Harmful Algae News

An IOC Newsletter on Toxic Algae and Algal Blooms

No. 47 - May 2013 · www.ioc-unesco.org/hab

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

In the Land of Bibimbap, Kimchi, and Impeccable Hospitality

Records of red tides and HABs are scattered through Korean archives for nearly 2000 years. **Changkyu Lee** [1] lists 38 between 161 AD and 1820 AD with fish mortalities and human victims. Accounts of what was probably PSP in Jinhae Bay are recorded for the years 1770 and 1820, in the same period as descriptions elsewhere (Vancouver, western Canada in 1793; Combe, eastern Scotland in 1827).

In more modern times, there have been broad shifts in the dominant HAB species in Korean waters. *Karenia mikimotoi* and *Chattonella* spp were more important in the 1970s and 1980s, there was a *Cochlodinium polykrikoides* 'period' from 1993 to 2008, now, since 2000, in decline (**Hak-Gyoon Kim**). In Japanese waters, *K. mikimotoi* and *Heterosigma akashiwo* typically formed red tides in the 1970s, *Chattonella* species in the 1980s, and *C. polykrikoides* in the 1990s. PSP records increased in Japan from 1978 to 1999. There are comparable trends elsewhere, e.g., a period of range expansion of *Pyrodinium bahamense* in the Philippines from 1983 to 1998 (**Rhodora Azanza**). *Gymnodinium catenatum* progressed seasonally from south to north along the Portuguese coast from 1985 to 1995, and again since 2005, but no blooms were recorded between 1996 and 2004 (**Ana Amorim**). Multiannual signals appear in *Heterosigma akashiwo* and *Prorocentrum triestinum* in Narragansett Bay (**Ted Smayda**).

Some of these patterns may be related to climate signals like the Pacific Decadal Oscillation or ENSO, or, as **Patricia Glibert** argues, to the growth of nutrient loading. Both eutrophication and ENSO were invoked to account for changes in a 50 year record of cyst numbers from Changjiang Estuary sediments (**Dou-ding Lu**). But blooms of *Alexandrium* in Japan and *Pyrodinium* in the Philippines do not appear to be related to nutrient loading, and may increase in importance when the latter is reduced (**Yasuwo Fukuyo**). In Japan, *Karenia* blooms have been recorded at least since the 1930s, and *Chattonella* cysts are common in pre-eutrophic sediments. *Alexandrium catenella* appeared in Marlborough Sound, New Zealand in March 2011 for the first time in 18 years of monitoring, and again in 2012 (**Lincoln MacKenzie**). This is a long interval

Photo of Patricio Díaz

to survive without a bloom, but results from Cork Harbour in Ireland suggest that blooms of *Alexandrium minutum* at 7-8 y intervals provide enough cysts to maintain local populations (**Sarah Cosgrove**).

Participants in the Crete conference will recall that the potential impact of anthropogenic acidification on phytoplankton was a theme there. We now learn that natural acidification (pH 6.8 to 8.1) due to fumaroles at a site in the Gulf of Naples has no apparent impact on the local *Ostreopsis* populations (**Davide di Cioccio**).

Older food chain models consisted of diatoms, copepods, and fish; real ones are a great deal more complicated. What was once food (building materials, calories) is now much more, and a consumer must decide what part of its food to digest, and what part to keep for its information value. Serial endosymbiosis is not just something that happened billions of years ago, it is going on all the time (**Veronique Séchet**). The organisms are small, but have 'big bites' [2]. Plastids and genes are passed around like playing cards (**Miran Kim**), trophic chains are really trophic and information networks, the boundaries between autotrophy and mixotrophy dissolve (**Hae Jin Jeong**, **Per Juel Hansen**), genomes no longer belong to species, and species have multiple genomes. Cryptophytes, for example, have four, the host nuclear genome, mitochondrial and plastid genomes, and a residual nuclear genome (nucleomorph) from the endosymbiont. Bottom-up and top-down regulatory mechanisms blur as more and more 'lateral' processes are identified, and convergence complements divergence.

Graph theory, pioneered by Paul Erdös, is used to explore the architecture and dynamical properties of food webs and other networks. His co-authors are assigned an Erdös number of 1, those who co-authored papers with Erdös' co-authors have an Erdös number of 2, and so on. We might then give *Mesodinium* (and *Karlodinium*) a Teleaulax number (T) of 1 since they acquire it directly, *Dinophysis* a T of 2 since it acquires it from *Mesodinium*; for predators of *Dinophysis* (tintinnids, *Noctiluca*, mussels), T = 3, and for patients with DSP, T = 4. *Dinophysis* derives information from *Teleaulax* via an intermediary which provides sustenance, a more complex linkage pattern than any so far tackled by graph theorists; it may be that such dynamics are beyond the present reach of this approach.

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Information on the distribution, abundance, and physiology of dinoflagellate cysts continues to accumulate. *Alexandrium tamarense* cysts are abundant in the Chukchi and E Bering Seas (**Masafumi Natsuike**), there are significant interannual variations in *Alexandrium catenella* cyst numbers in Puget Sound (**Cheryl Greengrove**) – and, it is claimed, no evidence of an endogenous clock – and *Gymnodinium catenatum* cyst abundance is low in Portuguese shelf sediments (**Teresa Silva**). Other reports dealt with cysts in Manila Bay (**Elsa Furio**), cyst numbers in Alexandria Harbour (**Amany Ismael**), and the effect of cyst age on germination rates of *Alexandrium catenella* and *Scrippsiella* (**Kirsten Feifel**). "Pelagic seed banks" in the Taiwan Warm Current (East China Sea) are proposed to account for blooms of *Prorocentrum donghaiense* (**Xinfeng Dai**). A qPCR count of *Heterosigma akashiwo* cysts compares well with direct counts (**Joo-Hwan Kim**). Dormant stages of diatoms also received attention (**Mineo Yamaguchi**). *Skeletonema marinoi* resting stages are viable for more than 60 years (**Karolina Härnström**).

The rôles of dormant stages in population dynamics continue to challenge us. In Crete, we heard of temporal discrepancies between peak laboratory excystment rates and the appearance of blooms. The mystery continues: field studies in Korean waters now seem to confirm that maximum germination rates of *Alexandrium tamarense*, *Scrippsiella trochoidea*, and *Anabaena flos-aquae* do not coincide with bloom development there (**Myung-Soo Han**). But in Sorsogon Bay, Philippines, model and field studies of *Pyrodinium bahamense* suggest excystment is closely linked to bloom formation (**Aletta Yñiguez**); this may also be so for *Heterosigma akashiwo* (**Elizabeth Tobin**).

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Contributions to this conference series in the past are usually grouped into a few broad categories – organisms, environmental conditions, bloom dynamics, chemistry and pharmacology of toxins, management. The range of organ-

Photo of Patricio Díaz

Photo from www.hab2012.kr

isms has expanded continuously, and the categories have broadened. In his summing up, **Allan Cembella** referred rather negatively to the branching out or dilution of HAB conference topics. This was already a concern at the Rhode Island conference in 1991. Ted Smayda worried then [3] that the community might "splinter into subgroups"; but the splintering is not apparent yet. We certainly belong to various interest groups, but we still attend ICHA conferences and talk to each other.

The HAB community has managed to cope with the addition of all kinds of new species and novel toxins, with brown tides and green tides, with outreach and high tech gadgets and climate concerns, even with the omics revolution (metabolomics, transcriptomics, ecogenomics, see Francisco Rodríguez in this issue). But the focus, to understand and manage HABs, remains intact. It might be that this conference series is one of the few that pioneered cross-disciplinary research, long before it was fashionable. **Øyvind Moestrup** noted that algal toxins have been killing domestic animals for 150 y (in fact much longer), *and we still do not know what they are for.* **Sandra Azevedo**'s review of what cyanobacterial toxins might be for reinforces Moestrup's point.

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The ecology of *Cochlodinium polykrikoides* in Korean waters was reviewed by **Chankyu Lee**. It was reported from Mijo Harbour (**Bum Soo Park**) and Jaran Bay, Korea (**Chang-Hoon Kim**), on the Wenling coast, China (**Douding Lu**), in the Straits of Hormuz (**M.S. Mortazavi**) and Kuwait (**Wafa'a Al Rashed**), in El Salvador and Guatemala waters and in Banderas Bay, Mexico (**Sergio Licea**). Elsewhere in this issue, blooms of this species are described in the Colombian Caribbean, by Andrés Malagón and Laura Perdomo and in El Salvador waters (Jaime Espinoza and others). Temporary resting cysts and planozygotes of this species have been produced in culture (**Ying Zhong Tang**).

Alexandrium records include *tamarense* and *catenella* from southern Korean waters (**Ji- A Park**), *catenella* from Changjiang Estuary, China (**Yunfeng Wang**), and *tamarense, catenella* and *ostenfeldii* in the Beagle Channel, Argentina (**Marcelo Hernando**). An un-named species of *Alexandrium* has bloomed in recent years in the Philippines (**Garry Benico**). Bacteria on *Zostera* may inhibit growth of *Alexandrium tamarense* (**Yuka Onishi**).

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Printing, a prerequisite to widespread literacy, was pioneered in Korea by means of movable metal type in the 13th century, 200 years before its appearance in Germany. More recent Korean enterprise gave the world the first MP3-phone and the first touch screen mobile phone, harbingers of an emergent multimedia neo-literate (post-literate?) era in which images and icons complement or even compete with words, with an accompanying threat of illiteracy. Now humans and some of our machines live in a kind of symbiosis and, we may ask, will science be able to prosper in a world without texts? Shall we tweet our (crowd-sourced?) science to each other with only 140 electronic characters? It would at least ease the information overflow now swamping peer review and editorial filters! Are electronic proceedings a way station en route to intellectual dystopia? And will everything disappear anyway because libraries no longer have funds to archive?

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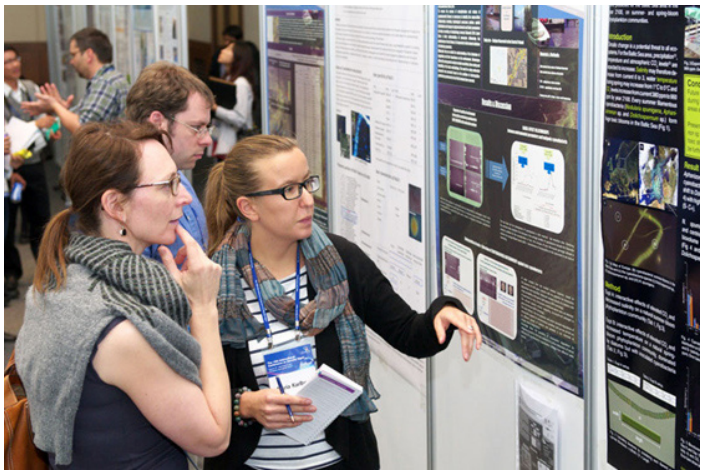
T. Wyatt

Photo from www.hab2012.kr



Top and bottom, photos from Patricio Díaz. Middle from www.hab2012.kr

Top and bottom, photos from Patricio Díaz. Middle from www.hab2012.kr

Some highlights from Gyeongnam

The XVth International Conference on Harmful Algae, ICHA, was held from Oct 29 to Nov 2, 2012 in Gyeongnam (South Korea). It was attended by participants from more than 40 different countries. It was obvious throughout the conference that almost every single detail had been carefully planned in advance by the local organizing committee, chaired by Dr. Hak-Gyoon Kim, and they had taken their responsibilities toward participants very seriously at every moment. Their commitment to timetables and general information about transport and accommodation was excellent. All the "*lost in translation*" problems that might have arisen during our stay in Gyeongnam were averted thanks to the guidelines on the ICHA website. The facilities of the modern Convention Center (CECO) were ideal.

There were 133 oral and 244 poster presentations; only a small selection of them can be mentioned in these few paragraphs. The theme sessions covered all relevant issues concerning the physiology, ecology, life cycles, and modeling of HAB species. These sessions also covered the key issues centred on the relevance of phycotoxins, as illustrated by the fact that 5 out of 31 oral sessions (19 talks) were dedicated to toxin chemistry and toxicity.

In his conference summary, (**Allan Cembella**) noted that toxin research now seems to be a "mature field". He meant that recently no major groups of phycotoxins or human toxin syndromes have been reported. 'Hyphenated methods' (like LC-MS) are now optimized, and toxin specificity and modes of action are well understood. But the toxins of fish killing algae like *Cochlodinium*, raphidophytes and prymnesiophytes, as well as the cyanobacterial toxins, are important exceptions to this view.

New methodological developments and the incorporation of toxin chemistry in multidisciplinary studies (biodiversity, biodistribution, fate of toxins in the food web, grazing interactions, etc) were prominent issues at this meeting. One of these is the 'metabolomics' approach (**Philip Hess**), which couples gradient chromatography and high resolution mass spectrometry to detect metabolites in the cell at a given time and under given conditions. For each species, this procedure yields a large 3D data set, and offers a massive chemotaxonomic tool, the 'metabolome'. Before comparative metabolomics can proceed, the data set is analysed with the Molecular Feature Extraction (MFE) to characterize the metabolome of a given species. This approach identifies new bioactive molecules in microalgae and improves our understanding of the biosynthesis and biogeography of marine compounds.

The search for novel bioactive compounds is also pursued using phenotypic based chemotaxonomy followed by a dereplication step combining solid-phase extraction and ultra high performance liquid chromatography, UHPLC (**Thomas Larsen**). When likely unknown molecules are observed after an initial comparison against a comprehensive compound database, these can be then purified for characterization by nuclear magnetic resonance (NMR).

Molecular tools have been integrated in multidisciplinary HAB studies, not only as complementary taxonomic aids, but also in transcriptomics and ecogenomics. For example, the available saxitoxin (STX) genes are now applied in studies of toxin physiology, metabolic intermediates and regulation of toxins production in paralytic shellfish poisoning (PSP) producers like *Alexandrium catenella* (**Maria Wiese, Shauna Murray**)

Other genomic and transcriptomic studies are related to the use of molecular probes and markers in Amplified Fragment Length Polymorphism (AFLP), qPCR, microarrays and *in situ* hybridisation techniques; biogeographical studies at the population level (**Karin Rengefors, Anke Kremp**) and the assessment of intraspecific diversity associated with toxin production in *Alexandrium tamarense* (**Chen Jiang**) provide examples.

In parallel with the metabolomic approach, metagenomic analyses look likely to play an important role in the future, e.g.in plankton monitoring using Next Generation Sequencing (NGS) methods like Roche 454 (**Satoshi Nagai**).

Although no new toxins or syndromes were announced here, some organisms attracted special attention, like the macroalgal "green tides" in China, and the dinoflagellate *Cochlodinium polykrikoides,* due to the economical damage caused by their blooms; the impact of the latter has been minimized by clay mitigation in South Korean waters, to protect shellfish and aquaculture farms.

Since the isolation of the azaspiracid producing *Azadinium spinosum* in 2008 in the North Sea (east coast of Scotland), its global distribution, toxicity and phylogenetic position have been intensively studied. Some of these advances were summarized by **Urban Tillman** and **Bernd Krock**, and included the recent discoveries of *A. poporum* and *Amphidoma languida*, as well as new azaspiracid analogues in *A. spinosum*.

There is growing interest in the role of parasites in HAB population dynamics, as reflected in the increasing number of studies of their specificity, infectivity and genomics (**Marc Arancio; Lourdes Velo-Suárez; Yameng Lu**). The strangest report at this conference was the mixotrophic behavior of *Alexandrium pseudogonyaulax* and its "toxic mucus trap" (**Hannah Blossom**).

Myung Gil Park and co-workers have made major contributions in the last decade to our knowledge of the dinoflagellate *Dinophysis*, so not surprisingly this organism received special attention at the ICHA meeting. In the theme session on population dynamics of HABs, it was not only *Dinophysis* but necessarily also its ciliate prey *Mesodinium* who shared the "lights on the stage".

Talks on this topic included an update on *Mesodinium* taxonomy, physiology and cellular organization by **Per Juel Hansen**, a presentation by **Miran Kim** with the suggestive title "Is the ciliate *Mesodinium rubrum* a sole prey of the marine dinoflagellates *Dinophysis* spp?"– he provided evidence of *Mesodinium* eating *Chroomonas*, a "green" cryptophyte, whose plastids were not kept as kleptoplastids in *Dinophysis* but only included in digestive vacuoles – and an account by **Meng Meng Tong** of the growth and toxins in *Dinophysis acuminata* under different nutrient regimes. The plot showing that *D. acuminata* does not apparently take up inorganic nitrogen (once *Mesodinium* was removed from the medium, consumption stopped), is one of the most intriguing results from this conference.

Benthic harmful algae, mostly *Ostreopsis* and *Gambierdiscus*, pose a major threat to human and environmental health in warm areas, and have apparently spread to temperate regions too, as shown by **Adriana Zingone**. These genera and others like *Coolia* and *Prorocentrum* were the focus of a theme session and several posters, but benthic microalgae attracted less attention than at ICHA XIV in 2010. Among the presentations covering this issue, molecular studies included qPCR for detection of *Gambierdiscus* (**Tomohiro Nishimura**), and physiological and phylogenetical research on *Ostreopsis*, *Gambierdiscus* and *Coolia* in Korean and Japanese waters (**Chui Pin Leaw; Akira Ishikawa**). A new domoic acid producer, the diatom *Nitzschia bizertensis*, was reported in a Tunisian coastal lagoon (**Donia Bouchouicha**).

Other highlights include a digital holographic cinematographic study grazing behavior of copepods exposed to toxic microalgae (**Pat Tester**), the systematics of the pinnatoxins-producing *Vulcanodinium rugosum* species complex (**Kirsty Smith**), the first occurrence of DSP toxins above the FDA level on the East coast of the US (**Theresa K. Hattenrath-Lehmann**), general issues of global significance like ocean climate change and its influence on HABs, fisheries and public health.

This is only a sketch of a diverse and fruitful conference. HAB research is an active and dynamic field of science which blends basic and applied research as few others, from systematic studies of algae, modeling of their natural populations, behavior and physiology in the laboratory, to the continuous development of equipment and analytical methods for toxin detection, HAB forcasting, and *in situ* identification of microalgae. All this supports the evolution of international monitoring programmes, sanitary regulations, and a better understanding and protection of our environment.

Francisco Rodríguez-Hernández
Instituto Español de Oceanografia, Subida a Radio Faro 50, 36390 Vigo, Spain
francisco.rodriguez@vi.ieo.es

Blooms of *Botryococcus braunii* in Nozha Reservoir, Alexandria (Egypt)

Nozha Reservoir (31.19°N, 29.37°E) south of Alexandria city is an enclosed, nearly circular freshwater body with a surface area of about 5.5 km^2 and an average water depth of 2.1 m. It receives its water supply from the Nile via Mahmoudia Canal. The reservoir suffers from progressive pollution indicated by the ongoing increase in cadmium concentrations in its sediments [1]. It produces 200-250 Tons y^{-1} of popular fishes (Mullet, Tilapia, Bayad, Carp, and Catfish).

Irregular sampling occurred from January to June 2012 during water discoloration periods, and an intensive monitoring of the ambient physical, chemical conditions, abundance, distribution, and the amount of lipids of *Botryococcus braunii* was done at 5 sampling sites in the first month. Blooms of this unicellular photosynthetic cholorophycean that forms colonies resemble large floating mats on the water surface. According to [2], *B. braunii* converts 3% of the solar energy to lipids which can reach 70% of the algal biomass; when *B. braunii* oil is cracked, it yields a distillate containing 67% gasoline, 15% aviation fuel, 15% diesel fuel and 3% residual oil [3]. The lipid production ability of this alga has been investigated at different growth phases under different culture conditions [4].

Water temperature varied from 13°C in January to 28°C in June, salinity 1.4-2.5, pH 7.8-8.9 and DO 7.2-9 mg L^{-1}, affecting fish survival. The effect of thermal stratification was ignored due to the shallowness of the pond and the effect of prevailing winds.

A controlled fertilizer programme is applied to Nozha Reservoir to increase fish production. This also seems to favour the occurrence of recurrent harmful algal blooms, in 1986, 1994, and 2003, mainly of Cyanobacteria (*Anabeana, Microcystis*). However, there was a dramatic change in species composition in 2012 with predominance of *B. braunii*. It formed intermittent dense blooms between January and June. The green-yellow water turned to brown, and covered the entire lake during calm periods. This species was the only constituent of the phytoplankton community in January (maximum 55.3x103 colonies L^{-1}). The high alkalinity changes (300-420 mg L^{-1}) might explain these mono-specific blooms by damaging the phytoplankton diversity. These blooms caused massive fish mortality (losses about 3000 US $ d^{-1}); it is the first reported harmful bloom of *B. braunii* in Nozha Reservoir. The bloom peak in January occurred at 14°C, 2.1 salinity and pH between 8.7 and 9.5, raising dissolved oxygen content to 10.6-11.6 mg L^{-1}. Nitrate fluctuations (0.03-0.9 mg L^{-1}) seems unlimiting while, phosphate (0.04-0.1 mg L^{-1}) and silicate (0.1-4 mg L^{-1}) were of more importance. Up to 30-50% of the dry weight of *B. braunii* were long chain lipids.

Unless major changes in the physicochemical properties (especially pH) of the water take place, red tides pose a serious threat to the Nozha Reservoir ecosystem. The knowledge of how *B. braunii* can gain and maintain dominance is useful to those who intend to grow ponds of it as a hydrocarbon source in Egypt.

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Authors

Wagdy Labib[1], Samia K. Mikhail [1,2], Ahmed M. Kassem[3], Magdy El Kassas[3], Mahmoud M. Ahmed[3]
[1]National Institute of Oceanography and Fisheries, Alexandria, Egypt
[2]Corresponding author: Professor Samia Kamel Mikhail
Email: Mikhail_samia @yahoo.com
[3]Egyptian Company for Fishing and Fishing Equipment, Alexandria, Egypt

ISSHA President's Corner

The International Society for the Study of Harmful Algae (ISSHA) convened the 15th International Conference on Harmful Algae (15 ICHA), held in Changwon, South Korea (29 October-2 November 2012). A beautiful hand-embroidered ISSHA flag, a gift from our Korean hosts, waved in the wind by outside the Gyeongnam Conference Centre (CECO). During the closing ceremony, a very symbolic act took place when the ISSHA president took this flag from Dr Hak Gyon Kim (Chair of the Korean Organizing Committe) and passed it to Dr Lincoln MacKenzie, chair of the next 16th ICHA local organizing committee.

The ISSHA Council meeting was held on 28 October before the conference. The 8th General Assembly of the Society was celebrated on 1st November. Beatriz Reguera (President) opened the meeting. The minutes from the previous assembly were approved and ISSHA committee chairs summarized activities and achievements in the previous two years. Ian Jenkinson (Statutes and Bylaws) presented proposed changes to update functioning of the Society to modern times: electronic voting instead of ballots, election years coinciding with those of ICHA conferences, etc. All changes were accepted by the Assembly. Nina Lundholm (Treasurer) presented the financial statement, covering the period October 2010 to October 2012, and detailed expenditures and funds raised by member's fees, book sales, and the auction graciously conducted by Barrie Dale at the 14 ICHA (Crete). Clarisse Odebrecht (by correspondence) prepared the Travel Awards Committee report, thanking sponsors of student travel awards (FAO, SCOR, NOAA) and in particular the generosity of the Gyeongnam Provincial Government who provided free accommodation for young scientists at the dormitory of the Gyeongnam Officials Training Institute (GOTI) and daily transport to the Conference Centre. Altogether, 43 young scientists (predoctoral, master and post docs) from 20 countries presenting oral and poster communications at the Conference were given grants.

Vicepresident Gustaaf Hallegraeff (Chair of Publication and Dissemination Committee) announced that for the first time, a free on-line publication of the proceedings from the 14th ICHA (Crete, Greece, October 2010) is available through the ISSHA website. A lively debate followed on the convenience of making on-line proceedings freely available just to the ISSHA members during the first 1-2 years. But this would represent discrimination towards non-ISSHA members attending the conferences. After post-assembly discussions among the Council members, a decision was made that future proceedings will be available to ISSHA members and to all participants registered at that ICHA conference who will be given a complimentary member-like use of the website for 2 years.

Vicepresident Jennifer Martin (Achievement Awards Committee, co-chaired with Rita Horner) reviewed procedures followed by the Committee to examine nominations, by ISSHA members, for the *Yasumoto Lifetime Achievement Award* and the *ISSHA Young Scientist Award*. The same committee had the task of securing double evaluations for the 45 students who competed for the *Maureen Keller Award* for best oral and poster communications.

2012 was election year in ISSHA. Some members had to step down because their period of service for the same office was due, and some others had to do so due to changes in their professional situation. Results of the elections were presented by Karin Rengefors (Secretary and Chair of Elections Committee) and are listed at end of this report.

Lincoln MacKenzie (Cawthron Institute), on behalf of the local organizing committee, presented the venue for the 16th ICHA to be held in Wellington, New Zealand (http://www.icha2014nz.com/) from 27 to 31 October 2014. All ISSHA members attending the Assembly welcomed with enthusiasm the bid by Brazil (Federal Institute of Santa Catarina), presented by Dr Luis A. Proença and supported by well known HAB experts from the South America, to host the 17th ICHA in 2016 in Florianápolis (Santa Catarina State, Brazil).

The ISSHA Auction, now a tradition, followed the ISSHA General Assembly. Due to unexpected throat irritation, Barrie had to decline to act as auctioneer. But the auction took place with great success thanks to Sandy Shumway volunteering, who demonstrated her great skill acquired from years of practice at the National Shellfisheries Association auctions.

All achievement awards were announced during the luxurious farewell dinner buffet that followed the traditional folk dances and music organized at the CECO Convention Hall.

ISSHA wishes to express its gratitude for the hospitality and generosity of the Gyeongnam Provincial Government, for the clockwork efficiency exhibited during the conference at the CECO Convention Centre, and for the enthusiasm and dedication of the Korean Organizing Committee, in particular its chair Dr Hak-Gyoon Kim, from the Republic of Korea National Fisheries Research & Development Institute. A beautiful carving of *Cochlodinium polykrikoides* by Prof. Takayama was given to him as a symbolic recognition for all his efforts.

New ISSHA officers and council members

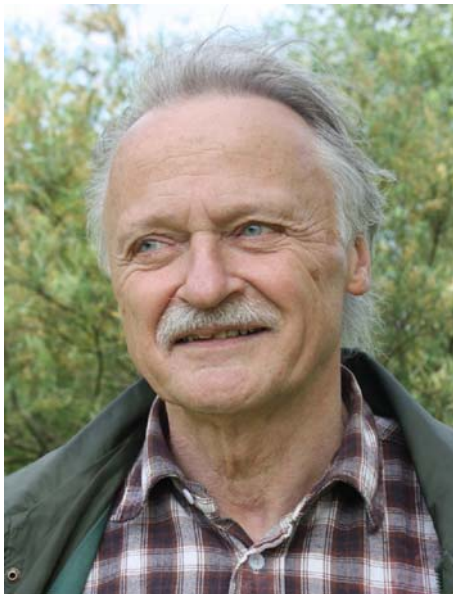
Officers
President: Beatriz Reguera (Spain)
Vice-Presidents: Robin Raine (Ireland) & Mingjiang Zhou (China)
Secretary: Karin Rengefors (Sweden)
Treasurer: Henrik Enevoldsen (Denmark)
Past-president: Patricia Tester (USA)

Council
Ana Amorin (Portugal)
Anke Kremp (Finland)
Anna Godhe (Sweden)
Aurelia Tubaro (Italy)
Christine Band-Schmidt (Mexico)
Esther Garcés (Spain)
Gustaaf Hallegraeff (Australia)
Ian Jenkinson (China, France)
Katerina Aligizaki (Greece)
Keith Davidson (United Kingdom)
Lincoln McKenzie (New Zealand)
Marta Estrada (Spain)
Philipp Hess (France)
Suzanne Roy (Canada)

Hak-Gyoon Kim receives his ISSHA present: a carving from Prof Takayama reproducing the Cochlodinium Conference logo (Photo from www.hab2012.kr).

ISSHA 2012 Achievement Awards

The *Yasumoto Lifetime Achievement Award* was given to Prof. Øjvind Moestrup (University of Copenhagen, Denmark). The *Maureen Keller Awards* for the best student communications were given to: Bum-Soo Park (South Korea), Hannah Blossom (Denmark), Theresa K. Hattenrath-Lehmann (USA) and Thanh-Luu Pham (Japan)



Prof. Øjvind Moestrup

Prof. Øjvind Moestrup received the *Yasumoto Lifetime Achievement Award* for excellence in his lifetime dedication to research on the biology, taxonomy and ultrastructure of microalgae. In addition, Prof Moestrup has made an outstanding contribution, through the *IOC Science and Communication Centre on Harmful Algae* in Copenhagen and the *IOC Reference List of Harmful Microalgae*, to training and communication on the taxonomy of HAB species.

Øjvind was born on 15 December 1941. He grew up on the island of Funen and in Jutland, Denmark, where his father was a vicar. He started studying biology at the University of Copenhagen in 1962 with particular interest in zoology and initially intended to do his Master's degree on the cytology of flatworms. However, in 1965, he was contacted by Prof Tyge Christensen, who asked him to consider a job as demonstrator at the Institut for Sporeplanter which he accepted. In 1967, Tyge suggested Øjvind accept an invitation from Prof Irene Manton, University of Leeds, England, the leading expert on ultrastructure of algae, to learn electron microscopy. It was there in 1968–1969, where Øjvind learned the techniques necessary for studying ultrastructural details of algae – and he has basically done this ever since. The topic for his Master's thesis was the ultrastructure of *Vaucheria*, and he revealed that the genus belonged to the golden-green algae. In the period 1970–1974, Øjvind was assistant professor at the Institut for Sporeplanter and studied spermatozoids in different groups of algae. In 1972, a new transmission electron microscope was sponsored by the Danish Research Council, and Øjvind moved onto nanoflagellates inspired by a bloom in Kaikoura, New Zealand, including the description of two new haptophytes (*Phaeocystis scrobiculata* and *Chrysochromulina novae-zelandiae*). Øjvind began reconstructing the flagellar apparatus of several different flagellates, such as *Chrysochromulina, Mamiella, Nephroselmis, Pyramimonas, Pseudoscourfieldia* and *Bicosoeca*, which ultimately turned into a whole issue of *Phycologia* and was used as a DSc thesis at the University of Copenhagen in 1983.

See more information on Prof. Øjvind Moestrup's achievements on "Trail Blazers" at the ISSHA website (www.issha.org)

Bum Soo Park

Best Oral to Bum Soo Park (Hanyang University, Seoul, South Korea) for his contribution on "*Distribution and seasonal fluctuation of diverse ribotypes of* Cochlodinium polykrikoides *in Southern Korean coastal waters*" (B.S. Park and M.-S. Han). Bum Soo Park is a PhD

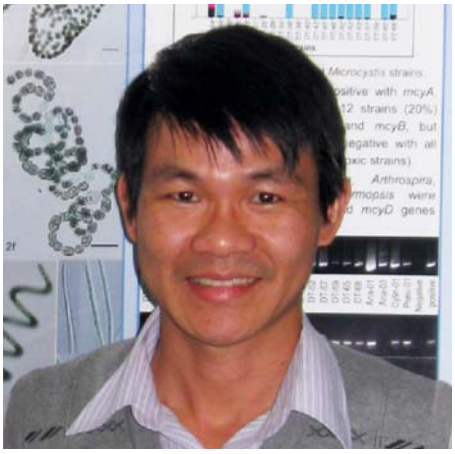
student (supervised by Prof. Myung Soo Han) at the Life Science-Natural Sciences Department in Hanyang University (Seoul, South Korea). He received his bachelor's degree from the same university in 2006 and was a visiting scholar at the University of Washington (Seattle, USA) in 2007-2008. He won best paper awards of The Korean Society of Phycology (2011) and the Korean Society of Environment & Ecology (2011). Park has worked with different microalgal groups (diatoms, dinoflagellates, small flagellates) and the importance of life cycles in their ecology. Currently he is interested in the application of advanced molecular tools in the study of the main HAB species causing socio-economic problems in Korea, in particular of *Cochlodinium polykrikoides.*

Hannah Blossom

Honorable Mention to Hannah Blossom (University of Copenhagen, Helsingør, Denmark) for her oral presentation "T*oxic mucus traps: a novel mechanism that mediates prey uptake in the mixotrophic dinoflagellate* Alexandrium pseudogonyaulax", coauthored by N. Daugbjerg and P.J. Hansen. This work was done as Hannah's Master degree, completed at the University of Copenhagen, under the supervision of Per Juel Hansen and Niels Daugbjerg. Hannah received her bachelor's degree from Northeastern University, during which she did an internship in Don Anderson's lab - sparking her interest in harmful algae. Hannah is now studying ichthyotoxic algae as part of a Danish strategic research project called HABFISH, led by Per Juel Hansen. She is currently focusing on *Prymnesium parvum* and its effects on fish as well as co-occurring algae. Hannah's research interests include the role of allelochemicals in phytoplankton interactions, particularly in mixotrophic harmful algae species. Originally from New York, Hannah has been living in Denmark for 3 years now, and has enjoyed the adventures of living abroad.

Theresa Hattenrath-Lehmann

Best poster award was given to Theresa K. Hattenrath-Lehmann (Stony Brook University, USA) for "*The Emergence of toxic* Dinophysis acuminata *blooms in a New York estuary*", co-authored by S.L. Morton and C.J. Gobler. Born and raised in Queens (New York), she left the big city to attend University of Maine at Machias (UMM) where she received her B.S. degree in Marine Biology with a focus on mariculture. The university is on the Gulf of Maine, a region well known for large-scale recurrent *Alexandrium* blooms and her professor, Dr. Gayle Kraus, encouraged her to attend a phytoplankton monitoring workshop at Bigelow Laboratory in Boothbay Harbor, Maine. There she became interested in HABs. Her Master's research, initiated in 2007, was on the effects of nitrogen loading on *Alexandrium* densities and toxicity, at Stony Brook University (advisor Christopher Gobler). Her studies concluded that nitrogen loads promote the intensity of PSP events and that nitrogen derived from a waste water source was promoting *Alexandrium* blooms in this system. In addition, she discovered that another harmful algal species, *Dinophysis acuminata,* was blooming in Northport Bay and that shellfish contained DSP toxins above regulatory limits. She is currently a Ph.D. student of Dr. Gobler. Her topic: to assess the causes of the onset, maintenance, and demise of blooms of *Alexandrium* and *Dinophysis*. Theresa would like to continue a career path investigating the root causes of harmful algal blooms, but is also interested in programmes concerning protection of human health against toxic algae. This last topic had been a large part of her Master's work and she found it gratifying to interact with NY state agencies and assist in monitoring programmes designed to prevent shellfish poisonings.



Thanh-Luu Pham

Honorable mention to Thanh-Luu Pham (University of Tsukuba, Japan) for his poster on "*Toxic Cyanobacteria and Microcystin Profile of Numerous Cyanobacterial Strains Isolated from Dau Tieng Reservoir, Vietnam*", co-authored with T.-L. Pham, T.-S. Dao, K. Shimizu, N. Sugiura and M. Utsumi. Thanh-Luu holds a M.Sc. degree from the Ho Chi Minh City University of Technology and is currently working toward his Ph.D. degree at the Aquatic Eco-Engineering Lab, University of Tsukuba, Japan. His Ph.D thesis research, supervised by Professor Motoo Utsumi, focuses on distribution of microcystins in aquatic environments, and covers several aspects including isolation of Cyanobacteria and detection of microcystin production, such as in the present poster. He also has a strong interest in discovering and understanding the mechanisms of distribution and bioaccumulation of microcystins in several aquatic organisms

Clogging up the works: a newly recognized aspect of HAB's

In the minds of most people, Harmful Algal Blooms are associated with phycotoxins and toxicity. This has certainly been the case among water engineers and policy makers in the exponentially growing desalination industry who have only relatively recently become aware of HAB's. Of course, the occurrence of algal blooms and red tides was not unknown to the desalination community but concern for possible residual toxic contamination in desalinated product waters deriving from HAB's in the intake waters of desalination plants has only recently been acknowledged. For example, the title of an invited plenary given by Don Anderson at the European Desalination Society meeting at Barcelona in 2012 was "The global problem of red tides and harmful algal blooms: an emerging threat to desalination plants". Obviously assessing whether current technologies adequately remove toxins in product waters destined for human consumption is of critical importance and has huge economic implications for the desalination industry.

However, there is another impact of HAB's on desalination facilities that has so far scarcely been considered. For years, plant operators have been aware of fouling problems caused by large quantities of biomass from occasional algal blooms entrained in intake water; these can usually be dealt with quite effectively by treating the incoming water with coagulants and/or filtration. Only recently, however, has there been recognition of a more problematic aspect of HAB's occurring in the vicinity of desalination intakes. As HAB's develop, and even more so when they decline, they generate large amounts of unseen organic microgel particles that are now known to be critical drivers of biofilm formation on filtration membranes and other sensitive surfaces [1,2]. Biofilm which leads to clogging of the reverse osmosis (RO) membranes is a major and extremely expensive problem for the desalination industry.

The most extensively studied of these microgels are transparent exopolymer particles (TEP), first described by Alldredge et al. in 1993 [3]. TEP are microscopic, deformable, organic gel-like particles visualized by staining with Alcian Blue, a dye specific for acid mucopolysaccharides. TEP range in size from >0.4 µm to 200-300 µm and appear in many forms; amorphous blobs, clouds, sheets, filaments or clumps (Fig.1). In addition to polysaccharides, many other substances, including proteins, nucleic acids and trace elements can be associated with these microgels. The presence of highly surface active polysaccharides explains the strong tendency of these particles to form metal ion bridges and hydrogen bonds. As a result, TEP are 2 to 4 orders of magnitude more "sticky" than phytoplankton or mineral particles, with a high probability of attachment upon collision. Thus small microgels may aggregate with each other or with other detrital fragments to form marine or lake "snow". In oceans and lakes. TEP are often colonized by bacteria and other microorganisms and may serve as the matrix for "hot spots", sites of intense microbial and chemical activity within the water mass. Extensive research has shown that TEP play extremely important roles in the ecosystem functioning of both marine and freshwater environments, especially in respect to the cycling and transportation of organic carbon [4,5]. Some examples of TEP occurring in Eastern Mediterranean coastal waters are shown in Fig. 1.

These particles may derive from numerous sources. In some aquatic environments, TEP form abiotically from dissolved organic exudation products by processes of coagulation and gela-

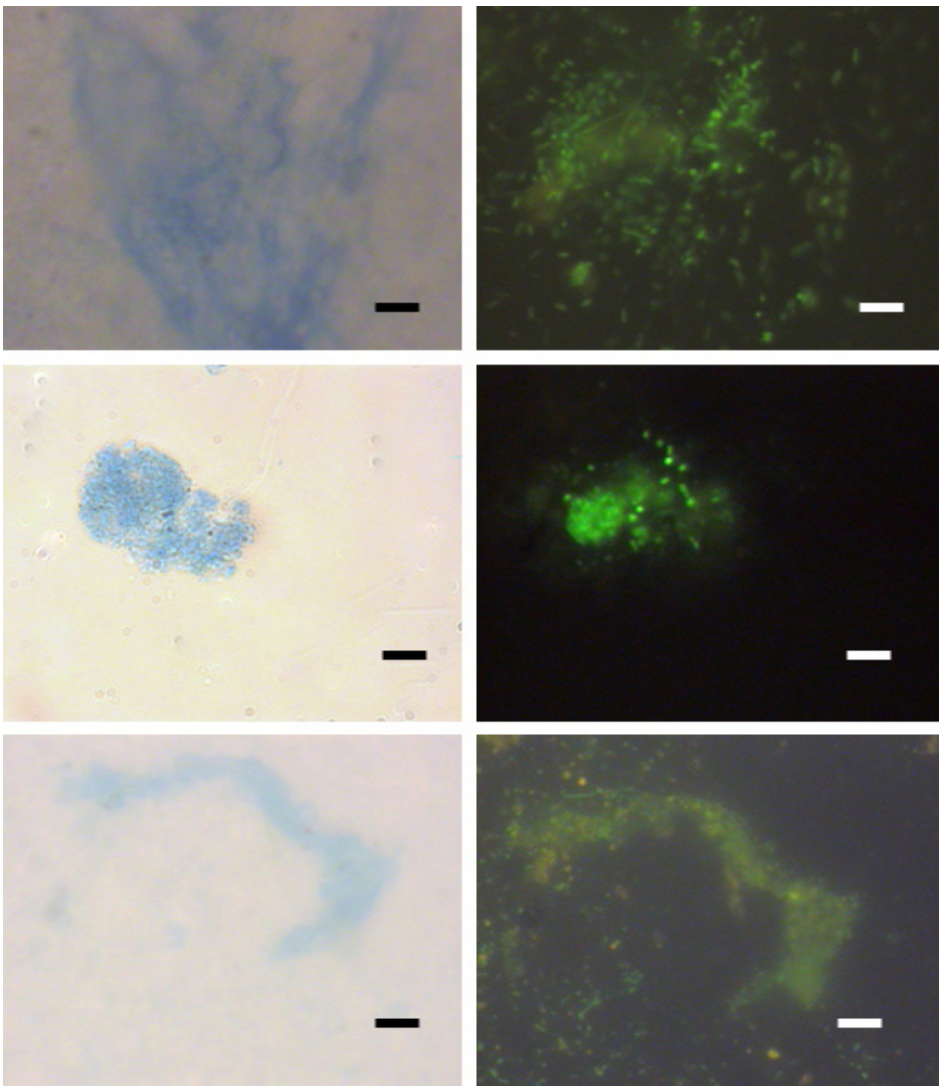


Fig. 1. Transparent Exopolymer Particles (TEP) in eastern Mediterranean seawater. TEP particles when stained with Alcian Blue dye are colored blue under regular microscope illumination (left panels). When the same microscope fields are viewed with ultraviolet epifluorescent illumination (right panels), bacteria staining with SYBR Green appear as bright green spots or rods. The faint green staining of some TEP is probably due to nucleic acids adsorbed to these particles. Scale bars 10 µm.

tion or by bubble adsorption. Considerable amounts of TEP are also produced from the gelatinous envelopes surrounding diatoms and other algae and from bacterial mucous. TEP is also released under stress conditions or at senescence by algae and cyanobacteria [6] or from the breakdown of marine or lake "snow". Therefore, the occurrence and subsequent demise of a HAB in the vicinity of a desalination plant intake would be expected to generate exceptionally high levels of TEP.

Recognition of the involvement of TEP in biofilm formation on filtration membranes and other sensitive surfaces is relatively recent. Based on the known characteristics of TEP, in particular their extreme stickiness and the observation that numerous bacteria were frequently observed on or in many of these particles Berman and Holenberg [7] first suggested that TEP should be involved in the biofouling process. However, it was only in subsequent experimental studies that TEP were actually shown to be actively involved in aquatic biofilm formation and that TEP levels in source water were significantly correlated with the rate at which membranes clogged [8]. Further studies by Bar-zeev et al. [1] led to a revised model for aquatic biofilm formation which included TEP as a significant player in the process. In this work, the term "protobiofilm" was proposed for large TEP heavily colonized by bacteria and other microorganisms on the basis that such microgels possess all the characteristics of biofilm with the exception of attachment to a surface.

Although relatively few data are available, the general impression is that present pretreatment technologies in desalination plants are not particularly effective in dealing with TEP, given the gel-like characteristics of these particles [2]. To date, experimental studies show reduction of only 30 to 50% in the concentrations of TEP reaching the reverse osmosis (RO) membranes that are the critical filtration step in most present day desalination facilitites. If indeed HAB's generate large amounts of these microgels then contamination of the intake waters by TEP may present a much more serious operational problem to the desalination industry than the extremely low levels of toxic residues apparently remaining in the processed waters. In any event, there now appears to be some awareness that HAB's of the biofouling potential of these microgels.

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Author

Tom Berman
Kinneret Limnological Laboratory
Israel Oceanographic and Limnological Research, Migdal, 14950, Israel

Tom Berman died this April while hiking in the Galapagos Islands. He was one of the founding fathers of the Kinneret Limnological Laboratory, its first director from 1967 to 1971, and again from 1986 until 1998, when he retired. He made major contributions to our knowledge of algal nutrient uptake and excretion, the microbial loop, organic nitrogen as a resource for algae, and recently transparent exo-polymers (TEP) and their role in desalination technology. A special session to commemorate him is being organised at the upcoming SIL congress in Budapest, 4-9 August.

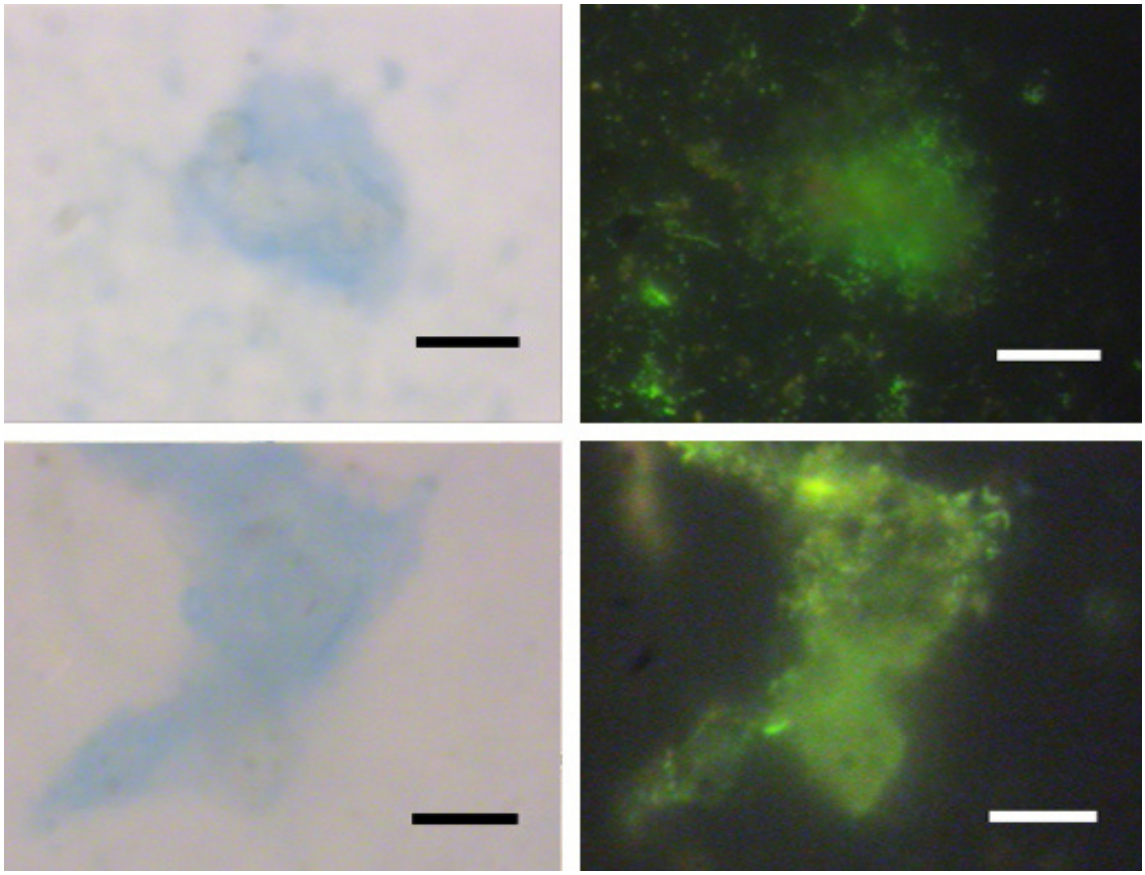

Fig. 1 continued. Transparent Exopolymer Particles (TEP) in eastern Mediterranean seawater. TEP particles when stained with Alcian Blue dye are colored blue under regular microscope illumination (left panels). When the same microscope fields are viewed with ultra-violet epifluorescent illumination (right panels), bacteria staining with SYBR Green appear as bright green spots or rods. The faint green staining of some, but not all, TEP is probably due to nucleic acids adsorbed to these particles. Scale bars 10 µm.

A *Cochlodinium polykrikoides* bloom in El Salvador

Figure 1. Sampling stations in El Salvador coastal waters, from east to west: Gulf of Fonseca, El Cuco, Puerto El Triunfo, Costa del Sol, San Diego, el Muelle, Punta Roca, El Sunzal, El Sonte, Chalpa, Taquillo, La Perla, Los Cóbanos, Garita Palmera. Cochlodinium polykrikoides *present (red), absent (green), (March 2012).*

A bloom of the fish killing dinoflagellate *Cochlodinium polykrikoides* that reached densities of 215 x 106 cell L^{-1} is reported from El Salvador coastal waters. Brown patches 3 km wide and 13 km long, visible by naked eye, developed in March 2012, causing fish mortalities, and social alarm amongst locals and tourists. Environmental conditions associated with this bloom were recorded at monitoring sites (from 13°10´N 87°54´W to 13°40´N 90°02´W) throughout the country.

El Salvador is subject to frequent paralytic shellfish poisoning events associated with blooms of *Pyrodinium bahamense* var. *compressum*. Even moderate cell densities of *Pyrodinium*, not visible to the naked eye, have caused human fatalities and deaths of sea turtles. PSP events in recent years (2010, 2011, 2012) have been detected by the Laboratory of Marine Toxins of the University of El Salvador (LABTOX-UES) and toxins analyzed with a receptor binding assay (1, 2). Blooms of *Cochlodinium polykrikoides* in the Eastern Pacific have been reported before in Costa Rica, Guatemala, Mexico and California (3, 4). During the second week of March 2012, brown patches approaching the surf area were observed by local people from La Libertad. By March 23, overlapping patches formed a 3 km wide band that extended along 13 km of coastline. This event coincided with Easter, when many tourists visited the area, and caused great social alarm. The high densities of *Cochlodinium polykrikoides* caused mortality of benthic fish populations, a putrid smell from decaying organic matter, and severe headaches to visitors and local residents. A member of a group surfing in the affected area who fell and swallowed water in the middle of the patch suffered from diarrhea for three days.

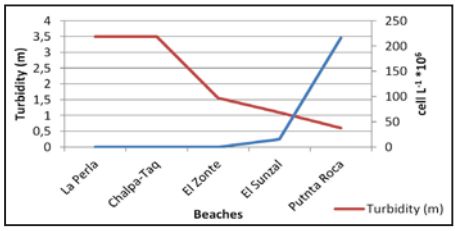

Figure 2. Cell counts and turbidity observed at La Libertad, the area most affected by the Cochlodinium polykrikoides *bloom.*

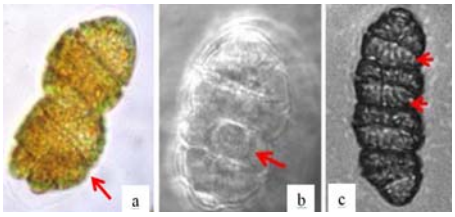

Figure 3. Micrographs of Cochlodinium polykrikoides *collected in El Salvador and criteria used for taxonomic identification. (a) Sulcus very close to cingulum; (b) Large nucleus located in anterior part of epicone; (c) Elongated rod-shaped chloroplasts.*

LABTOX-UES carries out routine monitoring of potentially toxic phytoplankton and phycotoxins along the coasts of El Salvador and had the opportunity to describe this event and warn the relevant health and fisheries authorities. Recreational activities were forbidden in the department of La Libertad to protect the public.

Water samples were taken with 10 L Niskin bottles at 1 and 2 m depth, and phytoplankton by vertical hauls with a 20 µm net. Cells were identified with a Carl Zeiss Axio Imager M1 microscope with phase contrast, and counted in Sedgewick Rafter chambers with an Axiovert 40 CFL inverted microscope. Salinity and temperature were measured with a HACH 56 probe and turbidity with a Secchi disc. Localities sampled are shown in Fig. 1.

During March and April high density patches (up to 215 x 106 cell L^{-1}) of the dinoflagellate *Cochlodinium polykrikoides* were observed (Fig. 1). Samples collected on 23 March revealed that the densest patches were found off Punta Roca and by the pier of La Libertad harbour. Lighter patches were found by the western beaches (Fig. 2). Cell counts decreased from 1.8 x 106 cells L^{-1} on March 29 to 3.3 x 105 cells L^{-1} on May 3. By April 11, patches extended along the entire coast to the Guatemala border. During April and May, a dense patch occurred off Cóbanos, a coral reef area. No *Cochlodinium polykrikoides* were found in samples from May 17 to June 19. Temperatures at 1 m depth were between 26.8 and 28.7°C, and salinity between 34 and 35 psu, characteristic of oceanic waters. Secchi disc depth varied from 0.6 to 3.5 m (Table 1). This is the first record of an ichthyotoxic red tide of in El Salvador waters. The cells were concentrated in the upper 2 m of the water, mainly between Puerto La Libertad and El Zonte beach.

Table 1. Environmental data from beaches affected by Cochlodinium polykrikoides *bloom in March 2012.*

Beaches	T (°C)	Salinity (psu)	Turbidity (m)
La perla	28.3	34.7	3.5
Chalpa	28.4	35.1	3.5
El Zonte	28.3	34.8	1.6
El Sunzal	28.7	35.0	1.1
Punta Roca	26.8	34.8	0.6

Acknowledgements

To CONAMAR, CENDEPESCA, MARN, MSPAS, Alcaldía Puerto La Libertad, the Navy, and students at LABTOX-UES. This work was partially funded by IAEA Technical Cooperation Project ARCAL RLA 7/014 (2009-2013).

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Authors

J. Espinoza[1], O. Amaya[1], B. Reguera[2], Y. Alvarado[1], G. Ruíz[1], D. Escobar[1].

[1]Marine Toxins Laboratory, University of El Salvador, LABTOX-UES, Faculty of Natural Sciences and Mathematics, University City, 25 North End Avenue, San Salvador, El Salvador
[2]Instituto Español de Oceanografía (IEO), Subida a Radio Faro 50, 36390 Vigo, Spain.

Cochlodinium polykrikoides bloom in the Colombian Caribbean

Figure 1

The occurrence of the dinoflagellate *Cochlodinium polykrioides* is reported for the first time in the Colombian Caribbean. Two blooms of this species, with maximal concentrations of $5x10^6$ cell L^{-1}, occurred in the rainy seasons of 2010 and 2011 in Santa Marta Bay. As a result of local currents and winds, the residence times of the blooms were brief, and no adverse effects on local wildlife were observed. The source of these blooms, associated with heavy and persistent rainy seasons and freshwater discharges, is unknown.

In the evening of 25 October 2010, a striking large brown patch of seawater delimited by froth was observed in Santa Marta Bay (Colombian Caribbean coast) (Fig 1). The patch, with a north to south orientation, was driven by the wind into the Bay and adjacent areas, and reached an extension of approximately 6 km^2. The same event recurred on 26 and 29 October and finally on 12 November, 2010. On 31 October and 1 November 2011 patches with similar features were again observed. Phytoplankton samplings were carried out following each patch sighting. Analyses of the samples revealed these patches were constituted by high densities of *Cochlodinium polykrikoides* (Table 1).

These rainy season blooms (October-November) of *P. polykrikoides* occurred with nutrient concentration values within the historic range for the area [1]; nevertheless sea surface temperature was above average and there were sudden declines in salinity. Figure 2 shows the annual variability of these parameters between 4 January 2010 and 23 April 2012. Ahh *et al.* [2] have shown that *C. polykrikoides* blooms in South Korea are associated with increased temperature; they hypothesized that heavy monsoon rains and typhoons, causing heavy floods and input of terrestrial nutrients, are key factors triggering the blooms.

Brown patches of *Cochlodinium* in Santa Marta were sighted in the afternoon and disappeared at night. A local current known as the Panamá-Colombia Countercurrent flows along the Colombian Caribbean coast from SW to NE [3]. This current is more intense in the rainy season, when the northeast Trades are weaker, reaching the coasts of La Guajira in October-November. Data from the meteorological station of the University in Santa Marta (Comarta) showed that when the patches appeared in the Bay, the predominant wind direction was from SSE to SE during the day, reversing to NNW to NW and W at night. All this suggests longshore advection of the blooms from south to north by the Panamá-Colombia Countercurrent as well as cross shelf transport: inshore in the evening, when they were driven into the Bay by local winds, and flushed offshore at night.

Little is known about the origin and causes of the blooms In Santa Marta Bay. Nevertheless the physical conditions associated with them (temperature, salinity, winds, currents, intense and persistent rainy season) suggest that they may have been initiated in the river plumes of the Ciénaga Grande de Santa Marta (a reserve of the biosphere) or of the Magdalena River, and advected to the north by the countercurrent until it reached Santa Marta Bay and the Tayrona Park coast. Persistent high-density blooms of *C. polykrikoides* in semi-enclosed embayments are reported to have a very negative impact

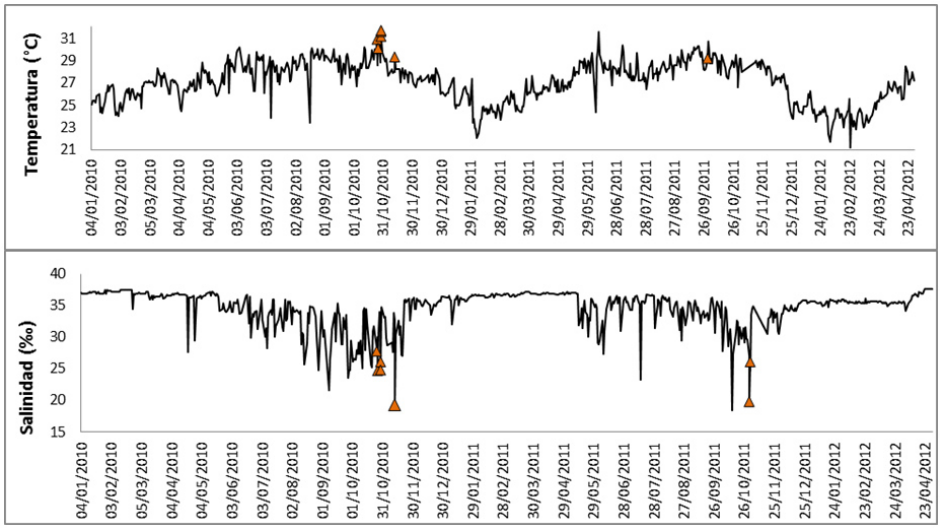

Figure 2. Sea surface salinity and temperature in Santa Marta Bay between 4 January 2010 and 23 April 2012. Orange triangles indicate when brown patches were observed in 2010 and 2011 (http://siam.invemar.org.co/siam/redcam).

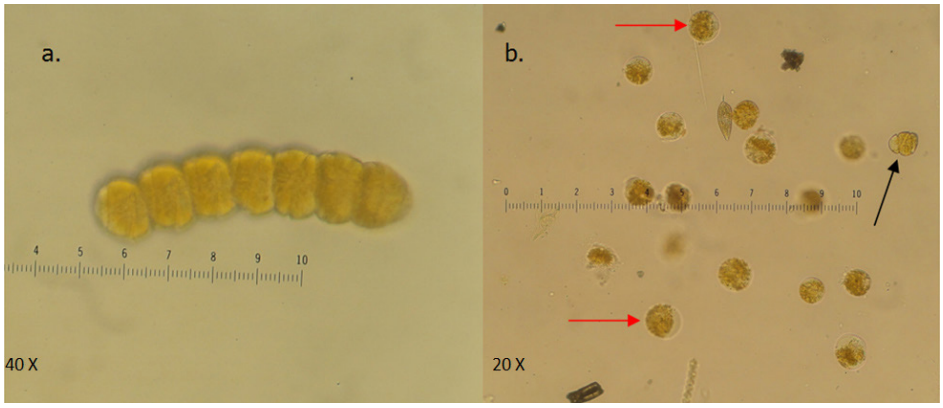

Figure 3. Micrographs of Cochlodinium polykrikoides *(Margalef) from brown patches occurring in Santa Marta Bay (Colombian Caribbean) a) Chain of seven vegetative (naked) cells. Each micrometre unit equals 2 µm. b) Different life cycle stages individual cells of* C. polykrikoides*: red arrows point at thecate and black arrows at naked or vegetative cells showing the cingulum around the cell. Each micrometre unit equals 4 µm.*

on cultivated fish in South Korea [4]. Blooms in Santa Marta were very short-lived and this may explain why harmful effects on the fauna were not reported.

Cochlodinium polykrikoides formed chains of 2-8 cells and sometimes solitary specimens were observed (Fig 3). Cells ranged in size from 30-40 µm long, with a two-lobed hypotheca. Cells were more and more oval as the number of cells per chain increased, but single or paired cells was ellipsoidal. Nucleus in anterior (epicone) position and numerous cylindrical chloroplasts; cingulum descended in distinct left-handed spiral nearly twice around the cell. This description corresponds to that of the naked or vegetative stage in the life cycle of *C. polykrikoides* [5]. Thecate specimens are an intermediate stage leading either to resting cysts or to vegetative cells able to grow in suboptimal conditions [6]. In the Santa Marta blooms, thecate cells became vegetative (naked) cells (Figure 2b), suggesting that the population was actively growing.

Acknowledgements

To the Instituto de Investigaciones Marina y Costeras (INVEMAR) for logistic and finantial support. This work was partially funded by IAEA Technical Cooperation Project ARCAL RLA 7/014 (2009-2013).

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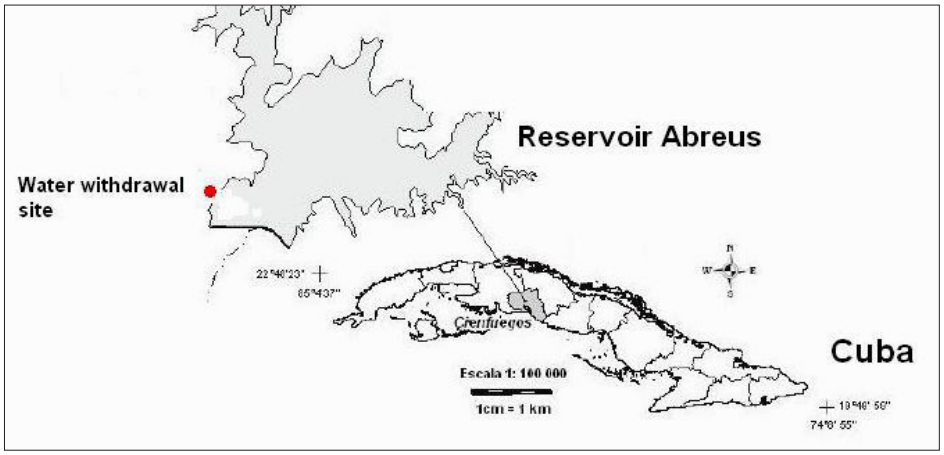
Authors

Andrés L. Malagón, Laura V. Perdomo
Instituto de Investigaciones Marinas y Costeras–Invemar, Cerro Punta de Betín, Santa Marta, Colombia. Apartado aéreo 1016.
anmalagon@yahoo.com.mx
laura.perdomo@invemar.org.co

"Red-tide world" Seoul National University, October 26, 2012

A special symposium on red tides "Red-tide world" was held at Seoul National University, Korea on October 26, 2012 just prior to the 15th ICHA held in Changwon, Korea. Eight talks were given by the invited speakers; "Forecasting the New England red tide: Current status, and ongoing efforts to improve accuracy and usefulness" by **Don Anderson** (WHOI), "Mixotrophy in red-tide organisms" by **Hae Jin Jeong** (Seoul National University), "Dense algal blooms, elevated pH, and regulation of estuarine biogeochemical processes" by **Diane Stoecker** (U of Maryland), "Long-term variations of *Heterosigma akashiwo* in Narragansett Bay and its environmental control" by **Ted Smayda** (U of Rhode Island), "The red-tide ciliate *Mesodinium rubrum* in the Korean coastal waters" by **Wonho Yih** (Kunsan National University), "Harmful algal blooms, Shellfish aquaculture and restoration" by **Sandy Shumway**, "New perspective on the relationship between harmful algal blooms and cyst germination in Korean waters" by **Myung-Soo Han** (Hanyang University), and "Taxonomy and phylogeny of the dinoflagellates. A new start based on ultrastructure and molecules" by **Øjvind Moestrup** (U of Copenhagen).

Cyanobacterial bloom in the Abreus Reservoir, Cienfuegos, Cuba



The Abreus Reservoir, constructed in 1985 is located on a very important basin in the Central Province of Cienfuegos. Its main tributaries are Damují, Jabacoa and Limones. The reservoir was originally planned for agricultural and industrial purposes, but in recent years supplies tap water to residential sectors of Cienfuegos.

In March 2009 a typical water bloom formed exclusively by *Microcystis paniformis* was recorded in the reservoir [1]. The event provoked a 'Territorial Investigation Project' to determine the water quality of the reservoir, and included a phytoplankton monitoring programme. As a preliminary result of this investigation, in October 2010 another water bloom with 1.1 x 107 cell L^{-1} was detected at the water withdrawal site of the reservoir (see Table 1), but now composed mainly of filamentous cyanobacteria, species of *Anabaenopsis, Arthrospira, Cylindrospermopsis, Dolichospermum* and *Raphidiopsis*: no toxic events were recorded.

The species found are known mainly from tropical regions; hence some taxonomical comments of these populations may be of interest.

Table 1: Some characteristics of superficial water from water withdrawal site, Abreus reservoir, Cienfuegos, Cuba. October 2010.

Conductivity	381,3 µS/cm
$O_2D.$	8,64 mg/L
pH	7,68
Temperature	27,5°C
$N\text{-}NO_3$	32 mg/L
$N\text{-}NH_4$	0,045 mg/L
$N\text{-}NO_2$	6,1 mg/L
$P\text{-}PO_4$	0,016 mg/L
Chlorophyll a	23,9 mg/L

***Anabaenopsis* sp. (Figs. 1-2)**

Characterized by solitary trichomes, 5.6-7.5 µm wide, short, irregularly coiled, constricted at the cross-walls, without mucilaginous sheaths; vegetative cells ± barrel-shaped to short cylindrical usually with aerotopes; heterocytes terminal, spherical, rare broad oval, 5,6-8,7 µm in diameter; akinetes absent. Abundance in the samples: 1.3 x 105 cell L^{-1}. No akinetes were observed, thus it is not possible identify the species, but some features suggest *A. arnoldii* Aptekar, but the mucilaginous sheath of this species is lacking in our specimens. *A. tanganyikae* (G.S.West) Wolosz. et Mill. has also been found in Cuba [2, 3], but is morphological quite distinct.

***Arthrospira* cf. *khannae* Drouet et Strickland in Drouet 1942 (Figs. 11-12)**

Trichomes with regular loose screw-like coils, solitary, free floating, blue green, 2.8-5.6 µm wide, unconstricted or very slightly constricted at the granulated cross-walls, spirals 22.4-30.8 µm wide, 44.8-58.8 µm length; terminal cells rounded, very slightly capitate; cell content usually with aerotopes. Abundance in the samples: 2.3 x 105 cell L^{-1}. This species is related to *A. khanne*, but the trichomes are not attenuated at the ends and the spiral dimensions are distinct, typically 20-22 µm wide, 20-35 µm long. Our specimens resemble *A. massartii* Kufferath, but this species is benthic, without aerotopes. The typical *A. khannae* has a tropical distribution, from India, Vietnam and Zambia [4].

***Cylindrospermopsis curvispora* Watanabe 1995 (Figs. 3-4)**

Trichomes slightly constricted at cross walls, irregular spiral coils, but circular in outline, 4.2-5 µm wide; cells cylindri-

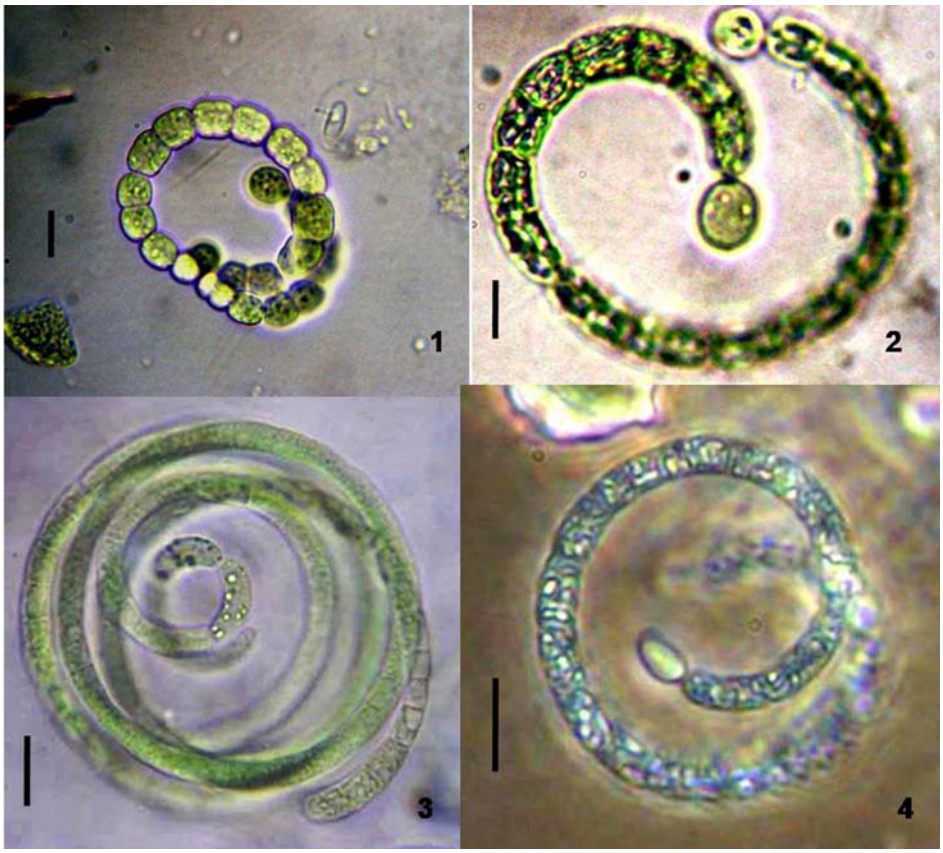


Fig. 1-4: 1-2) Anabaenopsis *sp., 3-4)* Cylindrospermopsis curvispora *Watanabe. Scale = 10 µm.*

cal, 7.5 x 5 µm; terminal cells cylindrical rounded; cell content usually with aerotopes; heterocytes terminal, conical ovoid, rounded at ends, 6.7 x 2.5 µm; akinetes 10 x 2.5 µm, cylindrical-kidney-shaped, sometimes curved, mostly in pairs, distant from heterocytes. Abundance in samples 1.4 x 106 cell L^{-1}. This species, known from central Japan, Sri Lanka and southern Africa [5], was previously recorded in Cuba from Laguna Las Canteras, Cienfuegos [1].

***Dolichospermum* cf. *flos-aquae* (Lyngbye) Wacklin et al. 2009 (Figs. 5-6)**

Trichomes solitary, free floating, constricted at cross walls, irregular spiral coils, but circular in outline, 5.6 µm wide; cells ± spherical, with aerotopes; heterocytes spherical, 7 µm in diameter; akinetes distant from heterocytes, cylindrical oval, 14-19.6 x 8.4 µm. Abundance in samples: 6.0 x 105 cell L^{-1}. This planktonic species is known from eutrophic water bodies around the world [6]. The Cuban specimens have smaller akinetes, not kidney-shaped.

***D.* cf. *solitarium* (Klebahn) Wacklin et al. 2009 (Figs. 7-8)**

Trichomes solitary, free floating, ± straight, constricted at cross walls, 5.6 µm wide; cells ± spherical, with aerotopes; heterocytes spherical, 5.6-8.4 µm in diameter; akinetes distant from heterocytes, cylindrical, 14-19.6 x 5.6-8.4 µm. Abundance in samples: 6.0 x 106 cell L^{-1}. Typical specimens with wider trichomes, vegetative cells citriform and akinetes larger, are known mostly from northern regions of temperate zones [7].

***Raphidiopsis curvata* Fritsch et Rich 1929 (Figs. 9-10)**

Trichomes solitary, free floating, isopolar, 2.8-4.4 µm wide, unconstricted at the cross-walls, irregularly curved to sigmoid, relatively long, gradually tapering to ends; terminal cells elongate pointed; cells cylindrical, one to two times longer than wide; akinetes cylindroids, 8.4 x 2.8 µm. Abundance in samples: 2.5 106 cell L^{-1}. Raphidiopsis Fritsch et Rich is ill-defined and polymorphic, and may be a non-heterocytous stage of other genera such as *Cuspidothrix* or *Cylindrospermopsis*. According to Li et al [8], *Raphidiopsis* and *Cylindrospermopsis* are phylogenetically related, but the identity of these genera is not yet clear [9].

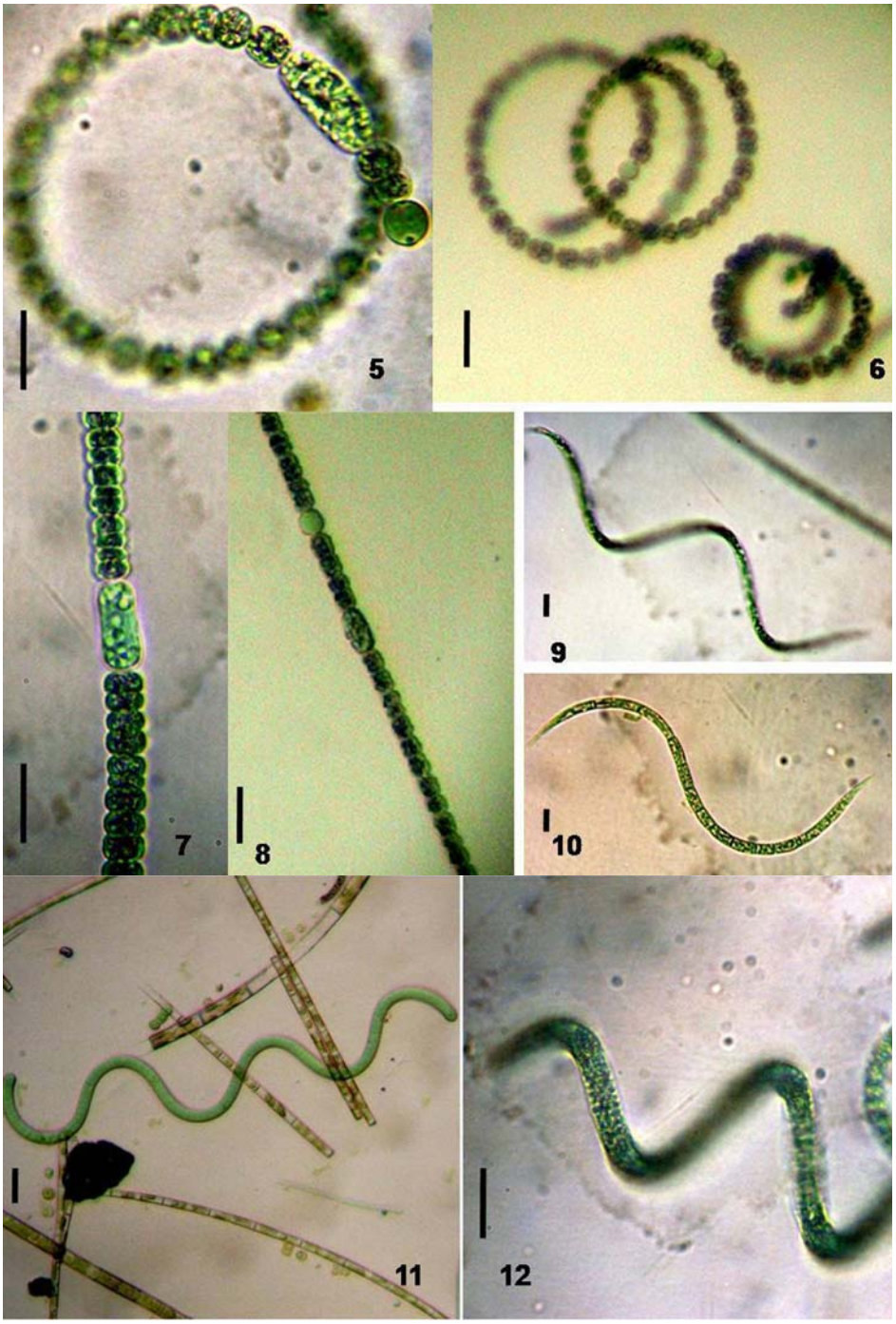


Fig. 5-12: 5-6) Dolichospermum *cf.* flos-aquae *(Lyngbye) Wacklin et al., 7-8)* D. *cf.* solitarium *(Klebahn) Wacklin et al., 9-10)* Raphidiopsis curvata *Fritsch et Rich., 11-12)* Arthrospira *cf.* khannae *Drouet et Strickland in Drouet. Scale = 10 µm.*

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Authors

Augusto Comas González & Ángel Moreira González
Centro de Estudios Ambientales de Cienfuegos (CEAC), Calle 17 esq. Ave 46 s/n. Reparto Reina, Cienfuegos 55100, Cuba.
Email: augusto@gestion.ceac.cu

Gambierdiscus and related benthic dinoflagellates from Madeira archipelago (NE Atlantic)

Ciguatera fish poisoning (CFP) has been of increasing concern in recent years since the expansion of this food-borne disease from tropical and subtropical areas of the Pacific and Caribbean to warm temperate areas like Macaronesia (Azores, Madeira, Canary and Cape Verde Islands) and the Mediterranean [1, 2]. Often associated with the CFP-causing dinoflagellates, *Gambierdiscus* spp., are the palitoxin-producing *Ostreopsis* spp., which were also recently recorded from new areas and of concern to human health issues [3].

In 2005, the occurrence of *Ostreopsis* cf. *siamensis* from Madeira (~32.5°N, 17°W) (Fig. 1) was reported for the first time [2]. The sample was collected in 2002 by Fraga (pers. communication) and cultured since then in Vigo, Spain, under strain numbers VGO610, VGO611, VGO613 and VGO614. After molecular studies, they were reassigned to *Ostreopsis* cf. *ovata* [4]. In summer 2007, several wardens from the Natural Park Madeira stationed at the Selvagens Islands, about 300 km to the south of Madeira Island (Fig. 1), suffered light ciguatera-like intoxications after eating locally caught fish (*Seriola* sp., *Sparisoma cretense, Serranus atricauda, Bodianus scrofa, Balistes capriscus* and *Pagrus pagrus*). A more severe CFP incident was reported by fishermen in summer 2008 after eating a *Seriola* sp., also caught around the Selvagens Islands [5]. Immediately after that episode, water samples were collected by the national monitoring laboratory INRB-IPIMAR, and *Ostreopsis* sp., *Amphidinium* sp. and *Prorocentrum* sp., were detected. *Ostreopsis* sp. was reported with cell concentrations as high as 700 cells L^{-1} [6].

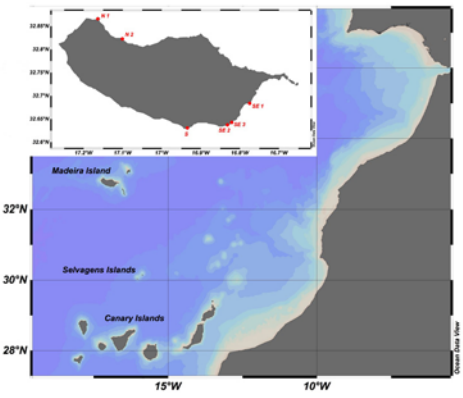

Fig. 1. Madeira archipelago including Selvagens Islands; insert: Madeira Island showing sampling sites.

Since then, CFP-like episodes have been reported almost every year [7, 8]. Several different ciguatoxins were detected in *Seriola* spp. caught in 2009 at the Selvagens Islands (a CTX of the CTX-3C group; a CTX analog, similar to C-CTX-1, C-CTX-2, I-CTX-1, and I-CTX-2; CTX-4A/B or an analogue) [9].

In light of these incidents, we sampled different seaweed around Madeira to enumerate *Ostreopsis* at 2 sites in the north (N 1-2), aad 3 sites in the southeast (SE 1-3), and at 1 site in the south (S) during occasional SCUBA-dives from 2008 to 2012 (Tab. 1, Fig. 1). All samples were preserved immediately with 4-5% formaldehyde in seawater until analysis. In the laboratory the samples were vigorously shaken during one minute to detach epiphytic dinoflagellates, and passed through a cascade of different meshes to retain the larger particles. *Ostreopsis* sp. (Fig. 2) cells were counted by light microscopy using a Sedgewick Rafter S52 counting chamber. The seaweeds were identified, blotted to remove excess water and wet weights determined; they varied from 0.5 to 77.6 g. Sea surface temperatures around Madeira usually range from 17°C in February/March to 24°C in September.

Cell numbers (Table) range from zero to 12639 cells per g wet weight, found in November 2009 at site S. Compared with other studies conducted during bloom phases, where cell numbers reached one to two million cells per g wet weight (e.g., 1.4 x 106 cells per g wet weight in New Zealand [10]), the maximum of present study is low.

Higher cell numbers were found at site S and very low cell numbers at sites N. At sites SE, both zero and high num-

Table 1 Ostreopsis *sp. cell numbers on seaweed substrate (cells per g wet weight ± 95% confidence limit) at sites around Madeira, 2008 to 2012.*

Sample #	Date	Site	Type of substrate	Cells g^{-1} WW
1	22.10.2008	S	*Stypopodium zonale*	158 ± 74
2	22.10.2008	S	turf (cf. *Jania*)	1176 ± 208
3	26.10.2008	SE 1	turf	0
4	26.10.2008	SE 1	Aufwuchs	11752 ± 2850
5	05.11.2008	S	Aufwuchs/*Sargassum vulgare*	1601 ± 380
6	07.12.2008	N 1	cf. *Lophocladia*	0
7	07.12.2008	N 1	cf. *Lophocladia*	0
8	07.12.2008	N 2	*Zonaria tournefortii*	86 ± 86
9	07.12.2008	N 2	*Gracilaria* sp.	5 ± 10
10	02.05.2009	SE 2	*Z. tournefortii*/cf. *Laurencia microcladia*	0
11	02.05.2009	SE 2	turf	0
12	05.06.2009	S	*Padina pavonica*	919 ± 141
13	05.06.2009	S	Aufwuchs/*P. pavonica*	3423 ± 437
14	05.06.2009	S	*P. pavonica*/*Halopteris filicina*/*S. vulgare*	2580 ± 188
15	08.06.2009	SE 3	*Corallina*	108 ± 43
16	08.06.2009	SE 3	turf	0
17	30.07.2009	S	turf	667 ± 162
18	03.11.2009	SE 3	Aufwuchs	2360 ± 357
19	03.11.2009	SE 3	*Dictyota*	2857 ± 415
20	18.11.2009	S	turf	4150 ± 340
21	18.11.2009	S	*Corallina*/turf	12639 ± 1105
22	08.06.2011	S	Aufwuchs	2962 ± 1140
23	12.05.2012	SE 3	*Dictyota*	1706 ± 194
24	12.05.2012	SE 3	*S. zonale*	417 ± 77
25	12.05.2012	SE 3	turf	702 ± 146
26	15.05.2012	S	*Stypocaulon scoparium*	487 ± 103
27	15.05.2012	S	cf. *S. vulgare*	303 ± 83
28	15.05.2012	S	turf/*Corallina*	8504 ± 487
29	14.08.2012	S	turf	11312 ± 1034
30	14.08.2012	S	turf/red algae	2856 ± 501

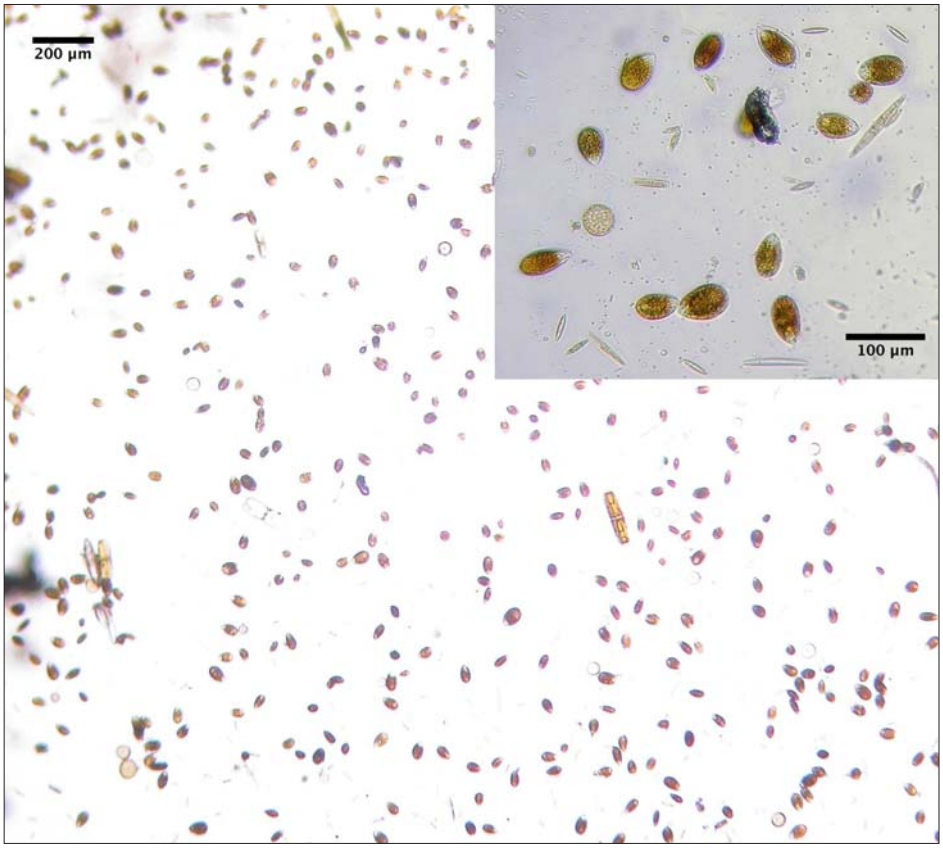

Fig. 2. Field sample of Ostreopsis *sp., LM, scale bar 200 µm, scale bar insert 100 µm*

bers of *Ostreopsis* cells were observed. It is likely that higher water temperature and a higher concentration of nutrients, as well as a less exposed environment favored the proliferation of *Ostreopsis* sp. [10-12] at sampling sites S and parts of SE. In general, the south coast is less exposed to the predominantly northeastern swell.

Higher numbers of *Ostreopsis* were found mainly on very thin layers or turf substrates and less on erect, frondose seaweeds. The higher surface to volume ratio might be the reason for higher abundance of *Ostreopsis* sp. on thin and turf seaweed layers [13].

During counting and when observing live samples, species of *Prorocentrum* (Fig. 4), *Amphidinium, Coolia*, and *Gambierdiscus* were detected. The latter was observed in a fixed sample from October 2008, and recently (August 2012) in a live field sample (Fig. 5). This is the first record of *Gambierdiscus* sp. in the archipelago. Further studies are needed to determine the species. Recently, *G. excentricus* was described as a new species in the Canary Islands [14], so similar assemblages may be present in the nearby Madeira archipelago where oceanographic conditions are similar.

Our findings confirm the recent spread of more tropical HAB-species into warm temperate waters, which may be a response to climate change, introductions by ship traffic, and eutrophication of coastal areas [15, 16].

Acknowledgements

During SCUBA-dives M. Maranhão, P. Wirtz, F. Alves, F. Peine, J. Packmor and P. Neves assisted. A. Neto confirmed the seaweed identifications.

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Authors

Manfred Kaufmann
University of Madeira, Marine Biology Station of Funchal, 9000-107 Funchal, Madeira Island, Portugal & CIMAR/CIIMAR – Centre of Marine and Environmental Research, Rua dos Bragas, 289, 4050-123 Porto, Portugal
Marianne Böhm-Beck
University of Bremen, Department 2, Marine Botany, Germany

Fig. 3. Ostreopsis *sp., LM, scale bar 25 µm*

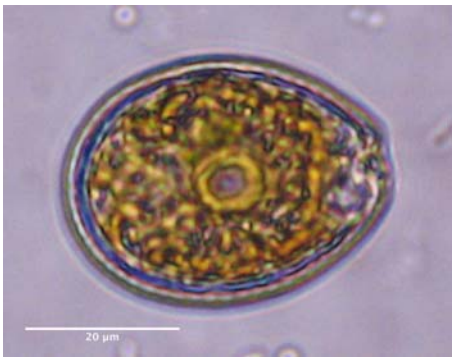

Fig. 4. Prorocentrum *sp., LM, scale bar 20 µm*

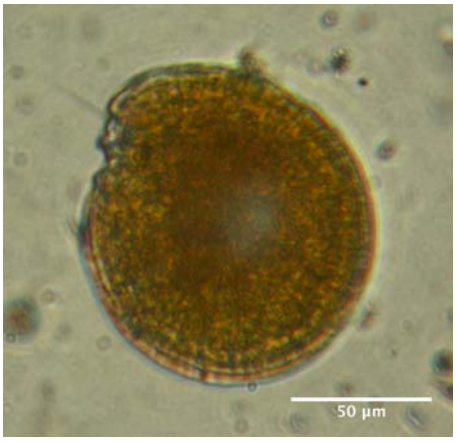

Fig. 5. Gambierdiscus *sp., LM, scale bar 50 µm*

A *Phaeocystis* bloom in the Cuban Archipelago

Fig.1. Cayo Largo del Sur, south-western platform of Cuba

The success of *Phaeocystis* and its impacts on marine ecosystems have been linked to their capacity to form large gelatinous colonies [1]. *Phaeocystis* colonies produce hemolytic substances, and are responsible for clogging fishing nets, repelling fish, for oxygen depletion, and for negative impacts on benthic life. These blooms affect tourism and recreational activities due to the water discoloration and deposition of thick layers of odorous foam on beaches [2].They also produce dimethyl sulphide, which produces cloud-condensation nuclei, increasing cloud cover and affecting regional climates [3].

During late March 2012, a bloom of *Phaeocystis* sp. occurred in Cayo Largo del Sur, a small cay in Los Canarreos archipelago, on the south-western Cuban platform (Fig. 1). This site is highly hydrodynamic coast. A red-brown water discoloration persisted for 15 days. The bloom occurred within 200 m of the shore and extended along 22 km of coast. As well as discoloration, abundant foam and mucilage were observed (Figs. 2-5).

Seawater was sampled for phytoplankton and physico-chemical analyses. The ratios Dissolved Inorganic Nitrogen/Dissolved Inorganic Phosphorus and Total Nitrogen/Total Phosphorus were calculated. The algal samples were studied using a light microscope Laborlux, Leica-Leitz with phase contrast equipped with Motic digital camera.

We observed large colonies formed by cells embedded in a firm gelatinous matrix. Cells are slightly kidney-shaped to irregular ellipsoidal to sphaeroidal, 5-6 µm in diameter, with two yellow-green to brownish, parietal plastids (Figs. 6-12). Our alga corresponds well with the generic description of *Phaeocystis*, however the species identification needs further studies.

High concentrations of ammonium and organic nitrogen were recorded along beaches. The high organic nitrogen/total phosphorus ratio indicates the presence of excess nitrogen (Table 1 and 2). *Phaeocystis* blooms are reported in nitrate-rich areas, either naturally or due to anthropogenic inputs [4]. High dissolved oxygen and chemical oxygen demand indicated the presence of a microalgae bloom with high production of organic matter.

Several factors could contribute to the bloom occurrence. Among them, sea surface temperature was slightly warm for this period, with temperature between 26.2 and 27.5 °C [5]. Strong winds (40 km h^{-1} with maximum intervals of 70 km h^{-1}); the velocity, dynamic, and direction of currents to the area (Fig. 13), from east to west could also increase nutrients (organic and inorganic) available in the Jagua depression [6]. But cultural eutrophication due to anthropogenic activities in Cayo Largo del Sur cannot be ruled out.

Sahara dust might be another factor involved in the occurrence of the *Phaeocystis* bloom. Two weeks before the bloom, the southern coast of Cuba

Fig. 2-5. Water discoloration and foam.

Table 1. N, P and Si nutrients at five beaches from Cayo Largo del Sur (DIN = Dissolved Inorganic Nitrogen, DON = Dissolved Organic Nitrogen, TN = Total Nitrogen, TP = Total Phosphorus).

Beaches	NO_2^- + NO_3^- µmol/L	NH_4^+ µmol/L	DIN µmol/L	DON µmol/L	TN µmol/L	PO_4^{3-} µmol/L	TP µmol/L	SiO_4^{4-} µmol/L
Sirena	0,07	0,35	0,42	35,99	36,41	0,04	0,25	1,78
Mal Tiempo	0,60	2,40	3,00	47,88	50,89	0,22	0,68	1,19
Blanca	0,28	0,04	0,32	42,39	42,72	0,07	0,51	1,53
Los Cocos	0,29	0,70	0,99	48,26	49,25	0,16	0,78	1,02
Tortuga	0,10	0,87	0,96	63,46	64,42	0,10	0,15	3,64

Table 2. DIN:PO43- and TN:TP ratios at five beaches from Cayo Largo del Sur.

Beaches	DIN/PO_4^{3-}	TN/TP
Sirena	11	144
Mal Tiempo	13	75
Blanca	5	83
Los Cocos	6	64
Tortuga	10	439

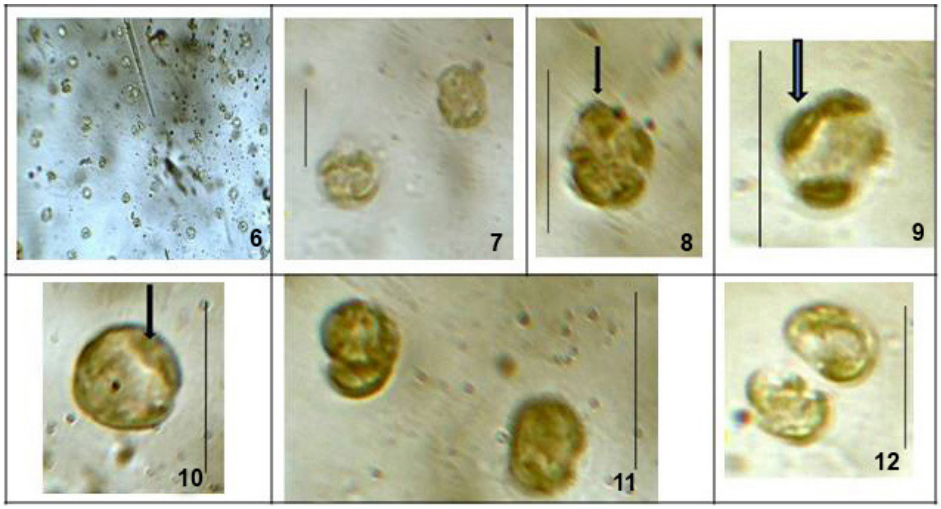

Fig.6. Interior detail of colonies. Fig. 7-12. Different forms of colony cells (arrows=chloroplasts). Scale = 10 µm

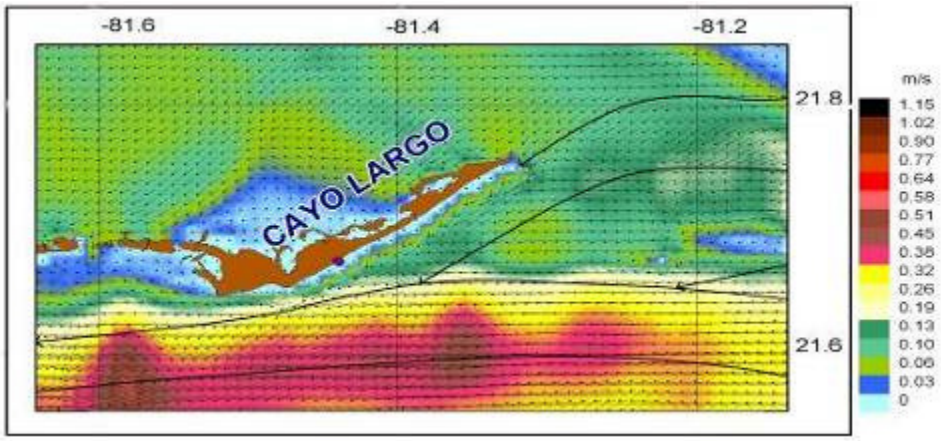

Fig. 13. Offshore currents around Cayo Largo del Sur, south-western platform of Cuba (March 22, 2012).

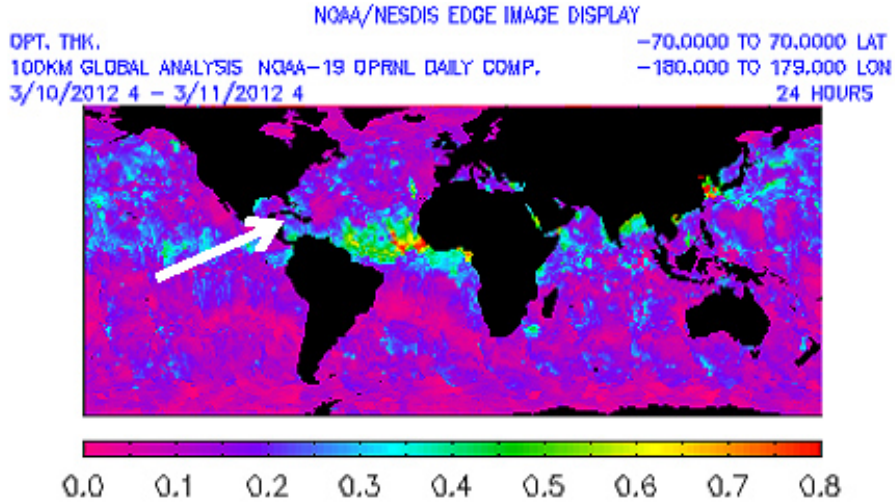

Fig. 14. Inputs of Sahara dust in the south-western coast of Cuba (during two weeks before the Phaeocystis *bloom).*

received inputs of Sahara dust (Fig. 14). It has been argued that desert dust provides iron which contributes to phytoplankton blooms in tropical and subtropical coastal environments [7].

No major mortality of marine organisms was observed in the key; however the bloom affected tourism because of the presence of abundant foam, water discoloration and bad odours. To our knowledge, this is the first record of a *Phaeocystis* bloom in the Caribbean islands.

Acknowledgements

To the Ministry of Tourism in Cayo Largo del Sur and Ministry of Science of Cuba. To the IAEA Regional Project RLA-7-014.

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S. Loza [1], A. Moreira[2], A. Comas[2], M. Sánchez[1], M. Carmenate[1], B. Álamo[1], V. Dobal[1], J. Montalvo[1], I. García[1], S. Lorenzo[1], L. Arriaza[1], M. Hernández[1], D. Montesinos[1], J. Gutiérrez[1] and P. Alcolado[1]

[1]Instituto de Oceanología, Avenida 1era, No.18406, Reparto Flores, 19100, La Habana, Cuba. E-mail: sandra@oceano.inf.cu
[2]Centro de Estudios Ambientales de Cienfuegos (CEAC), Apdo. 5, 59350, Cienfuegos, Cuba.

Gambierdiscus in Kuwait

The diversity of benthic dinoflagellates along Kuwait's shore has been well documented recently [1, 2], but with emphasis on sand-dwelling intertidal species and hyper-saline habitats [3]. During recent surveys of the coastal benthic biota along Kuwait's shore initiated by Kuwait Institute for Scientific Research (KISR) in November 2011 – December 2012, various coastal habitats were examined and numerous samples including intertidal and subtidal sediments, seagrasses, macroalgae, and algal turf were collected.

On 17 November 2012, subtidal sandy sediments were collected within small inshore Qit'at Julai'ah coral reef (28°53'4''N; 48°16'38''E) on the southern Kuwait's coast (Fig.1). Sediments and near bottom water were sampled by snorkeling at 2 m depth. In both sediment and water samples, we found low numbers of globular dinoflagellate cells that resembled *Gambierdiscus*. Similar cells were found epiphytically on floating brown macroalgae from two sampling sites to the south of the Khiran in a small semiclosed lagoon (28°38'6''N; 48°23'28''E), and on the shore (28°37'47''N; 48°23'30''E) on 24 November 2012 (Fig. 1). *Gambierdiscus* cells were present in two of the 16 macroalgae samples analyzed, and were observed in low abundance on *Padina tetrastomatica* (6 cells g^{-1} wet weight) and on *Sargassum oligocystum* (4 cells g^{-1} wet weight). *Gambierdiscus* was found at water temperatures of 23.5-25°C and at salinities of 41.2-42.4. It occurred along with other epiphytic dinoflagellates of the genera *Amphidinium, Coolia, Ostreopsis,* and *Prorocentrum*.

Examination by light and epifluorescent microscopy allowed detailed observation of morphology and plate tabulation (Fig. 2). Cells were photosynthetic with numerous golden-brown chloroplasts, globular in shape in lateral view, oblong in apical/antapical view and slightly laterally flattened, with dome-shaped epitheca and truncate-conical hypotheca. Cells had a depth of 54-73 µm, a width of 46-61 µm, and a length of 49-70 µm, with an average width-to-depth ratio of 0.86 and length-to-width ratio of 1.14. The pre-median cingulum was deeply excavated, descending, and was displaced by one x its width. The apical pore plate (Po) was elongated, characteristically fish-hook shaped and centrally located. The second antapical plate 2'''' was fork-shaped and rather large, with robust and beveled fork edges. Based on the morphological characteristics of the

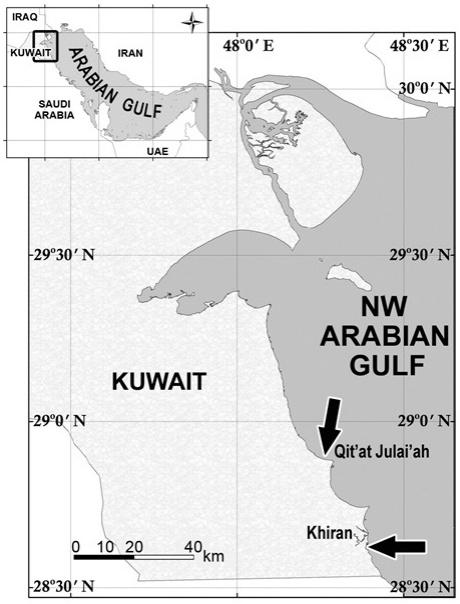

Fig. 1. Map of Kuwait showing where Gambierdiscus yasumotoi *was detected.*

cells, the globular *Gambierdiscus* species from Kuwait was assigned to *G. yasumotoi* Holmes 1998. Following the original description [4] and key to the species of *Gambierdiscus* [5], the cell shape, dimensions, plate tabulation and structure of the 2'''' from Kuwait's material confirmed the identification and allowed to distinguish our finding from the other globular *Gambierdiscus* species, *G. ruetzleri* [5].

Since the original description of *G. yasumotoi* from macroalgae on the coral reef of Pulau Hantu Island (Singapore) [4], its distribution has been referred to as tropical Western Pacific [5-7]. However, recently the globular *Gambierdiscus* species morphologically close to *G. yasumotoi* has been reported from coastal waters of Pakistan, sub-tropical North-West Indian Ocean [8]. The detection of *G. yasumotoi* in Kuwait's coastal area is the first record of the possible ciguatera agent in the Arabian Gulf, and the second report of this species from the Indian Ocean.

G. yasumotoi produces a maitotoxin-like compound lethal to mice [4], and its toxicity has been confirmed by detection of toxic strains in Indonesian waters [7]. The detection of this toxigenic dinoflagellate in Kuwait indicates the potential for future occurrence of Ciguatera Fish Poisoning (CFP).

G. yasumotoi was recorded in Kuwait coastal habitats in low numbers, but its density may be underestimated from our samples: it was found on various substrates, in sediment, and attached to floating macroalgae. While attached

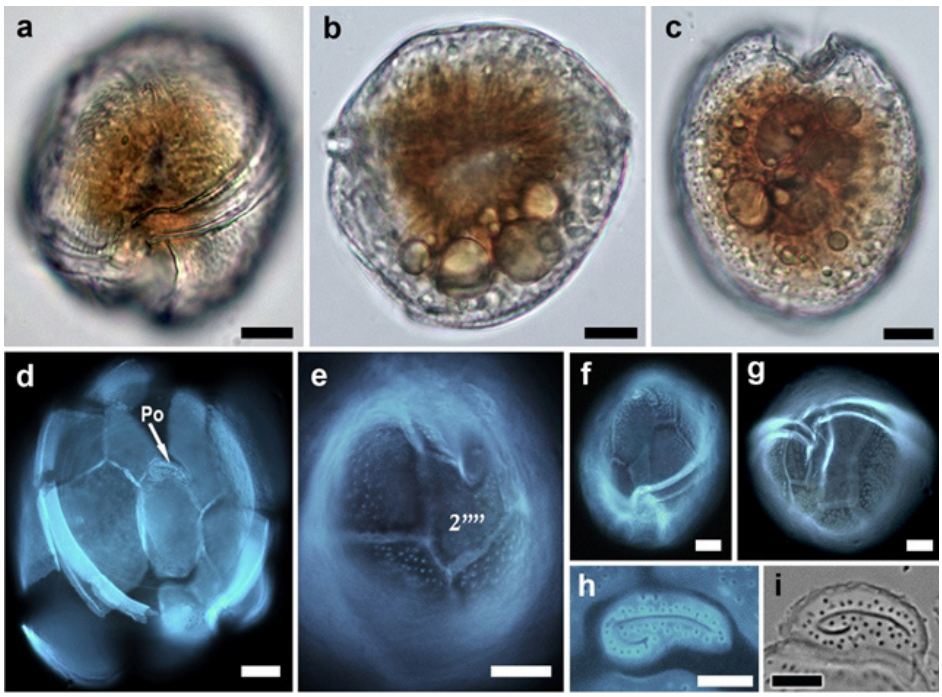

Fig. 2. Gambierdiscus yasumotoi *from Kuwait. a-c - light microscopy of cell in ventral (a), lateral (b) and antapical (c) view; d-g – epifluorescence image of squashed theca in apical view (d), cell in antapical view with the diagnostic 2'''' plate (e), and cell in apical (f) and ventral (g) view; h-i – apical pore plate (Po). Scale bar = 10 µm (a-g), 5 µm (h, i).*

macroalgae in shallow habitats are the prefered hosts of *Gambierdiscus*, their detached floating branches may be exposed to wave action, leading to detachment of the epiphytic dinoflagellates.

The southern part of Kuwait's marine area closely resembles the preferred habitats reported for *Gambierdiscus* [9] in terms of annual water temperature, salinity, hydrological features and available substrates to which cells can attach. Kuwait's coast stretches for about 180 km, occupying the northwestern corner of the Arabian Gulf in latitudes between 30°N and 28°30'N (Fig. 1). Hypersaline, shallow and subtropical environments provide a broad range of habitats, from extensive mudflats in the north to numerous sandy and rocky beaches extending to the Saudi Arabian border. Moreover, some of the world's most northerly coral reefs are located within southern Kuwait waters, and *Sargassum* grows in a discontinuous linear strip parallel to the coast where appropriate substrates exist. The average annual seawater temperature is 23.8°C, varying seasonally from 14°C during winter up to 30.5°C in summer, whereas the southern and offshore waters are generally warmer with an annual average temperature of 25.6°C [10].

Kuwait appears to be free of ciguatera; there are no official reports of CFP in this area to date [11], and *Gambierdiscus* has never been recorded here previously among other potentially ciguatera-related benthic dinoflagellates from the genera *Ostreopsis* and *Prorocentrum* [12, 13]. Our detection of *G. yasumotoi* in Kuwait is the first record of possible agent of CFP in the NW Arabian Gulf, and indicates a need for further studies of benthic dinoflagellate assemblages in the region.

Acknowledgements

We thank Tatiana Mikhailova (Komarov Botanical Institute, St.-Petersburg, Russia) for her help in macroalgae identification.

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Authors

Maria Saburova, Institute of Biology of the Southern Seas, National Academy of Science of Ukraine, Pr. Nakhimova, 2, 99011, Sevastopol, Ukraine, Email: msaburova@gmail.com

Igor Polikarpov & Faiza Al-Yamani, Kuwait Institute for Scientific Research, PO Box 1638, Salmiyah-22017, Kuwait

Bloom of *Pyrodinium bahamense* var. *compressum* in Ambon Bay, eastern Indonesia

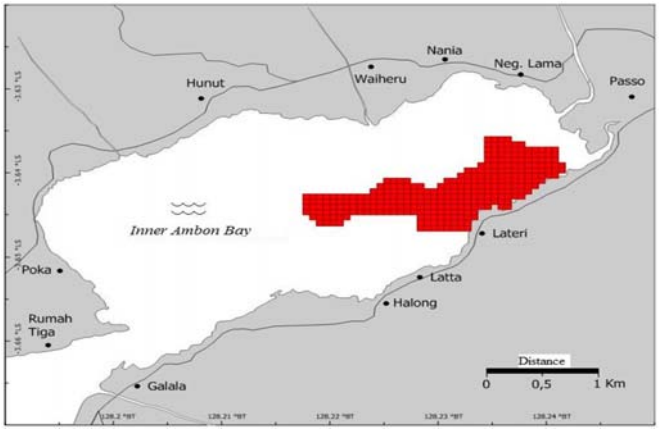

Fig. 1. Bloom area of Pyrodinium bahamense *var.* compressum *in Inner Ambon Bay, July 2012.*

Ambon is a small island located in eastern Indonesia with a population of about 600,000 people. It has a tropical climate influenced by northeast and southeast monsoons. Annual rainfall is approximately 600 mm occurring from May to September, associated with the southeast monsoon season when cooler air from Australia blows to this area. Dry season is associated with the northwest monsoon from October until March, characterized by increased air temperature and less rainfall.

Ambon Bay is an estuary with a sill; the inner bay averages 30 m depth and about 6 km^2 in area. The outer bay, which opens to the Banda Sea, is about 25 km long. The narrow and shallow sill, 15 m deep and 300 m wide, limits water circulation in inner Ambon Bay, making it susceptible to stagnation [1]. Increased development in the Bay and associated pollution inputs may alter the chemical regime of the inner bay.

Pyrodinium bahamense var. *compressum* is responsible for paralytic shellfish poisoning (PSP) in the tropical Pacific. This armored, bioluminescent dinoflagellate causes toxic red tides in coastal waters of several countries in southeast Asia; first event was reported in Papua New Guinea in 1972 [2]. It occurs in the Philippines, Malaysia, Indonesia and other countries in the region. In Indonesia, PSP caused by *P. bahamense* var. *compressum* was first recorded in Kao Bay in 1994 [3]. Subsequently, the first PSP followed in Ambon Bay which led to illness of more than 30 people and the death of 3 children after consuming shellfish collected in the area [4].

Beginning in 2007, monitoring of Ambon Bay water quality and plankton has been conducted to examine water quality alterations due to land development on Ambon Island. It has been reported that the land degradation produces massive sediment pollution which can trigger phytoplankton blooms in this area [5]. High precipita-

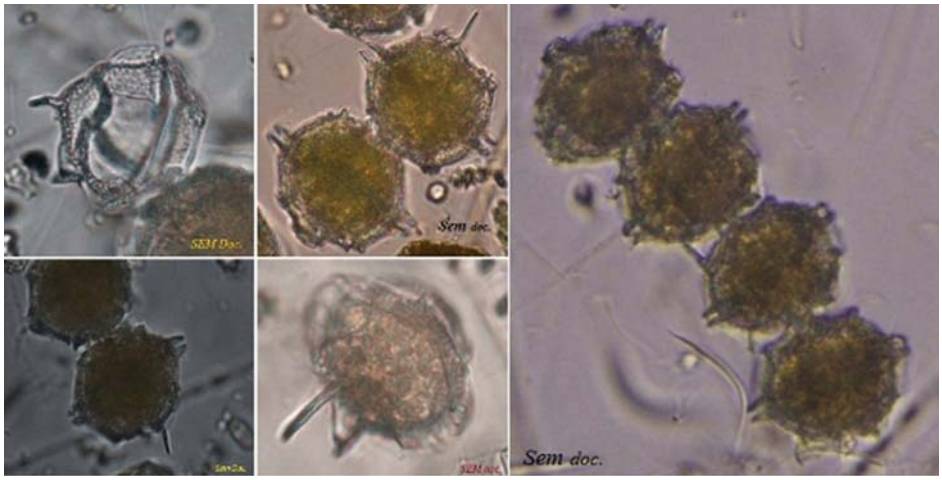

Fig. 2. Pyrodinium bahamense *var.* compressum *found in Ambon Bay, July 2012 (photo : Sem Likumahua, Plankton Lab, Ambon)*

tion in recent years has led to eutrophication in Ambon Bay during the rainy season. Monitoring showed that *P. bahamense* var. *compressum* is normally a minor component of the phytoplankton community between June and July [data unpublished]. The most recent bloom was encountered in July 2012. Two days with heavy rain trigged high runoff, and about 110 hectares of yellowish brown water occurred in the inner bay. The bloom was from 2 meters depth to the surface as shown by the CTD Chl-a profile. The bloom was dominated by *Pyrodinium* and the diatom *Chaetoceros* sp. which reached 2 x 106 and 1.2 x 106 cell L^{-1} respectively. A week later, on 1 July 2012, human illness was reported after consumption of shellfish collected in Ambon Bay. Seven people were nursed in the local hospital for a week before they recovered. The bloom killed hundreds of fish cultured in inner Ambon Bay. The Ambon city government issued warnings against harvesting shellfish in the inner bay. Monitoring of this species abundance and associated hydrological conditions need to be conducted continuously in Ambon Bay.

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Author

Sem Likumahua
Indonesia Institute of Science, Technical Implementation of Marine Life Conservation Ambon Maluku – Eastern Indonesia

Alexandrium tamiyavanichi on the north coast of Perú

Fig. 1. Distribution of Alexandrium tamiyavanichi*. Letters indicate localities where species has been recorded. (*) This report.*

Alexandrium tamiyavanichi Balech is a producer of potent Paralytic Shellfish Poisoning (PSP) toxins. It has been recorded in the Gulf of Thailand, Malaysia, Japan, Vietnam, Hong Kong, Philippines, South Africa, northeastern Brazil and the Mexican Pacific (Figure 1). Harmful algal blooms associated with toxicity in molluscs occur in Peruvian waters, causing human health risks and impacts on the shellfish industry. Therefore, shellfish production zones are monitored weekly under a national program. At least four *Alexandrium* species have been recorded on the Peruvian coast: *A. peruvianum* (originally described as *Gonyaulax peruviana*[1], the non-toxic *A. monilatum*[2] and *A. affine*[3] and recently, the potentially toxic dinoflagellate *A. minutum* (Baylón *et al*., in prep.). This report describes the occurrence for the first time of *Alexandrium tamiyavanichi* in Peruvian waters and the Tropical East Pacific, and its main morphological characters.

In October (spring) 2011 and February (summer) 2012, phytoplankton samples were taken in Paita Bay (05°04'17"S-81°07'19"W) and Máncora (04°06´29´´S-81°04´06´´W), north Perú (Fig. 2). These areas are not dedicated to commercial mariculture: they are influenced by subtropical equatorial waters and in part by upwelling, small estuaries and urban discharges. Samples for species identification were collected by vertical net hauls (10 µm mesh size) and preserved with 2% formalin. Samples for quantification were taken with a hose (15 m) or 5L Niskin bottle (5 m depth) and were fixed with Lugol solution. Surface temperature was measured with a digital thermometer. Counts were made by the Utermöhl method using a Nikon inverted microscope Eclipse TS100.

Qualitative analysis revealed an *Alexandrium* species forming chains of up to 10 cells, which had the same length and wide (40 µm average) and 35 µm transdiameter (Fig. 3A and 4A). This species was present in very low concentrations (≤ 40 cells L^{-1}). Surface temperature was 20°C in Paita and 25°C in Máncora. Microscopic examination showed an oval apical pore complex (APC) with large central comma-shaped foramen, various small marginal pores along the left margin, and a large anterior attachment pore (aap) near the right margin of the comma head (Fig. 3B). The first apical plate (1') was rhomboid and wide connected directly to the apical pore plate containing a ventral pore (vp) on the posterior part of the right margin (Fig. 3C and 4B). The anterior sulcal plate (S.a.) had a precingular part (p.pr.), almost triangular anteriorly (Fig. 4B) and a large sharp hook shaped

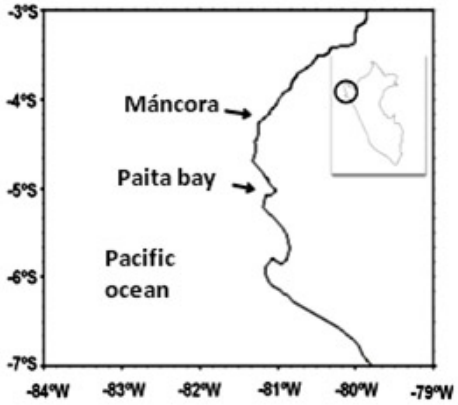

Fig. 2. North coast of Perú showing the two localities where A. tamiyavanichi *was found.*

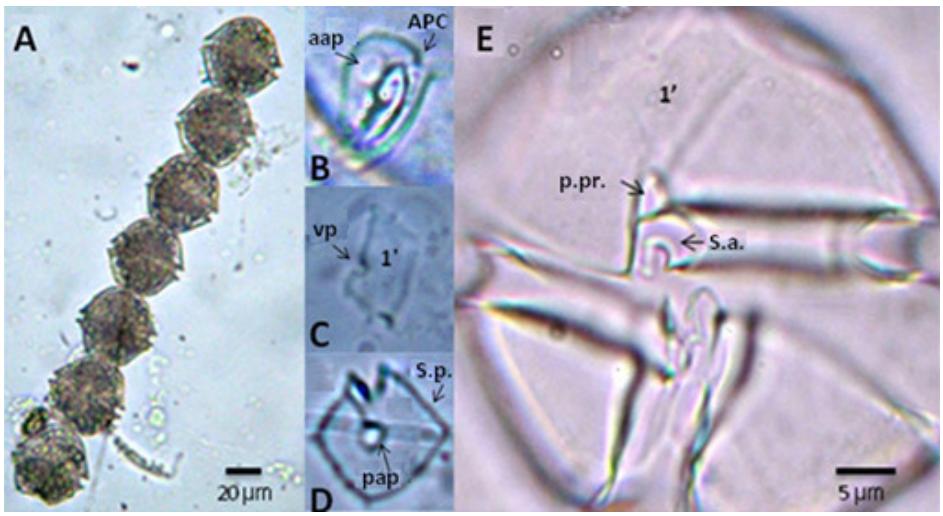

Fig. 3. Light micrographs of A. tamiyavanichi *from Paita Bay, Piura, Peru. A) Cells in chains. B) View of apical pore complex (APC) showing large anterior attachment pore (aap). C) View of 1' plate, wide and rhomboid with its ventral pore (vp). D) Detail of posterior sulcal plate (s.p.) showing posterior attachment pore (pap). E) Central view of intact cell showing 1' plate and anterior sulcal plate (s.a.) and its precingular part (p.pr.).*

apophysis posteriorly. Both regions are divided by a transversal furrow (Fig. 3E). A posterior attachment pore (pap), almost central, connected to the right margin plate through a narrow furrow (Fig. 3D) was observed in the rhomboid and nearly symmetrical posterior sulcal plate (S.p.) of *tamarense* type. Based on these characters and refs (5, 6), the species was identified as *Alexandrium tamiyavanichi*.

Differences between isolates of *A. tamiyavanichi* from Peruvian waters and those originally described by Balech in Southeast Asia were minimal. *A. cohorticula* is morphologically very similar to *A. tamiyavanichi*; Balech (1995) distinguishes the two according to the anterior sulcal plate (S.a.) which is larger and square, 1' plate is narrower and irregular and the posterior edge is right and almost horizontal; besides, it also has a wide sulcal fin. But the two species may be synonymous4.

Off the northern Peruvian coast in 06°S, there is a convergence of warm Tropical Eastern Pacific (TEP) water and cold Humboldt Current water. It is unlikely that *A. tamiyavanichi*, generally found in tropical waters, and stenohaline, extends far from the TEP, except during high environmental variability due to ENSO (El Niño Southern Oscillation). The presence of this dinoflagellate might be caused by current transport, or it may have been introduced by human-assisted dispersal such as ballast water transport. As far as we know, this is the first record of *A. tamiyavanichi* from Peru and in the TEP.

At present, cases of human poisoning due to PSP are unknown in Perú, despite sporadic occurrence of these toxins mainly in scallops from the central coast and associated provisionally with *A. peruvianum*[8]. Elucidation of the toxic nature of this species is still ongoing in Perú. The presence of *A. tamiyavanichi* presents a health risk via potential contamination of molluscs and an economic threat to the local seafood industry. At this time, its follow up should therefore be included in the national monitoring program. Furthermore, countries within the TEP subregion from Ecuador to Costa Rica should also be alert. Likewise, in Peruvian Northern coast we should be alert in north Peruvian coastal waters for the possible presence of other toxic dinoflagellates such as *Pyrodinium bahamense* and *Gymnodinium catenatum*, which occur in areas where *A. tamiyavanichi* is present.

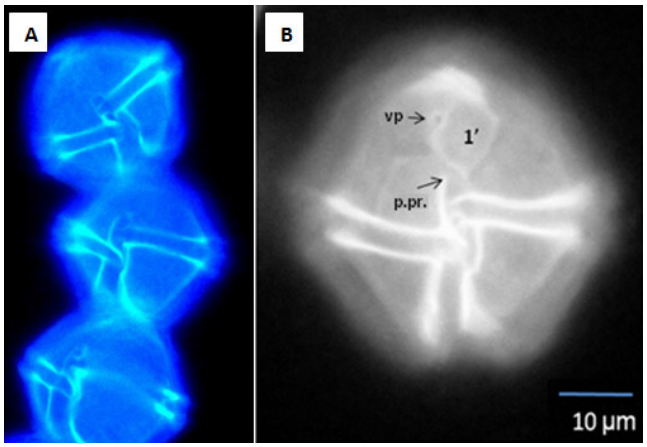

Fig. 4. Epifluorescence microscopy of A. tamiyavanichi*. A) Cells in chain. B) Details of an intact cell showing ventral pore (vp) and precingular part (p.pr.).*

Acknowledgments

We thank Santiago Fraga (IEO, Vigo, Spain) for confirmation of the species, CERPER-Piura for their support, and Carolina Fuentes Rivera-Trucíos for improving the English.

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Authors

Víctor Bárcena-Martínez[a,], Juan López-Hidalgo[a], Jenny Rojas-Fox[a], Maribel Baylón-Coritoma[a], Luis M. Sánchez-Ycochea[b], Omar Flores-Salmón[b]*

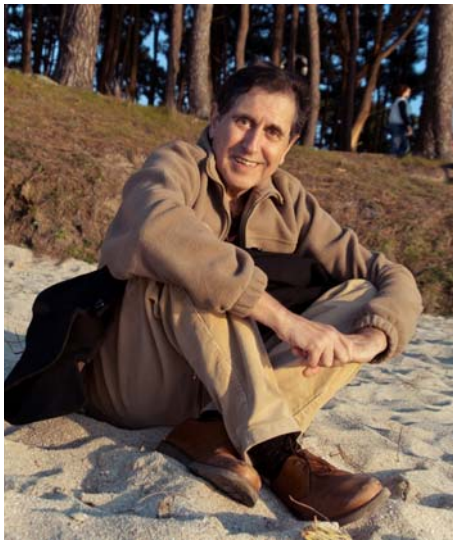
[a]Phytoplankton Laboratory, [b]Toxin Laboratory, Certificaciones del Perú S. A. (CERPER), Av. Santa Rosa 601, La Perla-Callao, Perú

E-mail address: fitopiura@cerper.com. (V. Bárcena-Martínez)

Manuel Zapata (in memoriam)

The changing colours of the sea make up one of the most captivating views of nature. For those who had the privilege of being co-workers of Manuel Zapata, the hues of colour and light at sea surface always bring to our minds the immense variety of plankton pigments, whose knowledge he contributed so much to enlarge. Unfortunately, his death in April 2012 abruptly ended a fruitful scientific career that provided us not only many concrete achievements but also a special mode of contemplating nature.

Manuel's life was always linked to the sea. Born in A Coruña (on the Atlantic edge of continental Europe), he received his BSc and PhD degrees in biology from Santiago de Compostela University. He worked for many years in the oceanographic research section of the Marine Research Centre (Galician Autonomic Government) and had recently (2009) joined the Institute of Marine Research (Spanish Council of Scientific Research). He was passionate about his scientific interests and everyone who listened to him talking about phytoplankton, marine ecology or algal



physiology was immediately fascinated with the topic. He made substantial contributions to our knowledge of algal pigments. These are summarized in 50 peer-reviewed publications and book chapters on the characterization of their chemical structures, the development of innovative chromatographic methods for their analysis, their distribution in plankton taxa and their use as signatures for characterizing natural phytoplankton communities.

A few examples associated with HAB species are the pigment studies of haptophytes (Zapata et al. 2001, 2004), dinophytes (Zapata et al. 2012) and the diatom *Pseudo-nitzschia* (Zapata et al. 2011).Many of these publications involved different Spanish and foreign laboratories, as he was always ready to engage new collaborations to widen the significance of his studies and learn from the expertise of other colleagues. They will surely miss his knowledge and irreplaceable contribution in the future. Manuel participated in several international HAB conferences, in the NATO Bermuda workshop celebrated in 1996, and in the more local Iberian meetings on HABs. He was an outstanding member of the HAB "club".

To his friends, Manuel was exceptionally bright, gifted with a brilliant (and rather surrealistic) sense of humour. He was the best colleague and the best friend. We will always remember him.

José L. Garrido, Santiago Fraga and Francisco Rodríguez

Ernesto Fattorusso Obituary 1937-2012

I am profoundly saddened to communicate to the HAB community that Ernesto Fattorusso, professor emeritus of Organic Chemistry at the University of Napoli Federico II, passed away on 7 July 2012. He was a distinguished scientist appreciated not only for his enthusiasm about science, but also for his brilliant personality, extraordinary creativity, communication skills, kindness, thoughtfulness, and great humanity. Ernesto was endowed with an elegant way of approaching people, deep humbleness in expressing his ideas, and great enthusiasm in passing on his knowledge. This is a huge loss not only for the Neapolitan group, but also for the entire marine natural products community.

Ernesto Fattorusso was born in 1937. He got his degree in Chemistry in 1960 and became full professor of Organic Chemistry in 1975. He served two terms as dean of the Faculty of

Pharmacy and he has long been Director of the Department of Chemistry of Natural Products at the University of Napoli Federico II. His outstanding research activity, which is documented by more than 320 papers, is impressive for the range and variety of contributions he made, many of them pioneering. He focused his research on the chemistry of bioactive metabolites from marine organisms. Over the past two decades of his career, he played a pivotal role in depicting the phycotoxin scenario across the Mediterranean Sea. With LC-MS detection and NMR-based structural elucidation, he identified many toxins belonging to the group of okadaic acid, yessotoxins, spirolides, and lately of palytoxins, among others.

Ernesto Fattorusso was held in high regard by the whole Marine Natural Products Chemistry community. Due to his scientific achievements in the field, he received a number of prizes, the last of which being the Paul Scheuer Award 2010, awarded for the first time to a European scientist.

Patrizia Ciminiello
Dipartimento di Chimica delle Sostanze Naturali, Napoli, Italy

Tribute to Phil Busby: 1947-2013

Phil Busby, in his capacity as Principal Advisor (Shellfish) at the Ministry of Agriculture and Forestry (MAF) in New Zealand, touched many in his productive career of putting quality assurance of molluscan shellfish on the world map. He retired from this position due to poor health on 31 October 2012 and passed away on 8 March 2013.

Phil initially wanted to become a plumber, but changed his mind to train as an Inspector of Health in the New Zealand Department of Health. He enjoyed many adventures doing field work in Wellington and Invercargill including rare trips to the mutton bird islands off New Zealand's southern coast. He gave up the field work to join the Department's Head Office to run the training course for health inspectors in 1983. Those who went through the course under Phil will never forget his no-nonsense marking style! From 1989 MAF took over running the New Zealand Shellfish Quality Assurance Programme and with it came Phil – initially on secondment from the Department of Health. He became responsible for all public health aspects of the New Zealand Shellfish Quality Assurance Programme, and in 1992 wrote the first New Zealand Shellfish Standard. The 1993 Karenia bloom in Hauraki Gulf and resulting Neurotoxic Shellfish Poisoning outbreak which led to New Zealand wide harvesting closures, catapulted him into the national and international spotlight. He did rise to the challenge, simply was always there, made a difference through persuasion and presence, and maintained his position of authority with grace, charm and flair. He effected major change through (sometimes polarising) debate and arguments, preferably over a few beers in a bar. Debates on human health significance of brevetoxins, gymnodimine, pinnatoxins, Phil has seen them all. He was responsible in 1996 for the requirement that Cawthron Institute acquire accreditation prior to phytoplankton being accepted as part of the New Zealand Marine Biotoxin Management Programme. Phil organised twice yearly Seafood Safety workshops through what is now called Ministry of Primary Industry. The informal proceedings of these meetings capture the significant changes in focus

as research and regulation on harmful algal blooms matured in New Zealand and tried to keep up with each other. In 2001 he was responsible for the validation and approval of LC-MS methods for ASP and DSP marine biotoxins, in 2003 for introducing mandatory hydrolysis of DSP samples, and in 2004 the approval of a LC-MS Screen Test Method for Brevetoxins. Each of these initiatives represented international trend setting decisions and showed the resolve of Phil to provide novel outcome based solutions to improve public health protection. In 2004 Phil was asked to chair the Joint FAO/IOC/WHO Expert Workshop in Dublin on Biotoxins in Molluscan Bivalves and he went on to Chair the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Molluscan Bivalves in Oslo. His role was facilitated through his position as Vice Chair of the UNESCO Intergovernmental Oceanographic Commission Panel on Harmful Algae Blooms and Chair of the Panel Task Team on Biotoxin Monitoring and Management. The respect in which he was held by his national and international peers showed in his re-election in 2009 to both positions. In 2006 Phil drafted and put in place the Bivalve Molluscan Shellfish Regulated Control Scheme (affectionately known as the BMSRCS) comprising new shellfish safety regulations and specifications to take the New Zealand Shellfish Programme through the years ahead and provide access to markets throughout the world.

Thank you for all your contributions, Phil. You will be a hard act to follow!

Gustaaf Hallegraeff
Institute for Marine and Antarctic Studies, University of Tasmania, Australia

Lesley Rhodes, Lincoln McKenzie
Cawthron Institute, New Zealand

Cath McLeod
South Australian Development Research Institute, Australia

Doug McLeod
Glenelg Shellfish, Australia

Dorothy J. McCoubrey
Dorothy-Jean & Associates, New Zealand

Jim Sim
Ministry for Primary Industry, New Zealand

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Charles S. Yentsch, Visionary Ocean Scientist, dies at 85

Charlie was honored as a leader and innovator. Yet he will be remembered as a "beloved maverick" by those who knew him best. Learning about the seasons, cycles, and ever-changing dynamics of the ocean was Charlie's life-long passion. Creating a positive and equitable learning environment shipboard, in the laboratory, and at home was his commitment. Compassion was his hallmark.

Since childhood, Charlie was a student of the seas. As a kid in Kentucky, he was fascinated with fossils of marine organisms in the limestone. As a California teenager, he studied waves from his post as a lifeguard and experienced waves as a surfer. He set up a business to free-dive for and market abalone. The diving triggered his fascination of sunlight interacting with water. As an adult, he turned his father's and uncles' mechanical skills with trucks toward the measurement of light in the sea and the tiny marine organisms dependent on sunlight. He held a reverence for all life and found the simplest forms most compelling. Always non-hierarchical in his belief of human potential, he put every person and every idea on a par to be tested. He cherished skeptically-minded colleagues.

He served in the U.S. Navy during WWII as a fire-fighting instructor in San Diego (1944-1946). The GI Bill and no job prospects encouraged his formal education at the University of Louisville (B.S. 1950), Florida State University in Tallahassee (M.S. 1953), and the University of Washington in Seattle. Employment included the Woods Hole Oceanographic Institution, Woods Hole, MA (1956-1967); Nova University Oceanographic Center in Fort Lauderdale, FL (1967-1970); and the University of Massachusetts Marine Station, Gloucester (1970-1974). In July of 1974, Charlie and wife Clarice founded the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, ME. Within a few years, this institution had become a world-class laboratory attracting innovative scientists from around the world.

Photo: Bob Mitchell

Charlie was regarded as one of the great pioneers of modern oceanography, and one of the first to conceive of and advise NASA on the potential of satellite observations of ocean biology. In 1985 he received a Ph.D. *Honoris causa* from Long Island University, Southampton, NY. The American Association of Limnology and Oceanography presented Charlie with a Lifetime Achievement Award in 1999. In 2010, The Oceanographic Society bestowed the honor of the Nils Gunnar Jerlov Award and also named him a Fellow of The Oceanography Society.

Charlie had a deep sense of time, space, and place. He enjoyed going to sea and world travel, with his family accompanying him when possible (despite a quote on his office door that read "Traveling with children is like traveling third class in Bulgaria"). Yet he was most fond of being home, defined by a wood fire, kids and pets, "tea" time, humor, a well-prepared meal fresh from the sea, and then the evening topped off with a puff on a Partagas and a good read. His descendents are three sons and two granddaughters. Charlie was proud that each one of the five is now an astute naturalist.

If you are so inclined, the next time you set sail, see sunlight reflecting off the ocean, dance the swing, listen to Dixieland, or sing Spirituals – raise a toast to Charlie.

Clarice Yentsch
Fort Lauderdale, Florida, USA

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen

Compiled and edited by Tim Wyatt,
Instituto de Investigaciones Marinas, CSIC,
Eduardo Cabello 6, 36208 Vigo, Spain;
Tel.: +34 986 23 19 30/23 19 73;
Fax: +34 986 29 27 62;
E-mail: twyatt@iim.csic.es
Lay-out: Department of Biology,
University of Copenhagen, Denmark

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Project Coordinator: Henrik Enevoldsen,
IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Øster Farimagsgade 2D, DK-1353 Copenhagen K, Denmark
Tel.: +45 33 13 44 46;
E-mail: h.enevoldsen@unesco.org

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ISSN 0020-7918