OBIS. Both *Alexandrium* and *Pseudo-nitzschia* include potentially toxic and non-toxic species.

#### 5.2.4.2 History

Since 1985, participants in the annual meetings of the ICES Working Group on Exceptional Algal Events (subsequently renamed the ICES-IOC Working Group on Harmful Algal Bloom Dynamics) have presented national reports of harmful algal events from their respective countries. Initially these reports were submitted and collated on paper. Due to the global interest in the impacts of HABs, it became clear that a database was needed to store the relevant information. IOC led the initiative to develop a database system, HAEDAT (<http://haedat.iode.org>). Since 2000, the HAB Section of the North Pacific Marine Science Organization (PICES) has joined as contributor to HAEDAT.

#### 5.2.4.3 Content

Data are summarized into individual events, with information on start and finish dates for the event, geographic coverage, maximum cell density, toxin concentrations in animals (expressed according to regulations) and/or other types of impacts. The coastline of each country is divided into sub-areas approximately 200 km in length, each with a unique area code that allows events extending over a large stretch of coastline and impacting multiple areas to be entered accurately. Ancillary environmental information such as temperature, salinity, and chlorophyll concentrations can also be entered, along with the name and address of the data provider (national editor or additional contacts) for each event, although in many instances these complementary data are not provided. Data are searchable by country, region, syndrome/nature and year, and can be downloaded as csv files for further analysis.

#### 5.2.4.4 Management

National editors, formerly including only participants of the ICES-IOC working groups, collate data on events from their countries and enter them into the database, which is maintained by the IOC IODE Project Office and funded by the Government of Flanders, Belgium. Many of the data come from statutory monitoring programs. Other data have been entered from peer-reviewed literature and accredited reports. Data are entered annually, although in some cases there have been delays of 1-2 years. Data have been inputted routinely in HAEDAT from ICES since the mid-1990s. Currently there are records from 102 countries, with some entering historic data back to the late 1800s. To date, a total of 8,399 events have been entered, with half of the countries reporting more than 10 events (Fig. 3). In some regions (e.g., Australia, New Zealand, and Italy) event records from the literature or other available reports have been recently entered, and more events from the literature continue to be entered into the database, stimulated by the GHSR initiative.

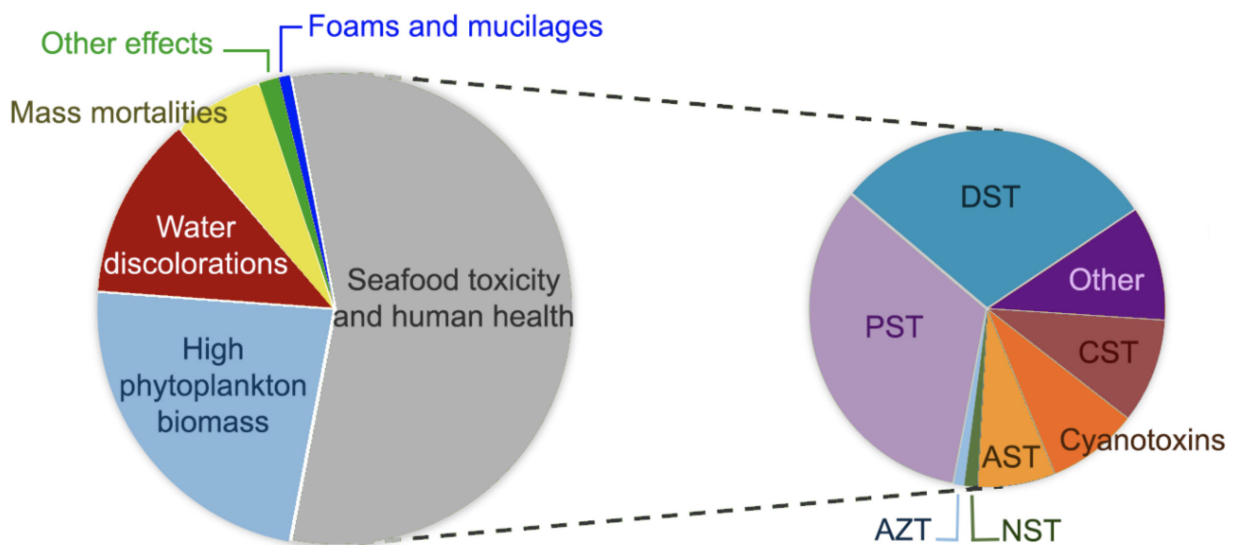


Fig. 3. Partitioning of 8,399 global HAEDAT records into types of events based on the number of events and further breakdown of seafood and other toxin types. Data as of 2/11/2020. Abbreviations: Amnesic (AST), Neurotoxic (NST), Azaspiracid (AZT), Diarrhetic (DST) and Paralytic (PST) Shellfish Poisoning and Ciguatera Seafood Poisoning (CST) toxins.

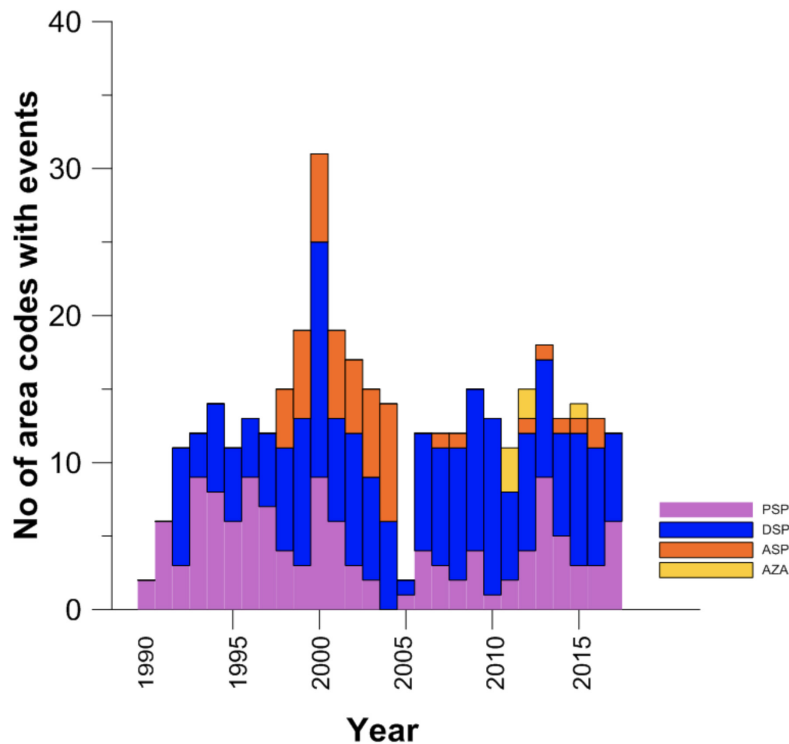


Fig. 4. Incidence of different seafood toxin syndromes in the United Kingdom between 1990 and 2017, based on the number of HAEDAT area codes with closure events. Such data represent the compound result of HAB events as well as changes in monitoring and regulatory approaches. DSP monitoring started in 1992, ASP in 1998, AZA in 2011, while the data gap in 2005 reflects reduced monitoring effort 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 resulting from changes in EU legislation (EU854/2004) facilitating the shucking and subsequent sale of the adductor muscle of *Pecten maximus* (compiled from Marine Scotland, Food Standards Scotland and Food Standards UK data).

#### 5.2.4.5 Strengths and weaknesses

HAEDAT is unique as a collection of HAB events and information mostly derived from statutory monitoring operations which, at least in Europe, go through strict accreditation processes and/or quality assurance proficiency testing (e.g., Quality Assurance of Information in Marine Environmental monitoring – Quasimeme: <https://www.wepal.nl/en/wepal.htm>) for toxin analyses and for identification of potentially toxic species. As HAEDAT is open access, easy to interrogate, and to download data from, it can provide a global overview of the incidence of harmful algal events. Aggregated data in HAEDAT summarize large and complex datasets, and make the information available to a wide audience including students, researchers, managers, policy makers, industry, and members of the public. For some events such as fish kills, HAEDAT is the only database holding these data. HAEDAT also has the capacity to map events. Should more information be required about events or over a regional scale, national editors or the IOC can be contacted.

Oceanographic data/information concomitant with the events are at times included in the reports, but not consistently. HAEDAT also has a number of other limitations that must be considered, and data users are advised to read available metadata information or contact regional editors to ensure that they have interpreted the dataset correctly. Key issues include:

**i) Absence of events and lack of monitoring is not recorded:** In HAEDAT, the absence of events, such as where toxin concentrations do not exceed regulatory thresholds, is not recorded. Areas with little or no monitoring are not flagged and so it is not possible to distinguish between areas where there are no events and areas where there is no monitoring. Some events not directly related to human health, e.g. water discolorations, fish kills and benthic mortalities, can be reported in an *ad hoc* manner in some countries and may be under-recorded in HAEDAT.

**ii) Changes in monitoring effort influence HAEDAT data:** Trends in HAEDAT data may reflect changes in monitoring effort or methods driven by changes in legislation or aquaculture activities in an area. Thus an observed reduction in events in HAEDAT could not only be a consequence of the actual decrease of events, but could also be caused by a reduction of monitoring effort. A clear example is the reduction in incidence of closures of offshore scallop fishing grounds in Scotland due to high concentrations of ASTs (Fig. 4). This was due to a change in EU legislation (EU854/2004), facilitating end

product testing, shucking and sale of *Pecten maximus* adductor muscle under defined circumstances, thereby eliminating the requirement for monitoring of offshore fishing grounds. As this kind of information is not readily flagged in the database, familiarization with the metadata and a contact with editors are needed to avoid misinterpreting HAEDAT data.

As a direct effect of monitoring effort, the toxin risk to human health in a region is not related to the number of events in HAEDAT. In fact, more events in one area reflect efficient management strategies to protect human health and thus may lead to a lower risk of intoxications compared to areas with insufficient monitoring and/or rare events.

**iii) Changing methodologies have affected the number of events:** The methods for monitoring algal toxins have changed over the multiple decades covered by HAEDAT. For example, when the mouse bioassay was the standard method for quantifying lipophilic algal toxins in shellfish, a positive result for DSTs was produced by the presence of a number of other toxins, such as yessotoxins, azaspiracids and palytoxins, co-extracted with okadaic acid derivatives, which are the actual toxins causing the Diarrhetic Shellfish Poisoning (DSP) syndrome. Unjustified closures of shellfish harvesting occurred because of the presence of yessotoxins, subsequently found to be non-toxic to humans. The advent of chemical methods for the quantification of individual toxins has allowed the different toxin groups to be distinguished and threshold concentrations identified for each of them to drive the closure of shellfish harvesting.

Changes over time of units for reporting algal toxins (e.g.,  $\mu\text{g STXeq } 100 \text{ g}^{-1}$  to  $\text{mg STXeq kg}^{-1}$ ) are recorded in the metadata and need to be considered by end users to interpret the data correctly.

**iv) Inconsistent spatial and temporal recording of early events:** During the early years, HAB events extending over wide geographic areas may have been entered as multiple events, and what constitutes an event is not easily or uniformly defined. Subsequently area codes were established for each country and events are currently entered as one event spanning different area codes. This can impact interpretation of the total number of events recorded. To circumvent this problem, the ICES-IOC Working Group on Harmful Algal Bloom Dynamics uses the number of years with events per area code as the metric for HAEDAT analyses, rather than the number of events per year. In some instances it can be difficult to categorize impacts from discrete blooms of toxin producing species into events. For example, when a bloom of a toxic species drifts in and out of an enclosed area there may be two separate periods of management actions associated with this same bloom. In instances such as this, categorizing management actions into an event can be subjective, and there can be inconsistency across countries/areas in how these events are assigned.

### 5.2.5 FUTURE DEVELOPMENTS AND IMPROVEMENTS NEEDED FOR THE THREE HAB DATASETS

Accounting for the strengths and weaknesses of the three main HAB-related datasets detailed above, we can make a number of suggestions to improve the databases and their use by researchers.

A single portal bringing the three databases together allowing unified access to the data is being developed. HAEDAT and HABMAP databases are already being prepared for integration within OBIS, with the development of a specific HAB user interface. Along with the *Reference Taxonomic List of Toxic Micro Algae* species in WoRMS, this will be launched as the IOC Harmful Algal Information System, HAIS (<http://hais.ioc-unesco.org>), within which the three databases will be interconnected and enhanced and made easily exploitable. Developments include ease of data entry and Quality Control (QC), improved mapping options combining data from HAEDAT and HABMAP-OBIS, and an overall improved user interface. A test version of the portal interface is presently accessible at <https://hab-dev.iode.org/>. Future improvements also include integration with the algal biotoxin database developed within the IPHAB 'Task Team on Biotoxins Monitoring Management and Regulations'



Several development needs for the three HAB-specific databases have emerged through the analysis presented in this section, and are described in the next subsections. It should be noted that the databases above have been established and developed mainly thanks to the voluntary activity and dedication of researchers, students and personnel from monitoring agencies finding the time in their work schedules. Future developments envisaged below are only possible when appropriate funding resources are identified.

#### 5.2.5.1 Developments for the IOC UNESCO Taxonomic Reference List of Harmful Micro Algae

Because the list is so widely used, an important advance would be to make it a complete taxonomic tool by including morphological information that would enable the correct identification of toxin producing species/strains and their distinction from similar non-toxic ones. The information added could consist of descriptions, images, drawings, and illustrated taxonomic keys. The inclusion of diagnostic DNA sequences (barcodes), possibly through links to the Genbank database, would be a natural expansion of the database towards becoming an effective identification tool, which could also promote a wider application of metabarcoding approaches to trace harmful species in the world's seas.

Information on toxins produced by the species on the list should be completed and kept updated, whereas cases could be highlighted where the production of toxins by cultured strains of the species has not been fully demonstrated. To this end, there are plans within the IPHAB 'Task Team on Biotoxins Monitoring Management and Regulations' to integrate the list with phycotoxin data.

The list would also benefit from a historical perspective, with reports of changes over time such as the exclusion of species initially thought to be toxic but then proven not to be. This information would allow the accurate interpretation of previous reports of toxicity, e.g., accounts of toxicity in *Prorocentrum crassipes*, before discovery of the true source of the toxin, notably *Azadinium spinosum* (Krock et al. 2009). A complete 'black list' of controversial cases and species 'suspected' but not proven to produce toxins could serve the same purpose.

Finally, an overview of species most frequently associated with non-toxic harmful events would be a useful addition to the list. The list could be restricted to species proven to be linked to an impact, and be compiled on a regional basis, because many beneficial microalgae have only caused adverse effects in specific areas.

#### 5.2.5.2 Developments for the HABMAP-OBIS database on the geographic range of toxin producing microalgae

The main development needed for this database would be to ensure better coverage in the areas where data are not available or not published. These include most African and south Asian coastal seas, and the Arctic and Southern Ocean. In recent years efforts have been made to increase the entry of data from these regions, but for some of them, e.g., western Africa, there is very little information about harmful species distribution and harmful algal events.

Besides the regions with little or no monitoring activities, data are also scarce for long stretches of coasts in regions quite extensively monitored or investigated, such as the European coasts. This could be for several reasons including: gaps in HAB monitoring in those coastal areas; the existence of data that are not made publicly available; data reporting may not be among the statutory activities of monitoring agencies; or there could be a reluctance to contribute to a negative image of an area by making data available on the presence of toxic algae, which could impact tourism or perceived safety of seafood. The reluctance to share data extends beyond toxic algae, and requires specific actions, as detailed in Section 5.4. Nevertheless, even if these data were made available by the agencies or research institutes, a large amount of work by expert people would be needed to organize them, assess their quality, and ensure their comparability before including them in HABMAP or other databases.

While harmful species data from microscopy counts are time-consuming and often difficult to compare among datasets, a huge amount of metabarcoding data has accumulated over the last decade, in which microalgal identification in environmental DNA samples is based on species-specific molecular signatures. For toxin producing species, these data need to be resolved at the species level. Many methodological issues concerning standardisation of sampling methods and analytical pipelines have not been resolved (Stern et al. 2018). Nonetheless, metabarcoding data are a valuable resource that could allow tracing individual harmful microalgae in areas of the world not covered by routine monitoring. Indeed, metabarcoding data of harmful microalgae are being published at an increasing frequency (e.g., Grzebyk et al. 2017; Liu et al. 2020; Esenkulova et al. 2020).

The HABMAP-OBIS dataset is primarily used to trace the presence of a given species at a given place. Incorporating species densities over their geographic range along with ancillary environmental data would enable the description of their ecological niches, which could be used in model forecasts of range expansion or retraction in response to climate change. Ideally, once the database is completed, it would be possible to analyse information on species presence and density together with physical and chemical data from the same records or from complementary datasets (see Section 5.4). Such analyses would provide insights into the environmental constraints on the distribution of individual species and of the drivers of changing distributions over time and under climatic variations. The date of the species occurrence or the bloom peak is already included in the database. More complete information on the temporal distribution of harmful species would allow insights into their phenological characteristics and on their long-term variations over their geographic range. All activities described above include a strong component of ecological research that could be developed in parallel with the location, collection and proper arrangement of relevant data.

#### *5.2.5.3 Developments for the HAEDAT database*

Key to interpretation of HAEDAT data is familiarity with the metadata, which should be improved and made more accessible. To this end, there is a need for a synthesis document published in a peer reviewed journal explaining how HAEDAT data are collected, along with a central repository for the metadata from each individual country to be stored and accessed by HAEDAT users. The specific criteria for defining events within areas in different geographic regions also needs to be made more explicit and recorded as part of the HAEDAT metadata for users. Information on monitoring effort would allow better understanding of HAEDAT data, improving interpretation and the ability to discern areas where HAB events are absent despite being subject to routine monitoring, from areas with a lack of or change in monitoring effort and instances where data have not been entered. The lack of routine monitoring in large geographic regions such as the Arctic and West Africa also needs to be more easily recognizable within HAEDAT.

For certain types of harmful events that are not linked to human health protection and statutory activities, such as high biomass blooms and fish and invertebrate mortality events, a mechanism to enter records in a more consistent and systematic way would be beneficial. Present data concerning these events need to be reviewed, while difficulties in extracting and interpreting these data need to be flagged in an overarching HAEDAT metadata document.

As for all the databases described in this section, data entry and QC should be facilitated, especially considering the substantial effort required when event data are collected by multiple agencies. The importance of this task needs to be recognized by agencies and given the dedicated resources. In addition, agencies also need to be acknowledged for their contribution as monitoring is often undertaken in an environment of limited resources.

Quantifying HAB impacts in terms of money and social impacts would be a great advance for the dataset, which would require assistance by economists, social scientists and statisticians. One recent assessment provides a strategy and recommendations for obtaining these critical data (Trainer et al. 2020).

## 5.3 Other datasets

### 5.3.1 THE CONTINUOUS PLANKTON RECORDER (CPR) DATABASE

The CPR data provide the most extensive coverage – with consistent methodology – of plankton in the global ocean in offshore waters > 25 m depth. The North Atlantic CPR survey was initiated in 1931 in the North Sea by Sir Alistair Hardy. His vision was to map near-surface plankton (top 10 m) in space and through time, to understand the changing fortunes of fisheries. The Sir Alister Hardy Foundation for Ocean Science (SAHFOS) started operating from the Citadel Hill Laboratory in Plymouth in 1990 as an international non-profit organization, and in 2018 merged with the Marine Biological Association. There are now CPR Surveys sampling in many of the world’s oceans, including the North Atlantic, North Pacific, Southern Ocean, seas around Australia and South Africa, and the eastern Mediterranean Sea (Batten et al. 2019). In 2011, the Global Alliance of CPR Surveys (GACS) was founded.

The CPR is towed behind ships of opportunity on their normal trade routes and the device has remained relatively unchanged over the past 80 years (Reid et al. 2003). Although CPRs have a relatively large mesh (270  $\mu\text{m}$ ), the leno weave and ‘hairiness’ of the silk mesh and the effects of net clogging allow CPRs to retain cells down to 10  $\mu\text{m}$  in size (Richardson et al. 2006). CPR data on phytoplankton should be considered semi-quantitative, but their strength is that ~100,000 samples are collected and counted each year (Fig. 5).

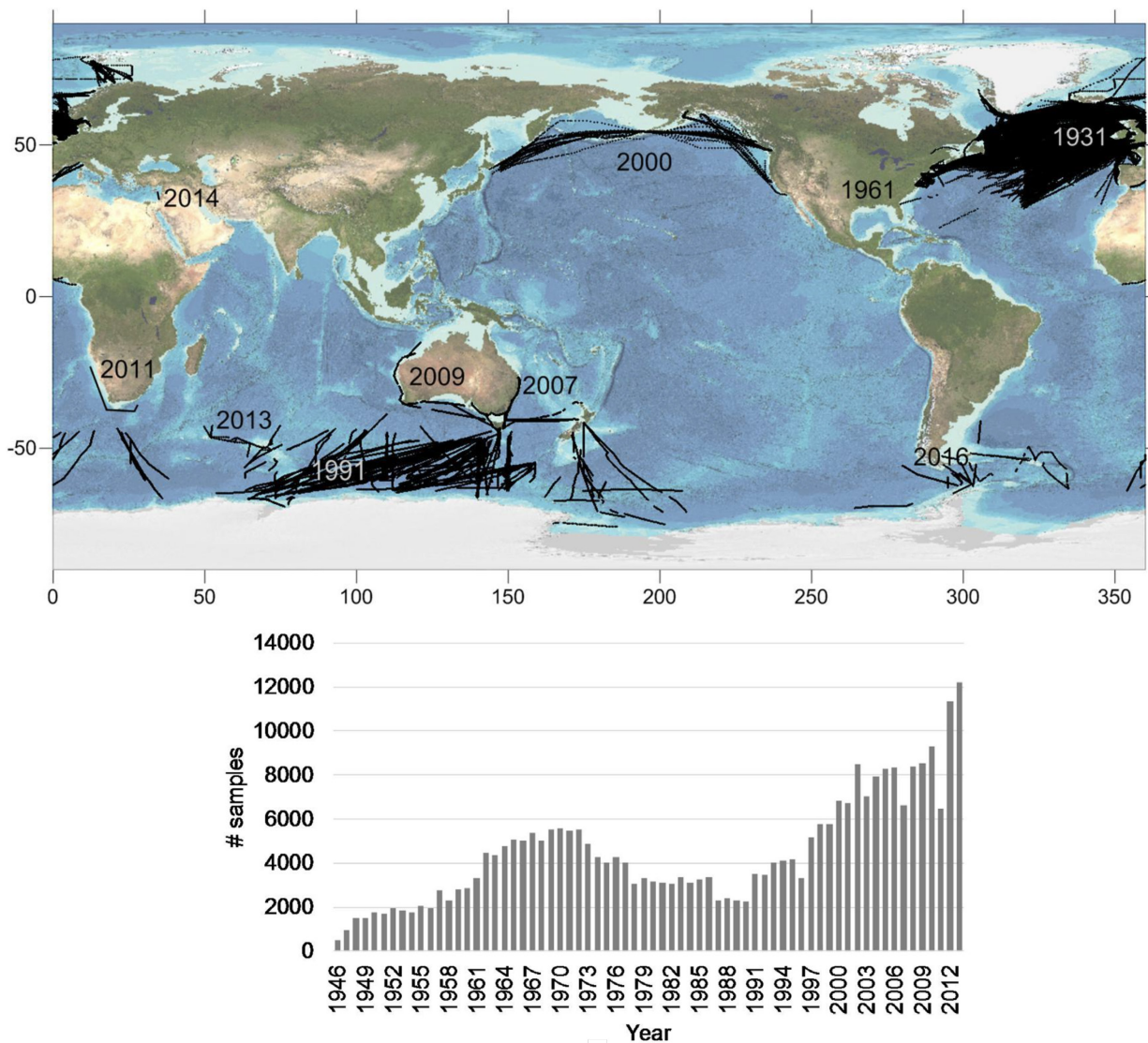
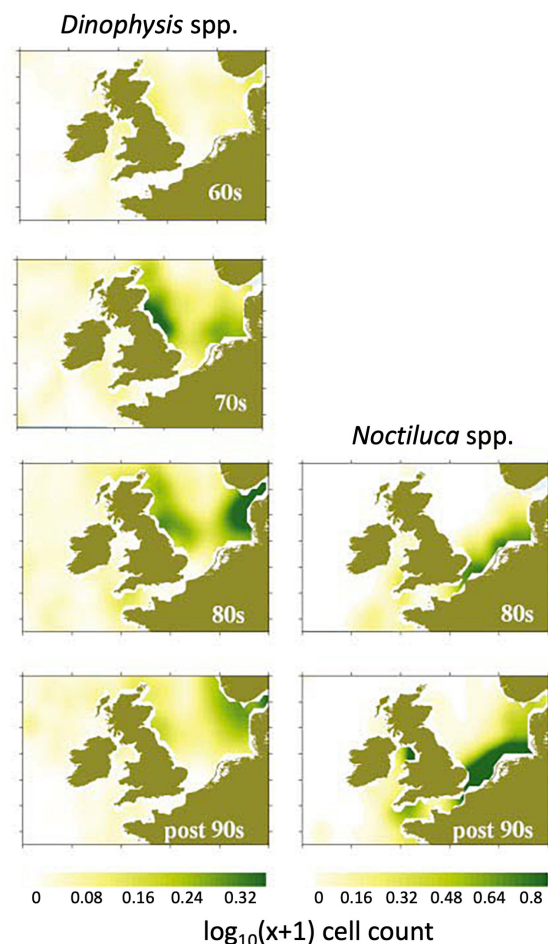


Fig. 5. Continuous Plankton Recorder samples. Top: Location and year of first sampling, and Bottom: Number of samples per year (from Batten et al. 2019).

Notwithstanding the limitations of light microscopy identification, the CPR collects data on several important HAB taxa (Fig. 6). In particular, many DSP-causing species, including *Prorocentrum lima* and several species of *Dinophysis*, and AST-producing *Pseudo-nitzschia* spp. (identified as 'seriata' or 'delicatissima' groups in some surveys) are commonly recorded. Among non-toxic but harmful species, data are available for *Cerataulina pelagica*, which can clog gills and asphyxiate fish, and for some *Chaetoceros* that can cause physical damage to gills. Data are also available for species that can cause water discoloration or produce odors, oil or foam, such as *Noctiluca scintillans*, *Phaeocystis* spp., *Cylindrotheca closterium*, and *Trichodesmium* sp.. As CPR data are internally consistent, they are excellent for describing spatial and temporal patterns, including blooms of these species. CPR data have correctly been used to identify repeated seasonal cycles of phytoplankton species and in showing changes in timing (phenology) in response to temperature (Edwards and Richardson 2004).

Despite coastal areas not being sampled, there are several approaches for using CPR data to answer questions related to HABs. First, CPR data can provide information on the oceanic populations of HAB species that are often only sampled in coastal areas. Second, the CPR data collected in open waters can be used to provide an independent test of potential drivers of blooms. For example, during the 1990s, there was substantial concern in European waters of increasing harmful and non- nuisance phytoplankton blooms in response to increased coastal eutrophication, but CPR data showed that the increase in phytoplankton biomass was widespread throughout oceanic waters of the Northeast Atlantic (Reid et al. 1998). This suggested that a larger-scale driver such as climate was responsible for the increase, rather than (solely) a more local driver such as cultural eutrophication. Third, CPR data can be used to provide longer-term context such as multi-decadal change for HAB species (Edwards et al. 2010). For example, long-term increases in *Noctiluca* and decadal changes in *Dinophysis* have been described (Edwards et al. 2006, Fig. 6). Fourth, data on other components of the plankton are available, including dozens of non-harmful phytoplankton species and grazers including copepods,

Fig. 6. Decadal distribution maps in the NE Atlantic based on the CPR data (from Edwards et al. 2006), showing range changes for *Dinophysis* spp. and an increase of *Noctiluca scintillans* in the 1990s.



euphausiids, salps and cladocerans. This can provide the broader context of the plankton community present. Last, because CPR data are collected over vast areas, they can document large-scale spread of HABs. For example, the CPR documented the spread of *Noctiluca* from the east coast of Australia around the south and north coasts and into the Southern Ocean (McLeod et al. 2012).

In terms of data availability, each CPR survey is run independently, so there are different data licensing agreements. However, >98% of all CPR data are freely available to researchers. For example, most information is downloadable from data portals for the North Atlantic survey (<https://www.cprsurvey.org/data/our-data/>) and the Australian CPR survey (see <https://portal.aodn.org.au/search>). Recently, the Global Alliance of CPR Surveys (GACS) has built a combined plankton database, including data on HAB species. Much of the global CPR data (as presence/absence) are included in OBIS.

### 5.3.2 THE AUSTRALIAN PHYTOPLANKTON DATABASE

In a collective effort by some 50 Australian phytoplankton workers, Davies et al. (2016) searched archives, contacted researchers, and scanned the primary and grey literature to collate 3,621,847 records of marine phytoplankton species from Australian waters from 1844 to the present. Many of these were small datasets collected to address local questions, but combined, they provide a powerful 170 years of data on phytoplankton communities in Australian waters. Units and taxonomy were standardized, obviously erroneous data removed, and all metadata included. This dataset was lodged with the Australian Ocean Data Network (AODN, <https://portal.aodn.org.au>) allowing public access. This dataset will continue to grow and will be regularly updated with new information and any further corrections to the data. If researchers or managers have additional datasets they would like to contribute, they can email [imos-plankton@csiro.au](mailto:imos-plankton@csiro.au), they can be included in future updates. The AODN portal will always direct the user to the most recent version, while the original version will remain available, and interim versions will be available on request.

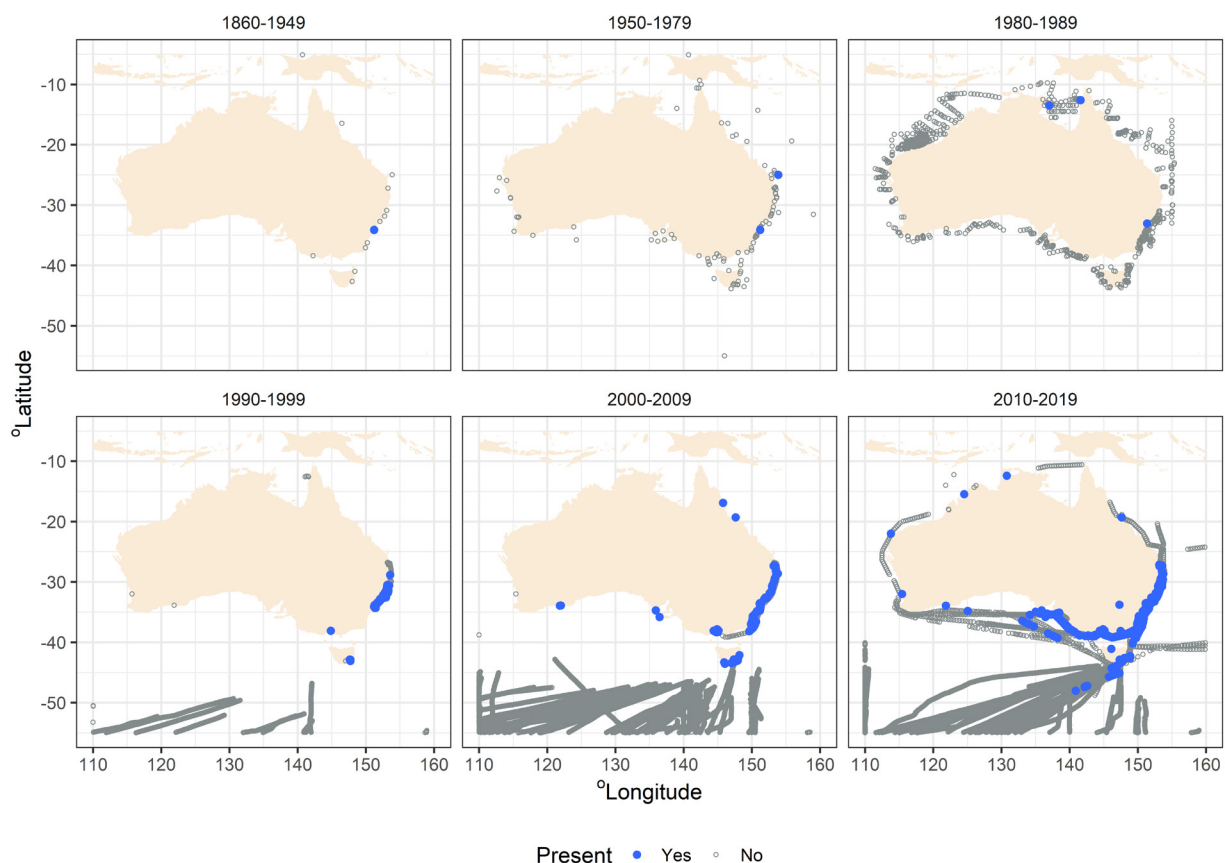


Fig. 7. Example output of the Australian Phytoplankton Data Base: Expansion in the distribution of *Noctiluca scintillans* in Australian waters (1860-2018).

Both the Australian Continuous Plankton Recorder (AusCPR) survey and the Australian Phytoplankton Data Base are part of the Integrated Marine Observing System (IMOS) which is a national collaborative research infrastructure, supported by the Australian Government.

The Australian Phytoplankton Database has proved invaluable for global change studies, as it allows analysis of ecological indicators of climate change and eutrophication (e.g., changes in distribution, Fig. 7; diatom:dinoflagellate ratios). In addition, the standardized conversion of abundance records to biomass provides modelers with quantitative data to initialize and validate ecosystem models of lower marine trophic levels.

### 5.3.3 THE DINOFLAGELLATE CYST DATABASE

Benthic resting stages play an important role in the seeding of dinoflagellate blooms, and fossil cysts in dated sediment depth cores can provide information on key environmental drivers and species adaptation over the long term (Chapter 4). The University of Bremen (Germany) website ([www.marum.de/en/Karin\\_Zonneveld/Modern-Dinocyst-Key.html](http://www.marum.de/en/Karin_Zonneveld/Modern-Dinocyst-Key.html)) has a valuable key to modern dinoflagellate cysts covering 94 taxa (Zonneveld and Pospelova 2015). This key will be continuously updated and improved by the authors. Importantly, this website also provides the open-access results of the 42 author international consortium that generated an atlas of modern dinoflagellate cysts based on 2405 samples (Zonneveld et al. 2013). The Atlas includes global maps of *Alexandrium 'tamarense'*, *Pyrodinium bahamense*, *Gymnodinium catenatum*, along with species of *Lingulodinium* (Fig. 8), *Operculodinium (Protoceratium)* and *Spiniferites (Gonyaulax)*.

The distribution of cysts of toxic and nuisance species represents a good complement to HABMAP-OBIS (section 5.2.3), which already holds records of HAB species based on cysts sampled from the sediments.

### 5.3.4 CYANOSITE AND GLEON

These two data initiatives record cyanobacteria and hence include information on several toxic species in this group. Cyanosite (<http://www-cyanosite.bio.purdue.edu>) was created in the Department of Biological Sciences at Purdue University in 1994 from an earlier LISTSERV, and represents the most complete information for cyanobacterial researchers. The site includes over 200 taxonomic images

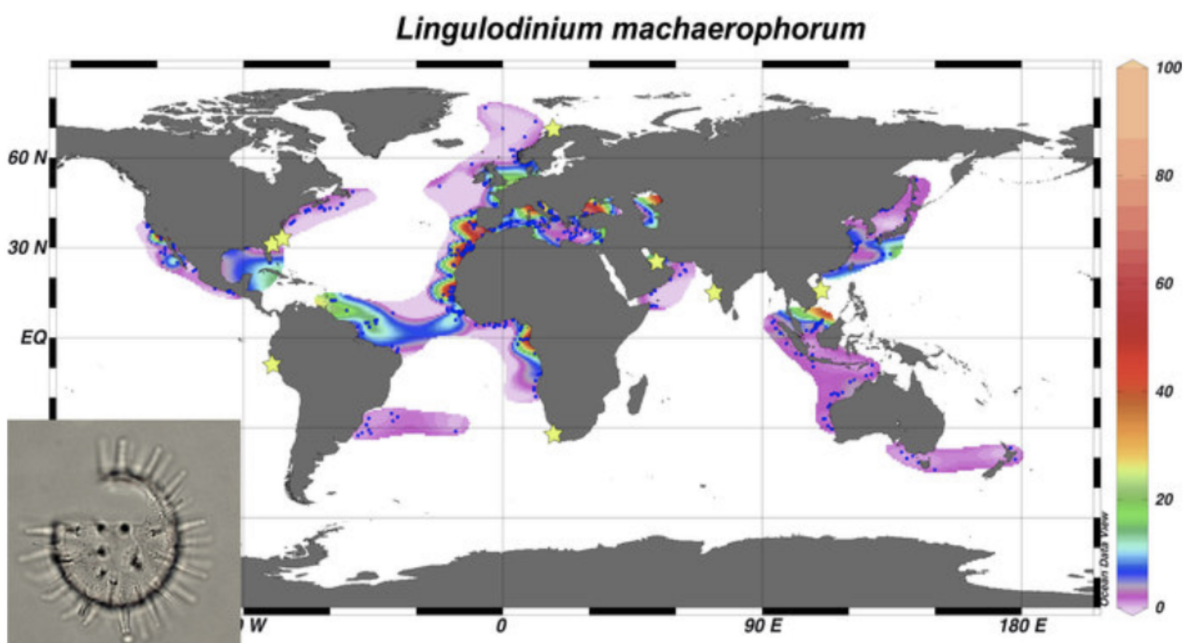


Fig. 8. Global distribution of the cyst *Lingulodinium machaerophorum* (Zonneveld et al. 2013). Stars represent reportings in the literature from regions that are not covered by the datasets incorporated in the Atlas.

and a searchable curated bibliography of 25,000 references. This material possibly offers the potential to be curated into a semiquantitative database of bloom events. Cyanobacterial bloom events have also recently been included in HAEDAT reporting.

GLEON, the Global Lake Ecological Observatory Network (<http://gleon.org/>), is primarily a network of 'people', with >500 members in 50+ countries and is growing rapidly (Fig. 9). About 30% of the members are students who belong to the *GLEON Student Association* (GSA), a vital part of the global network. GLEON is a network of lakes with more than 60 lake observatories across six continents. GLEON is also a network of data, including high frequency data gathered from sensors mounted on research buoys that are deployed in lakes across the globe. GLEON is the leading organization devoted to the study of lakes and reservoirs, and serves as a model for grassroots international research collaboration. Among activities that are relevant to HAB databases, the Cyanobacteria Working Group has developed a project aimed at compiling a global database on the spatial/temporal occurrence of microcystin, including physical and chemical data, to determine if toxic cyanobacterial blooms are indeed becoming more frequent and/or changing in concentration worldwide, and to develop predictive and forecasting models.

## 5.4 General considerations

The databases described in this chapter make a significant contribution towards addressing key questions in the quest to understand, manage and adapt to HABs and their impacts. Their analysis facilitates a description of the current status and changes in HAB occurrence and HAB events across the world seas, an issue that needs to be addressed to define a baseline for identifying future changes (Hallegraeff et al., 2021a). Investigation of shifts in the spatial ranges and temporal windows of harmful species and any trends of impacts from HABs can contribute to identifying clear cases of changes in species biogeography and bloom development. Finally, the databases can be used for the testing of models of climate drivers on HABs and their impacts, thus helping to address the questions outlined in this manual.

Extensive information to assess the status of HABs could be obtained directly from the vast literature and data-reports produced by scientists and monitoring agencies. Yet these data are difficult to access because they are provided in many different formats and spread across multiple scientific journals, countries and institutes. A unique benefit of global databases is that species ranges, similarities-dissimilarities, and changes in HABs and their impacts can be interpreted across wide geographic areas and over time. These data, if freely and easily available to the scientific community, have great potential to enhance our understanding of HAB dynamics and to the development of tools (e.g., rapid test methods, predictive models and risk alert systems) to address mitigation of HAB impacts in the future. Global databases also support the global scientific community to work together with a multifaceted approach to focus on significant HAB issues impacting wide geographic areas, e.g., ciguatera fish poisoning. Global databases can also provide fundamental data for statutory assessments of marine ecosystems and add power to assessments and studies made by individual nations by placing them in a wider historic, geographic and global context.

Based on the three main HAB datasets presented in Section 2, a first description of the status and current changes of HAB occurrence across the world seas is being provided by the ongoing project Global HAB Status Report (Hallegraeff et al. 2017, Zingone et al. 2017, Hallegraeff et al. 2021a,b). Importantly, this data-driven global overview will be an essential step, establishing a baseline towards building clearer quantitative linkages between climate drivers and changes (increase/decrease) in HABs regionally. Robust evidence is needed to substantiate perceived trends in HABs, similar to other environmental calamities that are perceived as increasing, such as widespread hypoxia and jellyfish blooms (Duarte et al. 2014). This evidence is required for appropriate advice to be given to society and industries impacted by HABs in the decades to come.

Within the GHSR initiative, regional editors of HABMAP OBIS and other scientists involved in HAB research have been invited to contribute overviews of HAB status and trends, each group focusing on one of the regions delineated in Section 5.2.3.4. The overviews have been built based on the existing scientific and grey literature and have widely exploited the information collected within HABMAP-OBIS and HAEDAT concerning harmful species and events. These studies have also given a great impetus to the compilation of the databases, with new information entered for several regions that were poorly covered previously.

The most striking result of the regional overviews is the heterogeneity of HAB types and their trends among and within regions. While DST events have higher incidence in European seas, with either no apparent trend (Belin et al. 2021; Bresnan et al. 2021) or a possible decrease (Karlson et al. 2021), they are less common than PST events in Canadian waters (McKenzie et al. 2021) and along the Atlantic US coasts (Anderson et al. 2021), with a slightly increasing trend in the latter area. Ciguatera is mostly confined to the subtropical Pacific and the Caribbean (Chinain et al. 2021, Sunesen et al. 2021), with recent expansion in Macaronesia and east and south Asia. PST and DST are also a major problem in South America, along with caged fish kills associated with *Pseudochattonella verruculosa* and *Alexandrium catenella* in Chile and tuna deaths related to *Triplos furca* and *Chattonella* in the Mexican Pacific (Sunesen et al. 2021). Salmon mortalities in Norwegian seas and the Skagerrak-Kattegat area have been mainly caused by prymnesiophyte blooms (Karlson et al. 2021), while *Margalefidinium polykrikoides* and/or other fish-killing algae have had impacts in Australia, New Zealand (Hallegraeff et al. 2021c) and the Philippines. In the Philippines and Malaysia, blooms of the PST producer *P. bahamense* have been a great concern in the 1990s, but have stabilized or decreased since then, along with an increase of other PST-producing species (Yñiguez et al. 2021).

In the Asian Pacific area, finfish mortalities by *Chattonella*, *Cochlodinium*, *Karenia* and *Karlodinium*, and shellfish mortalities by *Heterocapsa circularisquama* are of greatest concern, although bloom frequencies have decreased (Sakamoto et al., 2021). Mass mortalities are among the main problems in the Benguela upwelling system, where they may be caused by anoxia produced by non-toxic species as well as by blooms of several ichthyotoxic species and by the so-called 'ecosystem disruptive blooms' of *Aureococcus anophagefferens* (Pitcher and Louw 2021). In areas with intense tourism, such as the Mediterranean Sea, the highest impact derives from high biomass blooms, discoloration and mucilages (Zingone et al. 2021). While no global trend emerges from these overviews, new types of outbreaks (e.g., *Ostreopsis* in the Mediterranean Sea, Zingone et al. 2021), range expansions (e.g., *Noctiluca* in Australia), and disappearance (e.g., *Alexandrium catenella* in Australia, Hallegraeff et al. 2021c) are also recorded.

The highly variable picture emerging from the regional reviews reflects the wide taxonomic and ecological diversity of harmful species, intermingled with the complexity of coastal areas. In the context of HABs and climate change, the overall indication from these studies is that the analysis of trends and pattern of these phenomena and their links to multiple climatic or anthropogenic drivers should be addressed at the local and regional scale, and with a focus on species-specific ecological characteristics of the blooms. The other lesson learnt here is that databases can greatly benefit from large-scale scientific initiatives that demonstrate the usefulness of time-consuming, data collection efforts. These initiatives can also effectively attract researchers who can get involved in collaborative, high impact activities and publications, reinforcing the social and scientific visibility of HAB research in the respective areas along with that of the whole HAB scientific community.

The question remains if the datasets currently available or under construction are sufficient to answer the questions outlined in this book. They certainly represent a solid start, but several limitations will need to be addressed in future initiatives aimed at their improvement (see Section 5.2.5). It should be remembered that it is challenging to answer questions concerning HAB ecology, dynamics and long-term trends, even with existing databases. To improve our understanding of the environmental drivers



of HABs, especially in an age of rapid climate change, the HAB information in the databases needs to be matched with environmental data and investigated in the wider context of phytoplankton response to environmental changes (see Chapter 2). Along with some additional datasets described in this chapter (e.g., the CPR and the Australian Phytoplankton Datasets), several long-term phytoplankton datasets with ancillary environmental data could be exploited to this end, although their taxonomic resolution may not always be adequate to trace the individual toxin-producing species. Substantial effort and additional resources will be required in the coming years to collate microalgal distribution data from these time series, which presently have at least begun to be identified and located within several international initiatives (e.g., IOC IGMETS, IOC TrendsPO, ICES-IOC WGHABD, EMODNET, E-LTER DEIMS-SDR).

Although most of the HAB species distribution and event data do not have concomitant environmental data, global products are increasingly available that could meet this need. For example, environmental data from satellite, ships of opportunity, buoys and floats including sea surface temperature (e.g., <https://www.ghrsst.org/ghrsst-data-services/products/>), chlorophyll-a (e.g., <https://esa-ocean-colour-cci.org/>) and winds (e.g., <https://ghrc.nsstc.nasa.gov/home/measurements/winds>). Outputs from high-resolution hydrodynamic models can provide physical and chemical variables for analyses, including temperature and salinity throughout the water column, mixed layer depths and nutrient concentrations (e.g., <https://www.ncdc.noaa.gov/nomads/data-products>). Many of these datasets are daily in resolution and on the scale of a few kilometers, so there are often data available that might be sufficient to investigate mechanistic linkages of HABs.

Quality standards for oceanographic data have made considerable progress over recent decades (Tanhua et al. 2010), although difficulties and uncertainties have been recognized, particularly for coastal datasets (Campbell et al. 2013, Sabia et al. 2019). The issue of quality control is even more complex for biological datasets, particularly those deriving from expert classification and identification, for which specific methodological guidelines have been formulated (Karlson et al. 2010, HELCOM 2014). Specific requirements for metadata and a system of QFs for phytoplankton identification data have been proposed to ensure consistency of quality control of species level reporting (Zingone et al. 2015). Even in the case of peer-reviewed literature, data should undergo a quality control check and be flagged by experts, to ensure their correct use. HAB datasets need to be managed using the best available practices, i.e. using standardized vocabularies, units and methods and following existing quality standards such as ISO 17025. Comprehensive metadata for the datasets are also needed, so end users can understand how samples were collected, stored and processed, what tools and literature was used for identification, how abundance data were obtained, and any issues that may influence their interpretation (Zingone et al. 2015). This is essential for comparison among species and regions, but also to integrate different kinds of datasets, such as oceanographic data, toxin concentrations, economic impacts, human health and food safety. The current state of the art of which species are toxic, along with their correct nomenclature and relevant literature collected in the *IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae* (Section 5-2.1), represents a good start and a useful tool to standardize the information. The addition suggested here (Section 5.2.5) of reference identification keys with illustrations would be a further development of the List towards easier and sounder species identification. Datasets obtained through advanced technologies, such as reference molecular sequence databases, metabarcoding data and automated imaging system will require a different set of QC procedures and metadata, which should be designed along with the dataset and could not rely on QC of individual records, considering the huge amount of data produced through these approaches.

The retrieval of data from the literature, their revision and the standardization needed for any global collation require considerable time and resources. Over the years, the IOC and other organizations have greatly contributed to these efforts, also with the support of specific projects (e.g., the one of the Government of Flanders supporting the Global HAB Status Report), providing the essential framework and infrastructure. However, entering data in databases requires time and resources that are mostly

provided by expert scientists around the world who join data initiatives on a voluntary basis, making data available to the broader community to facilitate multiple uses over the years, in full agreement with the FAIR (Findable, Accessible, Interoperable and Reusable) data principles (Wilkinson et al. 2016). In the decades to come, technological developments such as metabarcoding of environmental DNA and in situ automated imaging systems are expected to improve the existing databases and their use and provide tools that allow drawing on multiple datasets to increase the level of information provided. Critical to all data-driven science and management in the future is that data producers and holders have adequate resources to deliver them in the appropriate way.

Global databases presented in this overview highlight the value of continuing the existing databases and establishing new ones that are more complete for use by the wider scientific community and policy makers to investigate impacts of climate variability and change on marine ecosystems. The first analyses (Hallegraeff et al. 2021) already have shown their value in addressing questions of global importance. It is only by increasing the use and application of these databases that we can create greater awareness and support for data initiatives by institutions and funding agencies.

## 5.5 Concluding remarks

The general considerations presented in this chapter can be summarized in the following series of remarks and recommendations:

- The design and extensive data housed by HAB databases have advanced considerably over the last years following substantial effort by the scientific community, led by a number of international agencies. They are starting to demonstrate their usefulness by supporting regional overviews and first data-driven global assessments of the status of HABs in the world seas. A number of other existing databases on phytoplankton distribution can also provide information on harmful species, their distribution and their trends, while environmental information from existing oceanographic and satellite dataset can be used to complement the HAB-related ones in the effort to understand drivers of harmful species distributions and trends.
- None of the available HAB-related databases include both spatial and temporal data of species densities and concomitant environmental conditions that are specifically needed for research on the links between HABs (and the phytoplankton community more generally) and climate change. This would be best investigated alongside the development of the aquaculture industries and other human activities in the coastal zone.
- Phytoplankton data, including harmful species density collected by multiple institutions, projects and individual scientists, should be made available to the scientific community. To this end, monitoring agencies and research institutions should be actively involved in data sharing projects fostered and funded by national and international agencies.
- International agencies such as IOC, SCOR, FAO and IAEA should reinforce their activities aimed at promoting data collation and exchange, as part of their mission, and further invest in the establishment and upgrading of data infrastructure and staff assigned to data management.
- To raise the profile of databases and share knowledge on their correct and optimal use, the above agencies, along with scientific societies such as the International Society for the Study of Harmful Algae (ISSHA), regional organizations such as ICES, PICES and the international Program GlobalHAB, should organize specific data seminars, courses and workshops. In these meetings, statisticians, harmful bloom experts and data producers would have the chance to work together to improve tools for the analysis of HAB data and produce guidelines for data collection and management.
- Data contribution to open access databases would be accelerated if scientists, institutions and agencies were supported by data management experts in the hard work of data reformatting and metadata documentation.

- The work and finances needed to collect, manage and quality control data should be acknowledged by involving data producers in the analyses of their data.
- Existing databases, including HAB related ones and more general ones such as AlgaeBase, have been mostly developed by scientists on a voluntary basis, but would best be supported by adequate resources to continue developing. This could happen through specific funding mechanisms in international agencies, for which specific calls should be solicited.
- Such efforts would align with the UN Decade for Ocean Science for Sustainable Development and the desired outcomes for open and equitable access to data, information and technology and innovation. This will facilitate the prediction of ocean states, enabling society to understand and respond to changing ocean conditions.

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## Appendix I

### Links to the databases and main websites cited in this overview

AlgaeBase, <http://www.algaebase.org/>

AODN: Australian Ocean Data Network, <http://portal.aodn.org.au/>

Old information at the portal <http://dx.doi.org/10.4225/69/56454b2ba2f79>

AusCPR: Australian Continuous Plankton Recorder, all data are contained in AODN, <http://portal.aodn.org.au/>

CPR: Continuous Plankton Recorder, <https://www.cprsurvey.org/>

Cyanosite, <http://www-cyanosite.bio.purdue.edu>

DEIMS-SDR: Dynamic Ecological Information Management System- Site and dataset registry, <https://deims.org/>

Dinoflagellate Cyst Database, [https://www.marum.de/en/Karin\\_Zonneveld/Modern-Dinocyst-Key.html](https://www.marum.de/en/Karin_Zonneveld/Modern-Dinocyst-Key.html)

E-LTER: Long Term Ecological Research in Europe, <https://www.lter-europe.net/lter-europe/data>

GACS: Global Alliance of CPR Surveys, <http://www.globalcpr.org/>

GLEON: Global Lake Ecological Observatory Network, <http://gleon.org/>

HABMAP-OBIS: Database on the geographic range of harmful species, <http://ipt.iobis.org/hab>

HAIS: Harmful Algal Information System, <http://hais.ioc-unesco.org>

IAPT: International Code of Nomenclature for algae, fungi, and plants (Shenzen Code),  
<https://www.iapt-taxon.org/nomen/main.php>

IGMETS: International Group for Marine Ecological Time Series, <https://igmets.net/>

IMOS: Integrated Marine Observing System <http://imos.org.au/>

ICES-IOC WGHABD: Working Group on Harmful Algal Bloom Dynamics  
[http://hab.ioc-unesco.org/index.php?option=com\\_content&view=article&id=11&Itemid=0](http://hab.ioc-unesco.org/index.php?option=com_content&view=article&id=11&Itemid=0)

IOC-ICES-PICES HAEDAT: Harmful Algal Event Database, <http://haedat.iode.org/>

IOC-IODE: International Oceanographic Data and Information Exchange, <https://www.iode.org/>

IOC Taxonomic Reference List of Harmful Micro Algae, <http://www.marinespecies.org/hab/>

IOC TrendsPO: IOC-UNESCO Working group to investigate Climate Change and Global Trends of Phytoplankton in the Ocean, <https://trendspo.net/>

QUASIMEME: Quality Assurance of Information in Marine Environmental monitoring,  
<https://www.wepal.nl/en/wepal.htm>

WoRMS: World Register of Marine Organisms, <http://www.marinespecies.org/>

# CHAPTER 6

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## Future Perspectives in Modeling Harmful Algal Bloom (HAB) Responses to Climate Change: Guidelines for HABs modeling

David P. Hamilton<sup>1</sup>, Clarissa R. Anderson<sup>2</sup>, Inga Hense<sup>3</sup> and Steven Chapra<sup>4</sup>

<sup>1</sup> Australian Rivers Institute, Griffith University, Brisbane, Australia

<sup>2</sup> Ocean Sciences Department, University of California Santa Cruz, Santa Cruz, California, USA

<sup>3</sup> Institute of Marine Ecosystem and Fishery Science, Universität Hamburg, Hamburg, Germany

<sup>4</sup> Tufts University, Medford, Massachusetts, USA

### 6.1 Introduction

Harmful algal blooms (HABs) have serious ecological, economic and human health-related consequences (Anderson 2014). There is concern about potential for future changes in the frequency and magnitude of HABs (Hallegraeff et al. 2021), under the individual and combined influences of climate change and altered nutrient loading from land use change (Hamilton et al. 2016), as well as increases in translocation of species that can become invasive in their new habitat under the influence of altered climate. Hence, there is a large societal interest in understanding and predicting the occurrence, magnitude, duration and area of coverage of HABs. Models are useful to address HAB dynamics, examine how HABs may be predicted to change in future environmental scenarios, and test operational strategies for their mitigation.

Before examining the details of the different types of HAB models, we should first set clear definitions for terms related to prediction time scales, as different terms have often been used interchangeably by modelers, sometimes resulting in confusion about the goals for a model application. In most applications, there is a **model hindcast** that is usually compared visually and statistically with a discrete set of site-specific observed data to demonstrate the utility of the model and its ability to capture the dynamics of one or more HAB events. In other cases, however, it may be desirable to have a single model application for an array of system types that have different hydrologic, morphometric, and biochemical characteristics (Chapra et al. 2017). In this case a *sensu stricto* validation across all systems is not possible, but the model may be used in a theoretical or risk assessment framework to assess the forcing inputs (e.g., air temperature) or model parameters (e.g., phytoplankton growth rates) to which the HAB event is most sensitive. This approach can be complemented with a sensitivity analysis which, alongside the site-specific **calibration and validation** procedure, is designed to generate confidence in the use of the model to simulate specific future events like climate change. This formalization of the modeling procedure is generally well established and agreed upon, unlike terms commonly used for model **predictions**. Here we adopt the terminology of Rouso et al. (2020) who refer to **forecasts** as focused mostly on short term events (days to months) that are often relevant to operational strategies (e.g., sampling a HAB or shutting off a HAB-affected water intake) versus **projections** generated from past or future forcing data (e.g., climate change), which themselves also include an



assumption of specific changes to the system (e.g., future socioeconomic and technological developments affecting CO<sub>2</sub> concentrations in the atmosphere; IPCC 2014). Predictions therefore include both forecasts and projections.

Models that include forecasts and projections most commonly include statistical and deterministic outputs, respectively. **Statistical models**, also known as data-driven models, take a variety of forms, including machine learning algorithms, artificial neural networks, genetic programming and Bayesian networks (Rousso et al. 2020). **Deterministic models**, also known as process-based or mechanistic models, attempt to represent the critical processes in a dynamic (time-varying) fashion. Statistical models generally provide probabilistic outputs of HAB composition and magnitude at a discrete point in time and are highly sensitive to initial conditions (Lorenz 1969, Slingo and Palmer 2011), while deterministic models can be used to understand long-term HAB dynamics and evolving trends in time and space. This separation of time periods in output from statistical and deterministic models is not exclusive and some statistical models can have application for climate change, while some deterministic models have been used in an operational mode, linked to short-term weather forecasts, for periods of a few days. Statistical models include diagnostic approaches that are based on logistic regressions or artificial neural network analysis. They may be driven purely by observational data (e.g. Velo-Suárez et al. 2007, Qin et al. 2017) or may use physical and biogeochemical variables from coupled ecosystem-hydrodynamic models (e.g. Brown et al. 2013). Often, HAB forecast systems employ combinations of different methodologies (Allen et al. 2008) such as field observations, numerical models, neural network models, and remote sensing (e.g., Brown et al. 2013, Wynne et al. 2013, Anderson et al. 2016). These blended approaches use the inherent capacity of a range of existing observational and model platforms to produce outputs extending beyond a single targeted forecast. For the purposes of this chapter, where we seek to provide guidance to the scientific community on HAB modeling strategies appropriate for climate change research, our main focus herewith is on deterministic models but not exclusively so, and we acknowledge aspects of overlap with statistical models.

The use of the term 'deterministic' refers to the non-probabilistic nature of outputs from these models, which produce only one simulation result from a given set of input data. A number of options are used by modelers to overcome this limitation, including: (1) **sensitivity analysis** to develop probabilistic outputs related to the risk of HABs (Chapra et al. 2017); (2) **Monte Carlo simulations** where forcing data and parameters are altered within pre-defined boundaries in repeated simulations with a single model, from which it is possible to generate a distribution of outputs; and (3) **model ensembles** that carry out statistical comparisons of outputs from different models using identical input data. Recent advances in computational power and methods (e.g., computational parallel processing) have greatly enhanced the capacity to run repeated simulations, often consisting of hundreds or thousands of model runs to support a probabilistic output. Ensemble outputs usually consist of individual runs from a small number of models to describe variations among models in simple statistical form, i.e., commonly a mean, median and range of the output (e.g., HAB biomass). Collectively, these three approaches help to communicate uncertainty in model output and can be converted to risk assessments and statistical probability for HAB occurrences. Nevertheless there may be limits to the capacity to communicate uncertainty generated from coupled model outputs and inputs (e.g., the response of HAB models to different catchment model outputs and climate change scenarios) as uncertainty is often considered to be multiplicative with the number of models, distributed outputs, and the pathways that are considered (e.g., Representative Concentration Pathways in climate change scenarios; IPCC 2014). Communicating uncertainty of model output remains an ongoing challenge for scientists, particularly where policy makers seek a high level of scientific robustness and the public may be skeptical of the use of model outputs to plan mitigation actions.

Deterministic models commonly include a hydrodynamic component, possibly an ecosystem component, initial distributions of relevant fields (**state variables**) over the model domain (e.g., HAB bio-

mass), **forcing functions** (e.g., wind, radiation), and analysis and visualization tools. A wide range of model systems has been developed and described (for an overview see, e.g., Franks 1997, Anderson et al. 2015, Davidson et al. 2016, Flynn and McGillicuddy 2018, Ralston and Moore 2020). Many of these model systems have been used to answer questions related to HAB dynamics and to provide guidance for their management. In this chapter we present an overview of current model approaches, their strengths and weaknesses and their range of applicability. We also define the state-of-the-art in HAB modeling and specify the requirements for future development of HAB models, especially for long-term predictions aligned with assessments of HAB occurrences under climate change. The chapter is separated into five sections, including this introductory section (1), a review of relevant hydrodynamic and ecological models (section 2), model input data (section 3), model calibration and validation (section 4) and a forward-looking section (5) to identify future challenges and requirements for HAB models.

## 6.2 Hydrodynamic-ecological models

In this section we examine different model systems differentiated by their numerical schemes for simulating water transport and mixing, coupled to HAB dynamics (e.g., hydrodynamic-ecosystem linkages), and hybrid modeling approaches that can collectively reinforce predictions of HAB dynamics. Several model systems have been established in recent years for short-term HAB predictions (e.g. Wynne et al. 2013, Anderson et al. 2016, Cusack et al. 2016, Dabrowski et al. 2016, Pinto et al. 2016). A major challenge is to choose the most appropriate model system for a given purpose, accounting for the specific modeling goals and the relevant temporal and spatial scales of interest. For the purposes of this chapter, our goal is to model impacts of climate change on HABs. Ralston and Moore (2020) identify a “window of opportunity” of increased risk of HAB development and toxin accumulation, which is altered by climate change. They suggest that aligning the model to this window is different from attempting to simulate specific HAB events or the phenology of phytoplankton communities, although many water managers may also require operational models with the ability to predict transient or acute events (e.g., the timing and magnitude of a particular HAB species; Harris 1997).

### 6.2.1 HYDRODYNAMIC MODELS

The basis of most HAB prediction models is an existing **hydrodynamic model** that simulates physical transport and mixing processes of water and its dissolved and particulate constituents, and provides flow fields suitable to determine the distribution of heat, solutes and particulate material for the region of interest (e.g., Zhang et al. 2013, Li et al. 2014, Aleynik et al. 2016, Dabrowski et al. 2016, Cusack et al. 2016). The model should include all the relevant forcing mechanisms (heat fluxes, freshwater fluxes, current and wind vectors and tides) for the model domain. The domain is generally delineated with a horizontal and vertical resolution adequate to capture the predominant variability of HAB populations. The domain of coastal and ocean model systems may be local (less than 10 km horizontally) or regional (up to a few hundred km), with grid spacing of a few kilometers in the horizontal and about one meter in the vertical, but often with spacing increasing deeper in the water column (e.g., Gulf of Maine; McGillicuddy et al. 2005). Models of HABs for the Great Lakes (e.g., Lake Erie; Ho and Michalak 2015) face similar challenges of scale to those for the coastal and open ocean but for smaller inland waterbodies and estuaries, it may be possible to apply models with horizontal resolution of the order of tens of meters, which is important for nearshore regions where there can be localized HAB accumulations and high variability of biomass (Fig. 1). Predictions of HABs in estuaries are complex because these areas experience high levels of anthropogenic pressure and strong physical forcings from tides, morphological variability and salinity changes that make these systems highly dynamic. For this case, coarser grids should not be used, as the representation of topography and coastline, fluid flow and tracer spreading may be compromised.

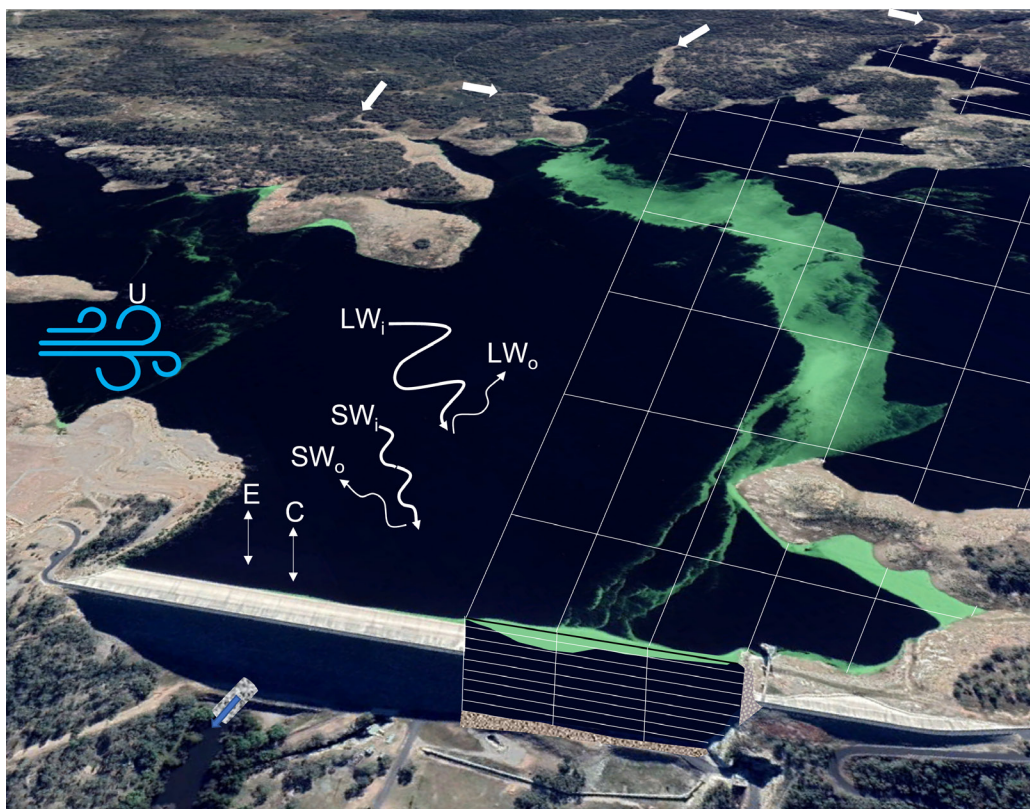


Figure 1. Conceptual diagram of a three-dimensional Eulerian model domain (right-hand side) for a deep eutrophic reservoir with a cyanobacteria bloom. Note the challenge of simulating the high variability of biomass across the reservoir. Model boundary conditions include inflows (white arrows in the catchment), outflow (blue arrow at bottom of dam wall), and climate ( $LW_i$  and  $LW_o$  are longwave radiation input and output,  $SW_i$  and  $SW_o$  are shortwave radiation input and albedo,  $E$  is latent heat flux,  $C$  is conductive heat flux and  $U$  is wind speed. Rainfall and sediment heat flux are not shown. Background image is from Google Earth.

Hydrodynamic modeling involves a number of considerations about the type of model that may be required. A **Eulerian hydrodynamic model** commonly uses a fixed framework to delineate rectangular grids of identical size (Fig. 1). More sophisticated curvilinear coordinate systems can circumvent some of the limitations of fixed grids using a localized concentration of grid cells, conforming them according to the morphology of the domain, and thus better representing effects of bays, land forms (e.g., islands) and constrictions that can rapidly disperse or concentrate HABs. This coordinate system has increased mathematical complexity and computational time. The Eulerian framework limits the ability to store attributes of particles (e.g., light history of phytoplankton cells) because properties of state variables are lumped with the water mass and momentum calculations, i.e., multi compartment models. By contrast, **Lagrangian hydrodynamic models** track the movement of parcels of water or individual particles. Hybrid Eulerian-Lagrangian models can be particularly useful for biogeochemical modeling as they combine the computational power of a Eulerian model to describe advection and dispersion, with the ability to explore tracer or particle trajectories in complex flow fields (e.g., eddies, surface or internal waves; Chenillat et al. 2015). Since most HAB species have a maximum doubling rate of approximately one per day or less, the approach is obviously limited to forecast periods of a few days to a week. Proper implementation of this approach requires numerous simulations with slightly different hydrodynamic and/or initial particle distributions for statistical analysis, to generate probabilistic distributions.

Compromises are sometimes made to model dimensions or resolution to reduce model run times, although nowadays access to high-performance computing has overcome some of the runtime limitations of complex hydrodynamic models. Most ecosystem models have a hydrodynamic model that uses a hydrostatic approximation that neglects non-hydrostatic pressure and vertical momentum,

reducing ability for realistic simulations of complex internal dynamics like internal waves and flow at topographic irregularities (Wadzuk and Hodges 2014). This simplification makes it possible to have fully coupled hydrodynamic-ecological models over large domains. In some cases, one or more of the dimensions may be excluded to reduce model run times and focus only on effects on HABs of diurnal or seasonal stratification. Such cases include one-dimensional horizontally averaged models (Hamilton et al. 2014) or two-dimensional models with longitudinal and vertical variation suitable for applications to canyon-shaped reservoirs (Mi et al. 2019).

### 6.2.2 ECOLOGICAL MODELS

Ecological models (mainly NPZD-type models, i.e., Nutrients-Phytoplankton-Zooplankton-Detritus) have been connected to hydrodynamic models in two modes. One is passive coupling in which the hydrodynamic model is run first to generate flow fields for the advection and dispersion of heat, solutes and particulate material. This mode can reduce run times because it avoids the computational cost of advection and diffusion of each biogeochemical state variable, redistributing these variables based solely on the output flow field from the hydrodynamic model, but it neglects potentially important physical-biological feedbacks such as changes in heat and radiation distribution through the water column from changes in optically active constituents (e.g., phytoplankton, colored dissolved organic material). The second mode is fully coupled hydrodynamic-ecological models that pass information dynamically between the constituent models during a simulation, slowing run times but capturing the important physical-biological feedbacks.

The NPZD models rely mostly on empirical models of growth, mortality and other biological and ecological processes (e.g., settling, motility or buoyancy control) (e.g., Li et al. 2014, Davis et al. 2014). Often, these models use compartments for nutrients, primary producers, consumers and detritus, and aim to achieve mass conservation of macronutrients at the atomic level (e.g., for carbon, nitrogen and phosphorus). This level of description and compartmentalization is required for a fully predictive model of HABs that integrates the bottom-up (e.g., nutrients) and top-down (e.g., zooplankton grazing) drivers of biomass. Some components of these models, however, may be individual based (e.g., Aleynik et al. 2016, Gillibrand et al. 2016, Ranjbar et al. 2021), i.e., following organisms through their life cycle and spatial domain (Hense and Beckmann 2015, Hieronymus et al. 2021). These types of models are relevant for HAB species life cycles and can allow heuristic functions for conditional branching (when to divide, how mutations will change the parameters of daughter cells), direct or indirect interaction rules, and the environment (detritus and nutrients), as well as stochastic processes (e.g., mortality) affecting individuals randomly.

Beckmann et al. (2019) define an **individual based model** (sometimes also referred to as an **agent based model**) as one that simulates the actions and interactions of independent individuals (e.g., HAB cells, filaments or colonies), with parameters to describe the key traits of the individuals. Many of these traits are related to reproductive cycles such as akinete differentiation (Hellweger et al. 2008) or encystment rate (Hinnners et al. 2019), nitrogen fixation (Hellweger et al. 2016), and stochastic processes like mortality that are designed to affect individuals randomly. Individual based models also provide opportunities to include evolutionary responses (understood here as adaptive; Chapter 4) (Beckman et al. 2019, Hinnners et al. 2019) at the strain level, as different strains can be selected under various water mixing (Li et al. 2018) or temperature regimes (Xiao et al. 2020). Such evolutionary (or adaptive) processes are likely to become important on time scales (e.g., extending to 2100) that may be used to examine HAB responses to climate change (see Chapter 4). The first steps to include HAB life cycle processes in addition to basic biological processes and biotic interactions have been made in local (one-dimensional) climate projection studies (e.g. Hense et al. 2013, Hinnners et al. 2015). However, the goal of these studies was not primarily to provide estimates of future HAB occurrences but to study the impact of intrinsic and extrinsic factors on future HAB development.

Table 1. Physiological and biological processes relevant to HABs including, where relevant, how they may be affected by climate change.

Process	Justification	Reference	Relevance to climate change
Nitrogen fixation including heterocyst formation	Some cyanobacteria use atmospheric N as a supplementary N source	Hellweger et al. (2016); Dutheil et al. (2018); Inomura et al. (2019)	Heterocyst formation has been linked to a discrete temperature range in some HAB species
Buoyancy control	Photosynthetic products act as a ballast to offset buoyancy	Visser et al. (1997)	Increased stratification due to increases in temperature may favor buoyant species or those that can regulate their position in the water column (e.g., by using flagella)
Colony formation	Important in buoyancy, grazing vulnerability, exopolysaccharide production, etc.	Serizawa et al. (2007)	Increased stratification due to increases in temperature may favor larger colonial species that are more buoyant
Trichome formation	Affects cell buoyancy	Yamamoto and Nakahara (2009)	Increased stratification from increases in temperature may favor multicellular trichomes that are more buoyant
Vertical migration (e.g., through use of flagella)	Migration is critical for water column position, front formation, accessibility to nutrients, light, etc.	Ji and Franks (2007)	Increased stratification from increases in temperature may favor motile species that are less frequently entrained in turbulent motions
Morphological changes	Changes in cell size from growth, change in temperature; colony or trichome morphology may be affected by turbulence	Li et al. (2018)	Cell sizes may decrease with increasing temperature (Zohary et al. 2021)
Evolutionary and adaptive traits	Physiological processes like encystment, excystment, etc. may be affected by environmental drivers, e.g., temperature	Hinners et al. (2019)	Environmental change, e.g., increased temperature, could favour adaptive or evolutionary changes to strains and populations
Programmed Cell Death (also linked to apoptosis and autophagy)	Cells spontaneously die or wither	Bar-Zeev et al. (2013)	Stochastic processes required to account for Programmed Cell Death
Carbon Concentrating Mechanism	Specialised bicarbonate and carbonate transport mechanisms, together with carbonic anhydrase, overcome carbon limitation at high pH	Price et al. (2013)	Increased magnitude of HABs may reinforce blooms by increasing pH and reducing availability of free CO <sub>2</sub> in freshwater; seawater less likely to be affected especially with ocean acidification
High affinity phosphorus uptake	Alkaline phosphatase and up-regulation of phosphorus uptake supports uptake from organic sources and under low phosphate	Bonachela et al. (2011)	Increased variability of phosphorus supply from greater rainfall variability could favor species with high capacity to store and regulate uptake of different phosphorus sources
Predation, chytrid infections or viral attack	Loss processes that are often not particularly well understood or represented in ecosystem models	Sigee et al. (2007)	Not known
Encystment (including akinete formation) and excystment		Hense (2010)	Temperature will affect differentiation of different functional cells in the life cycle
Quorum sensing	Influences interactions among and within species, and may be important in physiological processes including toxin production	Zhou et al. (2016)	Not known

The explicit representation of biological processes allows consideration of time scales beyond a few days used in some models that simulate dispersion of conservative particles as an analog for HAB cells (see section 2.1). Over longer time scales, growth and loss processes need to be included. Many other biological processes that act on longer timescales – weekly or seasonal – such as colony formation or resting stage duration (including, for example, germination), as well as the multiple interactions (e.g., competition, grazing; see Chapter 4) with other organisms (including viruses and parasites) through the microbial loop, make this modeling highly challenging.

Table 1 summarises some of the physiological and biological processes relevant to different HAB species and examines how adaptive responses could be altered by climate change. Many of the physiological responses have been observed over many years or decades, with algorithms developed to represent several of the responses. Burford et al. (2019) identify some fundamental challenges in predicting HABs in a changing climate, including lack of, or rudimentary sub-models (e.g., nitrogen fixation, colony aggregation and disaggregation, toxin production) that hinder capacity to simulate dominant HAB species and successional sequences. Only a modest proportion of algorithms is usually included in full ecosystem models, likely due to concerns about model complexity and parameterization (related to both over-parameterization and knowledge of parameter values), as well as inadequacies of process knowledge or model-developer skill. We therefore have somewhat incomplete descriptions of HAB physiology in most ecosystem models. Until this issue is resolved, we should not expect ecosystem models to output detailed phenological responses of HAB species and strains to climate change, except for relatively well known and largely empirical responses of increased growth rates at elevated temperature and greater near-surface accumulations with increasing water column stratification. This level of process description may help to differentiate when HAB species may out-compete non-HAB species under climate change, but more effort is clearly needed to include many of the physiological and adaptive responses of cyanobacteria when considering climate change (Carey et al. 2012).

### **6.3 Model forcing data for climate change simulations**

There is a growing need for long-term predictions that examine the effects of anthropogenic climate change on the frequency and magnitude of HABs (e.g., Dyble et al. 2008, Glibert and Burford 2017), even if current ecological models have limitations for differentiating HAB species and strains. The relevant time scales of HAB predictions for climate change are decades, often extending to 2100, but seasonal predictions are also relevant because climate variability acts over a number of time scales, e.g., due to the El Niño–Southern Oscillation (ENSO; see Doblas-Reyes et al. 2013), or extreme weather (e.g., Woolway et al. 2021), and these events are fundamentally altered by climate change. The future climate can be expected to manifest primarily as increases in average air temperature and alterations in the timing, magnitude and distribution of rainfall. Projections of future changes in wind speed are less certain but important for water column stratification. For example, atmospheric stilling has been identified as a component of increases in surface temperature and thermal stability for lakes across the Northern Hemisphere between 1980 and 2016 (Woolway et al. 2019).

#### **6.3.1 GLOBAL CLIMATE MODELS FOR CLIMATE CHANGE PROJECTION**

Climate projections are available from a number of models used by the IPCC, to drive ecosystem models at decadal and seasonal time scales, which is different from operational models commonly forced with weather forecasts on time scales of days. The current suite of climate change projections from the Coupled Model Intercomparison Project (CMIP, with CMIP5 derived from the IPCC (2014) fifth assessment report (AR5)) provides monthly perturbations of air temperature, radiation, wind speed, humidity, evapotranspiration and sea level at relatively coarse horizontal resolution (~100 km) across the globe relative to a recent 'baseline' (e.g., 1970-2000). A modeler interested in HAB predic-

tion needs to make decisions about (1) which variables to include as forcing input to hydrological, hydrodynamic and biochemical models used individually or collectively for HAB predictions, (2) use of regional downscaled models, either dynamic or statistical, that generate climate data at smaller scale (~5-10 km grid) and are designed to better represent meteorological conditions specific to a waterbody, and (3) Representative Concentration Pathways (RCPs) to indicate varying greenhouse gas emission mitigation efforts, from RCP2.6 with strong effort to reduce emissions to RCP8.5 with minimal reduction (see the Fifth Assessment Report of IPCC, 2014). CMIP6 will supersede CMIP5, with the IPCC sixth assessment report (AR6) making use of a substantially expanded number of climate models and providing a wider range of emissions scenarios (SSP1-2.6, SSP2-4.5, SSP4-6.0, and SSP5-8.5) in response to a greater potential diversity of policies for climate change mitigation. In an era when climate change models and output are increasing in complexity, and with successive CMIP iterations, partnering with climate scientists (see recommendations by Trzaska and Schnarr 2014) can help HAB modelers deal with complex decision-making processes for predictions of HABs using different climate modeling options.

### 6.3.2 HYDROLOGICAL MODELS FOR CATCHMENT RUNOFF AND COMPOSITION PREDICTIONS

Many lakes and smaller waterbodies are strongly influenced by forcing at boundaries, and therefore several HAB ecosystem models have also included a hydrological model to quantify the influence of climate change on inflow volume and composition in discharges from land (e.g., Me et al. 2018). Alternatively, models have ignored any influence of climate change on boundary hydrological processes and adopted identical boundary conditions to a baseline period used for model calibration and validation (e.g., Trolle et al. 2010; Elliot 2012). For example, one seasonal forecast study includes basic biological processes like growth and mortality, as well as life cycle processes (e.g. McGillicuddy et al. 2005), but simulations may not necessarily include all of the variability from a future climate due to reliance on historical relationships for hydrological and nutrient forcings (McGillicuddy et al. 2011).

Hydrological models represent our best tool to quantify how inflows are likely to be affected by climate change but there are also several critical assumptions that will affect the performance of these models. In addition to the challenges outlined above for climate models, hydrological models that derive input from climate models also themselves introduce uncertainty. Hydrological models used for prediction of effects of climate change are usually calibrated and validated over an instrumental period, but where there is land use change, stream channel modification or large changes in groundwater contributions to streamflow, the calibrated relationships may fundamentally differ from those for some future period (Vaze et al. 2010). This process is known as **non-stationarity** and it results in different relationships (e.g. rainfall-runoff) and dominant processes operating to those of the instrumental period.

Additionally, little is known about how nutrient concentrations in inflows will be affected by climate change, including the changes in nutrient stoichiometry that may be important in determining successional sequences of HAB and non-HAB species (Hamilton et al. 2016). A relatively small number of hydrological models include predictions of nutrients in catchment runoff (e.g., the Surface Water Assessment Tool, SWAT: Me et al. (2019); Integrated Catchments, INCA: Whitehead et al. 1998) and therefore ensemble modeling is generally limited or not possible for nutrient load outputs from catchment models that connect to HAB models through their input data. Hydrological-water quality models can be important tools to separate the effects on HABs of localized anthropogenic pressures from climate change (Burford et al., 2019). To ease some of the burden of interfacing climate-catchment-receiving water models, plug-ins have been developed such as SWAT2lake (Molina-Navarro et al. 2018) and the Water Ecosystems Tool (WET; Nielsen et al. 2017).

Based on one IPCC-based climate projection, a global long-term prediction for two HAB species for future time slices has been carried out (Glibert et al. 2014). These predictions used the Global Nutrient

Table 2. Components of a 'model train' showing a possible sequence to support HABs simulations under climate change. Different choices for a model user to consider are also shown.

Model	Considerations for selection			
<b>Climate model</b>	Global Climate Model (GCM) or ensemble of GCM models	Representative Concentration Pathways (RCPs)	Dynamic or statistical downscaling	Variables (air temperature, rainfall, wind speed, etc.)
<b>Catchment hydrological model</b>	Process- based or statistical model	Ensemble of models or individual model	Rain on grid for rainfall variability	Distributed or conceptual model
<b>Catchment particulate and solute model</b>	Statistical, empirical or process-based model	Variables (nutrients, suspended sediment, etc.)		
<b>Hydrodynamic model</b>	Spatial and temporal scale selection	Ensemble of models or individual model	Hydrostatic vs non-hydrostatic	Eulerian, lagrangian and numerical solution methods
<b>Water column biogeochemical model</b>	Dynamically coupled or independent use of hydrodynamic flow fields	Selection of state variables	Selection of processes and algorithms	Dynamic vs static sediment boundary condition
<b>HAB model</b>	Lumped or individual HAB species/group model	Solitary cells/colonies/trichomes	Selection of processes and algorithms	Toxin production of selected species
<b>Risk assessment and socio-ecological models</b>	Risk assessment of HABs	Decision support system for HAB mitigation and management	Socio-economic assessment and modeling	

Export from WaterSheds (NEWS) model (Seitzinger et al., 2005) to provide riverine nutrient loads to model domains for the NW European Shelf-Baltic Sea system, NE Asia, and SE Asia. The HAB species, however, are not explicitly resolved but statistically correlated to physical and biogeochemical variables from a coupled ecosystem-coastal ocean model. Extending this approach by coupling models across climate, hydrologic, and biogeochemical/ecological processes to socio-economic effects will support better understanding of how climate drivers influence HAB impacts (Table 2).

## 6.4 Model calibration and validation

Ability to provide initial conditions for model simulation runs, calibration (i.e., adjusting parameters so that model output matches observations) and evaluation considering validation and verification that the calibrated model provides a satisfactory fit to observations independent from the calibration are strongly dependent on the quantity and quality of observed data. Model validation is often not carried out as the calibration process can be time consuming, although automated calibration packages (e.g., the Parameter ESTimation Tool, PEST) may reduce time spent in manual calibration involving repeated parameter adjustments, running simulations and observing outputs (Doherty 2015). Parameter priors libraries can also help to define parameter values and ranges (Robson et al. 2018). The benefits of good observational data (Chapters 2 and 5) are also relevant to statistical modeling approaches, supporting the development of robust statistical relationships. These observational data are often not sufficiently extensive in space and/or time, nor do they generally represent the response/state variable of most concern, e.g. toxin concentration.

Initialization of models is challenging, and it is not possible to provide observed data for all state variables in all cells of a model. Data are usually interpolated or extrapolated from a combination of observations including vertical profiles and routine sampling (e.g., grab samples for nutrient analysis



and phytoplankton enumeration) at selected sampling stations, autonomous in situ sensor platforms located at key locations (or occasionally from autonomous underwater vehicles), and remote sensing of optically active constituents of surface waters. Autonomous sensors provide an opportunity for rigorous calibration of variables with high-frequency data (Hamilton et al. 2014), while satellite remote sensing can be used similarly to focus on spatial calibration over the surface-water model domain (Wynne et al. 2013).

Satellite images of chlorophyll have been utilized for comparisons with model output (e.g., Anderson et al. 2016) and particle tracking may also be included (Wynne et al. 2013). Any change in the HAB population is reflected through chlorophyll changes that are visible in the satellite images. Chlorophyll (Chl) is only a proxy for phytoplankton biomass (relationships vary with, for example, photoacclimation; Raven and Geider 2002), and HABs because Chl and C:Chl ratios vary, there can be high reliability of near real-time satellite estimates of Chl when ground truthing is conducted.

Both in situ autonomous fluorescence and satellite sensors have become more sophisticated in recent years, aligning with wavebands specific to phycocyanin and phycoerythrin. Hamilton et al. (2014) used high-frequency in situ phycocyanin sensor readings for comparisons with output from a 1-D model that included a cyanobacteria state variable, demonstrating how periods of diurnal stratification led to high accumulations of cyanobacteria (i.e., HABs) in near-surface waters. Fluorescence and sensor data should be carefully calibrated against proxies and variables (e.g., Chl) that these data represent, and checked for interferences like non-photochemical quenching (e.g., Bertone et al. 2019). Monitoring of HABs has used satellites like Sentinel-3, deriving images from wavebands aligned to the spectral emission band of phycocyanin (Ogashawara 2019). The opportunity now exists with autonomous sensors and remote sensing to vastly expand the data that is used for initialization, calibration and validation of ecosystem models.

The advent of extensive data sets for model calibration and validation has necessitated a more advanced statistical approach (i.e., supporting modeler skill levels) for model calibration and evaluation. Visual assessments are no longer satisfactory as the sole basis for model assessments, and interrogation of model performance should be made with complementary statistical tests (e.g., Moriasi et al. 2007, Bennett et al. 2013). These tests also reveal quite different performances among state variables, with decreasing performance from physical to chemical and biological variables. Wherever possible, performance assessments should not only include state variables but also key fluxes, after aligning units of flux measurements to those used in the model. For example, phytoplankton productivity is often expressed as a gross volumetric value ( $\text{mg C m}^{-3} \text{d}^{-1}$ ) but can be divided by phytoplankton biomass ( $\text{mg C m}^{-3}$ ) derived from biovolumes (e.g., using calculations from microscopic enumeration) to obtain a unit ( $\text{d}^{-1}$ ) suitable for model flux comparisons. These types of comparisons are valuable because they may help to resolve an issue related to **equifinality**, where a similar model outcome arises from different combinations of free parameters, i.e., parameter values may misrepresent the 'actual values' and lead to poor outcomes when the model is applied to a period of time outside of the calibration. Fluxes are infrequently used for calibration, partly because they are not standard output from model simulation runs. Chapter 3 provides information on experiments that can be used to better constrain parameters that are used in HAB models applied for the purpose of examining the effects of climate change.

## 6.5 Guidelines for modeling HABs in the future

Specific ecosystem models, including HAB models, will always depend on the research question and/or purpose, as well as user skill and availability of data. Chapter 2 highlights the importance of long-term databases to support models of HABs. These databases are particularly important for 'data-hungry' deterministic models. The requirements for breadth of disciplinary knowledge (e.g., of physical, chemical and biological processes), understanding of the relevance and quality of model ini-

tialization and calibration data, and desire to communicate outputs across diverse stakeholder groups are driving the formation of large, multidisciplinary teams that build upon existing model platforms using advanced software and hardware platforms (Trolle et al. 2012). In this section we do not consider all of these requirements, but we have identified some areas that should be the focus for future work to improve HAB models.

### 6.5.1 TIMESCALE-INDEPENDENT ASPECTS

- The chaotic or non-deterministic aspects of the Earth system and associated uncertainty of predictions of HABs requires multi-model ensemble runs to encompass the range of plausible outputs. Varying initial conditions and forcing functions can also be used to generate statistical variability for deterministic model outputs.
- Choices of numerical schemes in hydrodynamic models are very important as equations of motion are not exact analytical solutions and can introduce varying levels of numerical error (e.g., numerical dispersion) depending on the solution scheme adopted. When using a Lagrangian approach for particle tracking, for example, an advanced numerical scheme (e.g., a second or higher order Runge-Kutta scheme) should be used, because simple Euler-forward schemes can introduce large error terms (e.g. Gräwe et al. 2012). In addition, diffusion should be added, e.g., through random walk approaches (Hunter 1987). This approach requires an even larger ensemble of experiments.
- When using a Eulerian approach for representing a HAB population, sub-compartments (see Janowitz and Kamykowski 1999, Beckmann and Hense 2004, Hense 2010) are a suitable way to represent important aspects of HAB dynamics, e.g., internal quotas (for nutrients and toxin concentrations), different life cycle stages, nutrient uptake strategies or strains of organisms (see Hieronymus et al. 2021).

### 6.5.2 TIMESCALE-DEPENDENT ASPECTS

The length of the prediction time scale and the desired spatial resolution determine the specific requirements for forcing, boundary and initial conditions, and model components (Table 3). Our focus in this

Table 3. Model-specific features for short- and long-term predictions of HABs

	Short-term forecasts	Long-term predictions
<b>Resolution</b>	Horizontal: highly dependent on system size, from < 100 m in lakes to several km in coastal and ocean systems to resolve submesoscale hydrodynamic processes; vertical: $\leq 1$ m, possibly extending to several meters deeper in the water column	
<b>Atmospheric forcing</b>	Output from weather forecast models	Output from IPCC models under different RPCs, downscaling and models (including ensembles)
<b>Lateral boundary conditions</b>	Depending on the specific local and regional conditions (e.g. fresh water/nutrient loadings) and use of data-driven, statistical or hydrological model inputs	Depending on the specific local and regional conditions; additional ensemble runs with potential future evolution of relevant quantities with land use change) are needed using a hydrological model.
<b>Initial conditions</b>	As close as possible to real conditions by using field data, data from remote sensing, and operationalizing data assimilation techniques	Realistic, representative fields for the state variables of the ecosystem model (e.g. nutrient fields)
<b>Model systems</b>	Operational hydrodynamic model, particle tracking routines, and, for forecasts > 3 days, specific biological components (see Table 2 and timescale-independent aspects)  Coupled model systems with a hydrodynamic model, an ecosystem model, including HAB species, and a carbonate system model.	Conceptual, data-driven and other statistical models used for complementing deterministic operational models  Conceptual, data-driven and machine learning models, including Support Vector Machines (SVMs) and Convolutional Neural Networks (CNNs) (Rousso et al. 2020) can be used to complement long-term deterministic model runs

chapter has been on long-term prediction but for comparison we also provide information on short-term forecasts, some of which may be relevant (e.g., extreme events) to future climate.

Details of biological and physiological processes (see Table 1) that should be accounted for when predicting HABs are dependent on the length of the prediction time scale (Chapters 3 and 4):

- For forecasts <3 days, biological growth and loss processes are generally small compared with those attributed to hydrodynamic advection and dispersion, and thus can be neglected.
- Weekly forecasts should include basic cell and physiological acclimation processes of organisms.
- For seasonal forecasts, life cycle processes become important. On these time scales the seed population and colony formation that occur in many HAB species become relevant. Forecasts therefore require initial data for state variables that are constituents of the life cycle like seed bank concentration and distribution. Also, biotic interactions within the focal group or with competing organisms, as well as effects of predation, need to be included.
- Decadal studies require a major effort. On these time scales, life cycle and evolutionary processes (interpreted here as adaptation, e.g., Beckmann et al. 2019; Chapter 4) and/or different strains of species need to be included. A number of biogeochemical processes, including the carbonate system, must be resolved to account for pH changes, as they may affect HAB species (Chapter 2), and are relevant in the context of future global change.

## 6.6 Conclusions

Future modeling efforts should consider the way in which models can be used as an umbrella to link long-term observations and data sets, experimental methods and evolving understanding about acclimation and adaptation of HAB species and strains (see also Chapter 4). Focused efforts to separate physiological processes for HAB and non-HAB phytoplankton, as well as toxin-producing and non-toxin producing species and strains, will help to improve HAB predictions, but may require multiple iterations of submodels, e.g., for physiological processes like nitrogen fixation, to test assumptions. Predictions based on HAB-specific conditional constraints, such as sudden appearances of specific toxin-producing strains in certain regions are still rather speculative, however, and thus not currently within our reach. Additional work is also required to prioritize efforts using ensembles of multiple model runs or Kalman filter techniques, for both short-term operational forecasts and long-term predictions. These methods will help to produce statistically significant statements about important quantities such as occurrence, magnitude, duration and area coverage of HABs. Given the current state of knowledge, a coupled global HAB forecast is still not possible, especially given the need to carry out a large ensemble of model runs and to regionally downscale the results to meet stakeholder requirements. A collation of downscaled regional models to global scale is also unlikely. We envision that HAB prediction in the coming few years will continue to focus on local and regional scales since the majority of the impacts are experienced at this scale. Models at these scales can provide excellent tools to integrate field, experimental and conceptual studies that seek to provide insights into effects of climate change on HABs.

## 6.7 References

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