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The GlobalHAB mini-symposium on automated plankton observations

High-frequency observations of Harmful Algal Blooms (HABs) and grazers of HAB species are needed to be able to understand HAB dynamics, to develop predictive models of HABs and to produce robust warnings of HABs. In recent years, novel *in situ* instrumentation has been developed for automated high-frequency HAB detection in near-real time.

Automated plankton imaging-in-flow produces high quality images of phytoplankton larger than 5-10 µm including many harmful algae (Fig.1). There are four main approaches illustrated in Fig. 2: (1) Imaging-in-flow which is based on the morphology of organisms, (2) flow cytometry which is based on fluorescence properties (e.g., pigmentation) and scattering properties (e.g. size) of cells, (3) observations of bulk bio-optical properties (pigment based) of plankton communities, e.g. fluorescence, absorption and turbidity and (4) molecular methods. The mini-symposium was focused on the imaging-in-flow systems which give detailed information about HAB organisms, including some instrumentation which provided both imaging-in-flow and cytometric data. In addition, imaging-in-flow instruments for observing grazers, i.e., microzooplankton and multicellular zooplankton were included. These instruments are now being adopted in research and piloted in monitoring programmes. The aim of the mini-symposium was to bring together experts on, and users of, automated imaging-in-flow systems to present methods and recent results and to share experiences. Another aim was to carry out a comparison of results when analysing plankton communities quantitatively. Young scientists were particularly encouraged to attend the symposium. The mini-symposium/workshop on automated plankton observations took place at

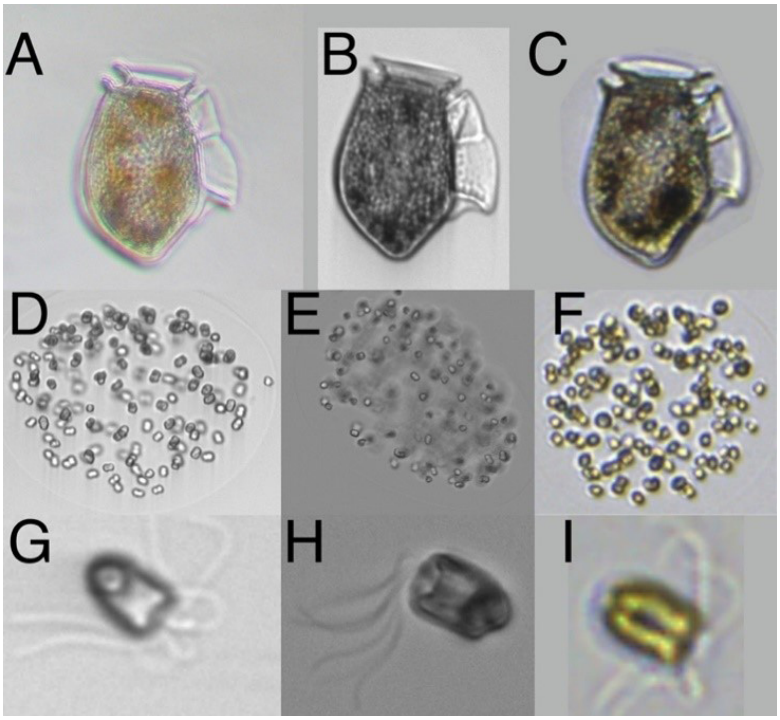

Fig. 1. Micrographs of phytoplankton observed during the mini-symposium. Scale is not consistent. A-C. Dinophysis acuta (field sample), A. Light microscope, photo: Mona Ring Kleiven. B. IFCB and C. FlowCam. D-E. Phaeocystis globosa (culture), D. IFCB, E. CytoSense/Cytobuoy and F. FlowCam. G-I. Pyramimonas sp. (culture), G. IFCB, H. Cytosense/Cytobuoy and I. FlowCam

Content

Featured article

High-Biomass HABs

Benthic HABs

Networking, Conferences and International Cooperation

Forthcoming

NEW !!

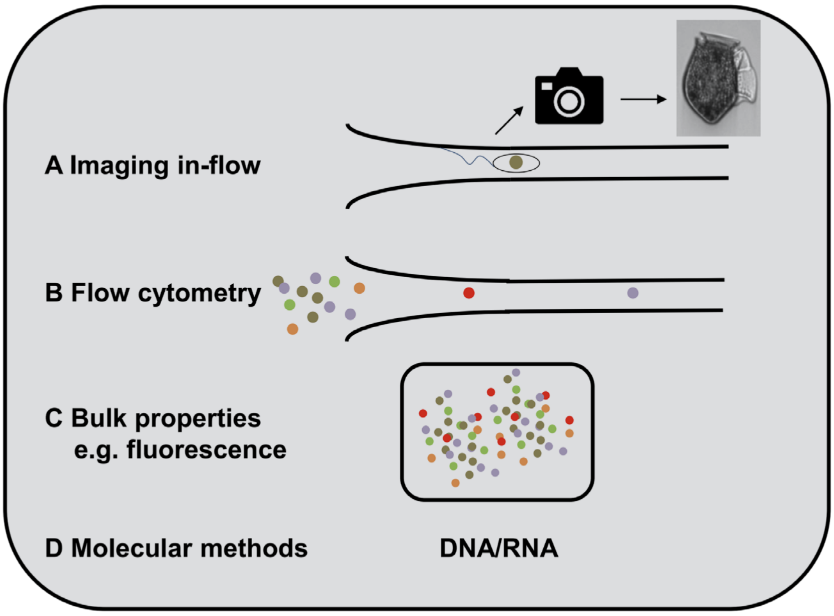

Fig. 2 Basic principles of methods of automated in situ observations of phytoplankton. A. Imaging-in-flow: Organisms are characterised based on morphology; cells are imaged. Camera is triggered by fluorescence or scattering. B. Flow cytometry: Organisms are characterised based on their light scattering and fluorescence properties (pigment content, target fluorochromes). C. The fluorescence or absorbance properties of all organisms in a certain volume of water give a signal of the bulk properties. D. Genetic material: e.g. in situ mini-laboratory for sandwich hybridisation (FISH)

the Kristineberg Centre in Fiskebäckskil, Sweden, 22-26 August 2022. The participants enjoyed nice weather and good company.

Instruments

Instruments from three manufacturers were demonstrated and used to analyse field samples and cultures (Fig. 3): 1. the Imaging FlowCytobot (IFCB, Mclane Research Laboratories Inc., Falmouth, USA), 2. the Cytosense/Cytobuoy (Cytobuoy Bv, the Netherlands) and 3. the FlowCam (versions 8400 and Macro, Yokogawa Imaging Fluidics, USA). The PlanktoScope was also presented, but this instrument was not available on site. There may also be other devices in development or available commercially. The instruments were operated in laboratories on land using field-collected and culture samples. The IFCB and the Cytobuoy are housed in water proof housings and can be operated *in situ*. These instruments are commonly operated in-flow-through systems in coastal locations or on ships, e.g., as part of Ferrybox systems. All instruments have strengths and weaknesses when compared to each other.

The IFCB and the Cytosense/Cytobuoy are true flow cytometers since they use sheath fluid to centre organisms in flow cells. The FlowCam is not a flow cytometer since water is simply pushed through the flow cell. This sometimes results in out of focus images. A unique feature with the FlowCam 8400 is that it produces colour images of the organisms while the other two instruments use grayscale cameras. In the FlowCam 8400 it is possible to change objectives with different magnifications. A high magnification results in images showing more detail but also in longer sample processing time. The IFCB images essentially all cells passing through its flow cell that are above a size threshold (~10 µm) and with a very high percentage of in focus images. The CytoSense/Cytobuoy produces images of only a subset of the cells passing through its flow cell. A large flow cell in this instrument makes it possible to cover a larger size range of organisms compared to the other instruments however a large flow cell may make it more difficult to centre organisms in the flow cell resulting in out of focus images. The Cytosense/Cytobuoy also use scattering properties and fluorescence fingerprints to characterize plankton. This makes it possible to quantify phototrophic picoplankton. The FlowCam Macro was used to count and characterize multicellular zooplankton.

The PlanktoScope *https://www.planktoscope.org*, a citizen science approach to automated imaging and identification of plankton, was presented during the mini-symposium. This is a low-cost alternative to the commercial instruments. The PlanktoScope can be used with objectives with different magnifications. At present, the PlanktoScope (version 4) is somewhat limited in functionality compared to the commercial alternatives and it may only be applicable to multicellular zooplankton and to larger HAB species at the moment. Future development may change the situation.

Images and automated identification of organisms

The instruments used produce high quality images of plankton larger than ~10 µm (Fig. 1). Smaller organisms are often difficult to identify automatically. Analysis of one sample often results in a few hundred or a few thousand images. To produce quantitative data the images are analysed using Artificial Intelligence machine learning (AI-machine learning). Supervised learning is applicable to HAB-organisms. This means that a phytoplankton identification specialist looks at many images and identifies the organisms. These annotated images constitute a training set for producing classifiers for automated identification of the plankton. The classifiers are used to analyse the main set of images acquired (Fig. 4). At present two main approaches are used for the AI-machine learning: 1. Random forest and 2. Convolutional Neural Networks (CNN). During the mini-symposium ongoing CNN-work in Finland, Scotland and USA were presented. There is also ongoing work on this approach elsewhere, e.g., in France.

Early warning systems

Systems for early warning of HABs using networks of imaging-in-flow devices were presented during the mini-symposium. Mike Brosnahan from Woods Hole Oceanographic Institution presented the HAB-Hub *https://hab-hub.whoi.edu* which includes a number of IFCB's operated on the east coast of the USA. Results are presented in near real time. Raphe Kudela, Kasia Kenitz and Clarissa Anderson (not present at symposium) presented the IFCB Network in California. Also, an emerging network in the Philippines was presented. In Europe an IFCB user network

Fig 3. Instruments used in the mini-symposium: Three Imaging FlowCytobot's (IFCB's) (top left) and two Cytosense/Cytobuoy's (top right), with their water proof housing removed during laboratory work, and the FlowCam 8400 (bottom left) and one FlowCam Macro (not shown) were used during the mini-symposium. The FlowCam Macro is shown in the photo. Bottom right: A PlanktoScope built by Maci Wigginton at Ocean & Earth Sciences, Old Dominion University. Source: www.planktoscope.org

has been established (Karlson, 2021, HAN 68, page 16) with IFCB's mounted in research vessels and in fixed locations. Felipe Artigas presented ongoing Cytosense work in the English Channel and the North Sea. Automated observations of phytoplankton is one task of the EuroGOOS Biological Observation Working Group (BIOWG).

Additional information

Due to space limitations only a selection of presentations and activities at the mini-symposium are mentioned here. Further reading recommended is listed below [1-7]. Most of the presentations made are available as pdf's on the GlobalHAB web site *http://www.globalhab.info/activities*

Some conclusions from the mini-symposium

1. Imaging-in-flow is a viable method for automated analysis of many harmful algae at the species or genus level.
2. High frequency sampling (~every 10-20 minutes) at fixed locations, on research vessels or on ships of opportunity (Ferrybox) results in very useful data.
3. Small species (< 10 µm) are difficult to identify using commercially available imaging-in-flow at present.
4. Very large species or colony forming species also pose a challenge since flow cells and tubes may be clogged.
5. The volume analysed (mostly 5-10 mL) is often too small to analyse rare harmful algae quantitatively. A solution is to pool data from several samples. The problem with a small volume analysed also applies to standard microscope methods, i.e., the Utermöhl method.
6. There is a need to join efforts in sharing annotated images of HAB-organisms and for producing classifiers for automated HAB analysis. There are initiatives in Europe and in the USA to accomplish this.
7. Citizen science initiatives for low-cost instruments such as the PlanktoScope are commendable.
8. Best practices are needed to establish routines for handling of instruments, images and data. At least two best practice documents about automated phytoplankton identification are at present in production, one in Europe and one in the USA.
9. **The successful application of these new instruments results from the intrinsic link between technology progress and phytoplankton identification specialists.**

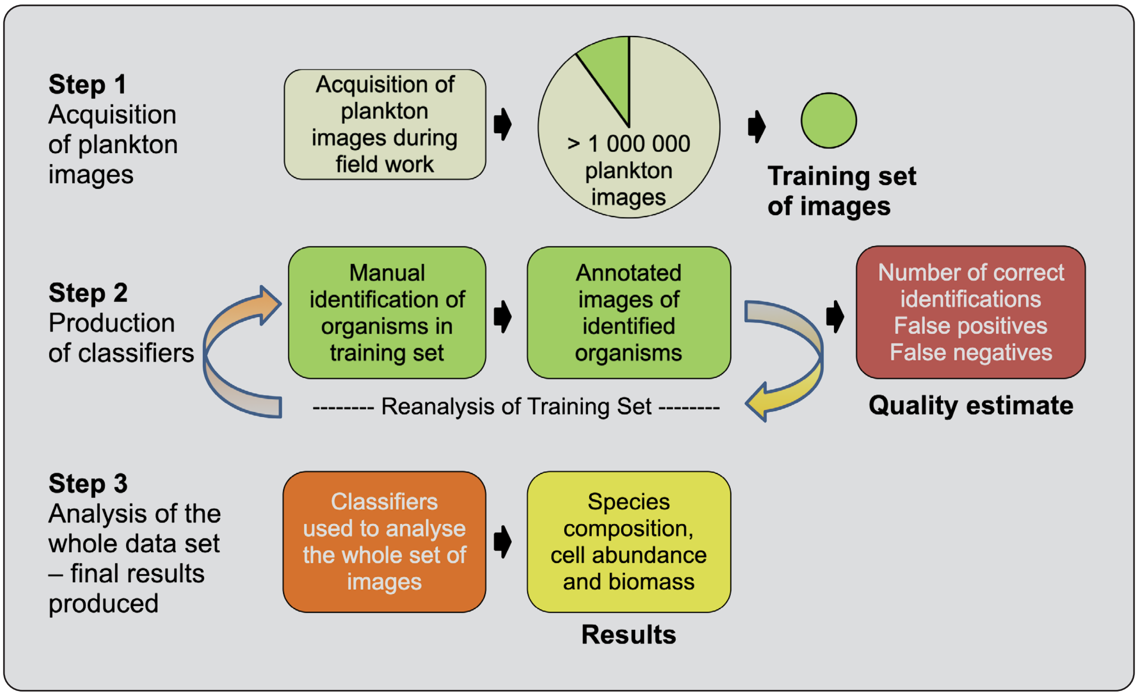

Fig. 4. The principle for using expert trained AI-machine learning for automated analysis of images acquired using imaging-in-flow instruments

Acknowledgements

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Authors

Bengt Karlson, Oceanographic Research, Swedish Meteorological and Hydrological Institute, Västra Frölunda, Sweden

Elisa Berdalet, Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain

Raphael M Kudela, Ocean Sciences, University of California Santa Cruz, USA

Email corresponding author:
bengt.karlson@smhi.se

Fig. 5. Participants of the GlobalHAB mini-symposium on automated plankton observations

High-Biomass Harmful Algal Blooms (HB-HAB) in the Chilean Fjords System: *Lepidodinium chlorophorum*

Harmful Algal Blooms (HABs) have become a recurrent problem in Southern Chile (Patagonian fjords) during the last decades, responsible for large scale events affecting public health and the aquaculture industry, especially salmon farming. Although High-Biomass Harmful Algal Blooms (HB-HAB) have negative socio-economic impacts, most studies in Chile have been focused on toxin-producing microalgae [1]. Here, we describe the development of an exceptional HB-HAB of *Lepidodinium chlorophorum* during late summer and early fall 2020. This bloom covered a large part of the northern Chilean Patagonia extending from Aysén to the Chiloé, Inland Sea including the Reloncaví fjord and sound system (Fig. 1A; [2]).

The National Fisheries and Aquaculture Service (Servicio Nacional de Pesca y Acuicultura, SERNAPESCA) have reported the occurrence of *L. chlorophorum* cells since February 10, 2020 in the Chiloé, Inland Sea, with cell maxima up to 2x10^{6} cells L^{-1} in Reloncaví sound, between 6 - 19 April, 2020. This report coincided with the detection of high concentrations of chlorophyll-*a* (Chl-*a*; 50 µg L^{-1}) in the middle reaches of Reloncaví Sound between March 31 -April 15, 2020 (records from an oceanographic buoy at station 3 www.cdom.cl; Fig. 1A). On April 2, 2020, an oceanographic cruise was carried out in Reloncaví Sound to characterize the physical-chemical conditions associated with the *L. chlorophorum* bloom. A single CTD profile (0-50m) was carried out at station 2 and water samples were collected with high vertical resolution (every 2 m between 0 and 20 m) for phytoplankton analyses. In addition, surface water samples were collected for phytoplankton analysis in station 1 (Tenglo Channel; Fig. 1A).

The results confirmed the presence of *L. chlorophorum* in the surface layer, reaching a maximum of 6x10^{6} cells L^{-1} in station 2 at 4 m depth (Fig. 2A) coinciding with the highest Chl-*a* concentration (115 µg L^{-1}; Fig. 2B). This thin layer of *L. chlorophorum* was recorded at temperature and salinity ranging 13.5-13.7 °C and 30-30.5, respectively (i.e. above the maximum gradient located between 8 and 11 m; Fig. 2B). Furthermore, green surface filaments with densities of ~500,000 cells mL^{-1} of *L. chlorophorum* characterized station 1 (Fig. 1B, C), possibly due to a semi-confined water masses.

Fluviometric registers indicated weak rainfall during summer and early fall 2020 and; negative outflow anomalies (~200 m^{3} s^{-1}; www.dga.cl) were observed for the Puelo River, the main freshwater input into the Reloncaví Fjord and Sound system

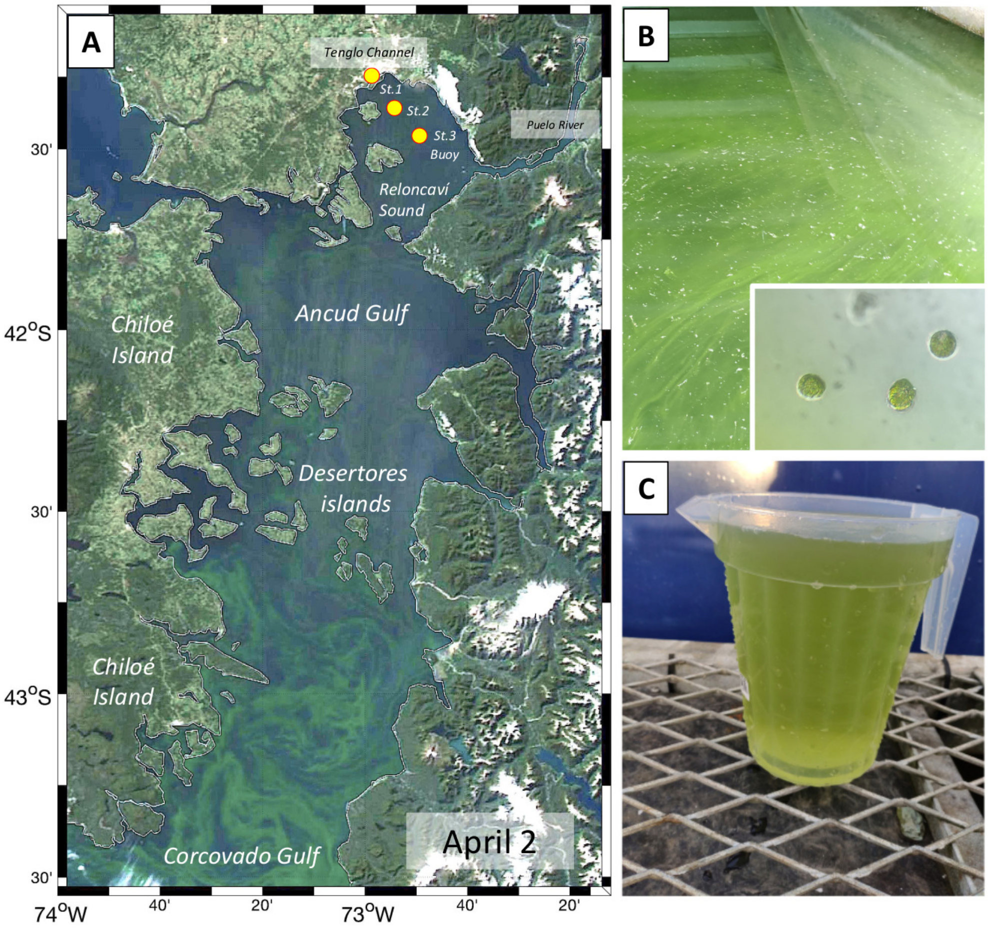

Fig. 1. A) True colour MODIS image of green filaments at the inner sea of Chiloé and the Reloncaví fjord and sound system. Oceanographic stations 1 and 2 and i~mar buoy (st. 3) are marked with yellow circles. B) Green filaments of L. chlorophorum in Tenglo Channel; C) Water sample from Tenglo Channel (st. 1).

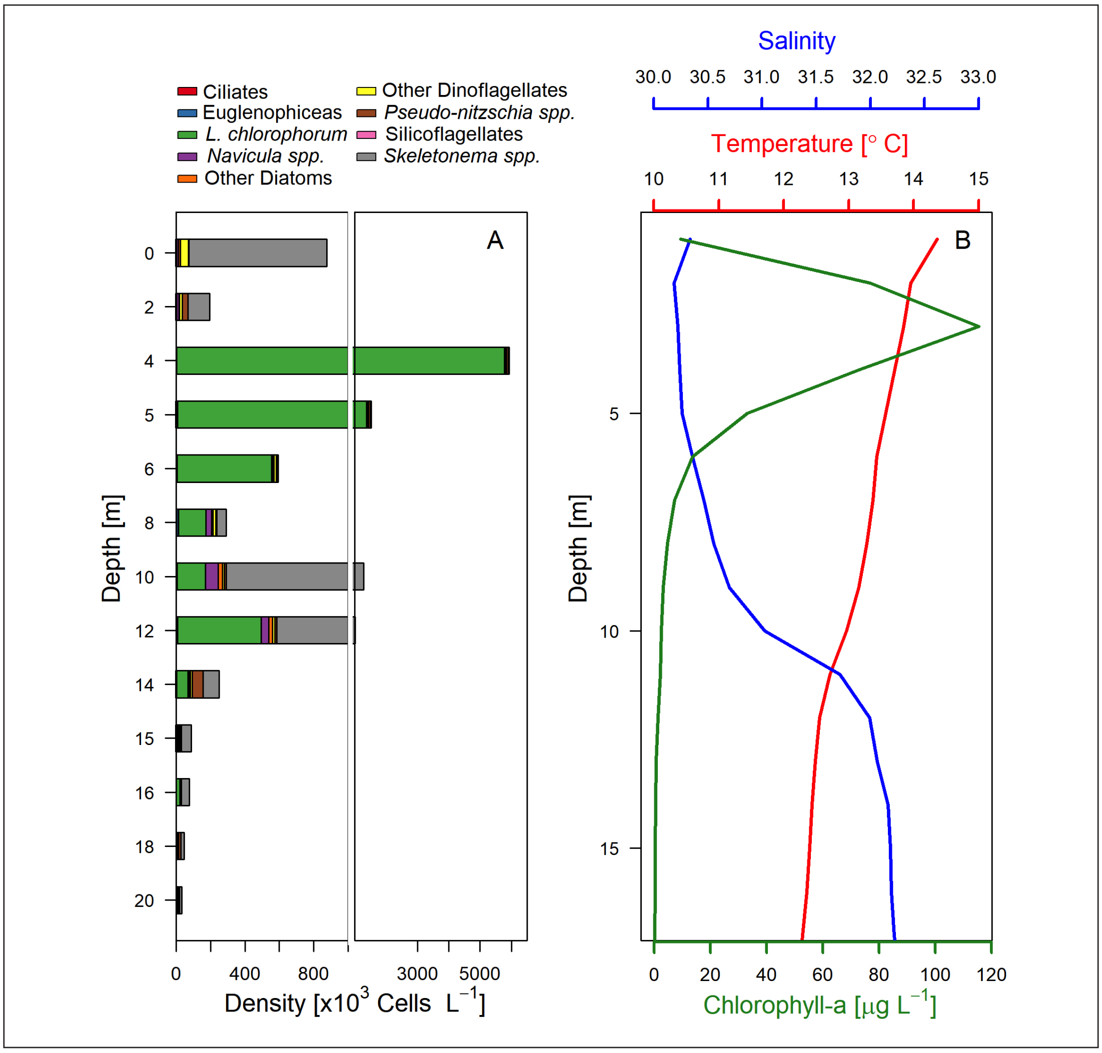

Fig. 2. Vertical distribution of A) phytoplankton cell counts, and B) temperature (red line), salinity (blue line) and chlorophyll-a (green line).

A similar event occurred in the same area during March and April 2003 (cell densities of 13x10^6 cells L^{-1} and Chl-a concentrations of ~1000 µg L^{-1}), causing a mass mortality of ~250,000 salmon [3]. Interestingly, these two events occurred with similar freshwater conditions and negative anomalies of river outflow. Similar events have been observed along the French Atlantic coast associated with an increase of nutrients, mainly ammonium [4,5] and resulting in a mass mortality of fish and shellfish [6,7].

We suggest the particular oceanographic conditions (especially high salinity), associated with reduced precipitation and weaker outflow from the Puelo River, favoured the proliferation of *L. chlorophorum* in Reloncaví Sound in 2020.

Although during 2020 there were no mortalities associated with this event, in March 2021, a bloom of P. chlorophorum in Reñihue Fjord, NW Patagonia (Los Lagos Region), caused a mass mortality of Pacific salmon (around 162 thousand fish) and losses close to 3.5 million US dollars.

This study highlights the need to pursue further studies for the understanding of non-toxic HB-HAB events, such as the *L. chlorophorum* events described above. These events represent a potential socio-economic, and environmental impacts, especially under current and future predictions of climate change including the decline of river discharges.

Acknowledgements

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Authors
Guido Mancilla-Gutiérrez, Patricio A Díaz, Iván Pérez-Santos, Cécile Pujol, Camila Schwerter, Robinson Altamirano, Sara Arenas-Uribe & Pilar Navarro, Centro i~mar, Universidad de Los Lagos, Casilla 557, Puerto Montt, Chile

Gonzalo S Saldías, Departamento de Física, Facultad de Ciencias, Universidad del Bío-Bío, Concepción, Chile

Email corresponding author:
guido.mncilla@gmail.com

Seaweeds of Rangitāhua hide toxic dinoflagellates

Fig. 1. Raoul Island (left), MY Dapple (right).

In November 2021 a team of scientists joined the Expedition Rangatahi to Rangitāhua/Kermadec Islands, a New Zealand territory approximately 1000 km north east of New Zealand. This was the first scientific voyage to Rangitāhua as part of the transformational MBIE Endeavour Programme, Te Mana o Rangitāhua, led by Ngāti Kuri and Auckland Museum. Using a holistic approach, the five-year funded programme combines indigenous knowledge and science considering both social and environmental elements. The project aims to understand the biodiversity and ecosystems of Rangitāhua from land to sea and identify indicators of ecosystem change to provide the tools for an iwi-led management of the Rangitāhua environment. The multi-disciplinary team spanned Auckland Museum, NIWA, University of Auckland, Massey University, and Manaaki Whenua. Research activities included selection of sites that will be regularly monitored during the programme, CTD profiles, ocean glider sampling and Triton Submersible testing. Scuba diving was a key activity with focus on community structure combined with environmental-DNA (eDNA) sampling and photogrammetry. The 18-day voyage to the Rangitāhua was on superyachts MY Dapple and The Beast and was made possible by generous philanthropic support (Fig. 1).

During the expedition 14 macroalgal samples were collected at Denham Bay Observation Site (GPS: -29.28472, -177.9536; Depth 8 - 16 m) for the isolation of epiphytic dinoflagellates. Macroalgae were shaken in tubes containing local seawater then removed and samples were transported back to the Cawthron Institute in Nelson, New Zealand, for isolation and culture of microalgae [1]. The macroalgal hosts were dried and kept as voucher specimens with subsamples preserved in silica gel for further molecular analysis. Dinoflagellates were isolated and the DNA sequence data prepared and analysed as described previously [2].

The dinoflagellate species isolated from samples collected at Denham Bay Observation Site are summarised in Table 1. *Gambierdiscus polynesiensis*, a

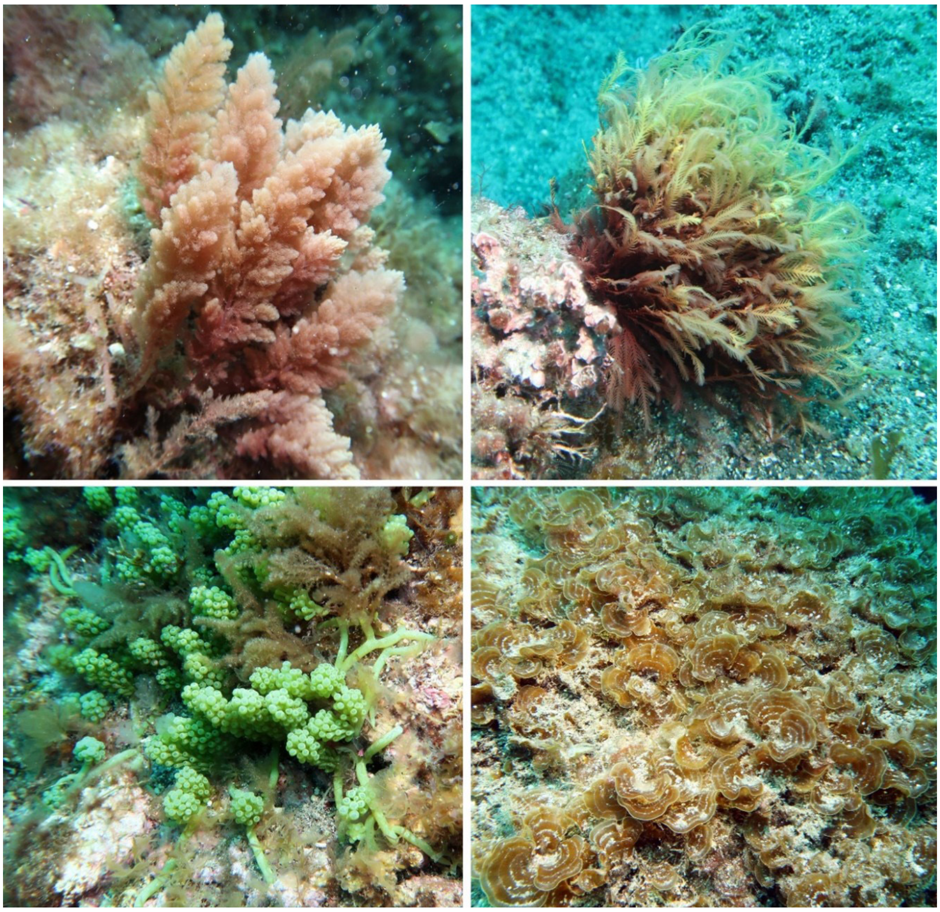

Fig. 2. Macroalgae hosting dinoflagellates: Asparagopsis taxiformis (top left), Delisea sp. (top right), Caulerpa racemosa (bottom left), and Distromium sp. (bottom right).

Table 1. Dinoflagellate species isolated from samples collected at Denham Bay Observation Site, Raoul Island, Rangitāhua Kermadec Islands, November 2021

Species	Macroalga host	CICCM code
Amphidinium carterae	*Dictyota* aff. *pfaffi*	ns
A. massartii	*Caulerpa racemosa*	CAWD377
Coolia canariensis	*Distromium* sp.	CAWD386, 387
C. palmyrensis	Cyanobacteria undetermined, *Distromium* sp., *Asparagopsis taxiformis*, *Caulerpa webbiana*	CAWD385
C. tropicalis	*Distromium* sp., *Caulerpa webbiana*	CAWD384, 388
Gambierdiscus australes	*Asparagopsis taxiformis*	CAWD381
G. polynesiensis	*Distromium* sp.	CAWD378, 380
Ostreopsis sp. 3*	*Plocamium* sp. *Delisea* sp.	CAWD383
Prorocentrum emarginatum	*Distromium* sp., *Actinotrichia* sp., *Dictyota* sp,, *Delisea* sp., *Plocamium* sp., *Galaxaura* sp.	CAWD392, 395
P. fukuyoi	*Dictyota* aff. *pfaffi*, *Distromium* spp. *Dictyota intermedia*	CAWD389, 390, 391, 393
P. hoffmannianum	*Dictyota* aff. *pfaffi*, *Caulerpa webbiana*	CAWD396
P. lima	*Dictyota intermedia*, *Dictyota* sp., *Ganonema farinosum*, *Delisea* sp., *Plocamium* sp., *Asparagopsis armata*	CAWD394

*CICCM: Cawthron Institute Culture Collection of Microalgae; *: Ref: [6]; not submitted to CICCM.*

known ciguatoxin producer, and *G. australes,* a maitotoxin producer, were isolated on previous expeditions [3] and were again isolated from samples collected on this voyage (Table 1). *Gambierdiscus honu,* which was first described from Rangitāhua [4], was absent. Associated dinoflagellates included species in the genera *Amphidinium*, *Coolia, Ostreopsis* and *Prorocentrum* (Table 1). Many of these isolates are now being maintained in the Cawthron Institute Culture Collection of Microalgae (CICCM).

The identification of *Gambierdiscus* species is of particular interest in relation to warming sea temperatures around Aotearoa New Zealand and the likelihood that these species will establish in New Zealand's sub-tropical north. This would bring with it the risk of ciguatera poisoning if established species produced ciguatoxins. In conjunction with isolating epiphytic dinoflagellates, metabarcoding of samples collected in Rangitāhua and Northland, New Zealand, will continue as a form of monitoring for such introductions [5].

The e-DNA samples collected from different habitats (macroalgae, turfing algae, barrens, and corals) have not been analysed yet. It is likely that further species of dinoflagellates will be identified indicating the need for further collections and study of toxic microalgae as well of poorly known macroalgae genera and undescribed species. In order to fully document the algal flora of Rangitāhua, the next voyage will be focusing on wider seaweed collections spanning different habitats, localities and depths and including the isolation of dinoflagellates from wider sampling.

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Authors

Roberta D'Archino, National Institute of Water and Atmospheric Research Ltd, Private Bag 14-901, Wellington 6241, New Zealand

Wendy Nelson, National Institute of Water and Atmospheric Research Ltd, Private Bag 14-901, Wellington 6241, New Zealand and School of Biological Sciences, University of Auckland, Auckland 1014, New Zealand.

Lesley Rhodes, Kirsty Smith, Lucy Thompson & Tomohiro Nishimura, Cawthron Institute, Private Bag 2, Nelson, New Zealand

Tom Trnski, Auckland War Memorial Museum, PB 92018, Victoria Street West, Auckland, New Zealand

Email corresponding author:
roberta.darchino@niwa.co.nz

First isolation of toxin-producing benthic dinoflagellates from the Kingdom of Tonga, including the ciguatera causing genus *Gambierdiscus*

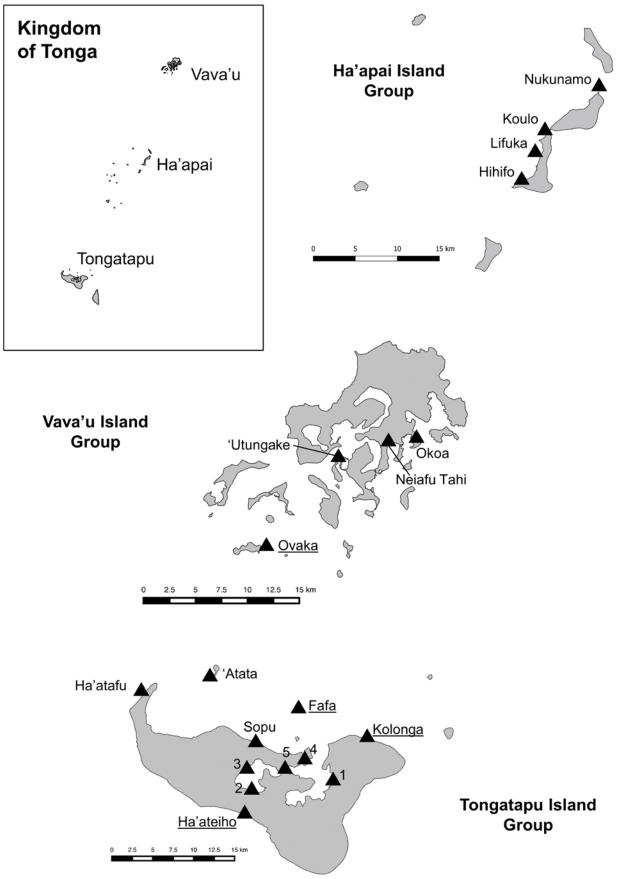

Fig. 1. Map of the Kingdom of Tonga showing study sites visited in June 2016 and 2017 across three island groups: Ha’apai, Vava’u, and Tongatapu. Sites where live isolates were obtained are underlined. Numbered sites on Tongatapu represent 5 sites sampled around the Fanga’utu lagoon.

Ciguatera poisoning (CP) is a serious and incurable human illness caused by ingestion of ciguatoxins (CTXs) in contaminated seafood [1]. CTXs are produced by *Gambierdiscus polynesiensis* (as definitively confirmed by liquid chromatography mass spectrometry; LC-MS) with other species displaying toxicity analogous to CTXs (reviewed in [2]). A number of targeted sampling efforts have documented the presence of at least twelve *Gambierdiscus* species across the northern and southern Pacific, including French Polynesia, Fiji, New Caledonia, the Cook Islands, Australia, Rangitāhua/ the Kermadec Islands, and New Zealand with a number of undescribed genotypes/ribotypes also requiring further investigation (reviewed in [2]). Other toxin-producing benthic and epiphytic dinoflagellate genera regularly found with *Gambierdiscus* Adachi & Fukuyo include *Ostreopsis* Schmidt, *Prorocentrum* Ehrenberg, and *Coolia* Meunier have also been documented across the Pacific [3, 4] and are of concern due to their human health, economic, and ecological effects. Harmful algal blooms have significant economic and health impacts in Pacific Island countries where fish consumption is part of the staple diet [5].

The Kingdom of Tonga is a southern Pacific nation where CP cases have been reported for at least 50 years [6]. There are very limited published records on the prevalence of CP despite local knowledge and awareness of the illness. It is likely that case numbers are higher than those reported due to difficulties in diagnosing CP and a lack of official reporting channels. Four previous reports have noted poisoning events associated with fish species from locations in Tongan waters [5, 6], with one reporting a total of 146 cases from Tonga between 2001 and 2005 [5]. Recently, four cases of CP were reported in New Zealand from people eating eel that had been brought back from Tonga; all four patients required hospitalisation [7].

Fig. 2. Artificial substrate samplers deployed in seagrass habitats in Tongatapu.

Fig. 3. (left) Phoebe Argyle working in in Fanga'utu lagoon, Tongatapu and (right) Neiafu Tahi, Vava'u.

Earlier reports of CP in Tonga indicate the presence of at least one toxin producing *Gambierdiscus* species, but there is limited information on the geographical distribution. During two comprehensive surveys in 2016 and 2017 (Argyle *et al.*, in prep), samples of benthic dinoflagellates were obtained for the purpose of making live cell isolations to establish cultures for identification, toxin analysis and future research.

Sampling occurred during June 2016 and June 2017 sites around Tongatapu, Ha'apai, and Vava'u island groups, Kingdom of Tonga (Fig. 1). Five sites around Tongatapu were sampled in 2016 and in 2017 nineteen sites were sampled across all three island groups. Untreated samples for isolations were taken at low tide directly from seagrass and macroalgae (2016) or from artificial substrates (2017) within the shallow subtidal habitats (Figs. 2-4). Established isolates were identified to species level by using amplification of the D1-D3 region of the large subunit ribosomal RNA gene (LSU) as previously described [8].

In 2016, three *Gambierdiscus* and one *Ostreopsis* isolate were successfully established in culture. Two *Gambierdiscus* isolates were collected from *Sargassum* at Kolonga and were identified as *G. australes* and *G. honu* respectively (Table 1, Fig. 5a-b). Another *Gambierdiscus* isolate, *G. pacificus*, was collected from *Padina* from Fafa Island (Table 1; Fig. 5c). One *Ostreopsis* sp. 3 isolate (Fig. 5d) was collected from *Sargassum* at Ha'ateiho. Seagrass habitats were sampled at Sopu, Fafa, and Kolonga, but

Fig. 4. Study sites from top to bottom: Hihifo (Ha'apai), Fafa Island, and Ha'ateiho blowholes (Tongatapu).

no *Gambierdiscus* cells were successfully cultured from these samples even though they were visually present in the samples.

In 2017, one *Prorocentrum lima* and one *Coolia tropicalis* (Fig. 5e-f) isolate were successfully established into culture (Table 1). The *Prorocentrum lima* isolate was collected from mixed turf algae on Ovaka Island, Vava'u, and the *Coolia tropicalis* from seagrass (*Halodule uninervis*) at Kolonga. Phylogenetic analyses strongly supported the species identification of the dinoflagellates. Representatives of all isolates were deposited in the Cawthron Institute Culture Collection of Micro-algae (CICCM; Table 1).

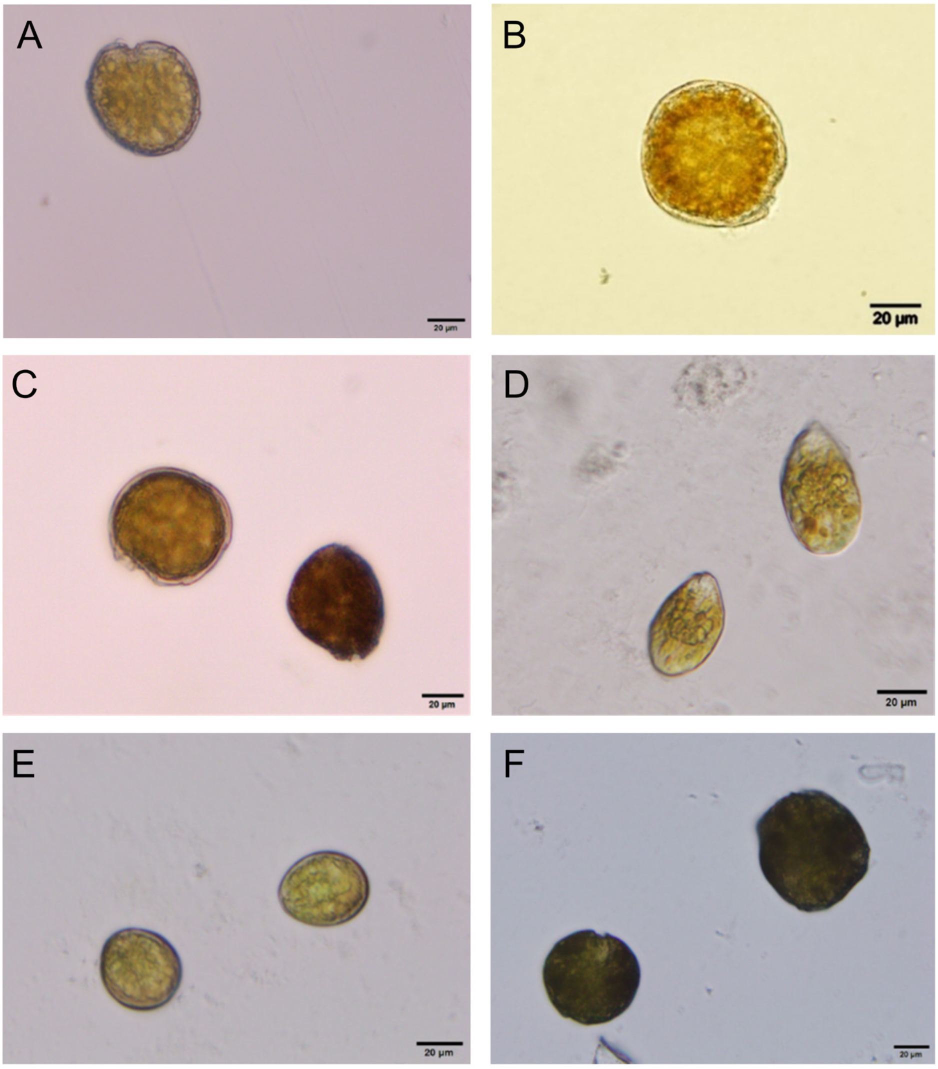

Fig. 5A-F. Light microscope images of representative cells of cultures made from isolates from around the Kingdom of Tonga in June 2016 and June 2017. **A** *G. australes* isolated from Kolonga (CAWD253), **B** *G. pacificus* isolated from Fafa Island (CAWD252), **C** *G. honu* isolated from Kolonga (CAWD250), **D** *Ostreopsis* sp. 3 isolated from Ha'ateiho (CAWD249), **E** *Prorocentrum* cf. *lima* isolated from Ovaka (CAWD271), and **F** *Coolia tropicalis* from Kolonga (CAWD277).

Gambierdiscus isolates were analysed for selected algal CTXs, maitotoxin -1 (MTX-1) and 44-methylgambierone (44-MG) (using the method described in Murray, Boundy [9]; Table 1). *Gambierdiscus australes* (CAWD253) produced MTX-1 (8 pg cell^{-1}) and 44-MG. This is comparable to a previously described *G. australes* isolate from the Cook Islands (CAWD149; 8.3 pg MTX-1 per cell [10]. The *G. pacificus* (CAWD252) and *G. honu* (CAWD250) isolates also produced 44-MG, which is produced almost ubiquitously by *Gambierdiscus* species but has low toxicity compared to other dinoflagellate toxins [11]. None of the *Gambierdiscus* isolates produced any of the algal CTXs that were monitored (CTX-3B, CTX3C, CTX-4A and CTX-4B).

Ostreopsis sp. 3 (CAW249) did not produce any PLTX-like compounds, as determined using the on-column oxidative cleavage method (Selwood, van Ginkel [12]; LoD < 0.01 pg cell^{-1}). *Protocentrum lima* clade 3 (CAWD271) produced both diarrhetic shellfish toxins (DTX-1; 0.3 pg cell^{-1}) and okadaic acid (OA; 11.3 pg cell^{-1}) when tested according to McNabb, Selwood [13]. The *Coolia* isolate was not tested for toxin production in this study.

The species of *Gambierdiscus* found in this study have all been previously reported in other studies from the South Pacific (reviewed by [2]). *Gambierdiscus honu* was first described in 2017, and has been isolated from the Cook Islands, Rangitāhua/the Kermadec Islands, and Heron Island, Australia, in the Southern Great Barrier Reef (also reviewed by [14]). *Gambierdiscus australes* has been found in the Cook Islands, French Polynesia, the Kermadec Islands, and Hawai'i [2]. *Gambierdiscus pacificus* has also been found in the Cook Islands and French Polynesia, in addition to Kiribati and the Marshall Islands [2]. Obtaining new representative strains of *Gambierdiscus* species is important as there are wide species and strain-specific differences and plasticity in growth and toxin production across the dinoflagellates [2].

Co-occuring with *Gambierdiscus*, other harmful taxa including *Ostreopsis, Prorocentrum,* and *Coolia* may pose further risks to human and ecological health. Blooms of *Ostreopsis* are associated with human illness through aerosol inhalation or skin irritation, particularly in the Mediterranean [15], and through consumption of seafood that has accumulated palytoxins and/or ovatoxins [15]. *Prorocentrum lima* (as found in Tonga) is a toxin-producing species that can cause diarrhetic shellfish poisoning; the route of poisoning is through bioaccumulation, in the same manner as for CTXs leading to CP [16]. *Coolia tropicalis*, the species identified in Tonga, has demonstrated toxic effects, and a Brazilian strain has been shown to produce 44-MG [17]. Whether this species poses a risk to human health requires further investigation.

Harmful dinoflagellates are increasing their range, and it is possible that illnesses such as CP may become more prevalent [2]. Identifying toxic species, their distribution, and their toxin production and toxicity is the first step towards designing monitoring programs that may assist in mitigating harmful impacts. A wider survey of benthic dinoflagellates in Tonga shows that there is high species diversity and that *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* are widely distributed across the

Table 1. Dinoflagellate isolates successfully established into culture from the Kingdom of Tonga in 2016 and 2017. Details include collection site, macroalgae species the cells were isolated from, species identification of the isolates, toxin production, CICCM identification code, and species identification confirmation details based on the D1-D3 region of the large subunit ribosomal RNA. 44-methylgambierone (44-MG) represents presence/absence as this compound could not be quantified at the time of the study.

Species ID	CICCM Code	Site	Substrate or habitat collected from	Toxin production
Gambierdiscus australes	CAWD253	Kolonga	*Sargassum hemiphylloides*	8 pg/cell MTX 44-MG
Gambierdiscus pacificus	CAWD252	Fafa	*Padina* cf. *sanctae crucis*	44-MG
Gambierdiscus honu	CAWD250	Kolonga	*Sargassum hemiphylloides*	44-MG
Ostreopsis sp. 3	CAWD249	Ha'ateiho	*Sargassum polycystum*	not detected
Prorocentrum lima clade 3	CAWD271	Ovaka	Artificial substrate deployed in mixed turf algae (unidentified)	0.3 pg/cell Dinophysistoxin-1 Okadaic acid 11.3 pg/cell
Coolia tropicalis	CAWD277	Kolonga	Artificial substrate deployed in seagrass bed (*Halodule uninervis* dominant)	not tested

islands (Argyle *et al.,* in prep). Methods for sampling and identifying benthic dinoflagellates for monitoring purposes are still being validated [18], but it is envisaged that monitoring programs can eventually be established in Tonga to detect blooms and warn of the risk to humans of harmful taxa.

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Authors

Phoebe A Argyle & Islay D Marsden, School of Biological Sciences, University of Canterbury, Private Bag 4800, 20 Kirkwood Ave, Christchurch 8041, New Zealand/ Phoebe A Argyle, Ministry of Marine Resources, Moss Rd, Avarua, Cook Islands

Lesley L Rhodes, Kirsty F Smith, J Sam Murray & D Tim Harwood, Cawthron Institute, Private Bag 2, 98 Halifax St East, Nelson 7042, New Zealand

Tuikolongahau Halafihi, Ministry of Fisheries, P.O. Box 871, Nuku'alofa, Tongatapu, Tonga

Email corresponding author:
phoebe.a@gmail.com

Aotearoa New Zealand – Japan joint research project on mapping the predicted distribution of ciguatera poisoning-related benthic dinoflagellates after forecasted global warming

Aotearoa New Zealand and Japan are maritime nations with strong cultural, recreational, and economic connections to seafood. There is an increasing demand for stable production and supply of safe and secure seafood in both countries.

Benthic dinoflagellates are ubiquitous in marine ecosystems and several species can cause benthic harmful algal blooms (BHABs). Some of these BHAB species produce toxins which can contaminate seafood, impacting seafood production and supply. In particular, toxins produced by the genus *Gambierdiscus* cause ciguatera poisoning (CP), the most common non-bacterial seafood poisoning worldwide. Although CP is historically reported in the tropical/subtropical zones, climate change effects, particularly the warming of sea surface temperatures, could result in the range expansion of CP into new coastal waters including temperate regions. Based on forecasted global warming of both air and sea surface temperatures under the Representative Concentration Pathway (RCP) 8.5 scenario proposed by the Intergovernmental Panel on Climate Change (IPCC) [1], coastal waters around Aotearoa New Zealand and Japan could warm by up to 2 °C between 2050 and 2099, above current average temperatures [Briscoe et al. unpublished]. This level of ocean warming could alter the habitable range for CP-related genera, *Gambierdiscus* and *Fukuyoa*, in these regions. It is therefore essential to reveal the current distribution of these toxic genera/species and predict the likely 'future' distributions for CP risk assessments.

To contribute to the current and 'future' CP risk assessments in Aotearoa New Zealand and Japan, researchers from the Cawthron Institute and Kochi University initiated a joint research project in 2021, jointly funded for two

Fig. 1. A group photo taken during the project's meeting at Cawthron Institute, Nelson, New Zealand, in August 2022. Lesley Rhodes, John Pearman, Lucy Thompson, Sarah Challenger, Sarah Finch, Haruo Yamaguchi (top, from left to right), Dana Briscoe, Sam Murray, Tim Harwood, Goshi Araki, Hiroshi Funaki (middle, from left to right), Natsumi Nishikawa, Jacqui Stuart, Tomohiro Nishimura, Kirsty Smith, Cath McLeod, Masao Adachi, and Takumi Iijima (bottom, from left to right). Photo credit: Cawthron Institute, AgResearch, or Tomohiro Nishimura.

years by the Royal Society of New Zealand (RSNZ) and the Japan Society for the Promotion of Science (JSPS). The project title is 'Mapping the predicted distribution of toxic benthic microalgae after forecasted global warming in Japan and New Zealand'. The project aims to develop a pipeline for assessing the future risk of CP in both countries and has several research aims, including:

1. Create comprehensive distribution maps of toxic BHAB species utilising a review of previous research and new data from multiple sources including molecular phylogenetic, morphological, metabarcoding, chemical, and toxicological;
2. Determine the growth characteristics of key *Gambierdiscus* and *Fukuyoa* species;
3. Collate environmental data sets based on available modelled products by the Mercator Ocean's GLORYS reanalysis version 12v1 [2];
4. Create current spatial distribution maps of the target species through modelling;
5. Collate environmental data sets based on available modelled products by the IPCC and create 'future' spatial distribution maps of the target species by modelling based on the growth characteristics.

This research project will develop a new methodology pipeline for modelling the distribution of harmful algal species, that can then be applied to other taxa in Aotearoa New Zealand, Japan, and across the Pacific.

The teams had a virtual meeting/workshop in August 2021 due to the difficulty of travel during the COVID-19 pandemic, and finally met in person in August 2022 for a two-day hybrid meeting held at the Cawthron Institute in Nelson, New Zealand (Fig. 1). This meeting brought together over 20 attendees for oral presentations, workshops, round-table discussions, and facility tours of the three research centres of the Cawthron Institute.

The teams have published several papers from this project: metabarcoding of *Gambierdiscus* [3], growth characteristics of *Gambierdiscus* [4], a literature review of BHAB species in Japan [5], and the structural characterisation of maitotoxins [6]. The project will continue with further modelling and more publications.

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Authors

Tomohiro Nishimura, Kirsty F Smith, Lesley L Rhodes, Dana K Briscoe & John K Pearman
Cawthron Institute, 98 Halifax Street East, Nelson 7010, New Zealand

Haruo Yamaguchi & Masao Adachi
Laboratory of Aquatic Environmental Science (LAQUES), Faculty of Agriculture and Marine Science, Kochi University, 200 Otsu, Monobe, Nankoku, Kochi 783-8502, Japan

Email corresponding author:
tomohiro.nishimura@cawthron.org.nz
or
aquariumuirauqa@gmail.com

Human Dimensions of the Citizen Science Oceanography Program Operated by the Pacific Salmon Foundation, Canada

Citizen Science involves members of the general public taking part in certain scientific work (e.g. data or sample collection), often in collaboration with or under the direction of professional scientists. There is great value in this approach as it allows for the collection of data in a very cost-effective way as well as increasing public understanding of specific issues to help foster stewardship within communities. Citizen Science is becoming increasingly utilized in the scientific community and sharing lessons learned from operating long-term programs can be very informative to other community based initiatives.

The Citizen Science Oceanography Program (*https://sogdatacentre.ca/atlas*) run by the Pacific Salmon Foundation (PSF) has been operating since 2015 and currently (2022) is in its eighth sampling season. This program includes several dozen trained citizens from different communities throughout the Strait of Georgia, Canada. Sampling (physical collection of ocean water samples, nutrients and harmful algae) is done at approximately 55 stations on a regular schedule (about 20 times a year). Detailed analysis of the harmful algae and oceanographic conditions in the Strait based on the program's dataset can be found in [1]. The datasets collected through this program are some of the most comprehensive oceanographic data obtained from this area, and are publicly available via the Strait of Georgia Data Centre (*https://sogdatacentre.ca/*). Several years ago, this program was endorsed by GlobalHAB, an international program by the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO.

After almost eight years of operating this program significant lessons have been learned, specifically related to operating oceanographic equipment, troubleshooting, communicating, and navigating unfavourable weather conditions. Overall, PSF has received tremendous positive feedback on both the methodology and longevity of the Program. We believe that the long-term success of this program is primarily due to our Citizen Science volunteers and their long-standing dedication and commitment.

All of the citizen science crew members are from local communities. They were initially selected because they have experience working on the water in some capacity (e.g. retired commercial fisherman, biologists, etc.) and own their own vessels (fuel expenses and a small honorarium are covered by PSF). Most of crews joined the program from the very beginning in 2015. An initial training on how to properly collect data and samples was carried out during a two-day workshop. Each team performed their first at sea sampling with a PSF biologist on-board for quality control. Each crew is accompanied by the PSF Program Coordinator about three times a year. Quarterly newsletters and regular updates via group e-mails and social media (*https://www.facebook.com/CitizenSciencePhytoplankton/*) are used to communicate updates or recent findings. An annual symposium is held to share citizen scientists' experiences and provide updates from the technicians and scientists who analyze measurements and samples.

For the 2022 annual symposium, citizen scientists were asked to take a survey to share and evaluate their experience with the Program. In the multiple choice questionnaire, all participants strongly agreed that they: have received adequate training, felt satisfied after sampling is done, and felt valued as a member of our team. Most of the participants (approximately 80%) felt that their work is important to the community and they were very likely to stay with the program for the next two years. It is clear that satisfaction with the program and feeling that their contributions make a difference, played a big role in high volunteer retention.

Citizen Scientists were also asked several open-ended, anonymous questions. The first one was – what was your motivation to join the program? Answers included:

- "I wanted to volunteer for a cause I cared about",
- "I am a marine biologist and wanted to put my skills to a useful scientific and community purpose. It was also a chance to go out in the marine environment again, something I had been missing",
- "Interest in doing something to help marine environment… Love of the ocean".

Most of the participants indicated that they were involved in other monitoring or work on the ocean for example: forage fish egg monitoring, eelgrass planting, and coastal waters monitoring surveys. Participants were also generally interested in ocean and marine life, particularly in fishes and marine algae. Survey participants were also asked if they learned any new skills or gained new knowledge. Respondents unanimously agreed with answers like, "Yes, absolutely", "Definitely – especially the technology side of our sampling", "Lots of new skills and knowledge, which I am grateful for", and "Working on the project has taught me more that I would have known". As for their favorite parts of being a part of the team, answers included:

- "contributing to important data collection",
- "knowledge that something is being done to better understand the marine ecosystem", and
- "contributing to something meaningful".

Finally, one piece of advice that the volunteers wanted to share is "Be open to new learning and adventures. The data we collect will make a difference".

There are a number of community-based harmful algae monitoring programs around the world. They fill in informational needs, enhance data baselines, improve bloom forecasting capabilities, increase awareness, and create new working relationships. Collected databases contribute to the current understanding of algae ecology and their impacts on marine life. From our experience, the success of the citi-

Cooperation scenes from the Citizen Science Oceanography Program, Strait of Georgia, Canada

zen science programs is the ongoing involvement of highly motivated volunteers, interested scientists who provide oversight and guidance, and continuing funding. With an apparent increase in intensity and duration of harmful algal blooms (HABs) and emerging threats, monitoring is becoming more important than ever. Establishing and maintaining long term programs will definitely benefit both scientific research and local communities.

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Authors

Svetlana Esenkulova, Nicole Frederickson
& Isobel Pearsall
Pacific Salmon Foundation, 1682-300 West
7th Avenue, Vancouver, BC, V6J 4S6, Canada

Email corresponding author: svesen@uvic.ca

12th International Conference on Modern and Fossil Dinoflagellates

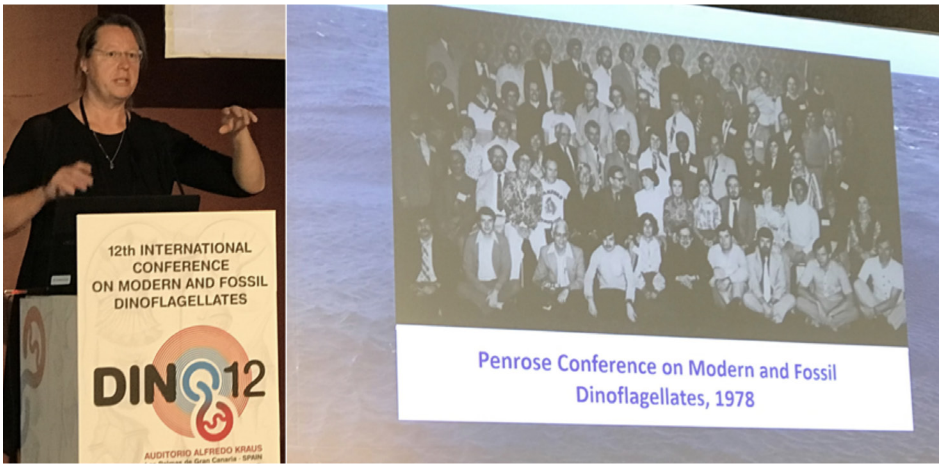


Fig. 1. Karin Zonneveld paying homage to the I Dino conference in 1978

Forty-four years after the Penrose Conference on Modern and Fossil Dinoflagellates (Fig. 1), the long awaited DINO12 conference was held 4-8 July 2022 at the Auditorio *Alfredo Krause* in Las Palmas de Gran Canaria (Fig. 2). The local organizing committee was chaired by **Emilio Soler Onís** with the support of the Canarian Observatory of Harmful Algae (COHAB) of The Canarian Science and Technology Park Foundation, University of Las Palmas de Gran Canaria .

DINO12 welcomed participants gathered (or joined remotely) for discussions on recent advances in the field of Modern and Fossil Dinoflagellates. The single session DINO12 format framed the topic of global warming while preserving the essence of the meeting in which the dinoflagellates and their cysts are the major protagonists. It provided a broad forum for basic and applied research including Climate Change, Ecology, Toxic Dinoflagellates and Biotoxins, Palaeoecology and Palaeoceanography, Mesozoic and Cenozoic Dynocyst Stratigraphies, Phanerozoic and deep time scales, Dinocyst Systematics, Life Cycles and Nutritional Strategies, Biodiversity and Systematics, Stratigraphy, Evolution, and Taxonomy.

DINO12's final presentation was an outstanding one presented by the Dale duo on *Ecological Signals in Dinoflagellate Biostratigraphy*. It brought together the richness of the fossil dinoflagellate records with concerns of modern environmental sciences using examples of studies in eutrophication, pollution and climate change. It was an elegant reminder of why the fossil and modern dinoflagellate researchers continue to meet in one forum.

Two workshops were also included in DINO12 activities. One was on Benthic HAB Sampling Methods (see separate article this issue) and Monitoring, Control and Management of Marine Microalgae. The DINO12 abstracts are available here.

Karin Zonneveld Center for Marine Environmental Sciences (MARUM) Geosciences Department, University of Bremen graciously agreed to host DINO13 in Germany. The conference venue and dates will be announced when plans are finalized. Contact Karin at *kzonneveld@marum.de* for further information.

Author
Emilio Soler Onís, Canarian Science and Technology Park Foundation (FCPCT), University of Las Palmas de Gran Canaria, SPAIN
Email: emiliosoleronis@fpct.ulpgc.es

Fig. 2. Participants of the DINO 12 conference (Las Palmas de Gran Canaria, 4-8 July 2022) at the Alfredo Krause Auditorium

DINO 12 Workshop on Sampling Methods for Benthic Dinoflagellates

Unlike planktonic species, there is no standardized, or even agreed upon, quantitative method for sampling benthic dinoflagellates. During the workshop, methods traditionally used for collecting benthic dinoflagellates (BHABs) were reviewed and new techniques using artificial substrates were demonstrated for participants whose interests ranged from implementing cell-based monitoring programs to curiosity about quantification of BHABs. Unlike pelagic phytoplankton where monitoring can be achieved by collecting samples from a uniform medium (either by integrating the water column or at discrete depths), benthic dinoflagellates are found in a variety of complex habitats adhered to a variety of substrate types including algal turf, macroalgae, rocks, coral rubble, sand or seagrasses and artificial structures such as concrete seawalls and pilings. As with planktonic sampling, BHAB cells are patchy and can vary by an order of magnitude between sampling sites only a few cm apart. So, many factors confound the methods to quantitatively sample BHABs for monitoring purposes.

Key decision points for standardizing protocols for monitoring *Gambierdiscus* are how, when and where to sample. Each of these topics were reviewed with the participant's individual sampling requirements used as examples during group discussions. If you are interested in using artificial substrates for sampling BHABs, please contact Juan Fernandez Zabala (*jfernandez@fpct.ulpgc.es*) for more information and a prototype sampling device. He has an ongoing project to validate the use of artificial substrates in as many different areas as possible.

Author
Juan Fernández-Zabala, Observatorio Canario de Algas Nocivas (OCHAB), Parque Científico Tecnológico Marino de Taliarte, Universidad de las Palmas de Gran Canaria, Las Palmas, Canarias, Spain

Email corresponding author: jfernandez@fpct.ulpgc.es

Dino 12 Workshop, Las Palmas de Gran Canaria, Spain. Top: participants at the workshop; bottom, benthic dinoflagellates sampling

XIV Iberian Meeting on Harmful Algae and Biotoxins (REDIBAL)

The XIV Iberian Meeting on Harmful Algae and Biotoxins was held in Lisbon, Portugal, from 1st – 3rd June at the *Magalhães* Auditorium of the Portuguese Institute of Sea and Atmosphere (IPMA). The event was organized in a joint cooperation between IPMA and the Marine and Environmental Sciences Centre (MARE). It was a long awaited in-person meeting after two years of COVID-19 shutdown that brought together over 100 participants, promoting scientific debate and collaboration among Iberian scientists.

A total of 44 communications were presented (29 oral and 15 posters) distributed amongst 7 thematic sessions: *i*) microalgae and toxins time series, *ii*) toxins and bioactive compounds from marine microalgae, *iii*) toxins detection methods, *iv*) toxins in seafood, *v*) effects of toxins on marine organisms, *vi*) microalgae bloom dynamics and vii) ciguatera. A plenary talk by Don Anderson covered comparative studies of large-scale *Alexandrium catenella* blooms in temperate and Artic waters giving evidence for range expansion of this species under current warming climate trends. The abstract book is available for download in the conference website (https://redibal.ipma.pt/livro-de-resumos-2022/).

Redibal 2022 awards.Top panel. Lifetime Achievement Award to Teresa Moita and Santiago Fraga; middle panels, Young Researcher Awards: left, Elisabet Cruz, Best Oral Communication on Harmful Microalgae; right, André Patricio, Best Oral Communication on Marine Biotoxins; bottom panel: Celia Costas, Best Poster.

During the XIV Iberian Meeting on Harmful Microalgae and Marine Biotoxins there was also the opportunity to pay a tribute to M. Teresa Moita and Santiago Fraga, recently retired, in recognition for their inspiring and outstanding contributions to HAB research both in the Iberian context and worldwide. This series of meetings has also traditionally been an ideal forum for students and young researchers to present their work (see http://redibal.org/ for details of previous Iberian Meetings). Following this tradition, a competition for young researchers best oral and poster presentations was held. Elisabet Cruz (University of Aveiro, Portugal) was awarded the best oral communication on "Harmful Microalgae" for her talk on "Transport pathways of *Dinophysis acuta*: from Portugal to Galician Rias Baixas"; André Patrício (University of Lisbon, Portugal) was awarded the best oral communication on "Marine Biotoxins" for his presentation on "Time-lagged correlation analysis of DSP toxicity across Portuguese shellfish producing areas, species and meteorological conditions"; Celia Costas (University of Santiago de Compostela, Spain) was awarded best poster for her work on "Early okadaic acid-induced ultrastructural alterations in the gut: role of serotonin". The awards were presented by M. Teresa Moita and Santiago Fraga.

Continued on next page

Group photo of participants of the XIV Iberian Meeting on Harmful Microalgae and Marine Biotoxins, Lisbon, 1-3 June 2022

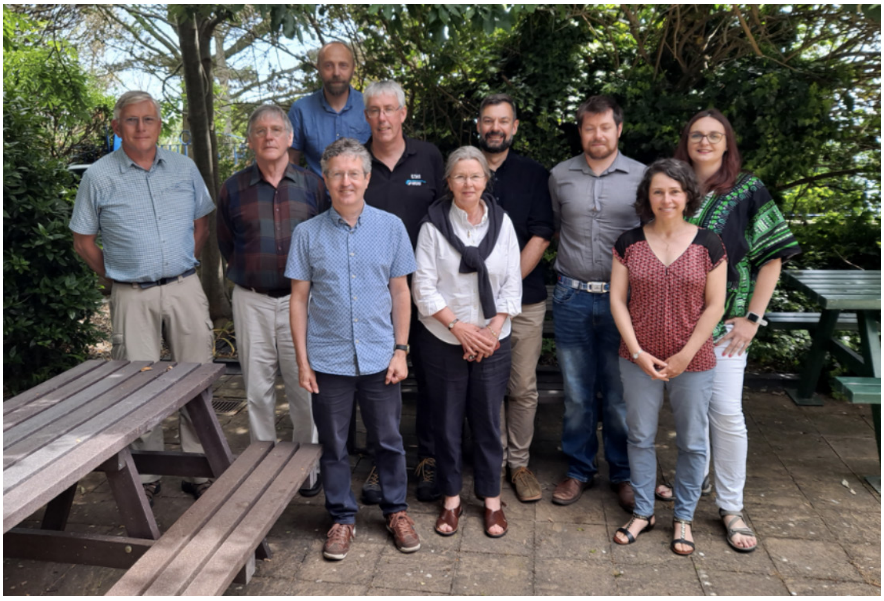
ICES-IOC Working Group on Harmful Agal Bloom Dynamics Meeting

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) is an important forum for ICES (International Council for the Exploration of the Seas) and the IOC of UNESCO to review and discuss HAB events and to provide annual advice and updates on the state of HABs in the region. It also facilitates interaction between scientists working in diverse areas of HAB science and monitoring and provides a forum for the interchange of various approaches to HAB research. The group reports to both the ICES Science Committee (SCICOM) and IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB).

The WGHABD annual meeting was held 14-16 June at the Centre for the Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, UK. This hybrid meeting facilitated members and invited guests to attend in person, with the capacity for online remote access to discuss a variety of related topics including progress on the WG's current Terms of Reference, new activities and findings, and national reports of HAB events during 2021 in the ICES Atlantic Area region. The meeting was opened and participants welcomed by Prof Stuart Rogers, Chief Scientist, CEFAS. In addition to the review and summary of WG activities from the current three-year cycle of the nine Terms of Reference, there were a number of presentations, including: i) Distribution of *Dinophycyeae* with a focus on HAB species in the Baltic Sea and the Kattegat-Skagerrak based on meta-barcoding and microscopy (Bengt Karlson, Sweden) and ii) Update on satellite ocean colour discrimination of Harmful Algal Blooms (Peter Miller, UK)

Annual information on HAB events reported by the WG members is publicly available in the Harmful Algal Event database (HAEDAT *http://haedat.iode.org/*) and also through Harmful Algal Information System (HAIS *https://data.hais.ioc-unesco.org/*).

Author
Dave Clarke, WGHABD Chair, Marine Institute, Galway, Ireland
Email: Dave.Clarke@marine.ie



Participants in person at ICES-IOC WGHABD 2022 meeting

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Finally, because art can bridge the gap between science and society, the Portuguese visual artist Diana Policarpo made a brief overview of her work on Ciguatera, which had already been presented in the Ocean Space TBA21–Academy Exhibition in Venice (*https://www.tba21.org/#item--dianapolicarpo-2317*).

The next REDIBAL Meeting will be held in the Canary Islands. See you in the Canarias in 2024.

Acknowledgements

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Authors
Pedro Reis Costa, IPMA, Instituto Português do Mar e da Atmosfera, Lisbon, Portugal

Ana Amorim, MARE, Marine and Environmental Sciences Centre, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

Email corresponding authors:
prcosta@ipma.pt; aaferreira@fc.ul.pt

CARMINA

The **CARMINA Project** (CARibbean MIcro-algae respoNsible for ciguAtera poisoning: Diversity, Toxicity and Toxin Production) with 20 participants from 11 island nations and mainland Caribbean countries, France and the USA held its virtual kick-off meeting (Fig 1).

The countries both in and surrounding the Caribbean Sea, suffer significant impacts from ciguatera poisoning (CP). Historically, CP incidences have been higher in the northern and eastern islands of the Caribbean. There, the CP rates there are second only to those in the south Pacific Ocean. Seven species of the benthic dinoflagellate genus *Gambierdiscus* potentially responsible for CP are found in the Caribbean yet the microalgal species causing CP remain(s) unknown. The CARMINA project, co-funded by the *French Development Agency* (AFD) and the *French Research Institute for the Exploitation of the Sea* (Ifremer) aims at identifying the microalgae responsible for CP in the Caribbean region and will develop protocols for monitoring toxic species. Scientific collaborators also include the *French Food Safety Agency* (Anses) and the *US National Oceanic and Atmospheric Administration* (NOAA). Monitoring will rely on environmental screening using qPCR assays and passive sampling for dissolved toxins and biomarkers. Cell isolation and culturing will be core activities, as well as toxicity assessments and characterization of toxin profiles of *Gambierdiscus, Fukuyoa* and *Coolia* species occurring in the Caribbean Islands, Central and South America.

The partner countries of the CARMINA project were selected according to their geographic locations and involvement in existing networks of the *Intergovernmental Oceanographic Commission* (IOC) of UNESCO and the *International Atomic Energy Agency* (IAEA), two of the agencies already involved in the monitoring of the marine environment and the development of its resources.

The purpose of the kick-off meeting was multifaceted and served to bring together researchers and resource managers who have worked together in the regional networks *Algas Nocivas in Caraibe* (ANCA), the *IOC Regional Working Group on Harmful Algal Blooms in South America* (FANSA) and shared IAEA workshop sessions to increase their sampling and detection capabilities for harmful algae and their toxins.

Participants were surveyed to provide a current list of capabilities and laboratory capacities for sampling, taxonomy, cell isolation and culture (including high biomass cultures) and toxin detection methods. Lecturers provided short presentations on these topics bringing everyone up to date on new research and techniques. Subject-specific workshops will be developed to enhance current skills. In addition to the skills and capacities inventory, activity schedules with specific tasks and timelines were agreed upon. Quarterly Zoom meetings will be held to assess progress.

A Twitter account has been created: @carmina_project and an Instagram account is under consideration.

Authors

Philipp Hess, Ifremer, Director Research Unit PHYTOX, Rue de l'Île d'Yeu, 44311 Nantes Cedex 03, France

Aurelie Boisnoir, Station Ifremer des Antilles, 79 Route de Pointe Fort -97231 Le Robert French West Indies

Nicolas Chomerat, Station de Biologie Marine, Place de la Croix, F29900 Concarneau France

Pat Tester, Ocean Tester, LLC, 295 Dills Point Road, Beaufort, North Carolina 28516 USA

Email corresponding author:
philipp.hess@ifremer.fr

Fig. 1 Participants at the virtual CARMINA kick-off meeting

ISSHA's corner

Invitation to ICHA 2023 Hiroshima

Dear ISSHA members and colleagues,

Please save the date! The 20th International Conference on Harmful Algae (ICHA) will be held at the lovely Grand Prince Hotel Hiroshima, Japan from the 5th to the 10th of November 2023. The organizing committee of conference is looking forward to welcoming many in-person participants, but will have some options for virtual streaming of selected presentations. Stay tuned for more information that will be posted on the ICHA2023 Conference website (*https://icha2023.org/*) soon!

The hotel is located approximately 20 min from the downtown Hiroshima train station and about 1 hr from the Hiroshima airport. Several excursions will be available for Wednesday afternoon, including Miyajima – a world historical heritage site, a trip to historical sake breweries as well as Onomichi and Shimanami cycling and Taishaku-kyo trekking to view fall leaf colors. This is the most beautiful time of the year to visit Japan.

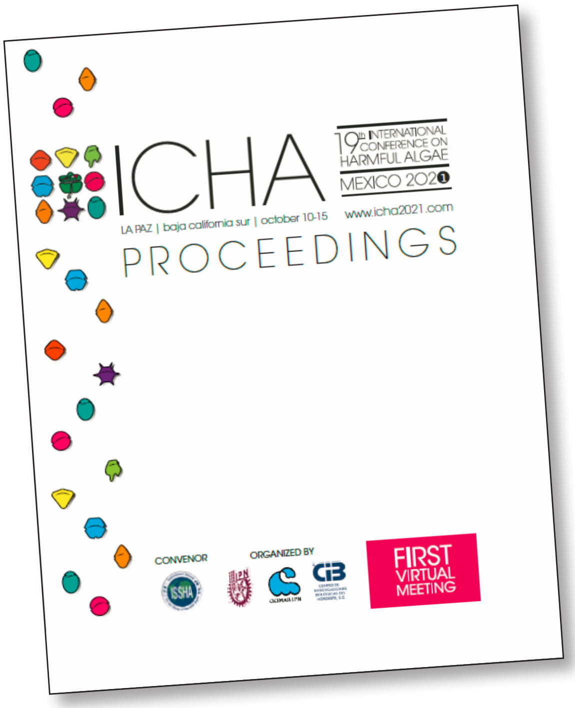
As the major host of the conference, ISSHA will support the following activities: student travel awards, achievement awards, and the popular ISSHA auction!

We are looking forward to welcoming you participants in Hiroshima. Please come and enjoy ICHA 2023.

Ichiro Imai
Chair of ICHA 2023
and members of local organizing committee


Venue at the Grand Prince Hotel, Hiroshima


Miyajima world heritage site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NEW ! The Proceedings of the 19th International Conference on Harmful Algae 2021 Mexico have been published. Each of the 47 contributions have been assigned a DOI identifier which can be found on the bottom of the first page of each manuscript. Eight case studies of Early Warning Systems for Harmful Algae are also included, based on a session, co-sponsored by FAO, IAEA and IOC, which was held at the virtual conference. Proceedings can be downloaded at this site: *https://issha.org/publications-resources/conference-proceedings/*

The documents should be referenced as follows:
Last name, first name, title, In: Band-Schmidt, C.J. and Rodríguez-Gómez, C.F. (Eds.). 2022. Proceedings of the 19th International Conference on Harmful Algae, La Paz, B.C.S., Mexico. International Society for the Study of Harmful Algal Blooms. 365 pp, DOI.

Christine Band-Schmidt and Carlos Rodríguez, editors of the proceedings, are thankful to all that contributed to this volume, and to the reviewers for their time and effort.

Contact: *cjband@yahoo.com*

Please feel free to contact any of the editors if you have article, ideas for article or special issues and we will work with you!

Deadline
Deadline to submit material for HAN 72:
February 1, 2023

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Compiled and edited by
Beatriz Reguera, Centro Oceanográfico de Vigo,
IEO (CSIC)
Subida a Radio Faro 50, 36390 Vigo, Spain
Tel: +34 986 492111
Fax: +34 986 498626
Email: *beatriz.reguera@ieo.csic.es*
and
Eileen Bresnan, Marine Scotland, Victoria Road,
Aberdeen AB1 9DB, Scotland
Tel.: +44 122 4876544
Fax: +44 1224295511
Email: *eileen.bresnan@gov.scot*

Project Coordinator
Henrik Enevoldsen, IOC Science and Communication
Centre on Harmful Algae, University of Copenhagen,
Universitetsparken 4, 2100 Copenhagen Ø, Denmark
Tel.: +45 23 26 02 46
E-mail: *h.enevoldsen@unesco.org*

Lay-out
Department of Biology, University of Copenhagen,
Denmark

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