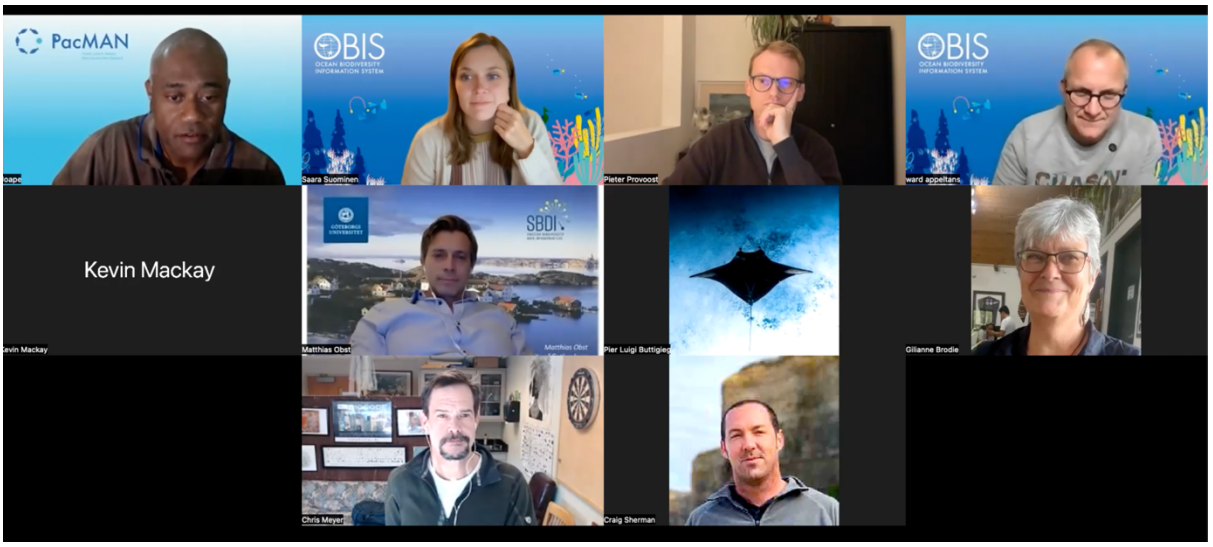
PacMAN
Pacific Islands Marine
Bioinvasions Alert Network

Second PacMAN Scientific workshop

20 October 2022

Online

UNESCO



Screenshot of virtual meeting. Names from left to right, from top to bottom: Mr. Joape Ginigini, Dr. Saara Suominen, Mr. Pieter Provoost, Mr. Ward Appeltans, Mr. Kevin Mackay, Prof. Matthias Obst, Dr. Pier Luigi Buttigieg, Dr. Gilianne Brodie, Dr. Chris Meyer, Dr. Craig Sherman

Table of Contents

1. Executive Summary

The second PacMAN scientific advisory board (SAB) meeting took place on the 20th of October 2022 as a virtual meeting. Five scientific advisory board members joined in-person, while the remaining members were given a chance to comment on the updated monitoring plan prior to the meeting on an online document. The meeting objectives were to give an update on the progress of the PacMAN project during the second trial year, discuss possible updates required for the monitoring plan and protocols and give an overview of the planned concept of the decision support tool.

Mr. Joape Ginigini the local project manager explained in detail the activities that had been done during the year as well as the changes made to the protocols. The stakeholder engagement of the project has been very successful and the project has received a lot of interest from regional stakeholders, while also bringing marine biosecurity to the forefront of policy in Fiji. The PacMAN project field protocols have been extensively tested and optimized during the second year of the project. Settlement plate depths have been modified to fit the depth profile and tidal cycle at the Suva port. Curved settlement plates are used instead of flat ones, which received interest from the SAB members. The amount of time that the settlement plates are incubated and the possible methods to collect motile organisms was discussed. PacMAN data formatting is being tested by Ms. Miriama Vuiyasawa the local research assistant and Mr. Kelly Brown the marine collection curator at USP. The importance of metadata collection and registration was emphasized by the SAB, especially in the case of biobanking samples.

Mr. Pieter Provoost the OBIS data manager introduced the first conceptualization of the PacMAN decision support tool and compared it to other available tools globally. The PacMAN tool will be similar to that of OSPAR-HELCOM, which evaluates species risk levels based on shipping routes and target species selection based on habitat suitability models. The SAB members shared their extensive experience with similar modelling done for example in Sweden. The SAB recommended keeping connectivity models which largely depend on human activity and habitat suitability models which are based on environmental measurements separate from each other. Modelling is largely used to pinpoint risk areas as well as find the original location of species detections, for further monitoring to take place. Hydrodynamic models at local scale can be used to identify the possible source of a detection, helping to find specimens to confirm important detections.

The meeting was closed on a discussion of future considerations for the next year of PacMAN. The importance of regional relationships was discussed. There is already good development in this direction with PacMAN taking part in regional workshops. It was also emphasized that it is important to manage expectations when working with eDNA, that managers and administrators have a realistic understanding of what eDNA can do, and which use cases it is best suited for. Awareness of developments in the regulation of Digital Sequence Information was also discussed. PacMAN was thanked for its efficient co-design approach with a strong effort in building a project that answers local stakeholder needs and takes into account local capacities.

2. Update on PacMAN project progress

Mr. Joape Ginigini welcomed all participants to the meeting and provided a progress report on the development of the PacMAN project. Monitoring has been conducted through four water and plankton sample collections, and three settlement plate collections, while the fourth plate retrieval will be done during October and November 2022. Some adaptations have been made based on issues that were encountered on sample collection and processing. Collections have been made in close collaboration with the ports authority of Suva, Fiji. Key achievements so far with regards to local stakeholders include close work with the national invasive species working group, and interest received from the local biosecurity, maritime and environmental agencies. PacMAN has been testing practical workflows that can be utilized by the local decision makers and has therefore received a lot of interest. The project has catalyzed and brought momentum to different institutions in Fiji (i.e. the Invasive species taskforce) bringing scientists together with administrators, managers, biosecurity, and academia, for marine invasive species management in the country. Mr Ginigini is optimistic that this will progress in the next project cycle.

Overview of PacMAN listed tasks of the last year shows that major tasks have been completed. This includes communication with a website, flyers, and brochures. The team has taken part in multiple international and local workshops where PacMAN has been presented. The establishment of the monitoring plan was successful and received a lot of support from the local stakeholders. The first training is planned for November for the technical teams that will be doing on the groundwork in relation to monitoring. The course will be a combination of online OTGA sessions with introductions to the material, that will then be taught in the face-to-face module. The objective is to introduce the local experts to what PacMAN is doing and what methods are used in PacMAN protocols.

Currently, the PacMAN fieldwork is still in its test phase. Lessons learnt so far include adjusting the depths of the settlement plates to the water depth at the sampling sites at the port so that the plates are submerged also at peak low tide. In addition, there is a depth limitation also for the collection of the environmental measurements due to the length of the probe cable. Materials for sample collection are autoclaved before they are bleached as an additional step for quality control and cleanliness. The dimensions of the settlement plates were kept at 15 x 15 cm, as larger plates were too much to process for specimen sorting. As PVC plates with flat surfaces are not available for purchase in Fiji, sections of curved PVC pipes were used instead. However there is considerable difficulty in photographing these, so that specimens can be identified from the resulting photos. Plankton collection was started with horizontal tows, but are now done with one vertical tow in triplicate at each site. Stronger anchors were set for the settlement plates, as there was entanglement of the ropes of the set-ups. The sample collections were split into two weeks, with two sites processed each week. This was to allow sufficient time for sample processing and specimen sorting. At each collection 18 settlement plates are retrieved (3 plates x 3 ropes x 2 sites); 6 of these plates are used for specimen sorting (one set-up from each site) and 12 plates are processed for DNA analysis. Specimen sorting has been developed and executed by Dr. Gilianne Brodie and Ms. Miriama Vuiyasawa. Species diversity on the plates has included bryozoans, colonial ascidians, barnacles, bivalves and tubeworms among others. A few crabs and bivalves have also been collected, but these were fortunately not from the species watchlist. Data collection and formatting to Darwin core has been started by Ms. Vuiyasawa and the marine collection curator Dr. Kelly Brown at USP. There has been a major roadblock in ordering materials for DNA processing to Fiji, but the DNA extractions have been optimized already, with good DNA yield achieved for all samples.

Discussion on the project progress was initiated with Dr. Pier-Luigi Buttigieg asking about what types of positive control samples are considered in terms of the needs of the local stakeholders. The types

of positive controls should be carefully considered to ensure that the detections are reliable, especially for detections that become more important in the region. He also recommended PacMAN data management to take into account environmental packages developed by the community (e.g. the Genomic Standards Consortium). Dr Chris Meyer commented that it would be interesting to see if there is metadata that the stakeholders would require, then maybe this should be added also. Dr. Buttigieg agreed, and added that there is no MixS extension for invasive species monitoring. Working on such an extension could be easily accomplished and an impactful output from this group.

Dr. Craig Sherman asked if there is a species that dominates the settlement plates like bryozoans and sponges. Dr. Brodie answered that because the plates are not in the water for that long, there is no 3-dimensional build-up, there are encrusting bryozoans and ascidians. Removing these destroys other communities, therefore the team is considering preservation of full plates. The team is trying hard to focus on species on the watch list: crabs, mussels, etc. with limited time. One mussel species was found and was confirmed by a specialist to not be a part of the watchlist species. The curved plate set-up received positive feedback from the SAB members, as different orientations of the plates is expected to harbour different species. Dr. Matthias Obst asked about the length of the plate incubation at the port, and if there is an idea of what the optimal time would be to catch species diversity. Dr. Brodie answered that the biomass coverage of the plates varies strongly between sites and different times. The time of incubation is about three months, as in this time the 3-dimensional structure is not yet in place, which would complicate specimen collection considerably, also with motile organisms. In Europe the community composition doesn't change much after three months, however this may be different in tropical waters. Dr. Meyer added that in Indonesia, the growth on plates in tropical environments is strongest between 3-6 months. Dr. Brodie added that salinity can change seasonally based on freshwater input into the harbour through rainwater. Background data on the hydrology at port (e.g. currents) is available, though it is not sure if this shows the depth of the freshwater lens.

Dr Brodie indicated that there are challenges in collecting motile organisms. Even some mussels don't stick on when taking plates out of the water. She asked if there is a way to collect those species that fall off during retrieval, and doesn't complicate work at the port. Dr. Sherman commented that they are not collecting motile organisms. If motile organisms of concern are found, he suggests that a targeted sequencing approach is taken for identification and classification of those individuals. There was agreement that it would be important to try to collect these organisms. How exactly this should be done for the set-up for it to remain practical was discussed. One collection net for each set-up would work, and this should be done in a standardized manner. Precautionary biobanking of samples of organisms is being done, as it doesn't require a lot of material. For example, the shell of a mussel would be kept for identification and the soft tissue can be banked for possible further DNA analysis.

Mr. Ginigini asked which type of preservation liquid was being used for biobanking of the specimens. Dr. Obst and Dr. Meyer mentioned ethanol, or extraction stabilization buffer which is a guanine-biocyanate based buffer. For unextracted slurries, supersaturated DMSO (25%) is being used for preservation, and samples are kept frozen. The importance of data management with these samples was emphasized once more. With this, Mr. Ginigini thanked the scientific advisory board for the discussion.

3. Development of PacMAN decision support tool

Mr. Pieter Provoost, the OBIS data manager, introduced the PacMAN decision support tool concept to the scientific advisory board. The purpose of the tool is to help local authorities set priorities and allocate resources for monitoring and mitigating biological invasions. To guide the discussions a conceptual framework was built (Annex 1). In risk assessment there are generally three high-level

components: hazard identification, risk calculation and risk management. Hazard identification is identifying all harmful things, in risk calculation you assign risk to all those harmful things, and that information is used in risk management to identify management actions and decide on the risk level that you are willing to accept. For invasive species this means identifying the possible impact each species can have and looking at the likelihood of those outcomes. A high impact outcome with a low likelihood would therefore represent low or moderate risk, while an extreme impact and high likelihood would be assigned an extreme risk. The impacts of invasive species include environmental, economical and impacts on human health. The likelihoods would then be calculated for the invasion process including transport, introduction, establishment, and local spread. Many frameworks and tools are already available, for example the Cefas Aquatic Species Invasiveness Screening Kit (AS-ISK), that has about 50 questions on possible impacts based on species traits that are used to calculate risk scores and therefore risk levels for a species. Another example is the Ecological Risk Screening Summaries (ERSS) from the US Fish and Wildlife Service. This tool has an extensive SOP, that guides assessors to collect data and assign appropriate risk levels based on a decision tree with for example history of invasiveness and likelihood of invasion based on climate match. The Species biofouling risk assessment framework for the Australian Department of Agriculture, Fisheries and Forestry (DAFF) results in species datasheets, with inoculation likelihood and information on different impacts which are all taken into account to define a risk level.

These examples are all quite labor intensive, using a lot of data, but still requiring a lot of human expertise and intervention. Other tools are more automated for example the vessel check tool, which calculates risk based on shipping route, vessel type and antifouling measures. Another tool is the OSPAR-HELCOM Ballast water exemption decision support tool which looks at shipping routes but also environmental conditions at port. Impacts are also considered as it is based on a target species list. PacMAN will be conceptually similar to the OSPAR-HELCOM tool. PacMAN will consider shipping routes and will use environmental conditions to do species habitat modelling for species on target lists. In addition, species analyses will be done on species not yet on target lists by using information from for example the world register of introduced marine species (WRiMS, https://marinespecies.org/introduced). The system will be continuously fed with detection information from the monitoring program. Data will be used from OBIS (including PacMAN detections), GBIF and WRiMS. Environmental layers from Oracle and Copernicus data store as well as AIS and port call data will be used from the UN Global platform and commercial operators. Physical dispersion of species can also be evaluated through particle dispersion with Lagrangian trajectories, to look at origins and possible spread of detections.

A prototype decision support has been initiated with information on the sampling sites, areas of interest and target species list. Preliminary information on the tool is available at https://iobis.github.io/pacman-decision-support/.

Dr. Obst initiated the discussion by explaining his experience on similar work done in Sweden. He indicated that by including shipping and human activity to the models they override all environmental information, thereby the habitat suitability models are done without these human induced factors and are superimposed to the models for connectivity/shipping routes. One output of the models is connectivity and spread, and another is habitability; i.e. if a species spreads, what is the likelihood that it will establish. Connectivity is modeled through oceanographic models and suitability with environmental information. Another issue is species with very few occurrences, more sampling frequency might be needed to address this. Mr. Provoost agreed that this is a big challenge as often models are trained on distribution information from the native area, which does not consider the different ecological dynamics and pressures in possible invasion areas. The important

thing is to model information from those that are observed to how many are likely to establish. Mr. Obst indicated how Sweden did this for high-risk species that were found in surrounding waters but not yet occur in Sweden. Species distribution modelling was done for these to identify areas with high likelihood for establishment, to pinpoint places where genetic monitoring should take place. In this way there is continuous feedback between modelling and monitoring.

Dr. Brodie commented that there is currently no data for the lagoon area in Suva, let alone the port. This can restrict the area where modelling can be done and collecting this data would be a huge undertaking. Mr. Provoost answered that the habitat suitability modelling is looking at regional scale patterns, so a very fine scale is not necessarily required for this purpose. Dr. Sherman agreed that the important aspect is to identify the major risk pathways for incursion of risk species. It is important to think about where the detection originated from as it is possible that the detection is not where the species is. A physical specimen is usually required to confirm a detection, therefore understanding the biophysical dynamics at the sampling site can help with finding the possible origin of a genetic detection. Dr. Brodie indicates that the PacMAN team will investigate what hydrodynamic data is currently available for the port.

4. The future of PacMAN

Linking PacMAN to regional work is seen as highly important and was partially discussed at a recent workshop in Papeete, Tahiti, with representatives from Samoa, Hawaii and French Polynesia. PacMAN workflows receive a lot of interest from these participants. It was also important that PacMAN stays in close contact with SPREP for regional work.

Discussion was made on what are the different scenarios where eDNA is the most useful and how this should be communicated to the management community. eDNA is often either overhyped or underhyped. There are many advantages for specific situations, but we must be clear also on the challenges and what eDNA cannot do. For example, eDNA is not best suited for ballast water monitoring as ballast water is treated to kill organisms, but not remove DNA. Therefore, DNA signals can be detected that originate from dead organisms and there is no way to differentiate this. It is also important to keep in mind how eDNA complements other methods, for example modelling. Citizen science can be useful with tracking and mitigation of invasive species. PacMAN can state that we have found a specific use-case where eDNA is the best tool, and as the project is established, we can connect to other methods which are better for further use cases. Finally, a combination of different methods will be the most powerful way forward. Awareness on future developments in DSI (Digital Sequence Information) policy is required. These changes can be prepared for by properly recording metadata linked to the provenance of samples, collections and permits that were required for sampling.

Mr. Appeltans thanked the PacMAN team for their work on the project. Dr. Buttigieg considers that PacMAN is more ready to scale up than other eDNA projects thanks to the integrative approach where the focus is on what is actually needed in the region, and what the region is capable of sustaining in terms of the needs of the stakeholders. This is different from trying to fill the needs of external researchers that come in. Therefore, the relationships that have been built through PacMAN will help in scaling up due to considering the local needs. Dr. Brodie thanked for this comment and indicated that PacMAN has already contributed to a paper that discusses co-development of the project. Scaling up of the PacMAN project could be started in one other country, which most

naturally could be Samoa, where there is already a survey on the harbour area that can be used as base information.

5. Summary of the meeting outcomes

The PacMAN scientific advisory board made the following recommendations:

- Addition of nets on the settlement plate setup below each rope setup to capture motile organisms e.g. crabs
- Use of precautionary principle to capture juveniles yet to develop adult features which are diagnostic. DNA vouchers to be kept for later reference work if needed
- Keep multiple reserved samples for Biobanking with good bookkeeping for the metadata collected to link samples to origin
- Careful consideration on the layers chosen to include in modelling e.g. ecological vs economical (Habitat Suitability modelling vs. Connectivity and shipping routes)
- Risk species of wide tolerance limits should undergo strict monitoring and modelling cycles to detect their presence
- Importance of mapping hydrodynamic information or access geospatial work on the harbour to proactively predict hotspots that can be settlement sites
- Importance of avoiding an isolated approach for Fiji and to attract interest from other teams in the Pacific with similar strategies
- eDNA for Ballast water may not be useful due to false positives and calibration time to detect positives or false positives. The risk of not declaring the uncertainties is real.
- Track and trace option and sustainable practices can be covered through citizen science and can complement eDNA methods
- Potential risk species discovered in French Polynesia to be noted and incorporated into query searches

6. Annexes

Annex 1: The agenda for the Second PacMAN scientific advisory board meeting

PacMAN Scientific advisory board meeting
20 October 2022 9-11 pm CET
[ONLINE]

Meeting Objectives

The objective of this meeting is to discuss the experiences of PacMAN after the first year of trial sampling in Suva, Fiji. The discussion will be guided with the updated monitoring plan, as well as specific questions that have arisen during the year.

In addition we will have the first introduction to the decision support tool, and we will welcome first comments and feedback on the scope and concept of the current plans.

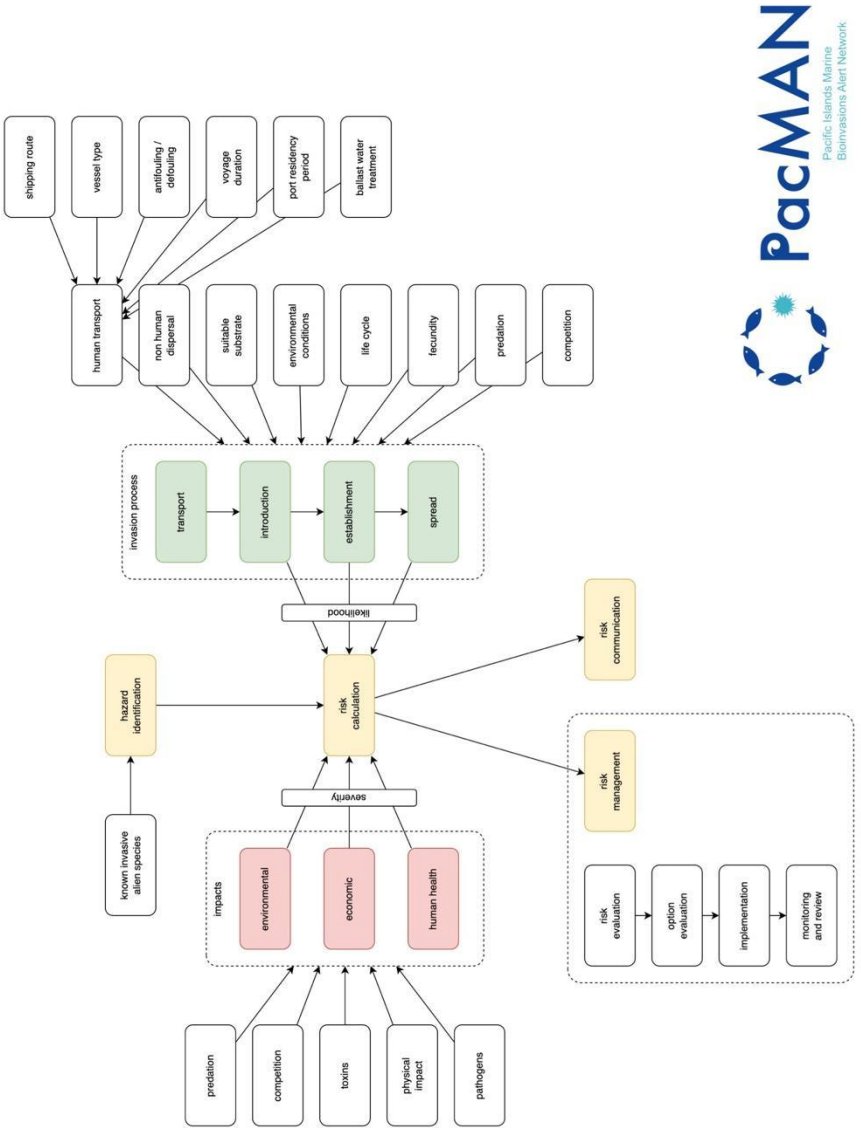
The agenda:

Time	Topic	Presentor
9.00-9.05	Welcome	Ward Appeltans
9.05-9.20	Update on PacMAN	Joape Ginigini
9.20-10.00	Discussion on monitoring plan	
10.30-10.35	Intro to decision support tool plans	Pieter Provoost
10.35-	Discussion on the decision support tool	

The meeting is to support open discussion around the experiences of PacMAN so far, so the agenda is a preliminary guide, but the timings are not strict.

We will provide material for reading before the meeting.

Annex 2: The risk analysis concept for the PacMAN decision support tool



Risk analysis
and decision support

Annex 3 Participants list the Second PacMAN Scientific Workshop, 2022

Scientific Experts

Mr. Kevin MACKAY
Marine Data Manager
Environmental Information
National Institute of Water and Atmosphere
Private Bag 99940, Newmarket
Wellington
Auckland 1149
New Zealand

Dr. Pier Luigi BUTTIGIEG
Data Scientist
Helmholtz Metadata Collaboration
GEOMAR | Helmholtz Centre for Ocean Research Kiel
Duesternbrooker Weg 20
24105 Kiel
Germany

Christopher MEYER
Research Zoologist and Curator
Invertebrate Zoology
National Museum of Natural History, Smithsonian Institution
12th and Constitution Ave., NW
Washington DC, 20560-0163
United States

Assoc. Prof. Matthias OBST
Scientist
Department of Marine Sciences
Göteborg University, Department of Marine Ecology
Box 461
40530 Gothenburg
Sweden

Assoc. Prof. Craig SHERMAN
Associate Professor
School of Life and Environmental Sciences
Deakin University
Deakin University, Pigdons Road
Waurn Ponds VIC 3216
Australia

PacMAN project team

Dr. Gilianne BRODIE
Deputy Director IAS
Institute of Applied Sciences

University of the South Pacific, Faculty of Science Technology & Environment, Institute of Applied Sciences
Lower Laucala Campus
Suva
Fiji

Mr. Joape GINIGINI
Senior Scientific Officer, Institute of Applied Sciences
Institute of Applied Sciences
The University of the South Pacific
The University of the South Pacific
Institute of Applied Sciences, USP Marine campus,
Suva
Fiji

Mr. Ward APPELTANS
Project Manager OBIS, GOOS Biology & Ecosystems, IOC Capacity Development
UNESCO / IOC Project Office for IODE
Wandelaarkaai 7
Pakhuis 61
8400 Oostende
Belgium

Mr. Pieter PROVOOST
OBIS Data Manager
UNESCO / IOC Project Office for IODE
Wandelaarkaai 7
Pakhuis 61
8400 Oostende
Belgium

Dr. Saara SUOMINEN
Scientific Officer
OBIS
UNESCO / IOC Project Office for IODE
Wandelaarkaai 7
Pakhuis 61
8400 Oostende
Belgium