First report on a bloom of marine dinoflagellate Pyrophacus steinii (Schiller) Wall and Dale from the South Eastern Arabian Sea

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Harmful Algae News

An IOC Newsletter on Toxic Algae and Algal Blooms

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High sea surface temperature, the potential trigger of mass mortality of fish, exceptional toxin producing HABs, and other socio-economic impacts in Uruguay

During February-March 2015 (Austral summer), sea surface temperatures up to 27.8 ºC were recorded *in situ* in Chuy, Department of Rocha, at the northern limit of the Atlantic coast of Uruguay. Values up to 28ºC were also recorded in surface coastal brackish waters from the Departments of Montevideo and Canelones, in the inner parts of Rio de la Plata estuary (10m mean depth). Satellite images of SST (Sea Surface Temperature) and of SST anomalies in relation to the 1971-2000 mean for the area (30º-40º S) [1] showed SST anomalies of +1-2ºC (Figs. 1 and 2). Anomalies were even higher in some coastal areas within Rio de la Plata estuary.

Bright green foam-producing blooms of a *Microcystis*-like cyanobacteria developed in February off Mon-

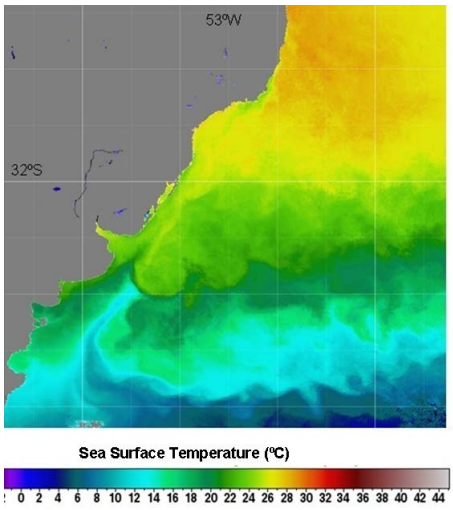

Fig. 1. Aqua MODIS image of sea surface temperature (SST) off the south western Atlantic Ocean (29°-36° S/51-56°W) shelf waters off Brazil, Uruguay and Argentina, March 2015.

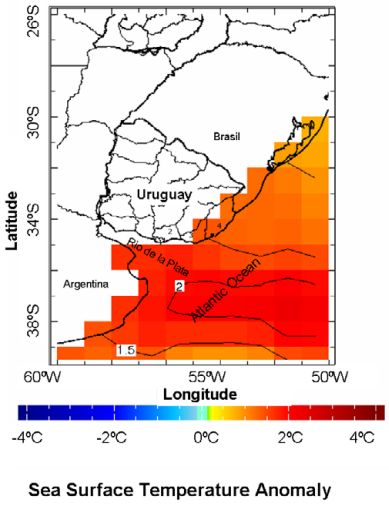

Fig. 2. Distribution of SST anomalies, in relation with the 1971-2000 mean, in Rio de la Plata estuary and shelf waters off Uruguay, March 2015. 1 = Montevideo, 2 = Canelones, 3 = Maldonado and 4 = Rocha.

Fig. 3. Bright green Cyanobacterial blooms in Montevideo coastal waters. Reddish colour is due to resuspended material from the river. (Photo from City Council of Montevideo).

Content

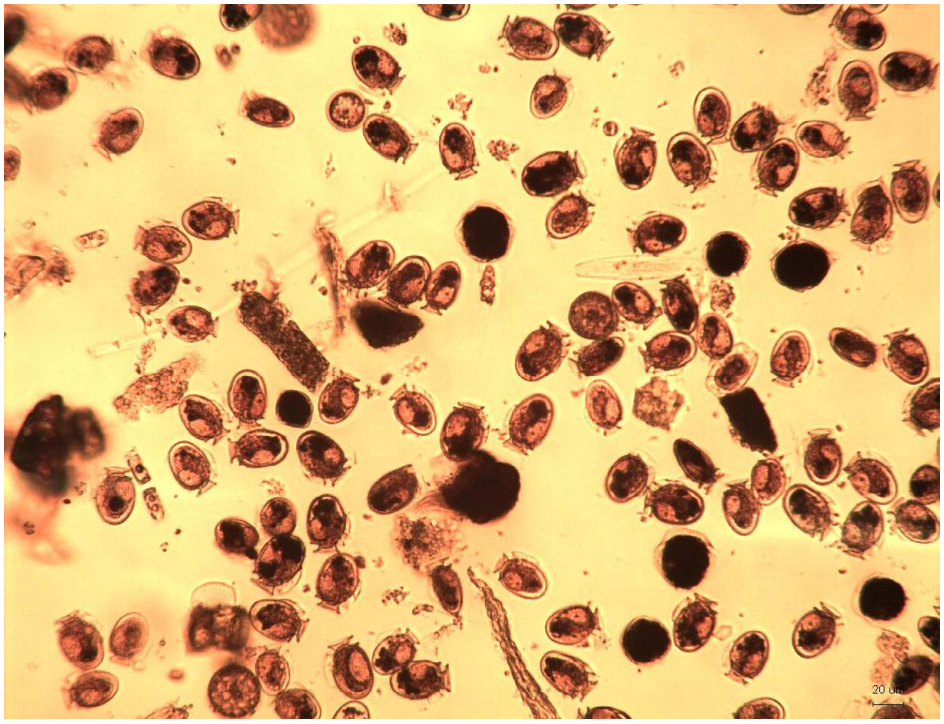

Fig. 4. Micrograph of the almost mono-specific bloom of Dinophysis *cf* ovum *in Uruguay coastal waters, March 2015. (Photo: Silvia Méndez)*

tevideo and Canelones (Fig. 3). Health authorities were prompted to display "sanitation" flags (red coloured with a green cross in the middle) to warn sunbathers about the risk of swimming there. It is worth mentioning that Montevideo beaches are permanently monitored and their water quality certified according to ISO 14001 standards by the City Council of Montevideo.

Late February and March, a massive bloom of the diarrhetic shellfish poisoning (DSP) toxin producer *Dinophysis* c*f ovum* was found in coastal areas with higher oceanic influence (Departments of Maldonado and Rocha) (Fig 4). Historical maximum densities were found in Punta del Este (83 x 10^3 cells L^{-1} on March 9) at salinity 32 and temperature 20°C and La Paloma (156 x 10^3 cells L^{-1} on March 16), with salinity 31.4 and 23°C in temperature. Shellfish harvesting was banned when DSP toxins were detected in the Departments of Maldonado and Rocha. Still, in the beginning of the bloom, about 20 cases of people, (pers.com. of local mussels vendors) were suspected to be intoxicated, suffering from gastrointestinal disorder after eating mussels. Harvesting closures, which started over 3 months ago, were still enforced at the time this article was written.

On March 5, the beaches of Montevideo and Canelones were covered by stranded dead fish of a single species locally called "Lacha" (*Brevoortia aurea*) (Fig. 5). Over 200 t of dead fish were collected by the City Council. Environmental analyses and examination of dead fish were carried out by the National Direction of Aquatic Resources (DINARA) and the University of the Republic of Uruguay.

Fig. 5. Mass mortality of fish in coastal waters of Uruguay, March 2015. (Photo: L. Carreño)

Harmful algal blooms were not detected in the affected area. *Pfiesteria shumwayae* and *Pfiesteria piscicida* are well known fish killers in United States, Australia and New Zealand. Work has been carried out to track these species with molecular tools in estuarine phytoplankton from 22 countries, including Uruguay, Argentina and southern Brazil; the two species were detected in Rio de La Plata [2]. *Pfiesteria* species are very difficult to distinguish under the microscope because of the multiple forms adopted through their life cycle. To test if these species had been the causative agents of the present fish kill, new sediment samples were taken from three different beaches (Buceo, Carrasco and Shangrilá located at Montevideo (1- Fig. 3) and Canelones (2- Fig. 3) affected by the mortality two weeks after the event and analyzed at the University of North Carolina at Greensboro, USA. Neither *P. piscicida* nor *P. shumwayae* were detected by PCR from the new DNA extractions. Salinity in Montevideo waters that week was 27, which is exceptionally high for the inner estuary waters according to previous studies in the area [3]. During spring–summer periods, conditions in the Rio de la Plata are dominated by offshore winds that deflect the river discharge plume towards the SW [3]. Low rainfall after January 2015 and the predominant NE winds for the season would have facilitated a marine intrusion deeper into the estuary [3]. The fish species affected by this episode is estuarine [4], and probably its optimum salinity space was reduced under this dry summer period. The main hypothesis is that the mass mortality was caused by a decline of dissolved oxygen levels associated with the positive temperature anomaly when fish shoals, pushed to inner parts of the estuary, were very dense.

The events described above were independent and with different locations

Continued on page 9

First report of *Alexandrium globosum* on the Pacific coast of Central America

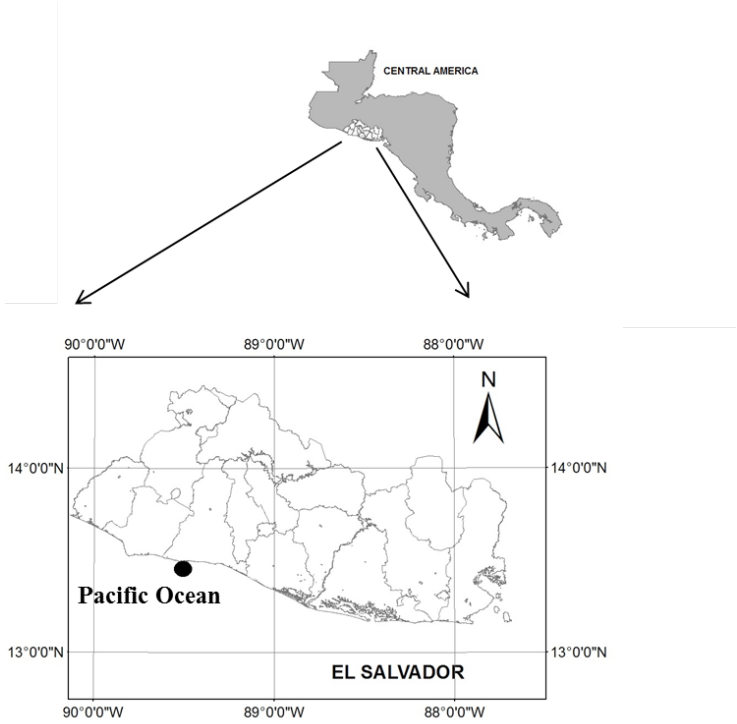

Fig. 1. Sampling area at department of La Libertad, El Salvador.

Harmful algal blooms of the dinoflagellate *Alexandrium* can have considerable socio-economic impacts. Amongst the 30 species described for this genus, more than half are known to be toxic. *Alexandrium* species are unique on their capacity to produce at least three different kinds of toxins: saxitoxins, spirolides and goniodomines [1]. Little is known about the impact of *Alexandrium* in Central America.

Between August and September 2014, an intense bloom of *Cochlodinium* cf *polykrikoides* developed which lasted 21 days and mainly affected the central and western coasts of El Salvador (Eastern Pacific). The observation of reddish-brown patches was associated with fish kills along the rocky shores of La Libertad. In addition, putrid smells from decaying algae affected the tourist industry. Cells of an unidentified *Alexandrium* species co-occurred with this bloom.

Plankton net (20-µm mesh) hauls and 10-L Niskin bottles samples were collected on 2 September 2014 off Puerto de La Libertad (13°29'11.6'' N; 89°19'12.6'' W), fixed with Lugol's solution and taken to LABTOX-UES for analyses. Samples were stained with Calcofluor, observed under the epifluorescence microscope (Zeiss Axio Imager) and cellular density estimated from Sedgewick Rafter chamber counts with an inverted microscope (Zeiss 40CF). *Alexandrium* spp. reached densities over 49 x 10^3 cells L^{-1}.

The genus *Alexandrium* is characterized by the Kofoidean plate tabulation formula: Po, 4', 6'', 6c, 10s, 5''', 2''''. Some important features for morphological classification of this genus are cell shape, size, chain-forming capacity, thecal ornamentation and shape of the cingulum, sulcus, cingular displacement and shape of the apical pore complex (APC), plates 1', 6' and some sulcal plates [2]. Plate tabulation of the cells in this work was Po, 4', 6'', 5''', 1'''', 1sp. The epithecal plate 1' was wide and asymmetrical with no direct connection with the APC (Fig. 2a); a characteristic ventral pore between plates 1' and 4' was present, plate 6' was rectangular and no connecting pores were observed either on the epitheca or the hypotheca (Fig. 2b). Morphology of the *Alexandrium* specimens corresponded to that of *Alexandrium globosum* as described by Larsen & Nguyen [3].

Fish kills were attributed to *Cochlodinium* cf *polykrikoides*. Nevertheless, we cannot rule out that *Alexandrium* cf. *globosum* contributed to the fish kills, because other species of *Alexandrium* have been described as producers of PSP and/or hemolytic toxins devastating fish farms [1].

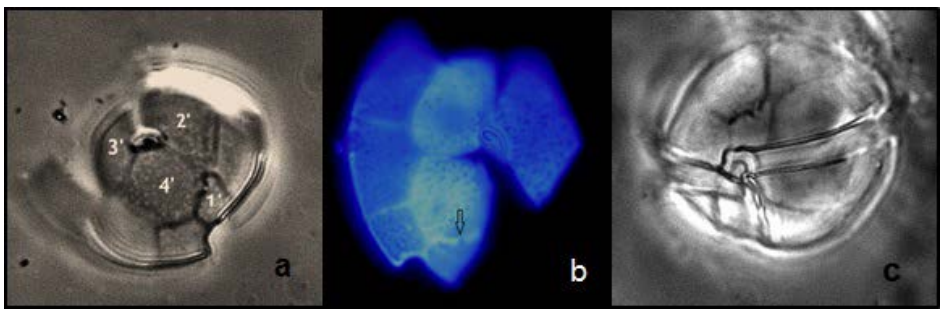

Fig. 2. Micrographs of Alexandrium globosum *a) Epitheca and tabulation of the apical plates; b) Stained cells showing the ventral pore; c) Ventral view. Samples from La Libertad, El Salvador, 2 September 2014.*

Acknowledgements

We thank Jacob Larsen for confirmation of the species identity and CENDEPESCA (La Libertad) for sampling help. This work was supported by the International Atomic Energy Agency (IAEA) projects RLA/7020 and ELS/7007.

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The Bay of Brest (France), a new risky site for toxic *Alexandrium minutum* blooms and PSP shellfish contamination

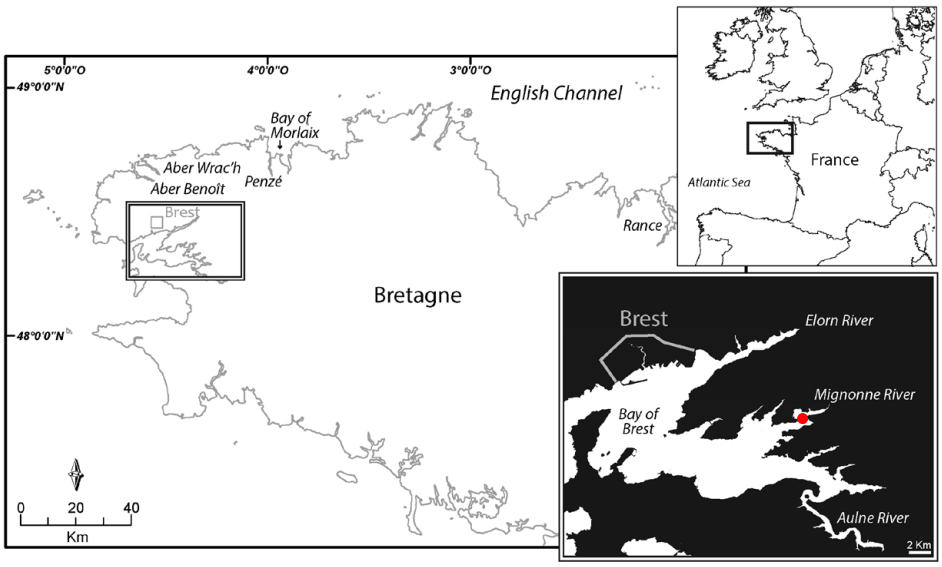

Fig. 1. Map with location of the Bay of Brest and other Breton estuaries where A. minutum *proliferates (● by the Mignonne estuary represents location of the sampling site)*

In summer 2012, a new toxic event occurred in the Bay of Brest (Fig. 1): a major PSP contamination event led to the prohibition of shellfish harvesting. It was the first time shellfish from the Bay of Brest were contaminated with these toxins. The maximal toxin level recorded in shellfish (over 8000 eq STX·kg^{-1}) was 10 times the sanitary threshold and *A. minutum* bloomed up to 41 x10^6 cells L^{-1}. This first PSP event was followed by additional *A. minutum* blooms leading to shellfish toxicities in 2013 and 2014.

A three-year survey of the Bay of Brest (Daoulex project, 2012-2014) has been carried out with water sampling twice a week in the Mignonne estuary around high tide as soon as water temperature exceeded 15°C. Data issued from this survey, associated with those from the phytoplankton and biotoxin monitoring network REPHY [1] (since 1984) and the Oyster network VELYGER [2] (since 2008) have shown that *A. minutum* has been observed in the Bay of Brest since 1990 only. This confirms results of a 1990 sediment survey showing the absence of *A. minutum* cysts in this bay [3]. From 1990 to 2008, *A. minutum* occurred at low densities (lower than the alert threshold of 104 cells L^{-1}). Conversely, since 2009, *A. minutum* has bloomed recurrently, surpassing this threshold and leading to PSP toxicities since 2012 (Fig. 2).

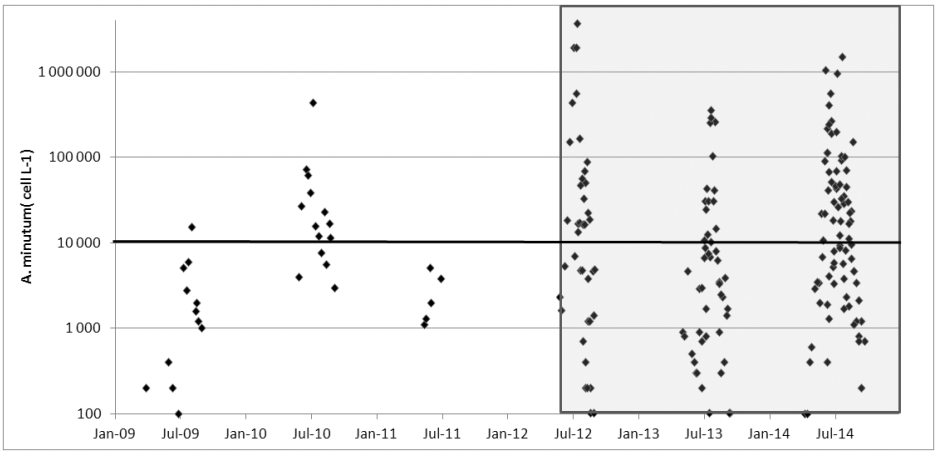

Fig. 2. Alexandrium minutum *abundances (data REPHY, VELYGER, DAOULEX) in the Bay of Brest. Grey box delimits the toxicity period. Black line stands for the alert threshold (10^4 cell L^{-1}).*

The unsafe bloom period (concentrations higher than the alert threshold) extended from mid-May to the end of August. In 2012 *A. minutum* developed from June 15 to August 16 with a maximum density of 41 x10^6 cell L^{-1} detected on July 15. In 2013, the bloom period was shorter (from July 5 to August 5) and with lower densities (maximum 3.6 x10^5 cell L^{-1} on July 22). In 2014, *A. minutum* developed during a longer period from May 26 to September 4 and peaked up to 1.1 x10^6 cell L^{-1} on June 6 and again to 1.5 x10^6 cell L^{-1} on July 22.

Surface water temperature seemed to be the major abiotic signal correlated to increased cell concentration above threshold limits and therefore to the risk period for shellfish contamination (Fig. 3). The temperature of 15°C seems to be a pre-requisite to allow bloom development above the threshold in the Bay of Brest as well as in other Breton estuaries [4]. In fact, 2014 showed initiation of an early *A. minutum* bloom in mid May when the water temperature reached 15°C. In contrast, 2013 showed a cold spring with water temperatures below 15°C until June 1 and the bloom this year initiated only in July. 2012 was an intermediate year for water temperature as well as for bloom initiation, with temperature of 15°C reached by May 24 (Fig. 3).

The main areas impacted by *A. minutum* are the upper estuaries of the Bay of Brest (Mignonne and other estuaries of the south-eastern Brest Bay, the Aulne and Elorn river outflows, Fig. 1). These areas correspond to shallow, sheltered estuaries, strongly influenced by tides. Along the Mignonne estuary, *A. minutum* maximum densities were observed with salinity values ranging from 29 to 34. These are transition waters between nutrient rich Mignonne waters (mean nitrate concentrations of 27 µg L^{-1}, mean phosphates 0.07 µg L^{-1}) and the Bay of Brest waters, which are phosphorus (P) limited for phytoplankton growth during spring and summer [5]. Nutrient concentrations in this estuary are closely linked to river Mignonne run-off and may impact *A. minutum* blooms, notably through phosphorus availability. In 2012, high spring and summer high river discharges (Fig. 3)

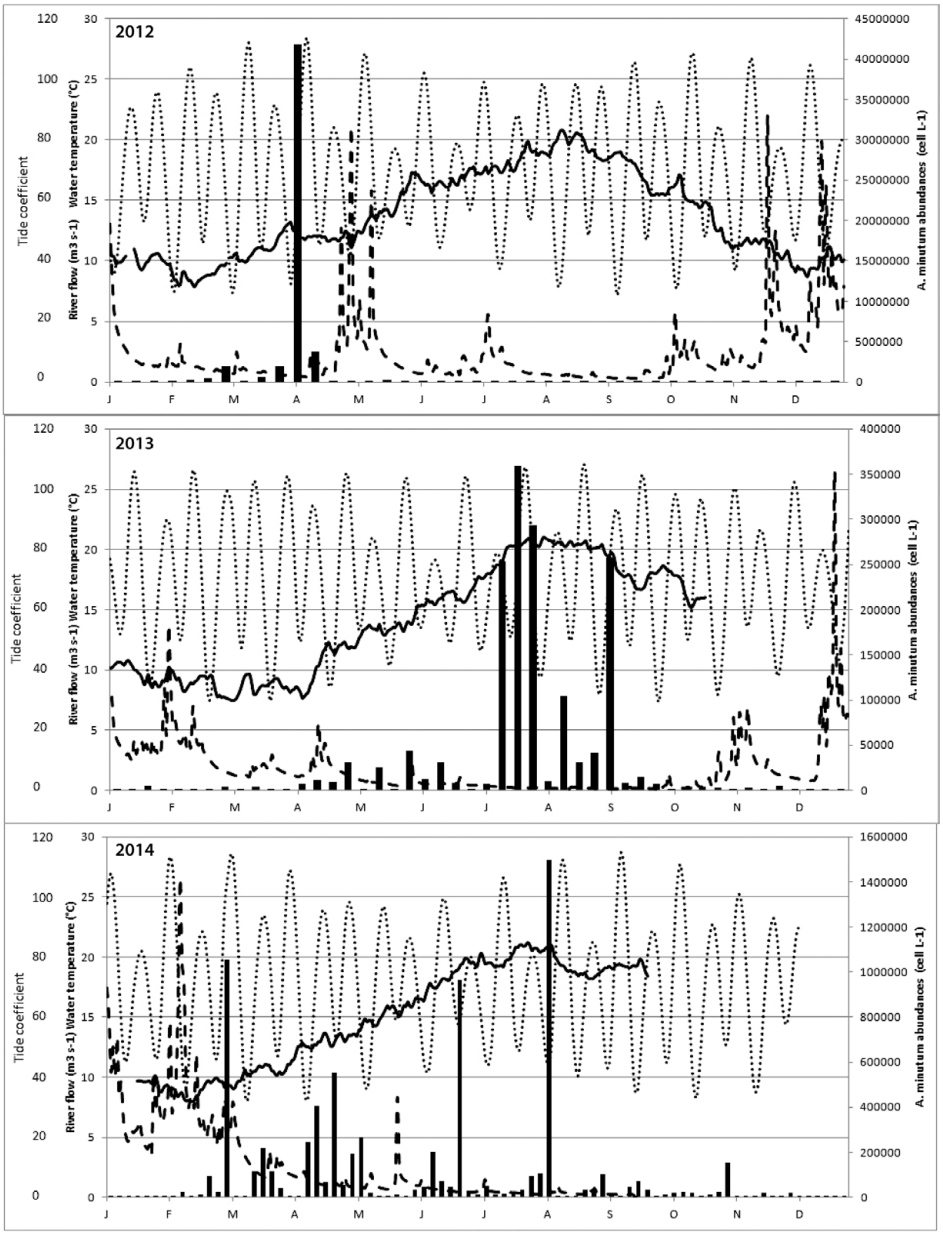

Fig. 3. Alexandrium minutum *densities (vertical bars, right axis), water temperature measurements (black line, left axis), river outflow (dashed line), and tidal coefficient (dotted line).*

may have been linked to the exceptional *A. minutum* bloom (>41 x10^6 cells L^{-1}). In 2013, river outflow was much lower, whereas 2014 presented intermediate river outflow in correlation with intermediate (between those of 2013 and 2012) densities of *A. minutum*.

Another environmental factor that seems to affect *A. minutum* maxima are tides. In fact, maximal abundances are observed when tides coefficients are lower than 80 (neap tides or little spring tides) (Fig. 3). This could be explained by lower bloom dispersion out of the estuary by lower tidal currents, allowing *A. minutum* to stay in its optimal (upper estuary) environmental condition for development.

In conclusion, the Bay of Brest represents today a new risky area for *A. minutum* blooms and occurrence of PSP toxins. These bloom events present similar temporal and geographical patterns as others that have already occurred in estuarine ecosystems of northern Brittany (Penzé, Morlaix, Rance, Aber Benoit and Aber Wrac'h estuaries, Fig.1) [4, 6, 7] as well as in Ireland [8]. They appear in rich-nutrient confined ecosystems, mostly estuaries, from mid-May to August, when nutrients, water temperature and irradiance support sufficient *A. minutum* growth to compensate mortality rates mostly due to tidal dilution.

Acknowledgements

The authors gratefully acknowledge the Region Bretagne which supported the Daoulex project for the in situ survey and the REPHY and VELYGER networks.

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Harmful Algae Blooms in Banderas Bay, Jalisco, México, 2013

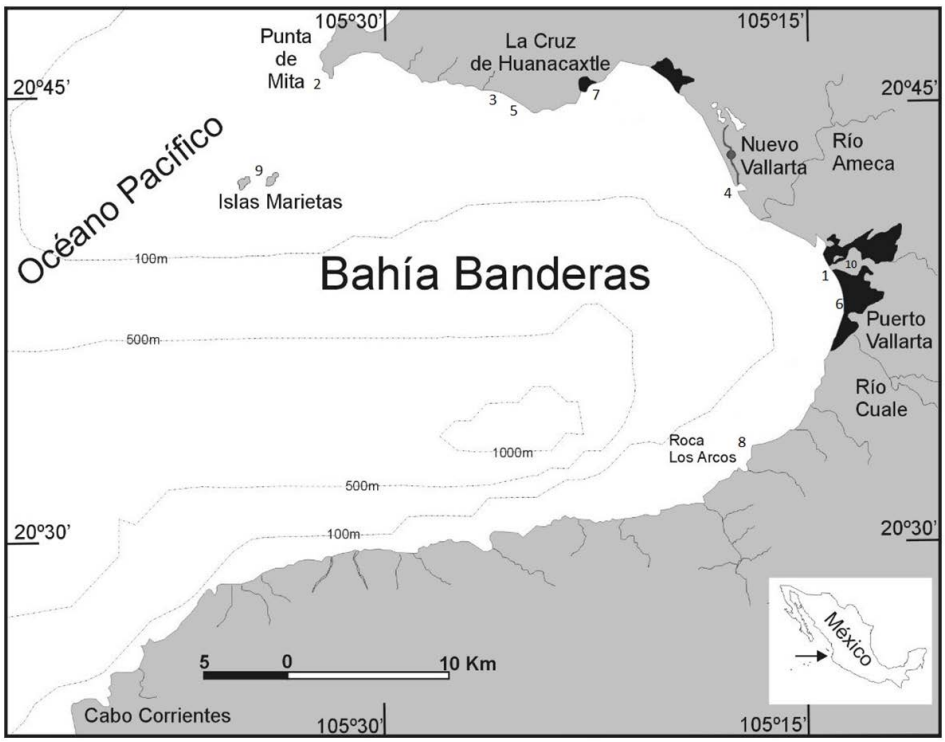

Fig. 1. Study area and sampling stations in Banderas Bay (May to November 2013).

Over the past decade Banderas Bay has been affected by the occurrence of 24 different toxic microalgal species responsible of Harmful Algal Blooms (HABs) [1]. Some of them have caused serious negative impacts to marine life, fisheries and the tourist industry in this locality where mollusks are a very important resource. Here we report data from the routine phytoplankton monitoring programme, carried out by the Centro Universitario de la Costa- Universidad de Guadalajara, during 2013. The main purpose of this HAB program is to monitor the presence and abundance of harmful algal species in Banderas Bay and surrounding areas to provide an early warning of HAB events and anticipate their adverse effect in human health and the environment.

Banderas Bay, the biggest bay in the Mexican Pacific, is on the western Mexican coast (20° 15´ - 20° 47´ N; 105° 15´ - 105° 4´ W) [4]. It has an area of ~ 975 km^2, and is limited by Punta de Mita in the north and Cabo Corrientes on the south (~40 km) (Fig. 1). Mean sea surface temperature (SST) is 26.4°C, with a seasonal range from 23.3°C during winter-spring time, to 30.0°C in summer-autumn. Located in a transitional area, the bay shows a complex hydrodynamics, with internal waves, upwelling pulses and large seasonal and interannual fluctuations. Three current systems converge there: (1) the California Current, which flows southwards bringing cool and low salinity water to the region; (2) the Costa Rica Costal Current, which flows northwards transporting warm waters of intermediate salinity, and (3) the warm dense waters from the Gulf of California, which are transported southwards into Banderas Bay [3].

Sampling areas were established, along the coast of Banderas Bay, in the inner harbor, Los Arcos, Playa Camarones, Estero El Salado, Islas Marietas, La Cruz de Huanacaxtle, Punta de Mita, Nuevo Vallarta, Destiladeras and Veneros. Surface phytoplankton samples from blooming areas were collected in March, April, May, October and November 2013 in 500 mL bottles, preserved with Lugol's solution and counted, with 100x and 400x magnification, using a 1 ml Sedgewick Rafter chamber. Temperature and salinity were measured in situ every 25 minutes using a HOBO (Pendant) data logger thermograph and a salinometer. Micrographs of the main HAB species were obtained using a digital camera.

Our results identified 10 sites where blooms were frequent. Four events causing visible seawater reddish discoloration, three during winter-spring and one during summer-autumn, were directly observed and monitored. The species involved in this outbreaks, such as the toxic dinoflagellate *Gymnodinium catenatum* (potential PSP agent), the ciliate *Mesodinium rubrum*, and the ichthytoxic dinoflagellate *Cochlodinium catenatum* (= *C. polykrikoides*), frequently associated with fish kills in Banderas Bay, are known to form mass occurrences elsewhere [4].

Gymnodinium catenatum showed maximal densities of 0.6 x10^6 cells L^{-1}, and PSP toxins exceeded the limits

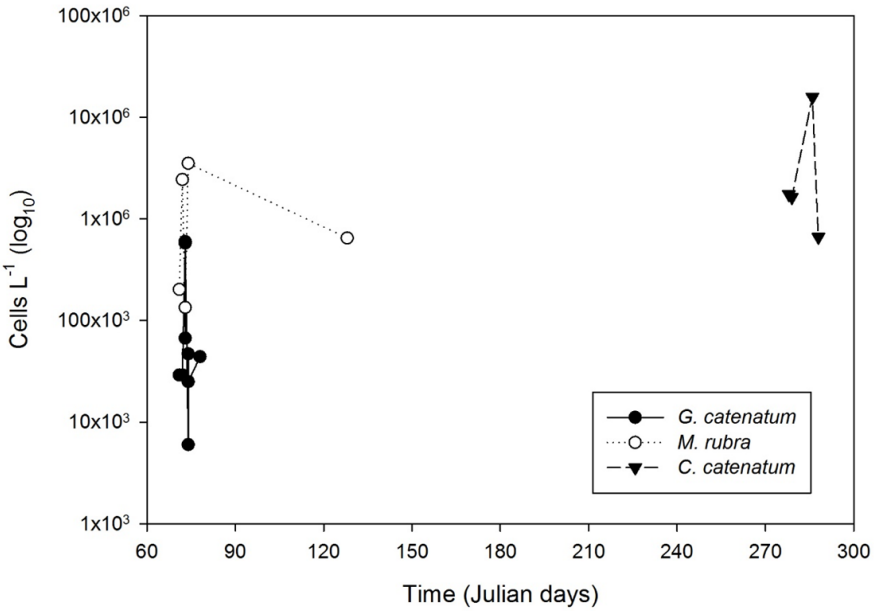

Fig. 2. Concentration of Gymnodinium catenatum, Mesodinium rubrum *and* Cochlodinium catenatum *(cells L^{-1}) in Banderas Bay Jalisco México, from March to November of 2013.*

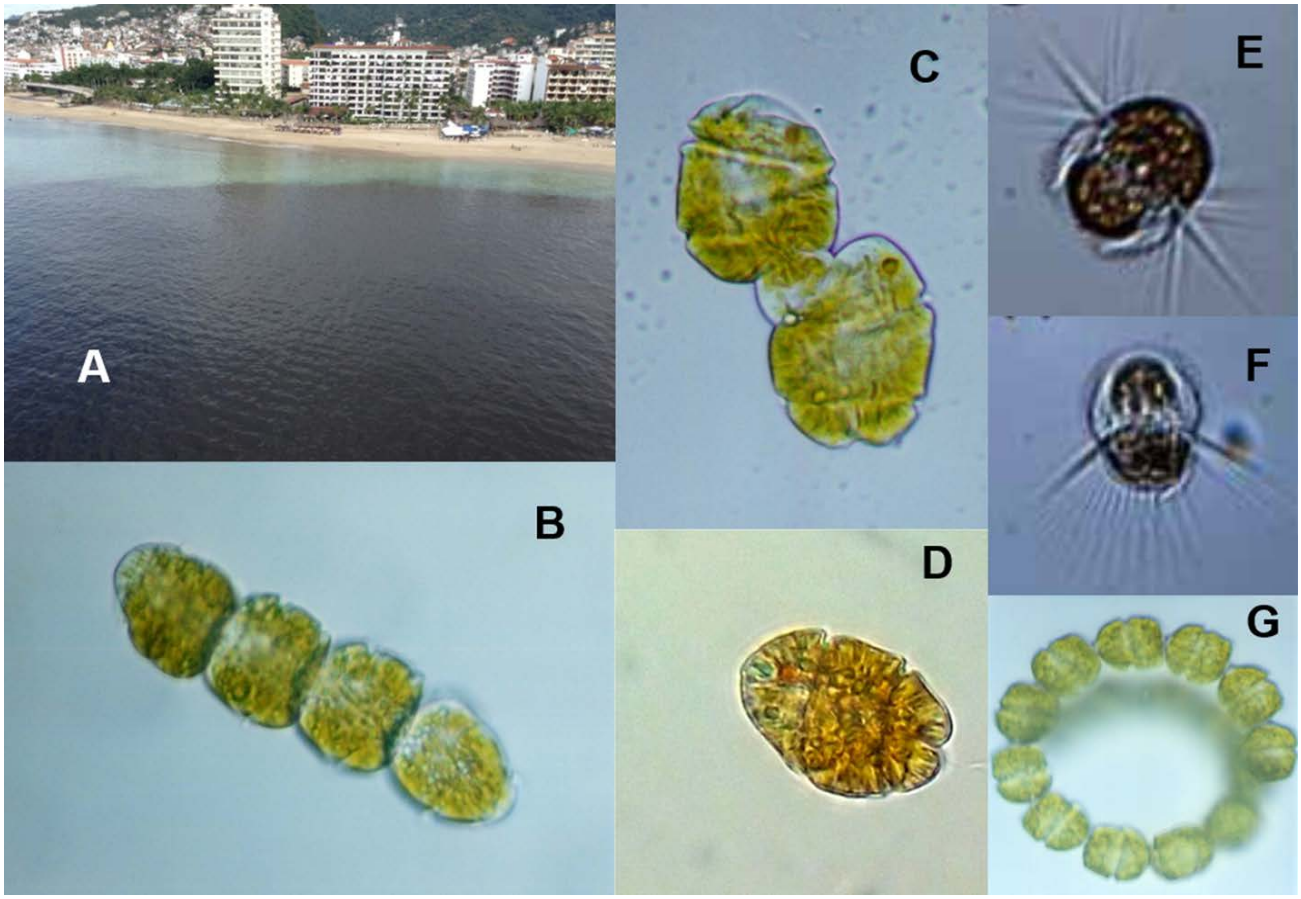

Fig. 3. Harmful microalgal species in Banderas Bay during 2013. A. Discolorations from Cochlodinium catenatum *bloom in November 14, 2013. B-C* Cochlodinium catenatum *(4 and 2 cells). E-F* Mesodinium rubrum*. G.* Gymnodinium catenatum.

established for human consumption. In consequence, COFEPRIS authority, through the formal notification advice COS/DEPE/2/OR/00066/2013 resolved to ban extraction, commercialization and consumption of bivalve mollusks coming from harvesting zones in Banderas Bay. This decision caused a hard impact on local people who make their living from these activities. Likewise, densities of *Cochlodinium catenatum*, which usually occurs after heavy rain, showed record values of 16 x10^6 cells L^{-1}.

Mean sea surface temperature records from the HOBO logger during the bloom in May was 23-24°C, whereas mean values for October-November were 29.12 to 30.03°C. Salinity values (33-35 psu) were within the data range found by other authors in this coastal area. Nevertheless, 2013 was an anomalous year concerning HABs intensity, in particular for the dinoflagellate *Cochlodinium catenatum* which exhibited very high concentrations (up to 15 x10^6 cells L^{-1}) in the coastal zone of Jalisco state throughout the year. There were no reports of affected consumers either within tourists or local residents.

Our results point out the impact of HAB species in Banderas Bay and highlights the importance of its HAB monitoring programme. This programme allows knowing the temporal and spatial distribution of blooming harmful species, providing early warning to decision-makers, such as COFEPRIS authorities, to protect public health, the tourist industry and the exploitation of marine resources.

Table 1. Main blooms reported during the study period.

Sampling date (2013)	Species	Density (cells L^{-1})	Permanence
March 17 - April	*Gymnodinium catenatum* *Mesodinium rubrum*	0.6 x 10^6 2.4-3.5 x 10^6	~20 days
May 8	*Mesodinium rubrum*	0.6 x 10^6	~7 days
October 14 -19	*Cochlodinium catenatum*	1.6 - 1.7 x 10^6	~10 days
November 4-14	*Cochlodinium catenatum*	1.6 x 10^6	~7 days

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First report of a bloom of *Pyrophacus steinii* from the South Eastern Arabian Sea

Pyrophacus Stein is the only genus of the family Pyrophacaceae Lindemann and has three species with very similar cell structure: *Pyrophacus horologium* Stein, *P. steinii* (J. Schiller) Wall and Dale and *P. vancampoae* (Rossignol) Wall and Dale. *P. steinii* cells are round, lens-shaped with an anteroposteriorly compressed theca. *Pyrophacus* species are distributed worldwide and are frequently observed in oceanic, neritic and estuarine waters. However, there is no record on their bloom formation and associated harmful effects, if any, on marine ecosystems in the World Ocean.

Phytoplankton production in the Arabian Sea (West coast of Indian EEZ), usually dominated by diatoms, is influenced by changes in physico-chemical and biological characteristics of the area determined by the two (SW and NE) monsoon cycles. Reports available on HAB events on the Arabian Sea are mainly on the occurrence of *Noctiluca scintillans* [1-3], *Gonyaulax* sp. [4], *Chattonella marina* [5-6] and *Trichodesmium erythraeum* [7-8] blooms. Here we report the first bloom of *Pyrophacus steinii* in Arabian Sea.

P. steinii occurs in very low numbers in Indian waters throughout the year.

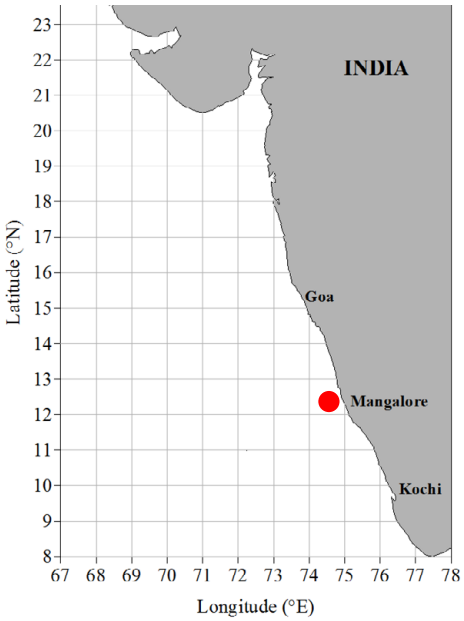

Fig. 1. Study area (red dot represents the bloom location).

A subsurface bloom of this species was observed off Mangalore (12°50.754N; 74°40.354E, Fig. 1) onboard the research vessel *Sagar Sampada* during the wane phase of summer monsoon (end of September, 2009). Hydrographic parameters (Seabird 911 plus CTD) and water samples for phytoplankton, chl *a* and nutrient analysis were collected from standard depths using Niskin bottles attached to a CTD-rosette. For phytoplankton, 30 L were filtered through a 20-µm mesh bolting silk and the samples immediately fixed in buffered formaldehyde-Lugol's iodine solution. Qualitative and quantitative analysis of phytoplankton were carried out using a Sedgwick Rafter counting chamber (Nikon Eclipse microscope) following standard identification keys; Chl *a* was determined by the standard acetone extraction method and nutrients (nitrate, phosphate, silicate) analysed using a segmented flow Auto Analyzer (SKALAR) onboard the vessel following standard procedures.

The bloom was without any noticeable surface water discolouration because the sea surface was slightly rough and the cell maximum (7.75x 10^5 cells L^{-1}) was at base of the pycnocline (10m) (Fig. 3). Almost 94% of the micro-phytoplankton population was composed of *P. steinii*, accompanied by *Chaetoceros* spp., *Thalassiosira* spp., *Rhizosolenia* spp., *Pseudo-nitzschia* sp. and *Ceratium* spp. The e round lens-shaped cells had an anteroposteriorly compressed theca with numerous golden brown chloroplasts. Dividing cysts and planozygotes were also found (Fig. 2). Vertical distribution of physical-chemical properties in the area, known to be well influenced by freshwater influx from the adjoining Nethravathi-Gurupur estuary [9], is shown in Fig. 3.

Intense upwelling during the summer monsoon brings up nutrients along the coastal waters of the South Eastern Arabian Sea through Ekman's transport [10] and high levels of nutrients may be found at subsurface layers [11]. Increased nutrient input and stability as a result of intense upwelling and more brackish waters at the surface may have favored *P. steinii* bloom formation. Mortality of fish and other marine organisms were not observed or reported from the bloom area.

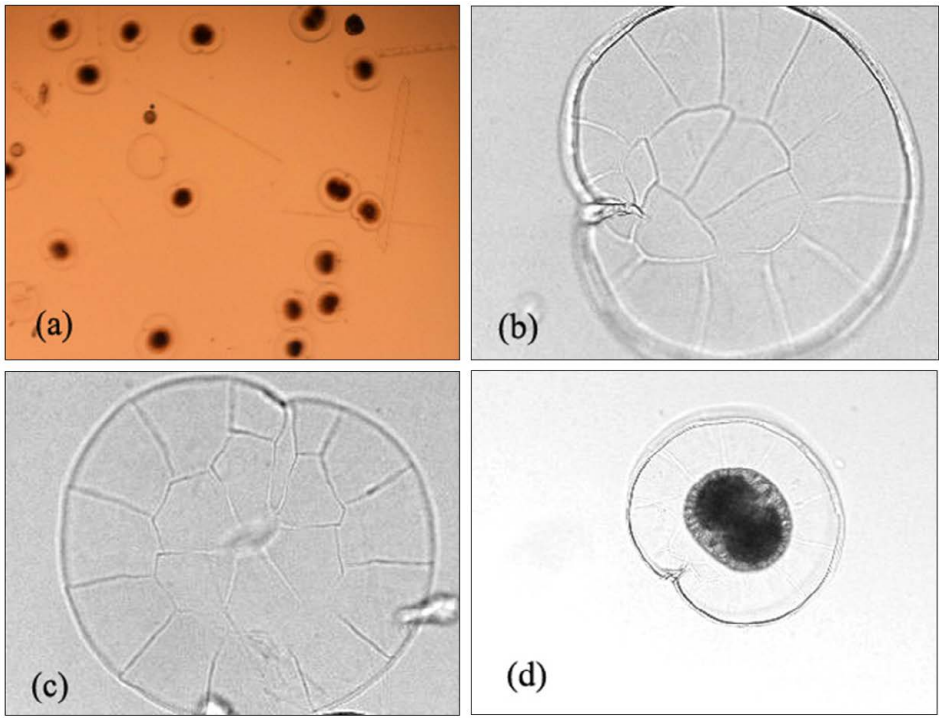

Fig. 2. Micrographs (a) Pyrophacus steinii *bloom (x40);* P. steinii *hypotheca (b) and epitheca (c) (x 400); (d) Binary fission (x 200).*

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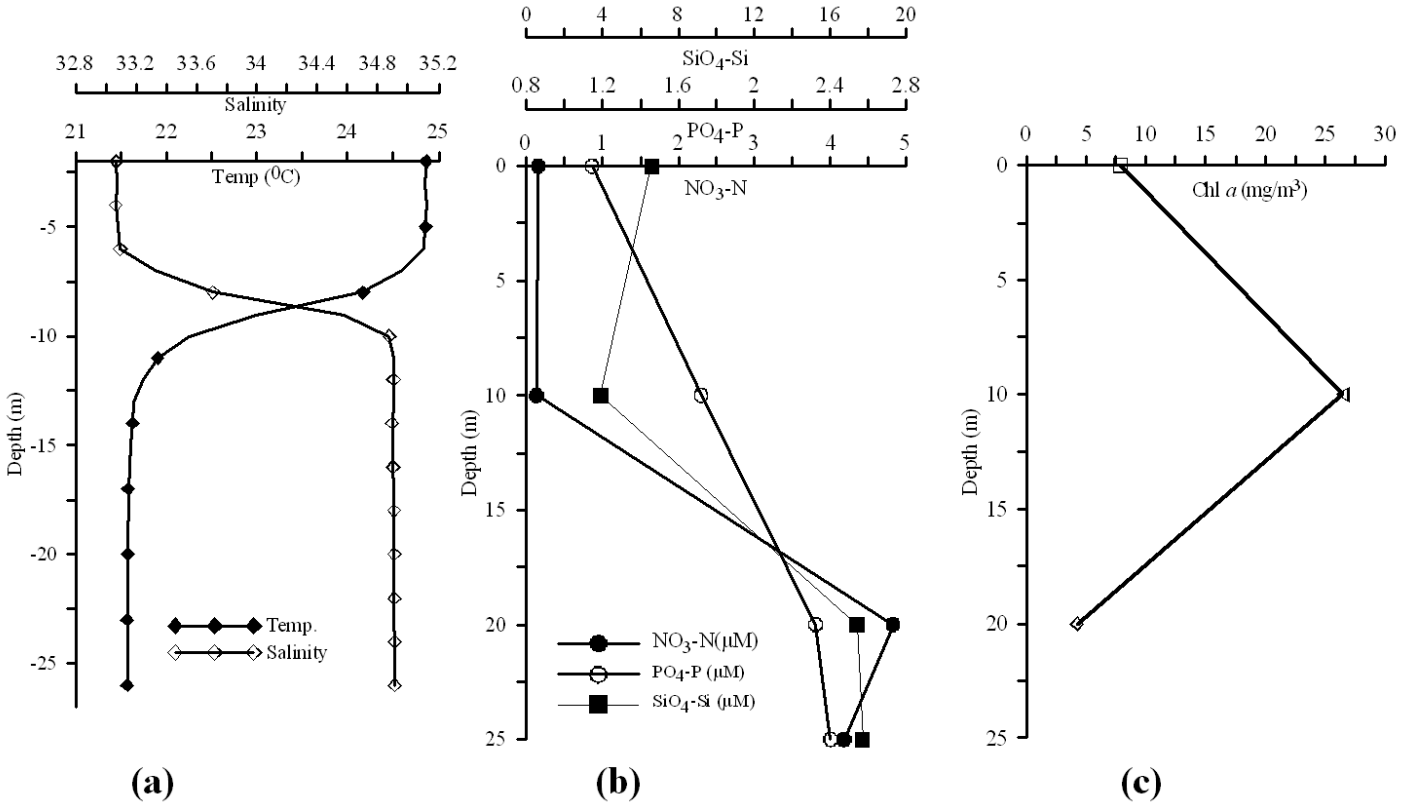

Fig. 3. Vertical distribution of physico-chemical and biological characteristics at the bloom area (a) Temperature and salinity (b) Nitrate, phosphate and silicate (c) Chlorophyll a

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Continued from page 2

and timing. Nevertheless, it is possible they were all triggered by the same environmental anomaly: the anomalous high temperatures observed in coastal waters of Uruguay during Austral summer 2015. Global warming may lead to increased frequency of this kind of events.

Acknowledgements

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French Polynesia Regional Workshop on Ciguatera Fish Poisoning field monitoring for Asia-Pacific countries. A joint IAEA, ILM, NOAA and IOC-UNESCO effort

Ciguatera Fish Poisoning (CFP) is now regarded as a potential threat on a global scale, due to the rapid development of travel, tourism, and fish trade from the tropics, as well as the expansion of the bio-geographical range of *Gambierdiscus* species and toxic fish.

Considering the major impacts of CFP on the health and economy of many countries of the Asia-Pacific region, and the need for capacity building in CFP field monitoring, the International Atomic Energy Agency (IAEA) Technical Cooperation Department organized a regional workshop at the Institut Louis Malardé (ILM), Tahiti (French Polynesia) from March 2-13, 2015 within the framework of the regional project RAS 7/026 project on "Supporting the use of Receptor binding Assay (RBA) to reduce the adverse impacts of Harmful Algae on seafood safety". This event was attended by 17 participants from China, Indonesia, Malaysia, Marshall Islands, Oman, Pakistan, Philippines, Thailand, Viet Nam, and Wallis & Futuna (sponsored by IOC-UNESCO) (Fig. 1).

The training team for this workshop included Jacob Larsen (IOC Science and Communication Centre on Harmful Algae), Patricia Tester (US NOAA-affiliate), Mireille Chinain, Taiana Darius, Clémence Gatti, André Ung (Institut Louis Malardé), Mélanie Roué (Institut de Recherche pour le Développement) and Gires Usup (University of Kebangsaan-Malaysia). The programme comprised a series of lectures coupled with field trips, hands-on training and demonstrations on the following topics: sampling techniques of benthic habitats including the use of passive monitoring devices (window screens, SPATT filters) (Fig. 2), identification of benthic HAB species using light microscopy techniques (Fig. 3), molecular approaches in dinoflagellate taxonomy and detection, isolation of benthic HAB species for culturing purposes, toxin extraction from different types of biological samples (Fig. 4) and toxicity analysis using the radioligand Receptor Binding Assay (RBA).

The training sessions were followed by a five days Project workshop led by IAEA officials Abdulghani Shakhashiro, project management officer (Technical Cooperation program, Vienna) and Marie-Yasmine Bottein, technical officer and research scientist (Environment Laboratories, Monaco), to update the work plan of the IAEA RAS 7/026 project to include new member state participants, to develop a plan for compilation and sharing of HAB event data and HAB species distribution data, and to plan in-detail field sampling of algae and fish and analysis of samples using the RBA. Frances Van Dolah (US NOAA) provided advice and guidance on the RBA work, and Patricia Tester and Mireille Chinain on field sampling of algae and fish. Henrik Enevoldsen (IOC-UNESCO) stressed the importance of HAB data compilation in HAEDAT and OBIS, and Clémence Gatti of implementing an accurate epidemiological survey of CFP towards the development of a Global HAB Status Report. This regional project and similar technical cooperation projects in the Caribbean and Africa using nuclear technics for a sustainable development of IAEA member states will lead to the provision of HAB data, basis for a first ever Global HAB Status Report.

Fig. 1. Group photo during the Tahiti island tour.

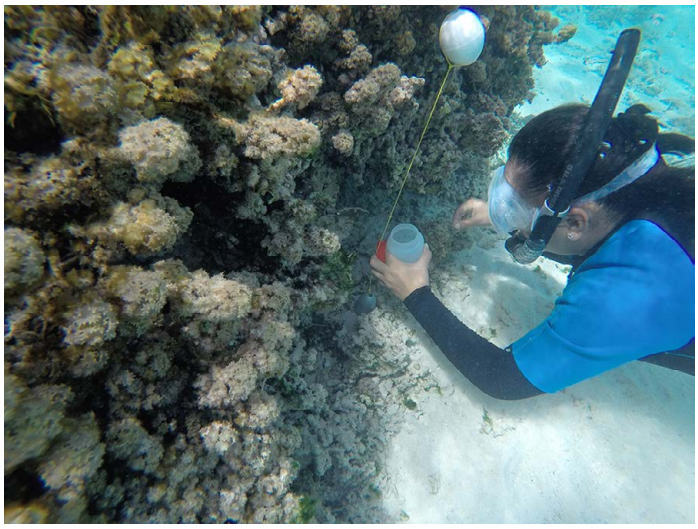

Fig. 2. Collection of window screen devices deployed in situ.

Fig. 3. Microscopy identification of benthic HAB species under the supervision of Jacob Larsen.

Fig. 4. Toxin extraction training.

Fig. 5. Strong coverage of this regional event by local media.

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HABs and Climate Change: A Report of Two Workshops

Two recent workshops have focused on providing a more rigorous assessment of purported links between anticipated climate-driven changes and HABs. The first was a 5-day international workshop held in Spring 2013 at the University of Washington Friday Harbor Labs in Washington State, USA, co-organized by Mark Wells, from the North Pacific Marine Science Organization Section on Harmful Algal Blooms (PICES HAB-S) and Bengt Karlson representing the International Council for the Exploration of the Sea / Intergovernmental Oceanographic Commission Working Group on HAB Dynamics (ICES/IOC-WGHABD) and jointly sponsored by PICES, US National Oceanic and Atmospheric Administration (NOAA), and Scientific Committee on Oceanic Research/ Global Ecology and Oceanography of HABs (SCOR/GEOHAB). A focused group of internationally-recognized HAB scientists having diverse expertise that bears strongly on climate change/HAB linkages reviewed what is known and unknown about HAB/Climate linkages (summarized in the PICES Press article "Harmful Algal Blooms in a Changing World", Volume 21, Number 2, July 2013). A seminal review paper identifying the keystone parameters and research infrastructure needed to test these purported linkages is accepted pending revisions and will be published in the journal *Harmful Algae* in summer 2015. This paper titled, H*armful algal blooms and climate change: what do we know and where do we go from here?* is authored by Mark L. Wells, Vera L. Trainer, Theodore J. Smayda, Bengt Karlson, Charles G. Trick, Raphael M. Kudela, Akira Ishikawa, Stewart Bernard, Angela Wulff, Donald M. Anderson, and William P. Cochlan.

The 2nd stage of assessment of an exploration of the links between climate change and HABs was a broader International Scientific Symposium on HABs and Climate Change that was held from May 19-22, 2015 in Gothenburg, Sweden, jointly sponsored by ICES, PICES, the Swedish Research Council (FORMAS), SCOR/GEOHAB, the Swedish Meteorological and Hydrological Institute, and the University of Gothenburg, and endorsed by IOC and ICES. This symposium brought together 58 participants from across the globe to discuss how to approach the formidable challenge ahead of ultimately providing a "market forecast" for changes due to climate impacts on HABs. The symposium participants considered what was known about the influences of environmental changes (e.g. nutrient fluxes, ocean acidification, temperature, stratification) on the character and magnitude of primary producers (including HABs) that may result in large-scale changes in HABs. The most likely of these included latitudinal range expansion of warm water HAB species at expense of cold-water species, and changes in seasonal windows of HAB outbreaks. However, substantial knowledge gaps on these and other potential effects prevent any clear assessment at present. Participants also worked to identify the key steps moving forward to improve understanding of HAB effects on fisheries and to develop forecasting abilities of HABs and other disruptive plankton systems. The symposium findings will

The Friday Harbor workshop group: (L to R), Stewart Bernard, Donald Anderson, Vera Trainer, Angela Wulff, Charles Trick, Bengt Karlson, Ted Smayda, Raphe Kudela, Mark Wells, Akira Ishikawa, William Cochlan.

Participants of the HABs and Climate Change conference in Göteborg, Sweden from May 19-22, 2015

be published as a PICES Press article, in a Special Report in the journal *Harmful Algae*, and a short synthesis document in a high-profile journal is being pursued.

There are key knowledge gaps in the underpinning of the intrinsic resilience and vulnerability of marine ecosystems to natural and anthropogenic forcing, how ecosystems of today in the future will respond to natural and anthropogenic forcing, and ultimately how societies will be affected by these altered ecosystems. It is reasonable to argue that the most uncertain of these gaps is constraining how planktonic systems shift from strongly sustaining to disruptive modes. To this end, several international organizations (see below) are continuing their efforts to coordinate observations and trend analysis of plankton data, and link these to climate change science, and to contribute to the newly developing United Nations Educational, Scientific, and Cultural Organization of IOC (UNESCO-IOC) Global HAB Status Report. The Scientific Steering Committee of the newly formed GlobalHAB, the replacement for GEOHAB, GlobalHAB, is being formed, It will include expertise on freshwater HABs, benthic HABs, satellite, observation systems, ecology, oceanography, toxins, human health links, economic links, and will have representation from all regions. Early discussions with IOC delegates have emphasized that PICES needs representation on the small executive committee to ensure a collaborative functioning with global committees that share similar aims, including PICES, IOC, SCOR, Intergovernmental Oceanographic Commission Climate Group (IOCCG), International Atomic Energy Agency (IAEA), and ICES.

Major partners contributing to the strategic focus to this international effort include Intergovernmental Panel on Harmful Algal Blooms (IPHAB), ICES, PICES, Oslo/Paris Convention for the Protection of the Marine Environment of the Northeast Atlantic (OSPAR), Helsinki Commission (HELCOM), Global Ocean Observing System (GOOS) and national and local programs including seafood safety monitoring and phytoplankton monitoring programs. Although long considered more of a unique threat to human health, HABs now are recognized to be a component of a broader array of disruptive plankton systems that influence and shape coastal and oceanic ecosystems. Greater coordination of HAB and Climate Science research will underpin not only the protection of public health in the future oceans, but also greater forecasting of climate change effects on wild and aquaculture fisheries and the broader sphere of ocean health.

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The 2015 meeting of the ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

WGHABD met from 13th – 18th April in Lisbon, Portugal. The meeting was hosted by Teresa Moita, Portugal and chaired by Eileen Bresnan, UK. It was attended by 19 scientists from 11 countries with three scientists from an additional two countries participating by correspondence. The report of the working group is now online and can be found at *http://www.ices.dk/community/groups/Pages/WGHABD.aspx.*

The group addressed 12 terms of reference (ToR) relating to harmful algal bloom dynamics. Members presented national reports on HABs during 2014 (ToR a). Highlights include the first report of concentrations of toxins responsible for paralytic shellfish poisoning (PSP) above the EU closure limit in *Mytilus edulis* Sweden and amnesic shellfish poisoning (ASP) in *M. edulis* in England. Closures of shellfish harvesting areas as a result of high concentrations of the toxins responsible for DSP were enforced in many countries in Europe and also in the USA. Cyanobacteria blooms were recorded in Poland, Finland and Sweden. A bloom of *Concinodiscus conncinus* produced nuisance slimes in Norway. ToR (d) examined the threshold/trigger limits for cell counts of shellfish toxin producing species/ genera in phytoplankton monitoring programmes which vary throughout the ICES area. ToR (e) presented new findings on biosensors (Environmental Sample Processor, Imaging Flow Cytobot) in the USA, citizen science and HABs in France, *Vicicitus globosum* in Sweden and modelling *Karenia mikimotoi* in Scotland. Tor (h) reviewed preparations for the ICES-PICES-IOC climate change and HABs symposium in Gothenburg in May 2015. ToR (i) examined molecular methods for the identification of HAB species. Methods discussed included FISH, qPCR, and metabarcoding. ToR (k) addressed the physical and chemical control of different HAB species. This year focussed on the dynamics of *Gymnodinium catenatum* in the Iberian Peninsula. The role of upwelling and cyst beds were included in discussions to explain the variability of this species in this region. ToR (j) reviewed the current status of β-N-methylamino-L-alanine (BMAA) and clarified the confusion between discrepancies in the literature.

ToRs (g) and (l) reviewed the use of HAB/nuisance species for the EU Marine Strategy Framework Directive (MSFD) and JAMP phytoplankton monitoring guidelines. An advice drafting group met at ICES in Copenhagen during June 2015 and incorporated the comments from WG HABD along with other WGs to produce an advisory document which was submitted to OSPAR. Three ToRs contributed towards the work of the IOC Intergovernmental Panel on HABs (IP-HAB). ToR (b) will produce a review of fish killing algae in the ICES area. This manuscript will be completed during the 2015 with a focus on northern Europe. As part of ToR (c) the WG held a one day workshop focussing on the Harmful Algal Event database (HAE-DAT) (*http://haedat.iode.org/*) on Apr 15th. This data is planned to be included in a Global HAB Status Report to be produced by IP-HAB. This workshop flagged issues with historic data to be addressed by group members before the 2016 WG meeting and prioritised issues for computer programmers to address to facilitate data extraction. ToR (f) addressed the production of a HAB status report which could act as an ICES contribution towards the IP-HAB Global HAB Status Report as well as a supporting document for the MSFD. During 2016 the WG HABD meeting will be hosted by Raeffele Siano in IFREMER, Brest, France from 19th -22nd April.

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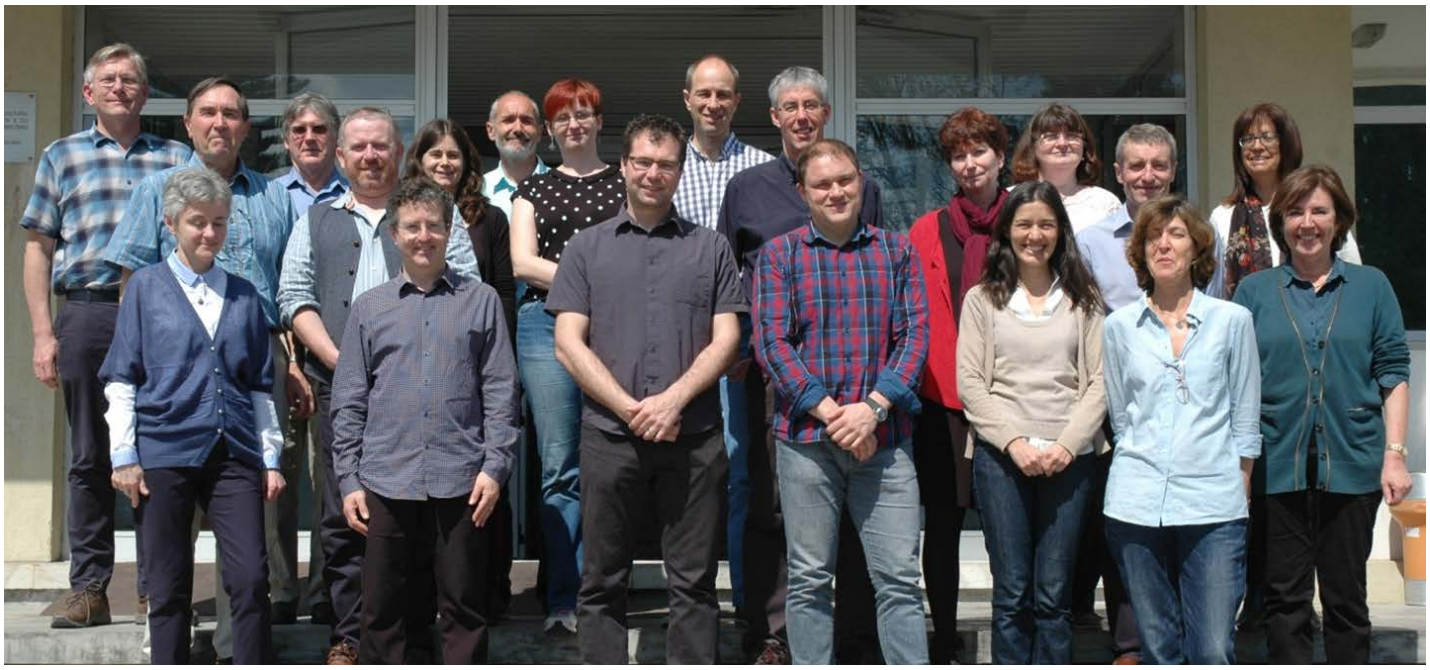
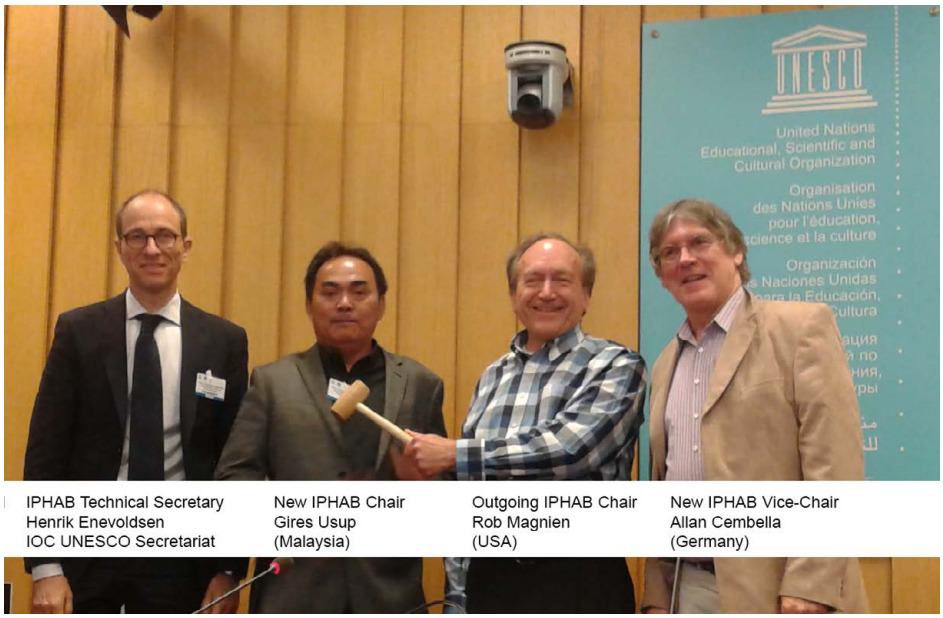


Participants at the ICES-IOC WGHABD meeting, IPMA, Portugal, 2015

XII Session of the Intergovernmental Panel on Harmful Algal Blooms (IPHAB)



IPHAB Technical Secretary Henrik Enevoldsen IOC UNESCO Secretariat | New IPHAB Chair Gires Usup (Malaysia) | Outgoing IPHAB Chair Rob Magnien (USA) | New IPHAB Vice-Chair Allan Cembella (Germany)

Rob Magnien (USA), IPHAB Chair (2011-2015), hanging over responsibilities to the new Chair, Gires Usup (Malaysia) and Vice-Chair Prof. Allan Cembella (Germany). 12th IPHAB meeting,

An Intergovernmental Panel on Harmful Algal Blooms (IPHAB) under auspices of IOC UNESCO has for more than 20 years provided the international framework for regional and global coordination and prioritization of capacity development, research and strategic initiatives on HAB.

The IPHAB met for its 12th session at UNESCO Headquarters, Paris, from 28 to 30 April 2015. The Panel reviewed the many activities and results completed during 2013-2015 and noted the continued broad engagement of the science and management community in activities.

The major achievements presented at IPHAB include: (i) the integrative synthesis of GEOHAB accomplishments; (ii) development of a research and monitoring strategy for Ciguatera; (iii) implementation of more than twelve training courses and training-through-research projects; (iv) continued development of the IPHAB-IODE Harmful Algae Information System; (v) continued publication of the IOC Harmful Algae News; (vi) convening an international conference on HABs and Desalination of Seawater; (vii) development of regional activities within ANCA, FANSA, HANA, WESTPAC-HAB and WESTPAC-TMO; (viii) supporting the work of the ICES-IOC WGHABD and ICES-IOC-IMO WGBOSV; and ix) ensuring the continued vital partnerships with SCOR, ICES, PICES, IAEA and ISSHA.

The IOC-coordinated HAB Programme is the only global intergovernmental effort to understand, manage and mitigate the harmful effects of algal blooms, and has therefore maintained a critical leadership role via IPHAB. Particularly noteworthy is the progess on inter-sessional IPHAB activities, including efforts by Task Teams to maintain an updated taxonomic list of HAB taxa and revisions to the nomenclature, identification and structures of novel phycotoxins, and associated toxicological effects. Combined with IPHAB-sponsored initiatives on mapping biogeographical information on HAB events, these databases are essential to sustain both research and monitoring strategies.

The IPHAB is recognized internationally as more than a static repository of HAB data with an event-reporting mandate. The Panel has shown flexibility and adaptive capacity since its inception, modifying its functions and tasks to address emerging global priorities for ocean research and monitoring as defined by IOC. The IPHAB has aligned its priorities to address the four IOC overarching themes: i. Healthy Ocean Ecosystems; ii. Early Warning for Ocean Hazards; iii. Climate Change Adaptation and Mitigation and iv. Enhanced Knowledge of Emerging Science. Exciting new elements of HAB research have benefited immensely from the recognition by IPHAB of the importance of linkages to ocean observational systems and the effects of global change.

The Panel made decisions for priorities 2016-2017 for international HAB activities to be implemented by the IOC UNESCO and IPHAB partners. The Decisions concern (i) HABs in a Changing World: '*A Global Approach to HAB Research to Meet Societal Needs: GlobalHAB*' (jointly with SCOR); (ii) continued strong regional HAB Programme development; (iii) formation of two new Task Teams, on a Global Strategy on Ciguatera for Improved Research and Management and, on Harmful Algae and Desalination of Seawater; (iv) revised terms of reference for IPHAB Task Team on the development of a Global HAB Status Report, Task Team on Biotoxin Monitoring, Management and Regulations, Task Team on Algal Taxonomy, and a Task Team on Harmful Algae and Fish Kills.

Dr. Gires Usup (Malaysia) was elected as new Chair and Dr. Allan Cembella (Germany) was elected as new Vice-Chair.

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Marine Institute
Foras na Mara

Proficiency testing on the abundance and composition of marine microalgae

Scientists have come under increased pressure in recent years to show that results they obtain arising from their scientific work are quality assured and stand up to scrutiny by independent expert auditors. This has meant that the methodologies used by laboratories involved in making these measurements have to be validated and fit for purpose and has led to the adoption of internationally recognised standard protocols.

These protocols must be underpinned by robust quality systems and must be accredited to an international standard. In order for laboratories to become accredited in particular methods, they have to fulfil a series of prerequisites but a compulsory one is the participation in a proficiency testing scheme. Proficiency testing schemes are independent assessor organisations which coordinate regular inter-calibration and inter-comparative studies between laboratories with a common purpose.

Microalgae are a very important ecological component of the marine ecosystem and have also become important ecological indicators of hydroclimatic change, ocean acidification and eutrophication. Member states of the European Union are obliged to monitor for toxic and harmful algae which can cause problems and devastation in the natural environment, have detrimental effects on human health if contaminated fish and shellfish are eaten, can cause huge economic losses to the aquaculture industry and impact directly in coastal communities.

The Marine Institute, Ireland under the BEQUALM/NMBAQC umbrella from 2005 and in close collaboration with the IOC Science and Communication Center on Harmful Algae, Denmark since 2011 has been producing an annual intercomparison exercise on the abundance and composition of marine microalgae using the Utermöhl cell counting method (Utermöhl et al., 1931, 1958). This collaboration involves the elaboration of a marine phytoplankton taxonomy quiz using an online platform called 'Ocean Teacher' (*http://classroom.oceanteacher.org/*) and the production of samples containing marine phytoplankton species of interest that analysts must identify and enumerate. The exercise is designed to test the ability of analysts to identify and enumerate correctly these organisms. In 2014, 64 analysts from 40 laboratories around the world took part in this intercomparison. The reports of these intercomparison exercises can be found and downloaded from *www.nmbaqcs.org.*

Also, as part of every intercomparison exercise, a training workshop is held annually to discuss the results of the intercomparison exercise and to provide training in some areas of interest on phytoplankton taxonomy to the participants. This workshop is a 2-3 day training workshop and was held in Denmark the 2-4 Dec in 2014.

But first a little bit of the history of the scheme may be helpful. The Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) project, funded by the European Union through the Standards, Measurements and Testing programme of the European Commission, was initiated in 1998 (*www.bequalm.org*). This was in direct response to the requirements of the Oslo-Paris Commission (OSPAR) to establish a European infrastructure for biological effects quality assurance and quality controls (QA/QC) for laboratories contributing to national and international marine monitoring programmes to attain defined quality standards. The original project consisted of twelve work packages (WP) processed by nine partner experts in measurements on biological effects and monitoring techniques, the phytoplankton component (WP 11) was entrusted to the Forschungs und Technologiezentrum Westkuste (FTZ) at Kiel University, Germany. They carried out two interlaboratory comparison exercises or ring trials in 2000 and 2001 (Reckermann and M., Colijn, F., 2000, 2001). These were the first intercomparison exercises in the enumeration and identification of microalgae at European level.

After the 2001 ring trial, there were no more phytoplankton intercomparisons at this level from FTZ. In 2003, INTECMAR in Galicia, Spain, organised a phytoplankton ring trial worldwide. This intercomparison was set up in 2003 under the name Iberia 2003 and the results were published in the proceedings of the IX Iberian meeting on toxin phytoplankton and biotoxins in 2007, Spain (Pazos *et al*., 2007).

Also, in 2003, BEQUALM adopted the UK NMBAQC (National Marine Biological Quality Control Scheme)

www.nmbaqcs.org as a model to progress the Community Analysis component which included phytoplankton analysis and launched the self-funded programme (participants pay a fee) in 2004. The BEQUALM phytoplankton work package initially comprised two parts: chlorophyll analysis and community analysis. The former was taken forward by QUASIMEME, whilst from 2005, the latter has been re-launched (initially for UK/Eire participants), through the BEQUALM/NMBAQC Scheme via the Marine Institute at Galway.

The participation in this type of scheme is becoming an essential requirement for National phytoplankton monitoring laboratories in order to achieve accreditation to international standards and also to support and assure the quality of their results. Many difficult decisions in policy are made based on quantitative or qualitative measurements made by scientists. It is a good working practise that these results contain some indication of their quality. Since 2008, the participation of individual analysts on the scheme is certified by issuing statement of performance certificates to each participant. The Marine Institute phytoplankton laboratory is accredited to ISO 17025 for marine phytoplankton identification and enumeration since 2005, and it recognizes that regular quality control assessments are crucial to ensure a high quality output of phytoplankton data.

This exercise and workshop has become an important forum for scientists working on phytoplankton monitoring programmes from around the world to convene and be able to discuss taxonomical matters related to monitoring, methodologies and techniques, new advances and finds, taxonomical nomenclature changes, looking at samples from different geographical regions and listen to relevant stories from other laboratories about issues regarding harmful algal events in their areas of great ecological importance.

Registration to this intercomparison exercise is done through the Marine Institute, (*Rafael.salas@marine.ie*). All the information regarding this scheme including past reports and workshop agendas can be found and downloaded from the *www.nmbaqcs.org* website and information on the new rounds of the exercise is also posted in the IOC Harmful algal bloom programme page under training *http://hab.ioc-unesco.org/*

The 2015 exercise is currently underway. If you are interested, keep an eye on the links above or e-mail me and I will add you to our distribution list of intercomparison participants.

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Sigma-Aldrich and Cawthron Institute align to supply Harmful Algal toxins worldwide

Pectenotoxin-11 is the 34-hydroxy analogue of pectenotoxin-2. In fact, at position 34, PTX-11 is chiral and so the additional hydroxyl can be left or right handed. Only the left handed S variant is produced by *Dinophysis acuta*. These subtle chemical realities may or may not be relevant to the biology, ecology and toxicity of Harmful Algal Bloom (HAB) species.

It is difficult for scientists from a range of disciplines to gain access to well characterised pure algal toxins, such as PTX-11, and so the relevance of these important molecular details can be difficult to investigate. The Cawthron Institute aims to make a wider range of toxins available to a global market through a partnership with Sigma-Aldrich to supply algal toxins via their renowned catalogue *www.sigmaaldrich.com*

Fig 1. Andy Selwood, Cawthron Institute

The partnership will focus on providing easy access to a wide range of previously rare and hard to source compounds for use in analysis, toxicology, ecology and other previously impossible to perform studies. The project is the result of improvements in the ability to isolate large quantities of these compounds from micro-algae. Andy Selwood (Fig. 1) is the scientist leading this work and further information can be obtained from Sigma Aldrich.

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ISSHA's Corner

Dear ISSHA colleagues:

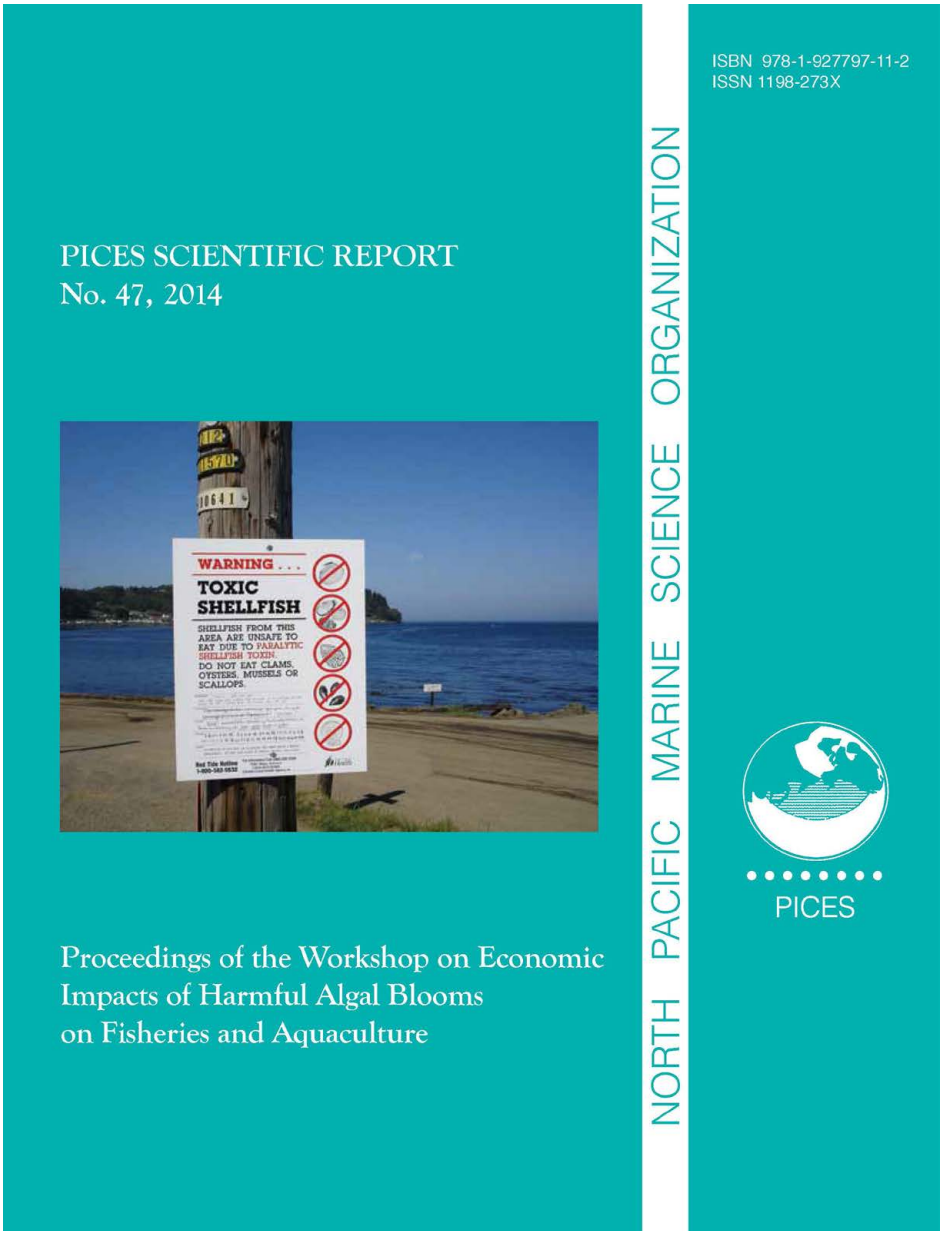
The ISSHA Council is pleased to present the new ISSHA logo! We have tried to incorporate some of the unique features of our past logo, but also to give it a fresh look. In the future, we hope to be able to provide some merchandise to purchase featuring the new logo.

In this HAN issue, you will find an article about a recent international conference in Göteburg, Sweden on HABs and Climate. Look for an article in the journal Harmful Algae that summarizes what we know, what we don't know, and the pathway to the future for HABs and climate change research. We hope that this will provide food for thought and encourage collaborations across the globe to enhance our knowledge on HABs and Climate Change.

As the HAB Section co-chair of the North Pacific Marine Science Organization (PICES), I would also like to bring your attention to a recent Special PICES Scientific Report, "Proceedings of the Workshop on Economic Impacts of Harmful Algal Blooms on Fisheries and Aquaculture" edited by Trainer, V.L. and Yoshida, T. 2014. PICES Sci. Rep. No. 47, 85 pp. This report can be downloaded from *http://pices.int/publications/scientific_reports/* Click on Report No. 47.

Don't forget that the ISSHA website is an important resource for job ads, future workshops and courses, HAB publications, past issues of Harmful Algae News and much, much more. Please visit it at *www.issha.org.*

Vera Trainer. President of ISSHA



Prof. Youssef Halim

(1925 – 2015)

We are sad to communicate that Prof. Youssef Halim (Oceanography Department, Alexandria, Egypt) passed away on Monday 18 May 2015. He was the most senior Oceanographer, a graduate from the first promotion who received its 2-years Diploma from the Oceanography Department, Faculty of Science, Alexandria University in 1950. In 1952, Y. Halim was awarded a French scholarship and moved to the "Station Zoologique" of Villefranche-sur Mer in southern France. Under the supervision of Prof. G. Tregouboff and Prof. L. Fage, he began working on his doctorate thesis, A Quantitative and Qualitative Study of the Dinoflagellates of the Bay of Villefranche. He defended his thesis at the University of La Sorbonne, Paris, in July 1956 and was awarded the degree of "Docteur ès Sciences" with the grade of Very Honourable "avec les felicitations du Jury".

Prof. Halim served as Vice-Dean of the Faculty of Science for Graduate Studies, a position he occupied from 1976 to 1982. He was the National Director and Principal Investigator for the UNESCO/UNDP Project for Aquatic

Environmental Pollution in Alexandria, 1980-1986. He won the UNEP's Global 500 Award in 1997. He published his first paper on this estuarine environment "Observations on the Nile bloom of phytoplankton in the Mediterranean" in 1960. Prof. Halim was the first scientist who studied Harmful algae in Egypt. He described several new species: *Peridinium tergouboffi* 1955, *Histoneis imbricata, H. rampii* 1960, *Ceratium egyptiacum* 1963, *Amphisolenia sigma* 1965, *Peridinium symmetricum* 1968 and *Protoperidinium elipticum* 1969. But the most important one, *Alexandrium minutum* n. gen, n. sp., a dinoflagellate of worldwide concern as a harmful microalgae, was isolated and described in 1960. Several other papers were to follow on the same subject. His last work, in 2012, was concerning benthic harmful dinoflagellates *Ostreopsis* spp. in the Egyptian Mediterranean waters.

Prof. Halim was a member of UNESCO Consultative Panel on Coastal Marine Ecosystems since 1978 (Biennial Workshops) and of GOOS-HOTO Panel. He was also a member of ISSHA (International Society for the Study of Harmful Algae) with many friends in the HAB community around the world. He was the key person to facilitate the establishment of the IOC-HANA Regional Group and its first Chair, from 2007-2012.

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HAB Dissemination

Scientific dissemination is an issue of outmost importance. More than ever, there is a need to communicate our research to a general audience and give back to society some of the resources it has placed at the disposal of the science system.

In 2011 I decided to open a popular science blog, **FITOPASION**, to share the most curious stories I learnt during my career. They do always share a common thread: the fascinating world of microalgae. *Naked or thecate, noxious or not,* all these astounding creatures have their place on this website.

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From issue 45 2012 and onwards, HAN issues are accessible via e-pages from where a PDF can also be downloaded.

United Nations Educational, Scientific and Cultural Organization

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Lay-out
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