Harmful Algae News

An IOC Newsletter on Toxic Algae and Algal Blooms

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25 YEARS

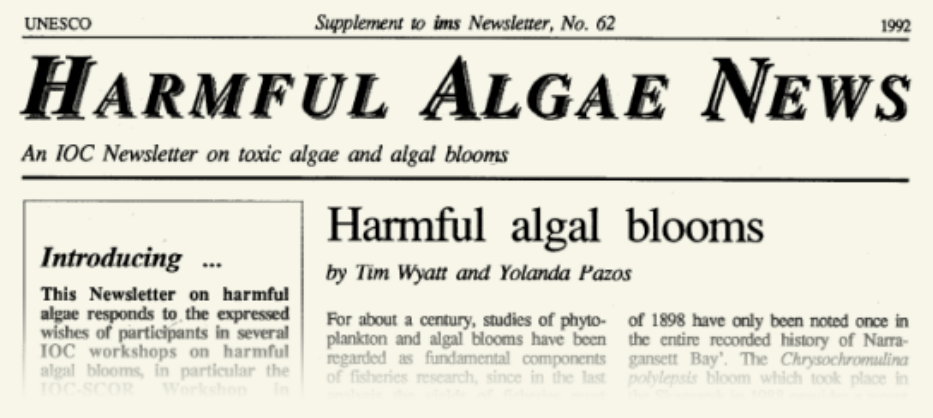
United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Content

Harmful Algae News was first published in early 1992 in response to requests from the participants at a number of IOC meetings and workshops on harmful algae, in particular the IOC-SCOR *ad hoc* Meeting in Rhode Island (USA) 2-3 November 1991, hosted by Ted Smayda where the final draft of the Harmful Algal Bloom Programme was prepared. At this time, e-mail was in its infancy, web sites still very simple but there was demand for a channel to disseminate information about harmful algal events and research, as well as to disseminate information on management/research programmes, conferences, meetings etc. The field of HAB science was new, the community multidisciplinary and at a national level, often scattered between many different institutions. The need for a communication channel to strengthen networks and co-operation was obvious and *Harmful Algae News* became a core element in the IOC Harmful Algal Bloom Programme.

Harmful Algae News started as an annex to the IOC UNESCO newsletter '*International Marine Science*' (IMS), with the enthusiastic support of IMS editor Gary Wright and assistant Michelle Turner. However, *Harmful Algae News* became independent and eventually survived the IMS which ceased some years later. *Harmful Algae News* has been in publication for longer than any other IOC newsletter and its viability reflects the sustained focus on harmful algae in the IOC programme, as well as the continued interest by governments, institutions, scientists and those im-



UNESCO *Supplement to ims Newsletter, No. 62* 1992

HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms

Introducing ...

This Newsletter on harmful algae responds to the expressed wishes of participants in several IOC workshops on harmful algal blooms, in particular the

Harmful algal blooms

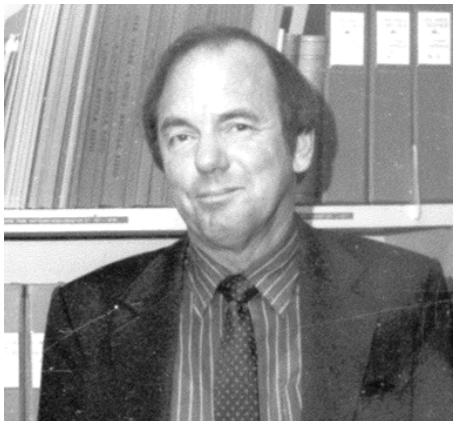
by Tim Wyatt and Yolanda Pazos

For about a century, studies of phytoplankton and algal blooms have been regarded as fundamental components of fisheries research, since in the last

of 1898 have only been noted once in the entire recorded history of Narragansett Bay'. The *Chrysochromulina polylepsis* bloom which took place in

Vladimir Ryabinin, Exec. Secretary, IOC

"The Decade of Ocean Science will be a unique ten-year, global, large scale cooperative programme to seek urgently-needed scientific solution to support effective ocean management, stewardship and sustainable development" *(continue next page)*

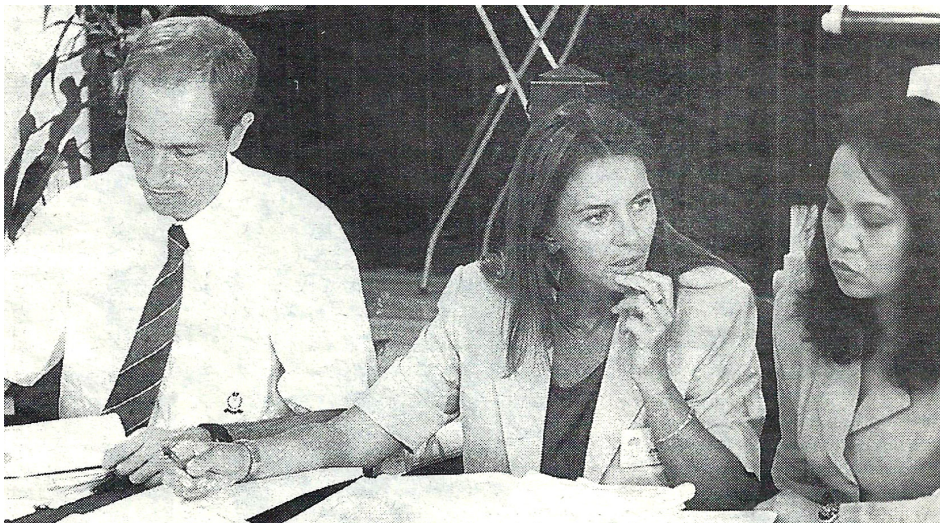


pacted by harmful algal events. Since *Harmful Algae News* turned 20 years old in 2012, it has been a web based e-newsletter which meant longer issues were possible and back issues easily accessible. We are currently working on a searchable index for all *Harmful Algae News* issues.

The start of *Harmful Algae News* would not have been possible without a dedicated professional editor, Tim Wyatt. Tim was already an experienced journal editor and author, and a main contributor to *Harmful Algae News* himself. In the early years he was assisted by Yolanda Pazos, also at the Institute for Marine Investigations (IIM) in Vigo, Spain. Later, staff at the IOC Science and Communication Centre at the Spanish Institute of Oceanography (IEO) in Vigo (Jorge Diogéne, Ángeles Aguilera, Mónica Lion and Cristina Sexto) provided invaluable support in the compilation and layout. Since 2012 Leif Bolding at the Department of Biology at the University of Copenhagen has been producing the layout and providing the appropriate server facilities. Tim Wyatt edited 48 issues of *Harmful Algae News* plus some special issues over his 22 years as Editor. When Tim decided to retire as Editor we were lucky to have Beatriz Reguera (IEO Vigo) and Eileen Bresnan (Marine Scotland) willing to take over and continue the same high standard. No doubt these standards have stimulated submissions and made *Harmful Algae News* what it is today. We hope the international HAB science and management community will continue to submit their news items and announcements and that *Harmful Algae News* can continue to serve all its readers including the International Society for the Study of Harmful Algae.

To subscribe to HAN, send an e-mail to "han@sympa.iode.org" with empty subject line and in the text body: subscribe han *name* e.g.: for Peter Black —> "subscribe han Peter Black" or just "subscribe han"

Henrik Enevoldsen, Intergovernmental Oceanographic Commission of UNESCO, Head, IOC Science and Communication Centre on Harmful Algae



Key people in the early days of the HAB programme. From top to bottom: Ted Smayda (USA), host of the IOC-SCOR Workshop, Newport, October 1991, where the HAB programme was outlined (photo DM Anderson); Tom Osborne, IOC Technical Secretary when the programme was launched; Bernd Dybern (Sweden), 1st IPHAB Chair, 1992, and last, Adriana Zingone (Italy), 2nd IPHAB Chair during the III IPHAB meeting in June 1997, Vigo, in the middle with Henrik Enevoldsen (IOC) on the left and Vice-Chair Rhodora Azanza (Philippines) on the right.

Dear Readers

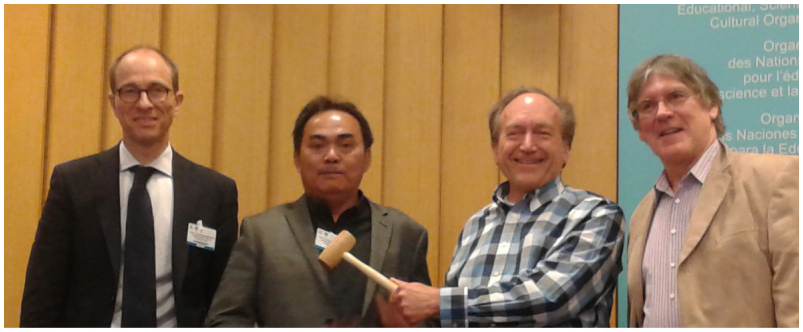
The fact that this year we are celebrating the 25th anniversary of the IOC newsletter *Harmful Algae News*, makes me, as Executive Secretary of the Intergovernmental Oceanographic Commission of UNESCO, proud of the strategic foresight of the Commission. Continuous publication of this newsletter shows the interest in the subject by the coastal zone management community; is evidence of successful delivery of new knowledge by the interdisciplinary research community; and is the result of effective prioritization of IOC work by its Member States. The harmful algal research domain is an example of ocean science which is of primary importance for sustainable development. Indeed, people's health, nutrition, availability of fresh water, and many economic interests are directly affected by the emergence of harmful algae. Successful research on and predictions of harmful algae demonstrate that oceanography is becoming more and more capable of responding to people's needs. To make decisive and major improvements in making ocean observations, research, sustaining services and responding to growing societal needs is the main objective of the United Nations Decade of Ocean Science for Sustainable Development (2021-2030). The Decade is going to shape much of the activity of the IOC and its partners in the years to come. Let me invite you all to engage and contribute.

Vladimir Ryabinin, Executive Secretary, Intergovernmental Oceanographic Commission, UNESCO, Paris

Intergovernmental Panel on Harmful Algal Blooms also turned 25!

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

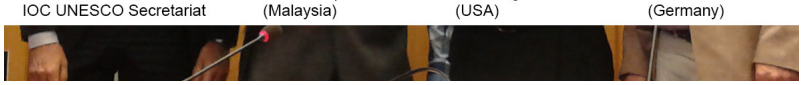


IPHAB Technical Secretary Henrik Enevoldsen IOC UNESCO Secretariat

New IPHAB Chair Gires Usup (Malaysia)

Outgoing IPHAB Chair Rob Magnien (USA)

New IPHAB Vice-Cha Allan Cembella (Germany)



During 1992, the same Year as the IOC published the first issue of *Harmful Algal News*, it also established an *Intergovernmental Panel on Harmful Algal Blooms* (IPHAB) which has met every second year since it was formed. The Panel is composed by IOC Member State representatives and representatives of organizations working on different aspects of HABs. The Panel identifies and decides on priorities for international cooperation on HABs and also works to identify resources to facilitate the diverse work schedule to solve some of the real problems caused by harmful algae.

Over the last 25 years the IOC Intergovernmental Panel on HAB has initiated a large number of initiatives and activities leading to training opportunities, manuals, guides, projects and cooperative research. A special issue of the journal *'Oceanography'* includes a paper on the history and role of the IOC HAB Programme in the development of international HAB science. The paper can be downloaded at *http://dx.doi.org/10.5670/oceanog.2010.25*.

We here wish to pay a tribute to those who have chaired the Panel, and their Vice-chairs, the past 25 years and their contribution to developing the HAB research and management community at a global scale.

From top to bottom: Beatriz Reguera (Spain) and Phil Busby (New Zealand), elected as IPHAB Chair and Vice-Chair in 2002; Flowers to the outgoing Chair (Adriana Zingone, Italy) (left) and Vice-Chair (Rhodora Azanza, Philippines) (right); Leonardo Guzmán (Chile) (middle), Chair from 2007 to -2011 welcomes the newly elected Chair, Rob Magnien (USA) (left) and Vice-Chair, Gyres Usup (Malaysia) (right) for the next term; Bottom: Results from last elections, at the 12th IPHAB Session, Paris, April 2015

A retrospective look at the early days of HAB cyst research, and a look to the future

On this occasion of the 25th anniversary of the publication of *Harmful Algae News*, several of us were asked to look backwards in time to some of the earlier days of HAB science. One area of study that has been a major part of my own research programme and that of many others in our community is the role of cysts and resting stages in the bloom dynamics of HAB species. Here, I offer a personal perspective on the early stages of development in that field and a brief look towards the future as well. My apologies at the outset if there are omissions of people or findings that should have been included – this is not meant to be a scholarly synthesis, but rather a personal retrospective.

To set the stage, let's go back nearly fifty years to a time when the term *harmful algal bloom* did not exist and when researchers worked individually or in very small teams in scattered locations without any major research programmes or international initiatives. In 1972 when I was just starting graduate school, there was a massive New England red tide caused by the dinoflagellate we now call *Alexandrium catenella*, but which then had multiple names, including *Gonyaulax tamarensis* and *G. excavata*. This outbreak dealt a devastating and unexpected blow to the New England region of the United States, causing shellfish closures along the coasts of multiple states. Considerable attention by the press and public covered this new and worrisome threat to public health and fisheries resources and ultimately motivated the *First International Conference on Toxic Dinoflagellate Blooms* in Boston, Massachusetts in 1974.

At the time of that conference, I was a long-haired graduate student in the Civil and Environmental Engineering Department at MIT (Fig. 1), searching for a thesis topic. People often ask me how someone in an engineering department could end up a biologist working on HABs, and the answer to that lies in part with the 1972 New England red tide, but also with the 1974 conference and a series of events and discoveries that came shortly before and after.

The conference was very small compared to the current ISSHA meetings, with approximately 100 participants from four countries versus 500 or more participants representing 50 or 60 countries at our current meetings. The focus of that meeting was almost entirely on blooms of *Alexandrium catenella* and *Karenia brevis* (the species was then called *Gymnodinium breve*), as the discoveries of diarrhetic shellfish poisoning (and its linkage to *Dinophysis* spp.) and amnesic shellfish poisoning (and *Pseudo-nitzschia*) were 10-15 years in the future. The ciguatera fish poisoning syndrome was known, but the identification of *Gambierdiscus toxicus* as the causative organism was also several years away. Among the contributions at that meeting, several are noteworthy in the context of the theme of this narrative. The first was by David Wall, a geologist at the Woods Hole Oceanographic Institution (Fig. 2). In the late 1960's and early 70's, Wall (working with Barrie Dale at the time) discovered that the fossilized cell walls of organisms thought to be extinct (called hystrichospheres) were in fact dinoflagellate cysts, and that many of the forms that they were using in paleontological reconstructions were still living in the ocean and producing cysts that could be germinated to establish taxonomic affinities. (This same line of work was concurrently being pursued by Bill Evitt and colleagues at Stanford University). In order to isolate and germinate living cysts from modern sediments, Wall and Dale developed a technique that is used to this day involving sonication and sieving – the former used to disaggregate sediments and the latter to separate size fractions containing cysts. In his paper in the 1974 conference proceedings and in other papers published near that time [1-3], Wall highlighted the important roles that cysts likely played in dinoflagellate bloom dynamics, including determining where and when blooms might originate, allowing survival through environmental extremes, and facilitating genetic recombination through sexuality. He told us "*Despite these seemingly important considerations, the encystment-excystment cycle in dinoflagellates has not been expressly studied in relation to any specific red tides or related instances of paralytic shellfish poisoning. Details of the cyst cycle have been accumulated independently of red tide research*….." [1]. This contribution and other papers at the time show considerable foresight that was fully validated through a sharp increase in the cyst-based HAB research of the subsequent decades.

Another talk at that conference was equally insightful, this one by Karen Steidinger (Fig. 3) ("Basic factors influencing red tides [4]"). In her talk and paper, Karen outlined the fundamental stages of blooms, focusing on initiation, transport, and development. Like Dave Wall, she also highlighted the importance of cysts: *"The recent investigations of Dr. David Wall ……lend credence to the speculation that pelagic, toxic dinoflagellate blooms might originate from*

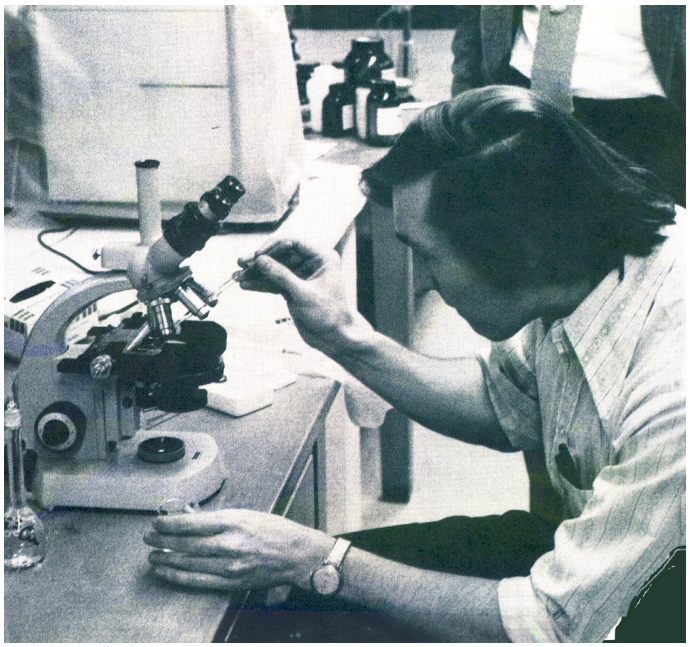


Fig. 1. Don Anderson as a graduate student in the Civil and Environmental Engineering Department at the Massachusetts Institute of Technology (MIT), 1975

dormant stages and that these stages might be associated with certain bottom sediments. This then brings up the question, if benthic resting stages of certain dinoflagellates actually "seed" coastal red tides, are there localized areas of accumulation, or what we could call "seedbeds"?.....The possibility of benthic seed populations and seedbeds for at least some Gonyaulax (=Alexandrium) and Pyrodinium has a much higher probability than for Gymnodinium breve..... It would seem that this avenue of research should have a high priority among phytoplankton systematists and ecologists."

As a graduate student sitting in the audience, I was motivated by these talks and papers and immediately started to look at my own research from a new perspective. At the time, I was in a water chemistry laboratory doing trace metal (copper) sensitivity experiments with my *Alexandrium* cultures, reasoning that there was some chemical process that could explain the link between *Alexandrium* blooms, river runoff, and low salinity coastal waters (I still believe that this is the case, but I dropped this line of inquiry and it has not been pursued to any significant extent since). Many of my cultures contained rounded, non-motile cells, which I thought could be cysts, but no cyst stage had yet been described for *A. catenella*. Fortunately for me, Woods Hole was a short drive away for me, so I was able to bring my cultures to Dave Wall. After a quick examination, he gave me the disappointing news that these were not what he would term true cysts, but rather a temporary stage with much thinner walls (he and I eventually named these "*pellicle cysts*" after the resistant layer that surrounds them [5]). Next, one of those pivotal moments in a career occurred in which luck or good fortune played a role. Instead of just sending me back to my lab with this negative news, Dave suggested that we sample a nearby salt pond where PSP toxicity had been recurrent in the years after the big 1972 bloom. We collected surface sediments with an old plankton net that was dragged across the bottom, and Dave demonstrated the sonication and sieving method. In short order, we were looking at a number of unknown cysts types that I set about trying to isolate and germinate. One produced an elongate cell (Fig. 4) that after germinating, ultimately divided to produce cells of *Alexandrium catenella*. I was already aware of the importance of this discovery after the talks and papers of Wall and Steidinger, and now I had proof that at least some of their speculations were valid. The door was suddenly open to some very exciting and new work with living cysts, and this new perspective on *Alexandrium* bloom dynamics totally changed the directions of my thesis research. Furthermore, I had found perfect study locations for my work – the Cape Cod salt ponds where these blooms occurred regularly every year, and in which they were localized due to restricted tidal exchange with nearby coastal waters. We continue to work in these systems today – they are essentially natural mesocosms. Several publications ensued [5,6], accompanied by a paper by Barrie Dale [7] who had left Woods Hole and was working independently on this same species using sediment from Norway.

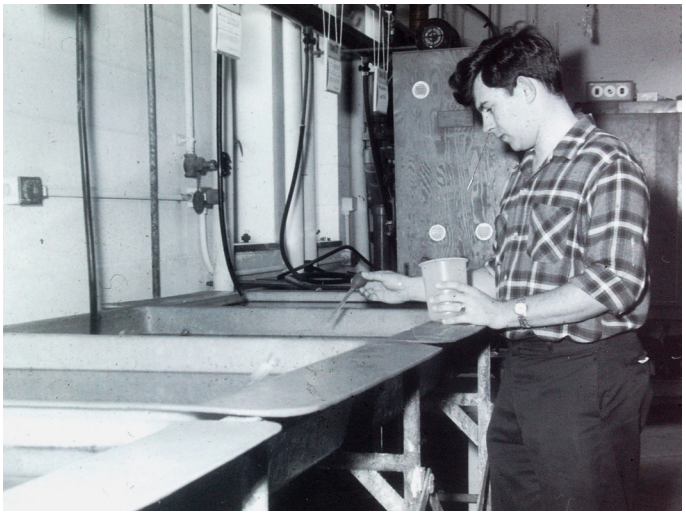


Fig. 2. David Wall, an early pioneer in dinoflagellate cysts, collecting cysts from settling trays in Woods Hole in 1974

In one of these papers [6], I was able to document the extreme seasonality of *Alexandrium* blooms in the salt pond, raising fundamental questions about the mechanisms underlying the patterns of excystment and encystment. I characterized the mandatory dormancy interval, which begins at cyst formation and can last several weeks to months until maturation is completed. Cysts were unable to germinate during this interval, thus explaining the absence of *Alexandrium* cells in the water column in the months following each spring bloom. What remained a mystery was why the bulk of the *Alexandrium* cysts in bottom sediment did not germinate later in the summer or fall or even early winter after they had matured, but instead remained in a resting state until the next spring. One part of the answer was found some years later when we showed that high temperatures can inhibit cyst germination [8]. Yet, the absence of a major bloom in the late summer and fall when temperatures had decreased enough to be favorable for germination remained a mystery. Only in recent years have we begun to understand that mature *Alexandrium* cysts can re-enter dormancy – one cause may be unfavorably warm summer temperatures. But we have also learned that exit from that dormant state into quiescence (a state in which cysts will germinate if conditions are favorable) occurs in response to a quantifiable amount of chilling. By quantifying the duration and severity of cold, *A. catenella* tracks the passage of winter, delaying germination until spring when the outlook is more favorable for bloom success. This groundbreaking work by one of my students, Alexis Fischer, demonstrates that dinoflagellate cysts possess physiological behavior that is similar to that observed in terrestrial plants where a period of chilling is needed for optimal seed germination, bud flowering, or bulb emergence [9]. We now know that *Alexandrium* cysts can cycle between quiescence and dormancy multiple times as seasons and years progress, and equally importantly, that the frequency and timing of the cycling may well be determined by the history of the chilling those cysts experience. Recent work by Cary López on *Pyrodinium bahamense* is revealing a similar environmentally-induced cycling behavior in tropical dinoflagellate cysts.

Those early days as a graduate student and then as a postdoc were times of rapid discoveries, as nothing was known about the distribution of *Alexandrium* cysts in the region, or of the role of temperature, nutrients, light, oxygen, and other environmental pa-

Fig. 3. Karen Steidinger, Penrose Conference on Fossil and Modern Dinoflagellates, 1978

rameters on the formation, deposition, and germination of cysts. One of several challenges at the time was that we were unable to produce true hypnozygotic resting cysts in our cultures. There was good reason to believe that sexuality was involved, so compatible mating types needed to be combined. Further, nutrient limitation seemed to be a trigger to initiate the process. We quickly learned that dinoflagellate species varied considerably in the ease with which they could be induced to form cysts in cultures. Species like *Scrippsiella trochoidea* produced prolific numbers of cysts without special precautions, whereas our *Alexandrium* cultures were far more recalcitrant. Eventually, with deliberate modifications to the content of the culture medium as well as the manner in which it was prepared (i.e., minimizing contaminants and precipitates) we were finally able to produce cysts in *Alexandrium* cultures [10], opening yet another door to new studies and discoveries.

It is clear that nutrient limitation can induce sexuality and cyst formation in cultures of *A. catenella* and other dinoflagellate species, but to this day, we still do not know if there are other factors at work – perhaps a density-dependent or quorum-sensing type of response, or even a response to the presence of grazers or parasites. Exploration of this response has long been limited by the constraints associated with laboratory cultures, but now we are fortunate to be entering a new era of in situ investigations thanks to biosensors like the Imaging FlowCytobot (IFCB), that allow us to observe the encystment process underwater in the field with extraordinary resolution. Among many other recent findings, IFCB observations by Mike Brosnahan [11,12] have documented mass gametogenesis (75 – 90% of all cells) at the end of *A. catenella* blooms under conditions that do not appear to be nutrient-limited. I believe that the tools are now in hand to resolve the longstanding mystery of what induces cyst formation in the field and suspect that the final story will be much more complicated than simple nutrient limitation.

Not long after our early successes with sexual induction and cultures, my attention shifted to studies of cyst distribution and dynamics in the field. We were documenting the abundance and dynamics of vegetative cells in the plankton during blooms, so I wanted to quantitatively link those observations to cyst abundance and distribution in the sediments before and after those blooms. At the time, however, studies of dinoflagellate cysts were still heavily influenced by geologists working with sediment cores. Typically, those investigations enumerate cysts at different depths in cores, expressing results in terms of cysts per gram of dry sediment. Although this approach is appropriate when one is studying vertical profiles of dead, empty, or fossilized cysts in sediments dating back hundreds or thousands of years, this type of parameterization is not useful for biological studies in which one wants to map out the distribution and abundance of cysts and estimate the number of germinated cells inoculating the overlying water column. To address the latter topic, we needed to know how many cysts were present per square meter of sediment, so we started doing experiments to see how reproducibly we could quantify cysts per cubic centimeter or ml of sediment. Our results were consistent and scientifically appealing so we published the method [13] and began to use it in the salt ponds and other nearshore locations. This approach was not well received, however, as I was repeatedly criticized at conferences when these results were presented. Geologists argued that the water content and lithology of surface sediments differs sufficiently from site to site, or from layer to layer, and that normalization of cyst abundance to a volume of sediment introduces substantial errors compared to the dry weight approach. I remember being told at an international conference that my results and the population dynamics models based on them were essentially meaningless! This disagreement went even further, as one publication [14] recommended that "*future studies use standardized methods based on measurements of cyst concentrations per gram of dry sediment*". This recommendation was repeated in training manuals for those working with living dinoflagellate cysts [15]. Despite this strong and vocal opposition, I was still firmly convinced that cysts could be quantified per unit volume of sediment, and so I initiated a study over multiple years in which we quantified cysts both ways (i.e., cysts per gram dry weight and cysts per ml). We did this in the Gulf of Maine across a wide range of sediment types and cyst concentrations, and found a strong and statistically significant relationship between the two parameters, for the top cm of sediment (Fig. 5). The relationship for the top three cm of sediment is even stronger. This of course does not mean that this relationship will apply in every location, but it does show that the hypothetical concerns raised about this

approach did not stand up to data at my study locations.

I raise this issue in this narrative because I want to correct what I feel are unjustified recommendations that may prevent those working on cysts from obtaining the type of biological data that can advance our understanding of certain types of HABs. With the proper precautions, we can map out cyst distributions in a way that is complementary to the manner in which we study the vegetative stages in the water column. There's no question that both populations are patchy and difficult to adequately represent with limited sampling, but it can be done. Knowing the cyst abundance per ml of surface sediments allows the distribution to be mapped in a way that has biological meaning, as has now been seen in many studies. If one assumes that the cyst germination flux comes from a thin layer at the sediment surface of a defined thickness, it is possible to calculate germling emergence per square meter, and thereby quantify the inoculum to the overlying water column. This is simply not possible if the cysts are expressed per gram of sediment, unless additional measurements are made that determine the density (grams per ml of the sample). Once the cyst distribution is expressed in terms of area, it is then possible to use laboratory- or field-derived germination rates to calculate the germination input to a body of water, an approach that is fundamental to bloom dynamics modeling efforts for cyst-forming species.

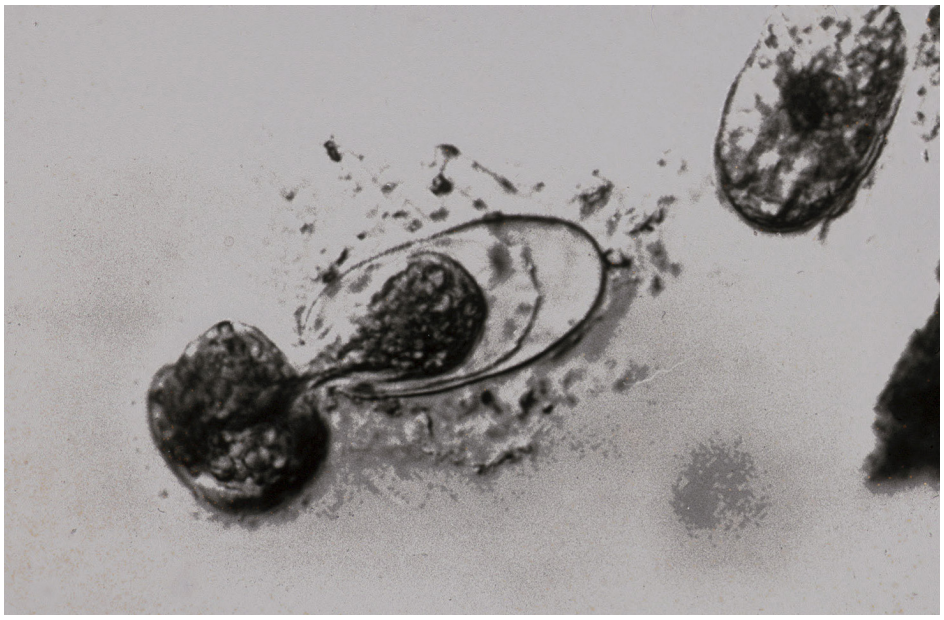


Fig. 4. Germinating Alexandrium catenella cyst. (Photo: Don Anderson)

My guidance to those working on cysts in natural sediments is to demonstrate that you can sample surface sediment reproducibly, and that the distributional data obtained makes sense in the context of bathymetry, currents, and sediment type. I hope that those working on dinoflagellate cysts recognize the validity and value of the two different approaches for cyst enumeration – cyst number per gram dry weight for paleontological reconstructions of past populations and sediment transport modeling, and cysts per unit area or unit volume for studies of the dynamics of living cysts. Another option would be to measure cysts per gram dry weight and determine the density of each sediment layer, as this would allow a simple calculation that would also provide cysts per unit volume data.

Armed with the knowledge that was possible to map out cysts over large areas, my team set about to do this in salt ponds, bays, and open coastal waters. These efforts confirmed Dave Wall and Karen Steidinger's original speculation – that areas of accumulation (seedbeds) existed and were a critical element in bloom dynamics. Some seedbeds were small, and others huge – like the ones we documented in the Gulf of Maine (see figure 5) that covered an area as large as 22,000 km^2, varying 3–4-fold in area and 10-fold in cyst abundance among years [16]. Those studies also demonstrated that the Gulf of Maine system has only two seedbeds with the bathymetry, sediment characteristics, currents, biology, and environmental conditions necessary to persist for decades or longer. The value of these mapping studies was even more evident when strong positive correlations were confirmed between the abundance of cysts in surface sediments and the size of the blooms and resulting toxicity in the subsequent year [16,17].

Our next challenge was to measure or estimate the flux of emerging cells that inoculates blooms. Efforts by my lab but in particular those of Ishikawa, Naksuike, and others [18,19] have yielded some promising devices and approaches that consistently indicate that germination is occurring in only a thin veneer a millimeter or so thick at the sediment surface, leaving the large

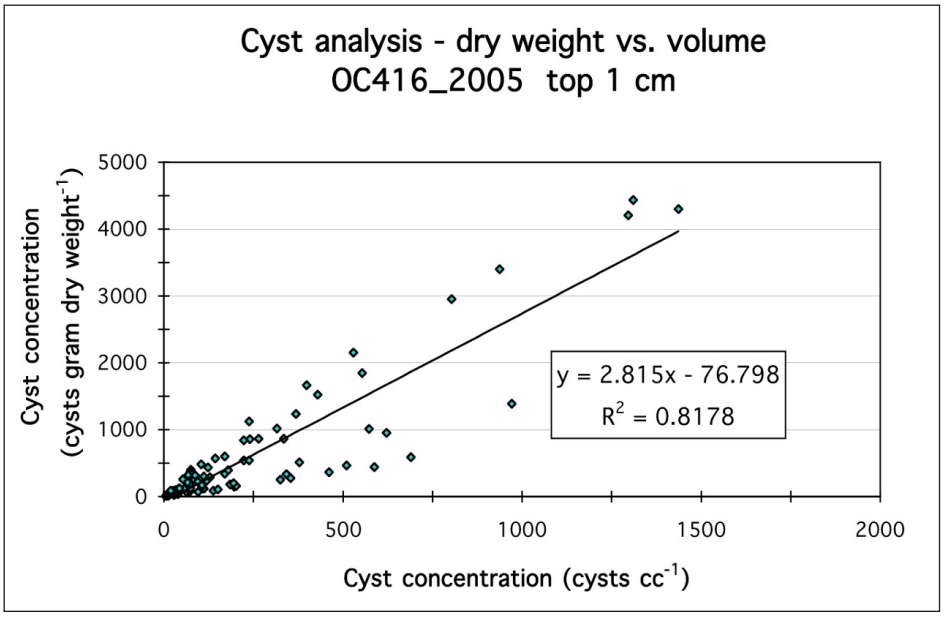


Fig. 5. Significant relationship ($p < 0.0001$) between cysts per cm^3 and cysts per gram dry weight in the top cm of sediment at 85 stations throughout the Gulf of Maine. From [16]

number of cysts in subsurface layers unable to germinate or emerge, presumably due to lack of oxygen or to the tortuous pathway posed by sediment grains and detritus. Many might think that major storms and waves can erode significant layers of sediment and transport cysts long distances, but here again, our studies in the Gulf of Maine have shown that other than in very shallow waters, even major storms only erode a millimeter or less of sediment [20] and that the cysts and other eroded material don't travel very far before settling again [21]. Specifically, the depth of sediment eroded ranged from about 0.05 mm at a sandy 70 m deep station, to about 1.2 mm in clayey-silt sediment at 250 m [20], with the majority of the resuspended material remaining within 20 km of the source locations [21].

I could go on, but lack of time and space argues that I bring this narrative to a close here. As I look back, I am fortunate and grateful to have been part of an era of discovery that has developed the methods and approaches to study cyst dynamics in a manner analogous to the way we study bloom dynamics in surface waters. It was not easy, as there was strong opposition to some of the methods that were used, but the scientific process and good data have overcome the speculation. There are certainly challenges and uncertainties ahead in the study of cysts and life history dynamics, but significant progress has been made and much knowledge gained. The future looks bright for future discoveries, facilitated by our growing recognition that marine dinoflagellate cysts have much in common with terrestrial plants and that their distribution and abundance can be quantitatively mapped – this valuable information will enable us to estimate deposition and germination fluxes. We clearly need continued study to help elucidate the mechanisms that trigger sexuality and cyst formation, and that will likely involve observations using in situ sensors like the IFCB. After more than 60 years, the study of living dinoflagellate cysts remains a vibrant and important element in HAB research, and I am grateful for the foresight and guidance of David Wall, Karen Steidinger and others, and to have been part of that evolution myself.

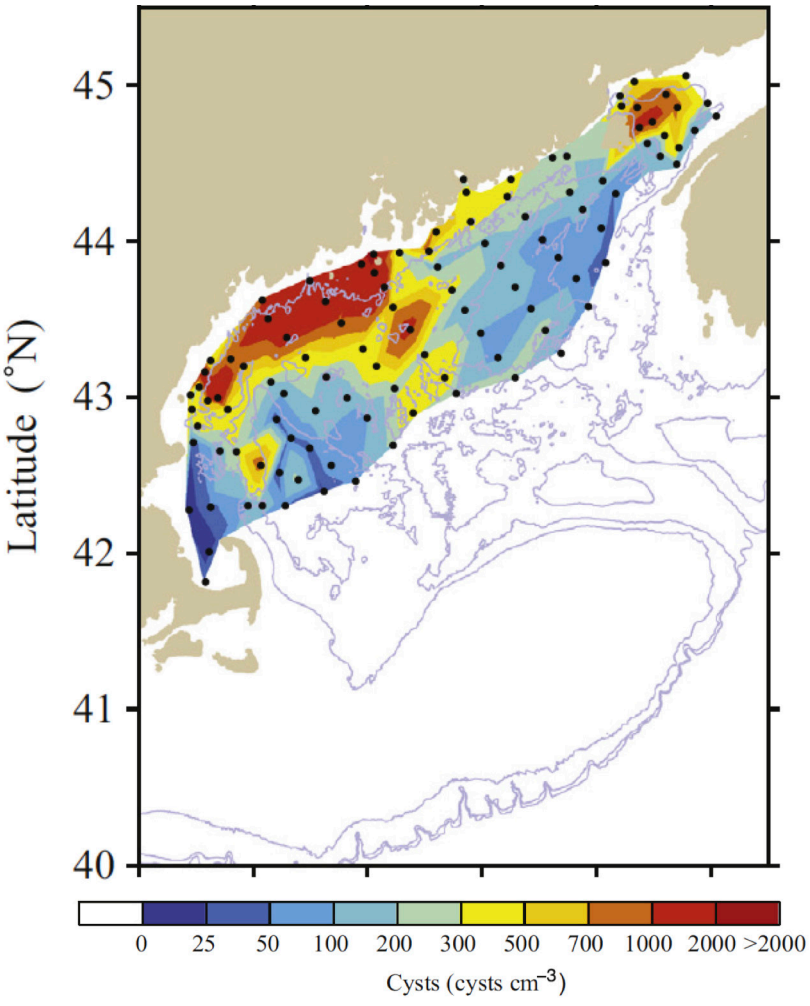


Fig. 6. Map of Alexandrium catenella cyst abundance in the Gulf of Maine. This image depicts a multi-year (2004–2011) arithmetic mean (cysts cm3) of the surface (0–1 cm) sediment-layer. Two seedbeds are clearly visible -one at the mouth of the Bay of Fundy in the north, and the other in mid-coast Maine to the south. From [16]

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How do algal blooms kill finfish and how can we mitigate their impacts?

Algal blooms, water discolorations and their association with fish kills have been recorded since historic times, such as the description in the Bible (1000 years BC) *"all the waters that were in the river were turned to blood. And the fish that was in the river died; and the river stank, and the Egyptians could not drink of the water of the river"* (Exodus 7: 20-21). In this case, a non-toxic bloom-forming alga became so densely concentrated that it generated anoxic conditions resulting in indiscriminate kills of both fish and invertebrates. Similarly, water discolorations and massive fish kills in Florida coastal waters have been reported by Spanish explorers since the 15th century (now known to be caused by the dinoflagellate *Karenia brevis*).

Finfish held captive in intensive aquaculture systems are extremely sensitive to HABs, and the impact of fish-killing algal blooms on human society thus has been exacerbated by the increase of finfish aquaculture. Shilo 1967 [1] first investigated problems for the Israel *Tilapia* pond aquaculture industry caused by the haptophyte *Prymnesium parvum*, but research efforts to understand fish killing mechanisms intensified in 1973 when *Chattonella marina* raphidophyte blooms (Fig. 1) devastated yellowtail aquaculture in the Seto Inland Sea [2] and in the 1980s when the dinoflagellate *Karenia mikimotoi* started to impact on the fledgling salmon farming industry in Norway [3].

Fish-killing algal species are responsible for much greater economic impacts (summarized in Table 1) than HAB events leading to seafood biotoxin contamination. The 2016 fish kills in Chile by *Pseudochattonella* and *Alexandrium catenella* which led to losses of US$800M triggered social unrest. It is therefore surprising that progress in our understanding of how HABs kill fish has been so slow. Financial support for and research interest in HABs by the fish farm industry has often been short-lived, and ranked lower than investments in fish husbandry, nutrition, and disease control. With the exception of *Karenia brevis*, so-called fish-killing toxins or "ichthyotoxins" are not known to have human health impacts and therefore also have not been a priority for seafood regulators. Scientific progress has been hindered by the use of widely different bioassay systems and lack of chemical analytical methods to quantify and characterize ichthyotoxins from seawater medium. Assay systems have included *Artemia* or *Daphnia* assays, the use of fish or mammalian erythrocytes, and a wide range of juvenile or adult fish (damselfish, sheepshead minnow, mountain minnow, zebrafish, salmon, sea bass) tested under different exposure regimes. The novel application of a standardised and highly sensitive and reproducible rainbow trout RTgill-W1 cell line assay in Australian, Chilean, Danish, Mexican and US laboratories has allowed significant progress to be made in the past 10 years [4,5]. This assay has been automated in a plate reader measuring cell viability dyes, and been successfully applied also to screen freshly collected natural seawater samples from fishkill events in Australian and Korean waters.

What is the precise mechanism causing fish gill damage?

While all high biomass algal blooms (>100 x 10^3 cells L^{-1}) can cause mechanical stress to the sensitive gill tissues of fish and trigger excess mucus production, of much greater concern for the aquaculture industry are the highly potent, taxonomically unrelated flagellate groups *Cochlodinium, Karenia, Chattonella, Pseudochattonella, Heterosigma* and *Prymnesium.* One feature that these algal groups have in common is that they are all fragile cells, which can lyse even upon impact on the gills of fish, especially during the end of blooms, or conditions of osmotic stress or during upwelling. For less fragile fish-killing algae such as *Karlodinium* or the armoured *Alexandrium* the conditions that cause cells to exude ichthyotoxins and/or cause cell lysis are critical, and ichthyoxicity by these genera tends to be more variable.

The first point of attack by all above algal groups are the fish gills (Fig. 2), resulting a generalized necrotizing degeneration of the epithelium of the secondary lamellae with associated sloughing, often accompanied by swelling of the primary lamellar epithelium and congestion of branchial vessels [3]. Fish gills tend to respond in a singular way and gill pathology is remarkably similar for different ichthyotoxic algae and in different fish species [3, 6-9]. Once the fish gills are compromised, algal neurotoxins if present can penetrate the blood stream, cause fish behavioural changes, and loss of the blood haemoglobin's oxygen binding affinity [2].

Several competing theories have been proposed as to the precise mecha-

Fig. 1. Mass mortality of (above) yellowtail fish in the Seto Inland Sea in Japan (photo courtesy Prof T.Okaichi) and (below) blue-fin tuna in South Australia (photo courtesy Barry Munday), both caused by Chattonella marina blooms. Higher potency of Australian blooms (kills occurred at 66,000 cells/L) compared to Japan (500,000/L) is attributed to higher sensitivities of tuna but also higher ichthyotoxicity by Australian high-light adapted algal strains [5].

Table. 1. Economic losses from algal blooms for finfish aquaculture in different parts of the world

HAB species	Country	Financial Losses
Chattonella *Heterosigma*	Japan	USD500M, 1973 USD135M,1980/90
Cochlodinium *polykrikoides*	Korea, China Canada	USD95M, 1995 USD2M, 1999
Heterosigma *Chaetoceros*	British Columbia	USD35M, 1980-90
Heterosigma	New Zealand	NZD12M
Karenia digitata	Hong Kong	USD32M, 1998
Karenia mikimotoi	Norway	USD6M, 1988
Alexandrium catenella *Pseudochattonella/* *A. catenella*	Chile	USD60M, 2002/ USD800M, Jan-Mar 2016
Chattonella *Karenia*	South Australia Tasmania	AUD45M, 1996 AUD3M, 2003

nism of how algae kill fish. These include: free fatty acids (FFA), reactive oxygen species (ROS), and phycotoxins such as brevetoxins, karlotoxins, gymnocins, or varying combinations of all previous compounds.

Polyunsaturated fatty acids (PUFA)

Shilo working with fish-killing *Prymnesium* pointed out how "lipid micels" released by the algae can impact on the fish gill lamellae and interfere with osmoregulation [1]. Okaichi, investigating *Chattonella,* focused on free fatty acids damaging fish gills [2]. This was pursued by French researchers including Patrick Gentien [10] working with *Karenia mikimotoi* (as *Gyrodinium aureolum*), who identified free fatty acids such as OPA (octadecapentaenoic acid) and EPA (eicopentaenoic acid) as having the highest ichthyotoxic potency. Mardones [11] also confirmed the ichthyotoxicity by DHA (docasahexaenoic acid) from *Alexandrium catenella.* Paradoxically these compounds are well-known to have a beneficial effect for human health, notably heart disease, when consumed in moderation and used to replace saturated fat. These PUFA compounds are prone to oxidative degradation, in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. In whole fish experiments it has been convincingly demonstrated that exposure to OPA and EPA did cause fish gill damage at concentrations in seawater of approximately 3ppm [7,12]. This is short however of what dense algal blooms would generate.

Reactive Oxygen Species (ROS).

The role of Reactive Oxygen Species in ichthyotoxicity has long been suggested from whole fish experiments with *Heterosigma* and *Cochlodinium*, where application of ROS mopping enzymes such as catalase and peroxidase significantly improved fish survival [13, 14]. Japanese researchers led by Oda [15] focused on the fish-killing raphidophyte *Chattonella* which is a potent producers of ROS notably when algal cells are ruptured. Several other fish-killing algae such as *Karenia* and *Alexandrium catenella* are also strong ROS producers, but for *Heterosigma, Karlodinium* and *Prymnesium* ROS on its own cannot explain ichthyotoxicity. Using a xanthine-xanthine oxidase chemical reaction to generate superoxide at concentrations equivalent to fish-killing *Chattonella*, Marshall [7] demonstrated that superoxide on its own does not kill fish. Similarly, Trick and co-workers [16] showed that hydrogen peroxide produced by *Heterosigma* did not explain ichthyotoxicity. ROS also exhibited negligible impact in the RT fish gill assay [5].

Synergism between ROS and free fatty acids

Pursuing the role of ichthyotoxicity by EPA, Marshall [7] demonstrated that when damselfish were challenged with EPA in the presence of ROS, this increased the potency of EPA by up to 15 fold (Fig.3). Similarly, DHA in synergism with ROS became 9 times more ichthyotoxic [11]. The precise nature of the lipid peroxidation products generated remains poorly known, and new analytical methods for the detection of such compounds in seawater during fish kills (e.g. using SPATT collectors) are much needed.

Role for true phycotoxins?

The dinoflagellate *Karlodinium* is known to produce chemically well-defined linear polyketide *karlotoxins*, which at ecologically realistic concentrations can account in full for their fish-killing potency [17]. While prymnesins from the haptophyte *Prymnesium* have attracted considerable interest [18] it remains to be shown whether these compounds can account for their ichthyotoxic potency. Similarly, cytotoxic chemical compounds such as *gymnocin* from *Karenia mikimotoi* remain to be shown to play a quantitative role in ichthyotoxicity [19]. Polyether ladder *brevetoxins* from the Florida *Karenia brevis* are well known as the cause of Neurotoxic Shellfish Poisoning in humans but purified brevetoxin PbTx2,3 exhibited limited ichthyotoxicity against RTgill cells [5], suggesting that peroxidation pathways may need be invoked in fish kills. The production of brevetoxins by raphidophytes is disputed [20]. Neuroactive compounds (but not brevetoxin) have recently been claimed also for the raphidophyte *Heterosigma* [21]. Mardones [11] found no evidence for a role of *saxitoxins* in fish gill damage, which instead could be explained by DHA and ROS synergism. Using the fish-gill ichthyotoxicity assay, karlotoxin and DHA could account for fish kills in their own right, but not EPA, STX, PbTX, OPA nor OTA. Synergisms between DHA x ROS and EPA x ROS were able to explain fish kills, and so could fatty acid aldehydes.

Implications for mitigation of fish-kill events

Several different strategies are currently practised in the fish farm industry to mitigate the ichthyotoxic effects of HABs. These include: cessation of fish feeding, towing away of cages from affected areas, perimeter skirts to protect against algal surface slicks, aeration or

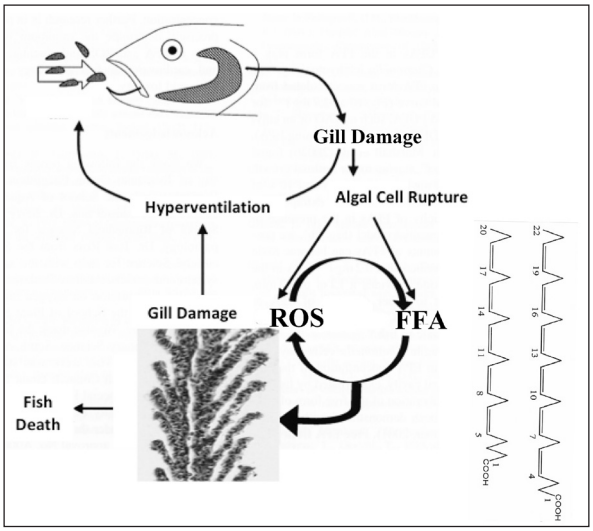

Fig. 2. Diagrammatic representation of algal bloom mediated fish kills, involving rupture of fragile algal cells to release a cocktail of Reactive Oxygen Species (ROS), Free Fatty Acids (FFA) but which rarely involves true Phycotoxins [modified after 7]. The chemical structures of two dominant microalgal polyunsaturated fatty acids EPA and DHA are indicated

airlift upwelling to dilute harmful algal concentrations, or clay flocculation to reduce numbers of harmful algal cells [22]. Mass fish mortalities lead to tonnes of dead fish being dumped in land fills (Port Lincoln, Australia, tuna mortality 1996) or dumped offshore (Chilean red tide 2016), but this material is rarely used for fish meal. As argued here, with perhaps a single exception (Florida *Karenia brevis*), none of these "ichthyotoxins" are of human health significance, meaning that recently killed fish are still fit for human consumption. Triggered by autonomous HAB monitoring systems, once fish start to die a decision thus could be made to instigate emergency harvest operations. To prevent the build-up of histamines, fish should be kept alive as long as possible during harvesting. This can be achieved by diluting algal concentrations via airlift upwelling, or by targeted in-pen emergency application of clays [23] that mop up ichthyotoxins at clay loadings significantly lower than those considered harmful to benthic marine invertebrates.

Laboratory experiments with fish gill cell lines or even whole fish tank experiments can only serve as proxies for real-life fish farm situations. HAB researchers need to forge better collaborative links with the fish farm industry to conduct research on fish kill events that involve the *in situ* measurement of ichthyotoxins in seawater using new analytical methods. Progress in understanding fish killing mechanisms by HABs will pave the way towards more effective mitigating strategies which are much needed to feed an ever growing world population with high quality seafood.

Acknowledgements

This overview is partially based on a keynote lecture presented at the ICHA17 Brasil Conference, a more complete write up of which can be found in the proceedings of that meeting. Numerous PhD students worked with me on fish-killing algae over 20 years, including Juan Dorantes-Aranda, Graeme Lush, Judi Marshall, Jorge Mardones, Ben Mooney, and Andreas Seger. I benefited from valuable collaboration with the late Barry Munday (fish pathology), Peter Nichols (lipids), Prof David Waite (ROS) and Prof Al Place (karlotoxins).

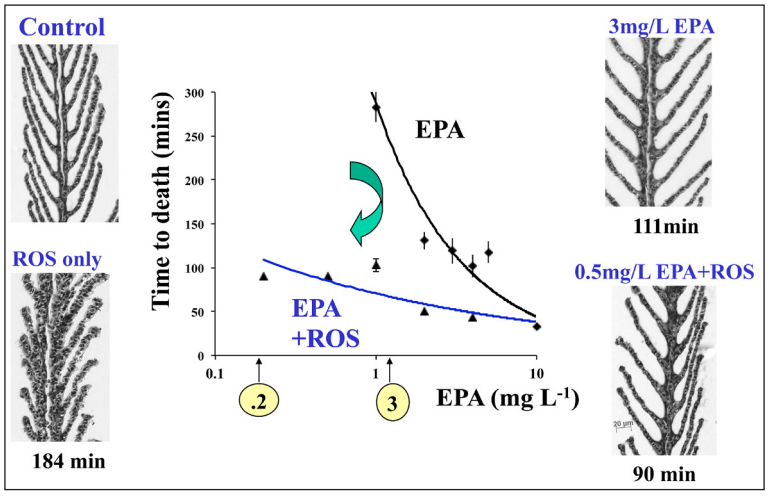

Fig. 3. Toxicity of the free fatty acid form of eicosapentaenoic acid (EPA) to damselfish: (top line) EPA on its own, requiring 3 mg L^{-1} to kill fish in 300 min; (bottom line) EPA in the presence of xanthine generated superoxide, where 0.2 mg L^{-1} EPA could kill fish in 100 min. Histopathology of damselfish gill tissues under selected treatment regimes is illustrated. Modified after [7]

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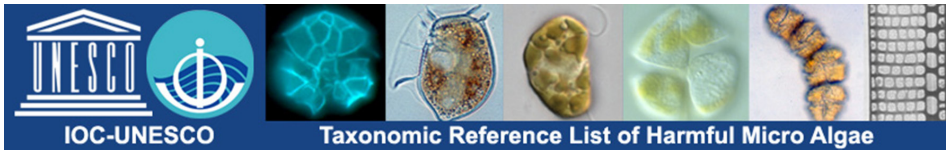

As part of the 25th anniversary issue of *Harmful Algal News* I am providing an overview of the *IOC-UNESCO Taxonomic Reference List of Harmful Algae* (*www.marinespecies.org/hab/*) and will highlight some of the problems which have faced or are facing the Intergovernmental Panel on Harmful Algal Blooms (IPHAB) *Task Team on Taxonomy* that is responsible for updating the list.

Historical timeline

1993

A task team on the taxonomy of harmful algae was established at the Second Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms in Paris in October 1993, in line with the HAB Programme Plan. The aim of the task team was to make taxonomic recommendations of harmful algal species, and membership comprised Y. Fukuyo, Japan, M. Elbrächter, Germany, and Ø. Moestrup, Denmark, the latter serving as chairman. During the 1993 session it was "encouraged" to create a computerized taxonomic data base of harmful species, an activity which at the time was planned between the *Expert Centre on Taxonomic Identification* (ETI) in Amsterdam and the *Botanical Institute*, University of Copenhagen.

1997

A major step forward was taken at the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms, which was held in Vigo, Spain 1997. There it was decided to establish a broader Task Team on Algal Taxonomy, with the following terms of reference:

I. to provide an agreed reference list of harmful algal species, including correct author citation, date of valid publication of the species, and a list of synonyms for each species
II. to provide guidelines to reduce nomenclature instability of harmful algal species
III. to organize round table discussions on nomenclature and taxonomy of harmful algal species
IV. to organize inter-calibration exercises to standardize the identification of harmful algal species

2000

The contents of the list were discussed in detail at the HAB Conference in Hobart in 2000, and it was decided to aim at including additional information in the list such as the basionym of each species (the first name applied to the species), reference to the article in which the species was described, reference to the article in which the species was given its current name, information on the type locality, information on the main harmful effects of each species, including up to three references with information on toxicity, toxins or toxic effects.

2002

The list was completed in 2002 and made available online on the homepage of the Department of Biology, University of Copenhagen.

Post 2002

During the following years the list was regularly updated, based on information from committee members and others. The list was also discussed at the meetings of the Intergovernmental Panel on Harmful Algal Blooms in Paris and suggestions for improvements were made.

2008

A major change took place in 2008 when the list became part of WoRMS, the World Register of Marine Species. This change was decided following a visit by Gert Hansen, Henrik Enevoldsen and me to the headquarters of WoRMS at VLIZ in Oostende, Belgium. The merits of becoming part of WoRMS were discussed and the technicalities of how to include and update species in WoRMS were explained and demonstrated. WoRMS is a very large database which includes almost half a million species of marine organisms. It was decided that the HAB list should both be included in WoRMS and be accessible as a separate entity of WoRMS. The WoRMS list is harvesting algal taxonomic data from ALGAE-BASE in Ireland. It was subsequently decided that the IPHAB Task Team should coordinate the List of Harmful Microalgae with ALGAE-BASE by using the same names of the relevant algal species. The names from ALGAE-Base are therefore automatically used in the List of Harmful Microalgae. When name changes become necessary, these are discussed and agreed with ALGAE-BASE and inserted in both lists.

Which species should be included in the list?

The list was born as a result of the growing interest in harmful algae. The many name changes which regularly took place for many harmful algae, following publication of new information, was/is a source of confusion to ecologists, toxicologists, people involved in routine monitoring and others, thus construction of an agreed and updated list was considered important. A decision had to be made on which species to include in the list. Early on the distinguished member of the Task Team, Prof Y. Halim from Egypt, of *Alexandrium minutum* fame, suggested that all harmful species should be included in the list, in other words the list should include also species which at some stage had formed blooms, causing low oxygen levels in the water at night, and leading to mortality of fish and/or or bottom invertebrates such as certain species of diatoms and silicoflagellates. While these were and still are very real issues, to include all such species in the list would have made the list too long and difficult to handle. It would have entailed including numerous non-toxic and usually harmless species. The Task Team eventually agreed on including only species known to form a toxin or suspected to do so. The lack of knowledge about toxins was particularly relevant for algae causing fish kills, as many of the compounds responsible for the fish kills were/are still not well known. The next problem was to decide on the definition of the term toxic, did it mean toxic to humans, fish, *Artemia*? It was agreed to concentrate on species toxic to humans or to fish. When in doubt, a comment was included under the relevant species.

Nomenclature problems

One reason for the development of the list was that incorrect author citations were recorded in the literature. In fact, the poor general knowledge of and interest in nomenclature is still a problem.

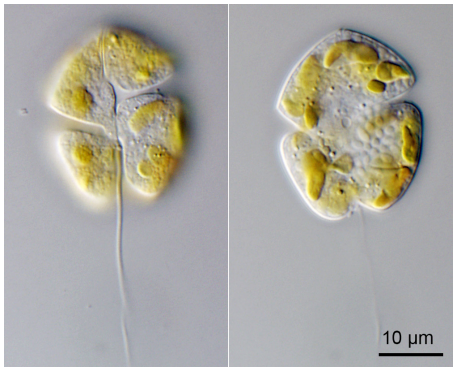

Fig. 1 Karenia mikimotoi (strain K-0260), a historic strain isolated in 1977, as Gyrodinium aureolum, from Oslofjord, Norway, by Karl Tangen (micrographs from Gert Hansen)

Thus mix-up of zoological and botanical nomenclature sometimes appears, even in major phycological journals. It may result in the creation of invalid species names, or it causes uncertainty about the validity of names. An example is the description of the genus *Stoeckeria*, which first appeared in 2005 in Journal of Eukaryotic Microbiology. The description lacked the Latin diagnosis required in botany at the time. The authors apparently intended this heterotrophic dinoflagellate to be described using zoological nomenclature. That is acceptable, a species which is validly described according to one Nomenclature, automatically becomes valid in the other. However, in the title of the article in which *Stoeckeria* was first described, the authors used the class name Dinophyceae, which is botanical, not zoological. Since the authors did not state specifically in the article which nomenclature they used, it caused some readers to conclude that because of the word

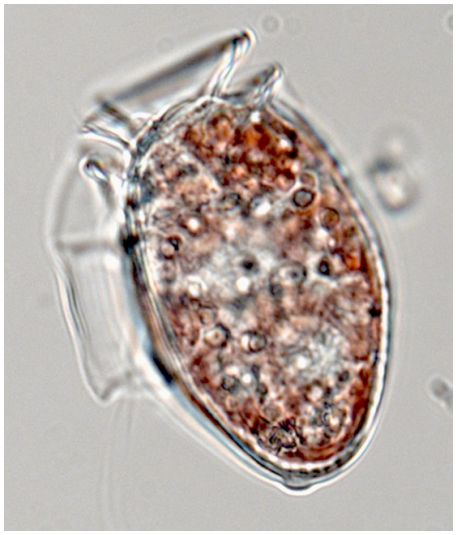

Fig. 2. Dinophysis acuminata from Mariager Fjord, Danish coastal waters (micrographs from Øjvind Moestrup)

Dinophyceae the authors intended the new genus to be described according to the botanical nomenclature. The lack of a Latin diagnosis therefore made it invalid. The confusion has actually still not been resolved, and the problem needs to be discussed and decided upon by the International Nomenclature Committee, before it can be fully resolved.

Name changes

One of the problems facing the Task Team on Algal Taxonomy that maintains the List of Harmful Microalgae, is name changes (e.g. *Karenia mikimotoi*) (Fig1). This phenomenon shows no sign of disappearing, and it has recently become an even more serious problem, following the increased use of molecular sequencing.

Invention and application of new techniques that allow species to be examined in more detail have commonly resulted in old species being split into two or more species. This happened when transmission electron microscopy (TEM) became a routine tool in the 1950s, used in detailed studies of small flagellates, morphological details of which are difficult or impossible to see using light microscopy (LM) but readily visible in the TEM. It resulted in the description of numerous new flagellate species, some harmful, others not. This became a problem for ecologists and others who did not use TEM in their investigations and identification to species level was no longer possible. A similar situation took place some time later, notably for diatoms, when scanning electron microscopy (SEM) became a general tool. In recent years, the problem has reached major proportions, following introduction of molecular methods. At the moment there is a tendency to split species into ever smaller units, discernible only by molecular methods. In other cases, some species have been merged, commonly based on studies of one or a few genes, or on observations of polymorphic life cycles (e.g. small cells of *Dinophysis* species) (Fig. 2). Controversy arises when certain genes of morphologically different species (morphospecies) are found to be very similar. Do such species in fact belong to a single species? This has become a major problem for the Task Team on Taxonomy, as it results in discussions on how to define species, genera, families, and in fact all taxonomic levels. Such decisions have always been a problem, but molecular techniques have enabled discrimination of very small parts of the genome, inducing some researchers to define only the genetically most similar strains as belonging to the same species. Categories, subspecies or varieties are presently rarely used by phycologists. If the genes examined are found to be slightly more different, the material is considered to represent different species, and if even more different, to belong to different genera, families, and so on.

Is splitting or merging more correct? There are no rules for this problem in the Code of Botanical Nomenclature; it is up to the researcher her/himself to decide on how to define what constitutes a species, a genus, a family, etc.

How to define a species has been discussed for longer that I care to remember, but there is still little agreement on this question. The same applies to the higher taxonomic levels. It is amazing that there is so much confusion, following so many years of careful study. Boenigk et al [1] in their discussion at the VI European Congress of Protistology in Berlin 2011, expressed it clearly: "*Scientists have been trying in vain for hundreds of years to find the 'correct' definition of species, but the simplest conclusion is that one does not exist*" (p 99 in [1]). The species concept is a practical means that allows us to communicate about our organisms. However, there are currently more than twenty definitions of what defines a species, and "*The advent of molecular data ... compels us to reconsider how species may be most effectively and informatively delineated*" (p 99 in [1]). The so-called biological species concept is often mentioned, also in phycology: if two populations can cross and produce fertile offspring, they belong to the same species. This concept has strong limitations, however, as shown in both mammals and vascular plants. The two known species of buffalo, the American and the European, readily form fertile offspring. If they were to be placed together, they would most likely soon merge into a single species. Yet, we prefer to consider them two different species. The same applies to many other geographically separated organisms. The situation is further complicated by some populations of

geographically widespread species being able to form fertile offspring, while other populations of the same species are not.

Molecular techniques have contributed very significantly to solving many taxonomic problems, but they have not resulted in the emergence of a finite species concept. We have to accept that all levels of classification are subjective rather than objective, and it has always been that way. To cite Charles Darwin: "*I mean by species, those collections of individuals, which have commonly been so designated by naturalists*" (cited by M. Ereshefsky in [1]).

At the generic level, splitting into many smaller genera is occurring rapidly at the moment. The genera containing a large number of species are presently being split into many smaller genera, and if this trend continues, these diverse genera will become a thing of the past. In some cases, some of the new genera are monophyletic rather than polyphyletic, and splitting becomes a matter of personal preference. In other cases separation into smaller genera can result into a better understanding of relationships of the species in this particular part of the old genus. All these problems obviously leave the work of the *Task Team on Taxonomy* difficult. One problem facing taxonomists is that results of taxonomic studies are used not only by academics themselves but also by non university people. With this in mind, the taxonomic systems suggested should not be overly academic; a pragmatic approach to taxonomy will help taxonomy to retain its usefulness in the general public.

A pragmatic approach is currently not held in high esteem, as exemplified by the case of *Cochlodinium*. *Cochlodinium polykrikoides* (Fig. 3) is a very serious fish killer in South-East Asia, but *C. polykrikoides* is not the type species of *Cochlodinium*, and evidence has existed for some years that the type species, whose morphology had not been studied in detail, is so different genetically that the two species should not be included in the same genus. If the genus were to be split, the type species, *Cochlodinium strangulatum*, would then retain the name *Cochlodinium*, while unrelated species presently included in *Cochlodinium,* including *C. polykrikoides,* would need to be given one or more new generic names. Such a change is bound to cause considerable confusion in countries like South Korea, where the name *Cochlodinium polykrikoides* is well known by the general public. There are, however, ways to solve such problems; it is possible to change type species of a genus if this is deemed to be critically important. It is a solution which has to be discussed and accepted by the internal *Nomenclature Committee*, but it has happened before (e.g. the type species of *Acacia*). In the case of *Cochlodinium polykrikoides* it would have been pragmatic to suggest that *C. polykrikoides* should become the new type species for *Cochlodinium*, as this would reduce confusion. There is no guarantee that the suggestion would have been accepted by the *Nomenclature Committee*, however, but in the meantime a new generic name, *Margalefidinium*, has been suggested for *Cochlodinium polykrikoides* [2].

A common problem facing the Task Team has been that in some cases sequencing of single or a few ribosomal genes have proved insufficient to decide on the species level to be used. This was particularly clear in the case of the dinoflagellate *Peridinium aciculiferum* complex, which comprises *P. aciculiferum*, *P. malmogiense* (as *Scrippsiella hangoei*), *P. baicalense*, and *P. euryceps*. The ribosomal genes of the four species are very similar [3], in fact Logares et al. [4] found no difference in ITS1, ITS2, 5.8S, SSU and partial LSU between *P. aciculiferum* and *P. malmogiense*. Yet these species are morphologically and ecologically different and it would be meaningless to merge them into a single species.

Studies on the ribosomal genes therefore do not always provide the final answer. Will these taxonomic problems be easier to solve when more genes or whole genomes become available for use by the taxonomist? Some changes are bound to occur, and an example of such a change is the case of the protist group Heliozoa, suggested by Ernst Haeckel in the late 1800s. The group was found to be polyphyletic based on sequencing of the SSU gene [5], and Haeckel's old name Radiolaria was therefore in for modification. However, subsequent studies of transcribed genes (single cell transcriptomics) resulted in the opposite result, Haeckel was now right once more; the group was again monophyletic [6].

Taxonomy is going through a difficult time at the moment. The Danish phycologist Tyge Christensen, whose name is associated with the *Tyge Christensen Prize* awarded annually by the *International Phycological Society*, wanted genera to be identifiable with the use of a hand lens or at least in a light microscope. If the present trend continues, most unicellular species and genera will eventually become identifiable only by using molecular sequencing. It will be a challenge to handle this situation in a pragmatic way.

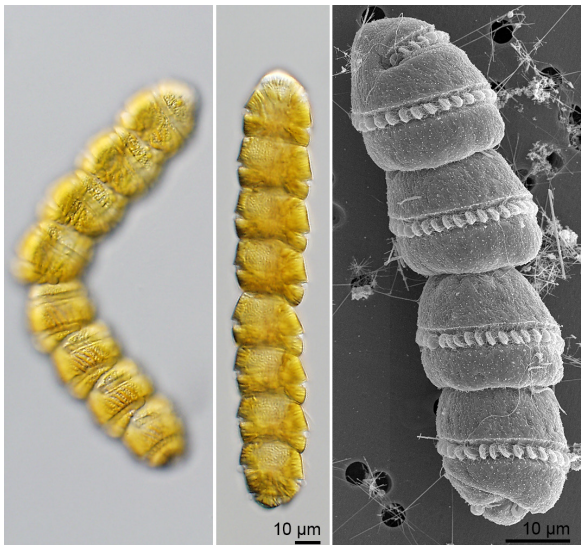

Fig. 3. Margalefidinium polykrikoides (strain K-1292) from Tsushima Island, Nagasaki, isolated by Mitsunori Iwataki in 2006 (micrographs from Gert Hansen)

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Algal toxin discovery, management and regulation over the last 25 years

Algal toxins in the dark ages (pre-1992)

From a historic perspective, knowledge about algal toxins can be divided into truly prehistoric occurrences such as known from paleontological studies [1-2] and more recent historic records. In these historic records, there are descriptions of poisoning incidents that clearly point towards the occurrence of the algal toxins centuries ago, such as the description of Captain George Vancouver, whose crew suffered from paralytic shellfish poisoning during the exploration of the Pacific Northwest in 1793 [3]. Other examples include ciguatera [4] and paralytic poisoning [5-6]. During the 19th century, modern taxonomy emerged as a science with developments in microscopy; by 1900, rather systematic studies of phytoplankton communities are common [7-10] and provide the basis for the biogeography of many toxic genera. In the early to mid-20th century the links between algae and toxins, or at least the toxic effects of algae, are being made [11-15].

By the beginning of the 1990s, many major algal toxins that cause acute poisoning had already been discovered, including brevetoxins [16-19], ciguatoxins [20-27], domoic acid [28-36], okadaic acid and analogues [37-40], prorocentrolide [41], and saxitoxins (STXs) [42-46]. Quite a few analogues of the main toxins had already been discovered [47-50], as well as several groups of compounds produced by dinoflagellates that provoke death in mice used for the mouse bioassay (MBA) but are not necessarily related to human poisoning events, such as pectenotoxins [50] and yessotoxins [51].

Discovery of toxins over the last 25 years

While discovery was mostly driven by human poisoning prior to the 1990s, afterwards the discovery of further compound groups that are produced by dinoflagellates and their metabolites in shellfish was facilitated by the introduction of the MBA for lipophilic toxins in routine shellfish safety testing in European legislation [52-53], as well as by several technological advances. One of the main technological drivers in discovery was certainly the onset of the use of liquid chromatography coupled to mass spectrometry (LC-MS) [54-56], which became quite widespread by the beginning of the 2000s [57-61].

The number of toxin groups that were discovered over the period from 1966 to 1990, (fifteen), was not much less than those discovered over the last 25 years, (nineteen) (Fig. 1). However, the number of analogues in each group has rapidly increased. A good example of this is the STX-group where a review in 1990 counted nineteen observed analogues with a further five predicted from plausible metabolization or chemical transformation pathways [62]. In 2010 a review reported over 50 observed analogues [63]. A simi-

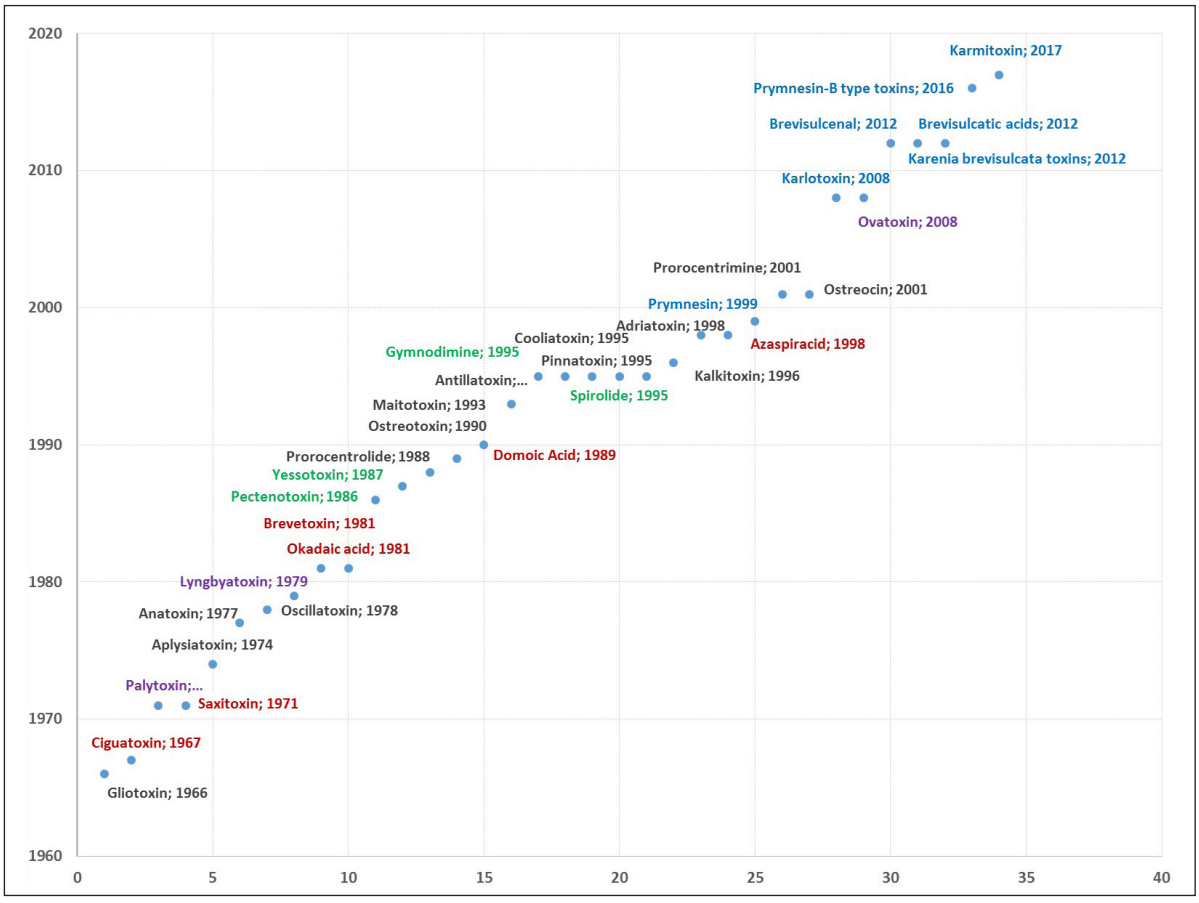

Fig. 1. Discovery or description of the structure of the first analogue of 34 toxin groups (1966 – 2017). Colour code: blue: toxins involved in fish kills; red: toxins involved in human poisoning, violet: toxins causing skin irritation or respiratory problems (BTXs should be red, blue and violet), green: toxins known for 20-30 years and not proven to have negative effects on humans or aquatic organisms, black: toxic compounds yet to be related to effects in humans or aquatic compounds. Nota bene: not many toxin groups relevant to human poisoning are being discovered while more and more toxins related to fish kills are (toxins of Karenia brevisulcata may be related to the Wellington Harbour syndrome)[79-108]

lar rapid increase in known analogues has been observed for the azaspiracid (AZA) group, with the first analogue described in 1998 [64] and a review in 2014 reporting 30 analogues [65]. Only three years later, over 50 analogues are known for this group, including novel phosphate derivatives [66-71]. This discovery rate could have been even more rapid if the causative organisms of AZAs had been elucidated earlier. However, the delay from the first poisoning report in 1995 [72] to the discovery of the culprit organism [73] in this case was likely due to: i) the initial misidentification of the heterotrophic dinoflagellate *Protoperidinium crassipes* (a vector of AZAs upon its feeding on *Azadinium*) as the causative agent, and ii) the difficulties in identification of such a small organism (<15 μm) by optical microscopy in water samples fixed with acidic Lugol's solution, the most common way to preserve samples in routine plankton monitoring.

Another phenomenon that has appeared repeatedly over the past 25 years is the discovery of slightly modified base skeletons for toxin groups. The ciguatoxin (CTX) or CTX1B (= P-CTX-1B) had been reported relatively early on and had been isolated from the moray eel [74]. The algal precursor CTX4A was only described in 1997 [75], yet a slightly modified base skeleton had been reported a few years earlier from *Gambierdiscus*, i.e. CTX3C [76]. Prymnesins are another example of such skeleton variation which is indeed very labour-intensive in natural product discovery as basically the full discovery pipeline has to be completed: bioguided fractionation and isolation of the compound, purification and structural characterisation including mass spectrometry, nuclear magnetic resonance (NMR), UV, infrared and potentially many other studies [77-78].

Finally, it should be noted that only a few compound groups discovered since 1992 have been clearly related to human health issues. These include AZAs (diarrhoea), ovatoxins and to a lesser extent the toxins of *Karenia brevisulcata* (aerosol and direct contact exposure).

There is a significant increase of compounds that appear related to fish kills, e.g. karlotoxins, karmitoxins, prymnesins (A, B and C-Type) and *K. brevisulcata* toxins. The need to clarify the agents involved in fish kills has also been highlighted by a recent systematic review of toxic and harmful algae [109], as well as by the Intergovernmental Oceanographic Commission of UNESCO (IOC) Intergovernmental Panel on Harmful Algal Blooms (IOC-IPHAB), that included the topic in its list of Task Teams.

The systematic inventory of toxins has also been updated [78-108] by the IOC-IPHAB Task Team on Biotoxins, Management and Regulation over the past few years and international databases, e.g. the Harmful Algal Event Database (HAEDAT) updated accordingly. This same panel also contributes to other IPHAB activities whenever chemical expertise is required (e.g. fish kills, HABs and desalination etc.).

Drivers of change in management and regulation

There have been many drivers of change in management practises (e.g. detection methodology) and regulation. These include (i) increased awareness by governments of poisoning events and fish and shellfish mortalities through IPHAB communication with member states (ii) increased pressure from shellfish industry against the MBA for lipophilic toxins due to its qualitative character, false positive results and delays in reporting (iii) technological advances. The conflicts caused by the disadvantages of the animal assays (mouse and rat) for lipophilic toxins has been subject to much debate [110] and decade-long efforts to produce the necessary standards and reference materials for the validation of alternative methods, which have been aided by researchers in Canada (e.g. Michael Quilliam), Ireland, Japan (e.g. Takeshi Yasumoto), New Zealand and many other countries [111-122]. Again the IPHAB panel played a pivotal role in pushing this issue at European and international levels for several years with the help of Phil Busby† (New Zealand Food Safety Authority), a long battle for which the international community will remember him.

Monitoring systems, management practises and legislative changes have been recently reviewed for different trade blocks [123-125]. A major step has been made with the switch from the mouse bioassay to chemical testing by LC-MS/MS for lipophilic toxins, first in New Zealand, then Europe [126] and most recently Japan.

Outlook

Several points can be raised looking foreward from the historic perspective. Climate change is one of the most striking challenges that has been raised with regards to prediction of harmful algal blooms (HABs), and while certain trends appear to manifest themselves [127], much more research is needed to fully anticipate the impacts of climate change on our ecosystems, HABs and their impacts on society [128]. As mentioned above, the need to improve our understanding of the impacts of micro-algae on other aquatic organisms, in particular those that serve as major food resources, i.e. fish and shellfish, has been recognised and requires major international efforts. The multiplicity of compounds in the marine environment only emerges with the recent onset of the omics and while recent studies have shown the feasibility to explore this chemical diversity in the marine environment with techniques such as metabolomics [129-131], more systematic studies will be required to effectively monitor our coastal waters to protect our resources and consumers. Finally, it should be noted that one of the longest-known groups of toxin, i.e. the ciguatoxins, still continue to cause the highest number of seafood poisoning globally [132] and thus deserves the attention of the scientific community over the next few decades.

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Butterflies in Brazil

Abstracts are not always reliable guides to authors' intentions. They are not expected to reveal a great deal about the evidence to be deployed in support of the science, evidence that may not even exist before deadlines for writing abstracts! Nevertheless, as examples of a minor art form, we are entitled to subject them to criticism. These comments were provoked by reading the abstracts of the Florianópolis conference. Here the focus is on two topics, i) climate change, and ii) suggestions that we follow Ben Schneiderman [1] into the convoluted procedures of "Science 2.0". A word count of the Florianópolis conference abstracts reveals that *climate* occurs 73 times, 33 times in the phrase *climate change*, 4 times in presentation titles [2].

Here are some incantations from the abstracts: "*Emerging*[1] *evidence suggests that climate change is impacting marine and freshwater phytoplankton communities"; "Climate change and anthropogenic activities in the coastal zone are increasing the risk of Harmful Algae Blooms"; "The worldwide distribution, frequency and duration of cyanobacterial blooms are driven by eutrophication and climate change"; "El Niño ... is increasing in frequency and magnitude due to climate change"; "As a result of climate change and non-climatic factors, algal bloom frequency, composition and spatio-temporal distributions are changing."* These quotations are mostly assertive; such formulae are not unique to the Florianópolis conference, but were much more prominent there than in earlier conferences in the series.

The idea that harmful blooms are a growing problem was expressed in the first HA conference by Anand Prakash who wrote; "*... there is evidence that paralytic shellfish poisoning outbreaks are increasing in intensity and spreading to new areas*" [3]. He invoked eutrophication to account for increased *intensity* of blooms, and cited Tokyo Bay and the inner Oslofjord as examples. His only example of *spreading* was that of PSP to the western Gulf of Maine in 1972, which was the primary raison d'être for convening the conference. In subsequent discussions of this theme, we often find some combination of the words *frequency, intensity* and *spreading*. Ted Smayda used the phrase *global epidemic* [4].

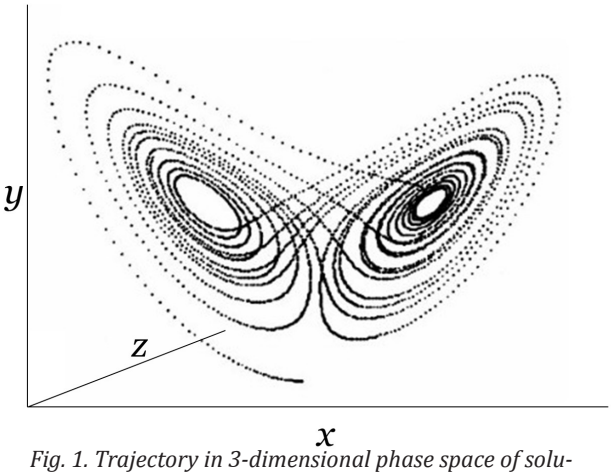

Fig. 1. Trajectory in 3-dimensional phase space of solutions of a simple model of atmospheric convection. Each point represents an instantaneous state of the system. The region to which the points are confined is called a strange attractor. This is Lorenz's butterfly.

In a widely cited statement of concern with climate change and harmful algae Gustaaf Hallegraeff wrote that "*public health and economic impacts of such events appear to have increased in frequency, intensity and geographic distribution*" [5]. Note here, it is not red tides that have increased, and the public health and economic problems *appear to have* (not *have*) increased. This distinction is often missed. Hallegraeff also discussed the possibility that there is a real epidemic (in Smayda's sense). Although he concluded that this too is apparent rather than real, he listed various putative causes, amongst them eutrophication and climate variations.

Revisiting the theme nearly two decades later, Hallegraeff [6] provided a succinct guide to what might happen under a conventional IPCC climate projection; he discussed the potential impact of warming seas, changes in mixed layer depth, wind regimes, runoff, upwelling rates and other variables – we can call them underwater weather – as well as various feedbacks, and overfishing. These variables are standard fare for marine ecology, appealed to routinely to explain the changing population dynamics of HABs as well as other marine species.

The key ideas of climate change are not always examined critically, nor with the natural suspicion that their frequently doctrinal character should excite. So what do we mean by *climate change*? On decadal to secular time scales, we can distinguish *mean* climates, *modal* climates, and *model* climates. Climates result from the interactions of many different elements within the atmosphere, hydrosphere, cryosphere, and biosphere, including phytoplankton. The future states of each of these systems are determined by many interactions within and between them, multitudes of signals with differing amplitudes and phase relations, positive and negative feedback mechanisms, in short, complex dynamics. Complexity really is complex! The mean climate of a region is the average of all the processes we call weather, conventionally calculated over a thirty year time span (World Meteorological Organization) from surface records of temperature, humidity, precipitation, winds, etc. But the mean deceives; it hides variations due to internal *modes*, the best known of which are the El Niño Southern Oscillation (ENSO), and the North Atlantic and Pacific Decadal Oscillations (NAO, PDO); so on decadal to secular time scales, regional climate fluctuations are largely due to modes. Model climates are those we build in our imaginations and in our computers.

Climate *changes* are persistent trends identifiable in meteorological records, usually considered as responses to changes in external forcing rather than modes; recent examples are the cooling from 1940 to 1970 or the warming from 1970 to 2000. "Anthropogenic global warming" (AGW) considers recent warming a result of industrial growth, and many AGW models focus on forcing by atmospheric carbon dioxide concentrations [CO_2]. As time scales grow from secular to millennial, we meet palaeoclimates with well known phenomena like Pleistocene Ice Ages and the Holocene climatic optimum. But the perceived HA epidemic is

1) *Emerging* is a fashionable prefix for evidence, as it is for markets, equities, roles, ... A sciencedirect.com search finds the phrase *emerging evidence* in nearly 30, 000 titles; time to give it a rest?

on a decadal time scale, and identified palaeoclimatic oscillations are not necessarily a useful guide to its interpretation.

An obvious obstacle to detecting climate signals in HAB data is posed by anthropogenic eutrophication. Another obstacle is the fact that phytoplankton respond directly to the increased [CO_2] blamed for warming in many models. Phytoplankton, macroalgae and sea grasses respond to raised [CO_2] by increased nitrate uptake and growth. Thus higher [CO_2] can lower the impact of eutrophication. Increased [CO_2] consumption by phytoplankton also raises pH, hence counters any tendency for acidification.

In the original examples of increased intensity of blooms (Tokyo Bay and inner Oslofjord), eutrophication seems a highly likely cause since both are semi-enclosed water bodies with increasingly urbanized watersheds; but eutrophication cannot have driven the sudden spread of PSP into the western Gulf of Maine, open waters with low human population density along the coast. The message from these examples is that we must analyse the frequency, intensity, and spreading of HABs as separate phenomena, on a species by species basis, and not lump them all together as manifestations of a global epidemic. The same message emerges from more recent examples, for example those summarized by Hallegraeff [6] from his antipodean perspective.

Edward Lorenz [7] asked, "*Does the flap of a butterfly's wings in Brazil set off a tornado in Texas? Is the behavior of the atmosphere unstable with respect to perturbations of small amplitude*?" There is a *nonlinear* relationship between the two variables, butterflies and tornados, meaning that an equation relating them is not of the first degree; nonlinear relationships are the hallmark of *dynamical* systems (Fig. 1). Lorenz wrote: "*Since we do not know exactly how many butterflies there are, nor where they are located, let alone which ones are flapping their wings at any instant, we cannot, if the answer to our question is affirmative, …, accurately predict the occurrence of tornados at a sufficiently distant future time*." A practical corollary of this sensitivity to initial conditions, called *the butterfly effect*, is that forecasts of the future are nearly impossible. Climate

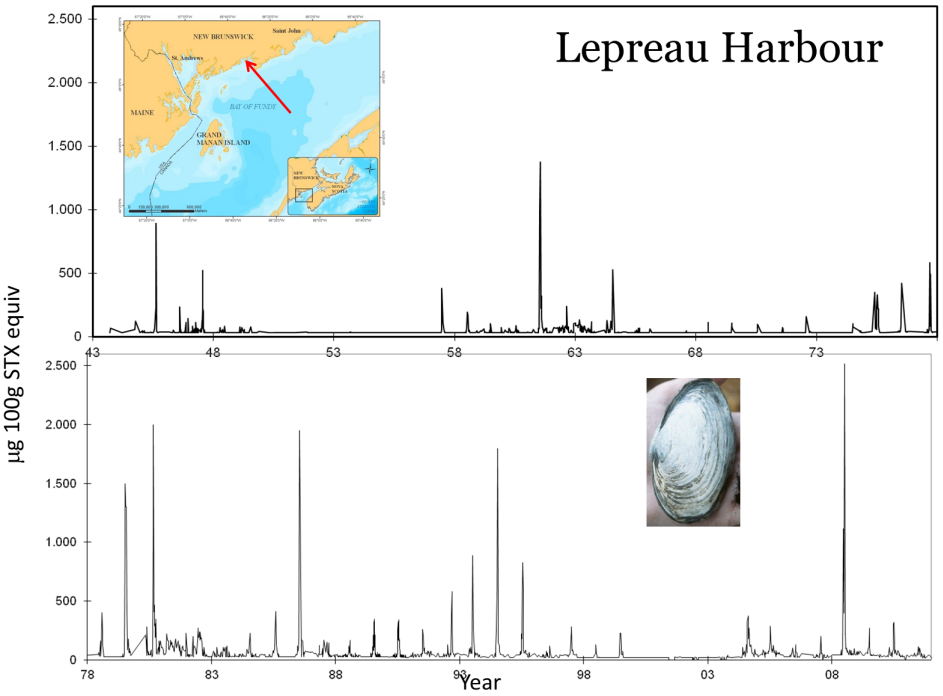

Fig. 2. PSP toxicity maxima identified by White [11] are centered on years 1945, 1961 and 1980; these lag minima of lunar declination in 1940, 1959, and 1978 by a few years. Had the trend continued, further peaks might have been expected around 2000 and this year. The Lepreau Harbour toxicity time series shown here has the three earlier peaks, and more recent ones in 1995 and 2009 which precede the lunar declination minima in 1997 and 2015. Clearly the nodal signal is not the whole story! (Figure courtesy of Jennifer Martin, St Andrews Biological Station, NB, Canada).

then is the prototype of chaotic systems.

We all know that algal growth and the likelihood of blooms are linked to wind regimes, water column stability, runoff, mixing rates, and so on, that is to the local weather, all modulated by regional climate variations. One might therefore anticipate that time series of harmful algal events contain signals provoked by multiannual trends in these oceanographic parameters, hence act as proxies for climate trends. But it seems to be very hard to extract unambiguous signals from records of HABs and algal toxins. In an issue of the *Journal of Sea Research* [8], 14 papers examine phytoplankton time series 1 to 4 decades in length, with special attention to climate variability and eutrophication. "*The initial hope that these data sets do contain significant signals, and that they can be identified and described, is not realized*", at least on these time scales; and, "*despite indisputable and often major changes in coastal waters caused by human activities, no clear signals are detectable in phytoplankton composition and dynamics, nor any which are distinguishable from changes in areas relatively unaffected by such impacts*." [9]. Claims to the contrary make much use of the subjunctive tense. But Kedong Yin & Jianzhang HE may have distinguished eutrophication and climate variation in a time series (1983 to 2014) of Hong Kong red tides [10].

In the case of toxins, PSP records from the Bay of Fundy (1944-1983) have yielded evidence of a multi-annual lunar signal, the nodal tide, with a period of 18.6 years (Fig. 2) [11]. This finding merits more attention than it has so far received given that the same signal has emerged from analyses of the PDO, the NAO, and many oceanographic and meteorological time series [12, 13]. Chaotic systems are conceptually remote from the clockwork universe of Isaac Newton and Pierre-Simon Laplace. But the nodal tide and other astronomical forcings are deterministic, they do run like clockwork, and can in principle provide forecasts of future climate variation.

The first conference in this series took place in Boston in 1974. From then until the Lund conference (1989), the word *climate* was not indexed in the proceedings. At the Newport conference (1991), El Niño was mentioned twice, and there were suggestions that as a result of hypothetical climatic

trends, *Karenia brevis* might appear more often in the South Atlantic Bight of the US and *Gymnodinium catenatum* bloom more often in northwestern Iberian waters. There was also a warning by Barrie Dale germane to such speculations, that large scale climate models cannot predict local changes.

Little more was heard of supposed links between HABs and climate until two decades later in Hersonissos (2010). The preface to the Hersonissos proceedings claims that "*…this conference included … a solid body of 50 papers and posters on climate change*"; if true, few of them appeared in the proceedings, where the phrase *climate change* appears in a single contribution about *Prymnesium parvum* in some Texan lakes, and the phrase *global warming* only twice. There were speculations that the appearance of *Chattonella globosa* and *Dinophysis tripos* in Norwegian coastal waters might be related to global warming, similarly for *Ostreopsis ovata* in the upper Adriatic.

Changing patterns of abundance on decadal time scales were reported at the Changwon conference (2012) for species of *Karenia*, *Cochlodinium*, *Chattonella*, and *Heterosigma* in Korean and Japanese waters, *Pyrodinium* in the Philippines, *Gymnodinium* in Portugal, *Prorocentrum* and *Heterosigma* in Narragansett Bay. As pointed out at the time [14], some of these patterns may be linked to regional effects of phenomena like the PDO or ENSO; and some almost certainly, as Patricia Glibert argues, to eutrophication. As already mentioned, disentangling the separate impacts of climate and nutrient loading is a major problem. AGW was not invoked, indeed it is clear that we do not yet have an adequate data base to do so [15]. Also at Changwon, Barrie Dale warned that "available time series plankton data are inadequate for establishing species' responses to natural climate variations and therefore offer no sound basis for predicting effects of climate". The Wellington conference (2014) abstracts contain the word *climate* only six times; in the two contributions in which climate change was addressed directly there, its impact on HABs was again enmeshed with eutrophication.

So what did participants at Florianópolis mean when they wrote of climate change? Was warming intended? From the abstracts alone, it is hard to know. A few clearly mean *weather* in the sense used above. Are others jumping on a bandwagon? If so, they might recall Gustaaf Hallegraeff's admonition: "*Crying wolf does not serve our discipline and we need to refrain from making unsubstantiated climate predictions*!" [16].

Science 2.0 was represented at Florianópolis [17, 18] by Jianguo Liu's "*coupled human and natural systems*" or CHANS [19] and Tim Lang's "*ecological public health*", EPH [20]. Both combine ecosystem models with other complex systems. CHANS are built by linking models of economic, social, hydrological, atmospheric, and biological subsystems and interactions among them. EPH combines "*more than just evidence, and includes the open pursuit of social values, highlighting the role of interest groups, and debate across society not just within restricted scientific circles*." Climate change is central to both schemes. The novelty then is to weld ecosystem dynamics with climate dynamics, add some flavours from outside science, run some models, and see what might happen.

Shneiderman's Science 2.0 combines applied science, engineering, and design and attempts to tackle problems like disaster response systems or environmental sustainability [1]. These are not purely scientific problems, and their complexity makes it necessary to break them into smaller pieces for analysis. One of Shneiderman's five strategies for doing this is classical reductionist science [21]. It is therefore impossible to accept the viewpoint of one participant in the conference, that taxonomy, physiology, biogeography, and population dynamics are no longer useful research topics, that "they will not adequately inform on purported climate change: HAB linkages" [22].

Even though complex models of the climate or an ecosystem can exhibit chaotic dynamics, with all the technical challenges entailed, Science 2.0, EPH, and CHANS propose to combine several such systems in search of solutions to economic and political problems. These aspirations read like recipes for "cybernetic gigantism" [23]. We can agree that solutions to complex problems demand multidisciplinary approaches, and that collaborative projects which combine expertise from diverse disciplines are potentially fruitful. But if science is to be part of these activities, it must be real science, not a debased imitation.

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Red tides in Kamchatka coastal waters (Bering Sea, Russia) are a barrier for the salmon fishery and Pacific salmon

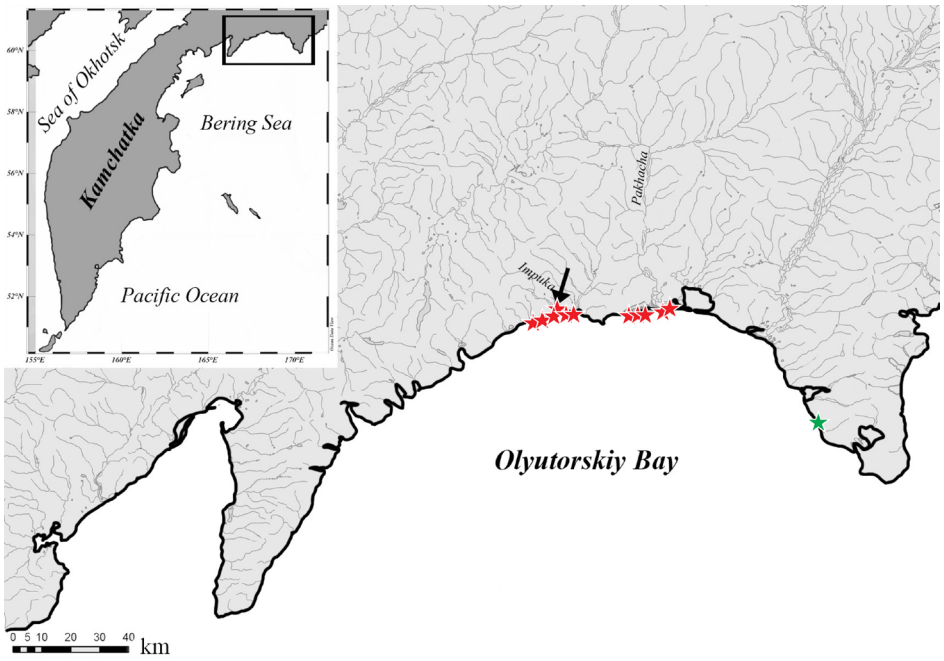

Fig. 1. Map of Olyutorskiy Bay (Kamchatka, Bering Sea) where a red tide, reported by fishermen, occurred in July 2017. The stars denote fishery sites: red, affected by the bloom; green, not affected; the arrow indicates the sampling area

In mid-July 2017 a red tide occurred in Olyutorskiy Bay, Bering Sea (Fig. 1). Fishermen of the company *Delfin* witnessed this phenomenon at their fishery sites (Fig. 2). The first sign of the bloom was observed on July 11th, following a flood during July 8th-9th caused by a strong cyclone. By July 15th the bloom extended along the central coastal zone of the bay, in a layer approximately 5-6 m in depth. The fishermen's attention was attracted by the unusual behavior of pink salmon (*Oncorhynchus gorbusha*) that were approaching the rivers to spawn and entered the bloom area: "*Fish were slack, looked tired and died soon in the trap net*". Additionally, natives of the Pakhachi settlement reported that nearshore waters were reddish-brown, had an unpleasant smell, and dead salmon were washed ashore.

To investigate this phenomenon, a surface water sample was taken on 15th July 2017 from the reddish-brown discoloured plume one mile from the mouth of the Impuka River (Fig. 1). Bright-field and epifluorescence microscopy [1] were used to identify the phytoplankton present. Saxitoxin content in seawater samples was determined by an immune-enzyme assay (IEA) with the help of the test-system RIDASCREEN® FAST PSP SC, R-Biopharm AG, Germany. The test-system detection limit is 5 µg kg^{-1} with specificity to different PSP toxins of: PSP, 100%; decarbomoyl PSP, 20%; gonyautoxin II and III, 70%; neosaxitoxin, 12%.

Alexandrium tamarense (Lebour) Balech was the dominant species (up to 1.32 x 10^5 cells L^{-1}) (Fig. 3). Other dinoflagellates, such as *Tripos fusus* (Ehrenb.) Dujard., *Protoperidinium* sp. and *Alexandrium* cf. *leei,* co-occurred. The diameter of *A. tamarense* vegetative cells ranged from 31 to 40 µm, and that of *A.* cf. *leei* was around 36 µm. *A. tamarense* was found both as vegetative cells (Fig. 3A-C) and in different stages of cyst formation (Fig. 3F, G).

Saxitoxin concentration of the seawater sampled was 0.33 µg L^{-1}. The neurotoxic impact of the bloom on fish in Olyutorskiy Bay was observed by the "slack" behavior of pink salmon passing through the bloom patches, and mortality of some individuals. There are no standards for saxitoxin concentration in sea and fresh water in Russia, but the established regulatory level is 800 µg kg^{-1} for the edible tissues of mollusks and crabs [2, 3].

Visual observation from the air of the Pacific salmon in the spawning

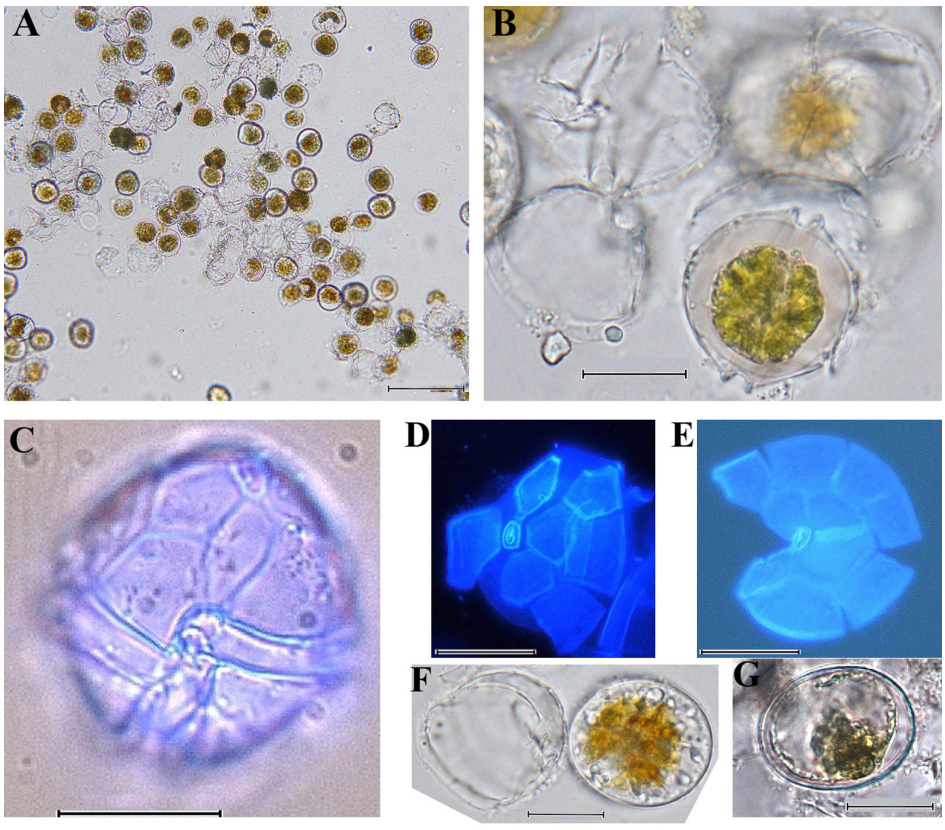

Fig. 3. Alexandrium tamarense in a water sample from Olyutoskiy Bay during the bloom event: A. General view of cells in the sample; B. Cells with content and empty thecae; C: Ventral view of the theca; D-E. Calcofluor-stained epithecal plates; F. Shedding of the theca (cyst formation); G. Cyst. Scale bar = 100 µm (A), 20 µm (B-G).

Fig. 2. Red tide in Olyutorskiy Bay on 15th July 2017

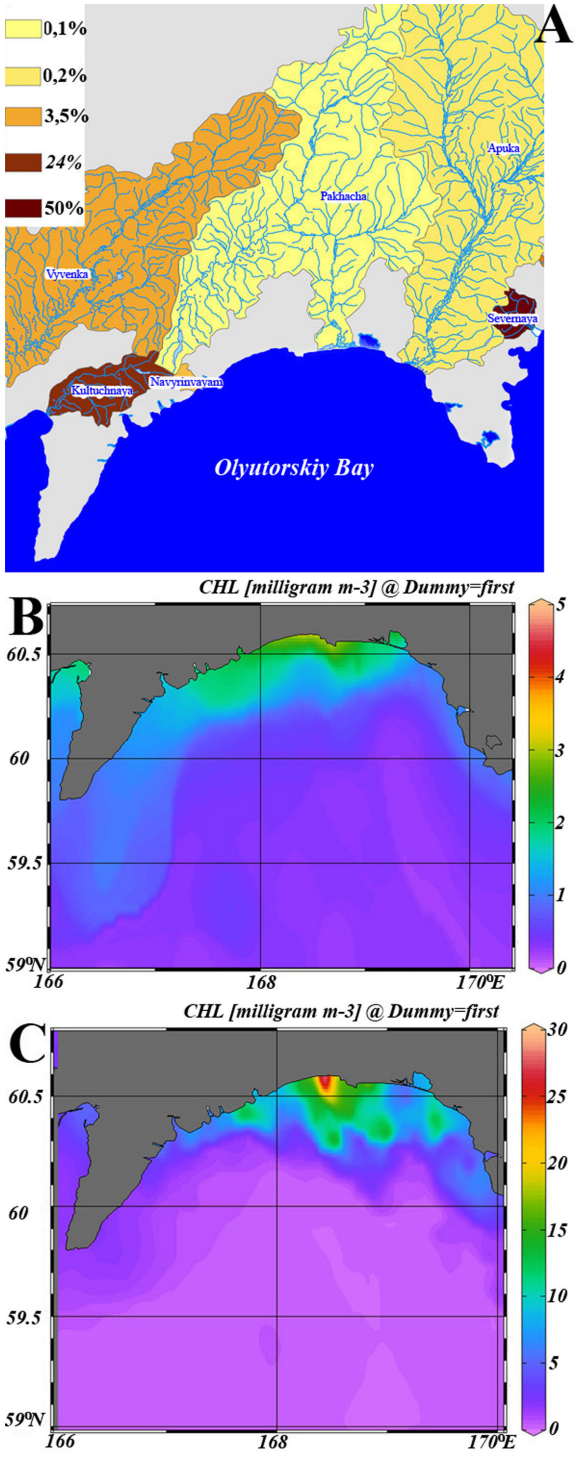

Fig. 4. Distribution of Pacific salmon in river basins (A) and bloom patches as detected from the chlorophyll-a concentration (B:15th July 2017; C: 21th July 2017; http://marine.copernicus.eu).

grounds of the Olyutorskiy Bay river basin performed during the second half of August showed an atypical distribution in the rivers. Maximal escapes were recorded in river basins located in the western and eastern parts of the Olyutorskiy Bay area, whereas in its central part, the abundance of the target species (*Oncorhynchus gorbusha*, *O. keta* and *O. nerka* (all called Pacific salmon) was extremely low (Fig. 4).

A map of salmon distribution over the spawning grounds (Fig. 4A) was compared with that of satellite images of chlorophyll-*a* (Fig. 4B, C) which suggested that the toxic bloom caused a re-distribution of the migratory paths for salmon, with most of them moving to the rivers adjacent to Olutorsky Bay. Changes in anadromous migratory paths of Pacific salmon caused some problems for the fishery in the bloom zone and additional unscheduled expenses.

This is the first documented event for Kamchatka, where a coastal bloom of the toxic microalgae appeared to be a barrier both for fisheries and Pacific salmon spawning migration into freshwater.

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First report of *Gambierdiscus* in the Western Mediterranean Sea (Balearic Islands)

Gambierdiscus (Dinophyceae) species are benthic dinoflagellates living in marine littoral zones of circumtropical areas and have recently been described in temperate waters [1]. Some species are producers of potent neurotoxins: ciguatoxins (CTXs) and maitotoxins (MTXs). Ciguatoxins are linked to Ciguatera Fish Poisoning (CFP). Ciguatera used to be restricted to tropical and subtropical areas, but since the last decade, it appears to be expanding to more temperate latitudes. For example, outbreaks of ciguatera have been reported in the Canary Islands and Madeira (eastern Atlantic Ocean), where several species of the genus *Gambierdiscus* have been identified [2].

In the Mediterranean Sea, no thorough evidence of cases of ciguatera exist. The only reports of CTX-like toxins in fish, which are not confirmed, were based on the Cigua-Check Fish Poison Test kit (Oceanit, Hawaii), a method that has proved to be unreliable [3]. Nonetheless, *Gambierdiscus* species have been reported in the last decade in Crete and Cyprus (eastern Mediterranen Sea) [4-6]. One species of *Fukuyoa (F. paulensis)*, a genus that includes species previously included in the genus *Gambierdiscus*, was reported in the Balearic Islands in 2015 [7]. Little is known about diversity, distribution and toxicity of *Gambierdiscus* spp. in the Mediterranean Sea. The present study confirms the presence of *G. australes* in the two Balearic Islands of Majorca and Minorca, and this constitutes the first report of *Gambierdiscus* genus in the western Mediterranean Sea.

In this study, microalgal samples were collected from macroalgae and rocky substrates in 19 stations in Majorca and Minorca in September 2017. Water temperatures ranged from 24 to 27 °C and salinity from 36.2 to 38.0. In the laboratory, samples were observed under the microscope and individual cells were isolated with micropipettes to establish cultures for morphological and molecular analysis. Calcofluor white stain was used for morphological identification. Cells were observed with a compound microscope equipped with epifluorescence at 630X (Leica DMLB). The *Gambierdiscus* cells observed were anterior-posteriorly compressed. Morphology of the epitheca and the hypotheca is shown in Fig. 1 in which the plate terminology employed follows Fraga and collaborators [8]. The epitheca has a rectangular-shaped 2' apical plate and the P_0 plate is ventrally oriented; the hypotheca has a narrow 2'''' plate equivalent to 1p plate in Chinain [9]. The cell surface is smooth. The cell length and width of 62 individuals were measured. Length ranged from 60.9 to 92.3 µm (mean of 75.6 µm) and width ranged from 64.1 to 90.8 µm (mean of 78.6 µm). The original description [9] described a length range of 76-93 µm and a cell width of 65-84 µm. Further morphological analysis will be performed using electron microscopy.

To facilitate molecular identification to species level, DNA was extracted from individual or a few clonal cells using the Arcturus™ PicoPure™ DNA Extraction Kit (Applied Biosystems, CA, USA). Afterwards, the domain D8-D10 of the LSU rRNA gene was amplified by a Polymerase Chain Reaction (PCR) using the pair of primers FD8 and RB [9], and products were sequenced. The D8-D10 sequences obtained in this study were deposited in GenBank under accession numbers: MG708117- MG708130. DNA sequence analysis of amplified rDNA fragments confirmed that all *Gambierdiscus* spp. corresponded to *G. australes*, which was in accordance with the morphological identification. *G. australes* was present in 10 out of the 19 sampling stations in Majorca and Minorca (Fig. 2), indicating that this species is well established at different locations around the coasts of both islands. It will be important to evaluate the temporal distribution of this species.

The first report of *G. australes* was in the Australes archipelago (French Polynesia). This species is also widely distributed in areas such as New Zealand and the Canary Islands, but it had not been reported yet in the Mediterranean Sea. Some studies mentioned that the spatial expansion of *Gambierdiscus* and CFP may be related to the increase of temperatures caused by climate change

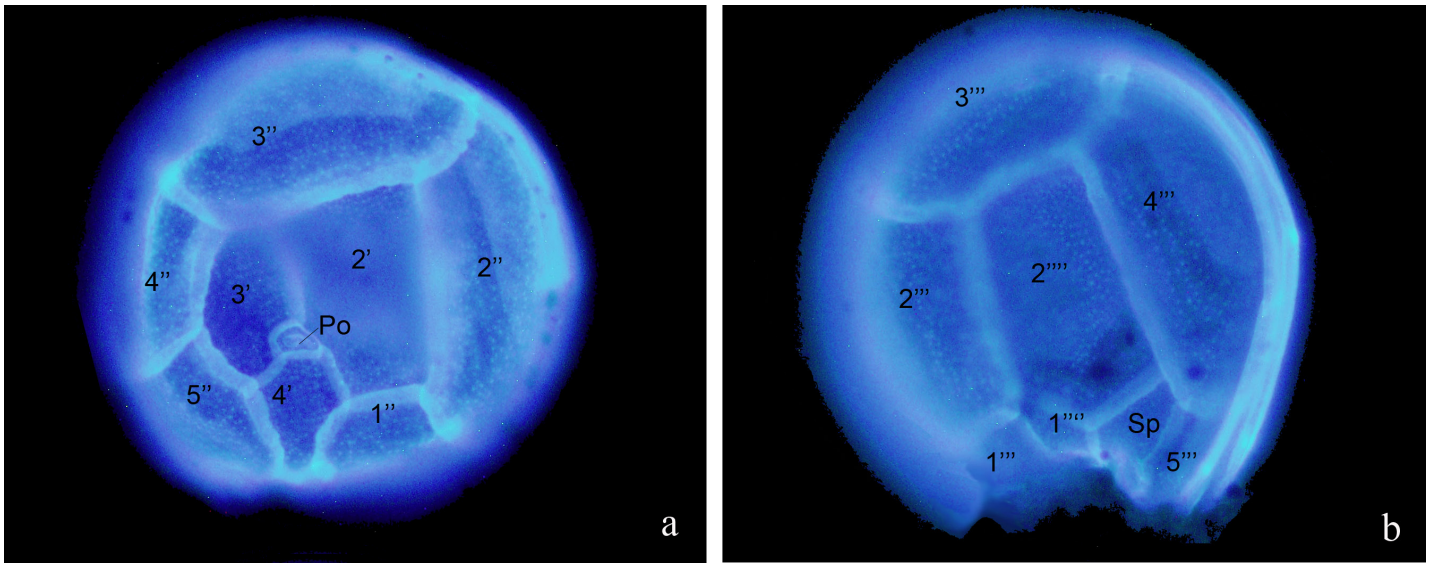

Fig. 1. Epitheca (a) and hypotheca (b) of Gambierdiscus australes cells stained with Calcofluor White.

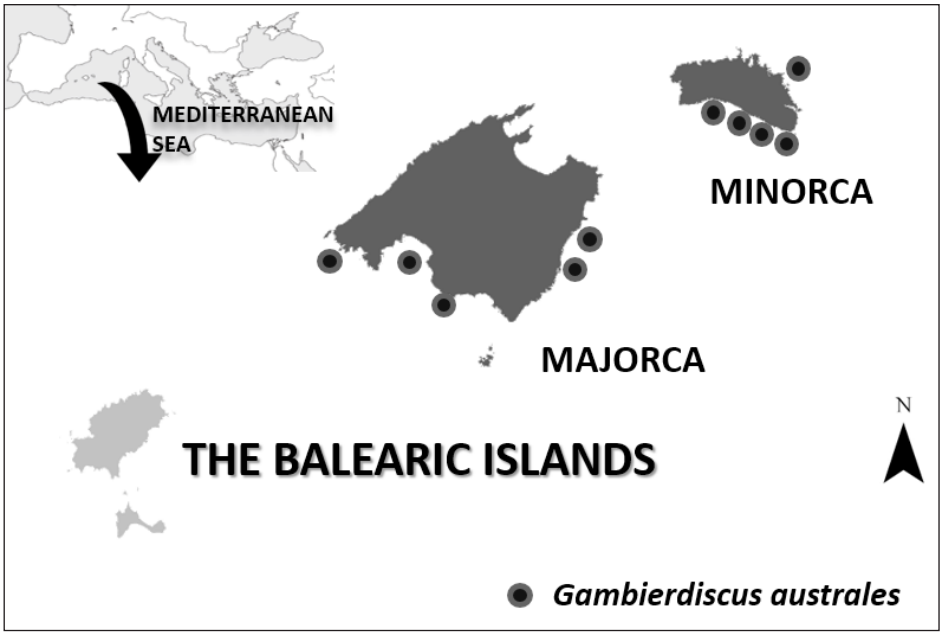

Fig. 2. Locations where Gambierdiscus australes was recorded in the Balearic Islands (39° 30'N, 3° 00' E), Spain.

[10]. The Mediterranean Sea, which is a semi-enclosed sea, seems to be one of the regions strongly affected by the rising of temperatures, and this makes this region more suitable for tropical species [11]. A recent study describes a high diversity of *Gambierdiscus* species in the Canary Islands which would suggest that this genus is not a recently introduced taxon in that area, although climate change may contribute to increase the populations density [2].

It will be important to understand the origin of *Gambierdiscus* in the Mediterranean and the effect that climate change may have on *Gambierdiscus* populations. Moreover, it will be necessary to study whether the Balearic Islands could be a new spot of ciguatera.

Improving our knowledge about diversity and toxicity of these benthic dinoflagellates will provide a better characterization of health risks taking into consideration climate change trends.

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Forthcoming Events

ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

The next ICES-IOC WGHABD, hosted by Margarita Fernandez-Tejedor at IRTA, Sant Carles de la Rapita, Tarragona, Spain, will meet from the 23rd – 26th April (inclusive) to work on the following terms of reference:

A. Deliver National Reports on harmful algal events and bloom dynamics for the year 2017.
B. Review progress and summary of fish killing algae activities underway during the reporting period 2017–2020.
C. Updating of the ICES-PICES-IOC Harmful Algal Event Database (HAE-DAT.
D. Global HAB Status Report for the North Atlantic area: report using data and products generated from HAE-DAT and supplementary time series data as appropriate.
E. New findings in harmful algal bloom dynamics.
F. HABs and the EU Marine Strategy Framework Directive (MSFD). Approaches in Europe to including HABs in the assessment of the Good Environmental Status for the EU Marine Strategy Framework Directive.
G. New results about how physical, chemical and biological interactions control the dynamics of selected harmful micro-algae.
H. Ciguatera Fish Poisoning (CFP) in the ICES area: review new developments in methodology to research the issue, modelling efforts, risk assessments to protect human health, initiatives in other bodies (IPHAB, PICES etc.).

Comparison by light microscopy and qPCR of potentially ichthyotoxic microalgae in Danish on-shore lagoons producing European flounder (*Platichthys flesus*): Pros and cons of microscopical and molecular methods

Fig. 1. Lagoon used for production of European flounder at Fishlab, Denmark.

Evaluation of phytoplankton communities is frequently used to determine the ecological status of water bodies. Hence, species diversity of phytoplankton has become an integral component of national assessment programs. The most commonly used technique for counting phytoplankton is the Utermöhl method [1] and the precision of the enumeration is evaluated with standard statistical analyses techniques [2]. Despite long-term reliance on the Utermöhl method, new techniques for algal identification and enumeration are continuously being explored. Particularly, the recent explosion in molecular tools has resulted in an influx of alternative methods (e.g. real-time qPCR, microarrays and FISH-FC). While many of these methods are promising, their results often differ from those of the conventional method that they were intended to supplement (or replace). Given the importance of algal community assessments, significant efforts have been put into the quality assurance of phytoplankton counts [3]. This has led to the development of standardised procedures.

This study aimed to compare cell counts of seawater samples by light microscopy (LM) and qPCR from on-shore production lagoons of European Flounder (Fig. 1). A total of six lagoons had been filled with untreated seawater from the nearby Limfjorden. Samples for phytoplankton analyses were taken twice weekly from 7 March to 18 May, 2017. A total of 55 samples were examined by LM and qPCR. Potentially ichthyotoxic species (Table 1) were identified either quantitatively or qualitatively by qPCR using species-specific primer sets and hydrolysis (Taqman) probes. Results from qPCR were compared to microscopic cell counts performed by Fishlab. Examples of two representative lagoons are provided in Fig. 2A-D.

Data gathered from all six lagoons revealed ca. 20 groups or species by LM (not shown) and the qPCR assays available detected 8 out of the 11 potentially fish killing species (Fig. 2A, C). There were very few cross overs in terms of species identification between the two methods. LM failed to detect seven of the potentially ichthyotoxic species and *Dictyocha* was the only microalga detected by both methods. Despite this, the cell densities differed markedly. The qPCR assay only detected *Dictyocha speculum* cells in lagoon six at a density of 2,600 cell L^{-1}, whereas LM detected much higher numbers of 56,560 and 36,210 *Dictyocha* 'sp.' in lagoons 1 and 6, respectively. *Pseudochattonella farcimen* and *P. verruculosa* were detected in all lagoons by qPCR and a succession pattern for *P. farcimen* to *P. verruculosa* was evident. When temperatures were low (8-9 °C) *P. farcimen* was dominant but as temperatures increased above 9 °C a switch occurred and a decline in *P. farcimen* concentration coincided with an increase in *P. verruculosa* cell numbers (Fig. 2A, C). During the production period, lagoons were replenished with an addition of ca. 10% of newly collected seawater. The additional water appeared to re-inoculate each lagoon with additional *Pseudochattonella* cells. Fish survival rates in the lagoons were between 0.5 and 13 %.

In terms of monitoring, the discrep-

Species	Lagoon number 1	2	3	4	5	6
Alexandrium tamarense						
Alexandrium ostenfeldii	+			+	+	+
Karenia mikimotoi						
Prymnesium parvum				+	+	
Pseudochattonella farcimen	+	+	+	+	+	+
Pseudochattonella verrculosa	+	+	+	+	+	+
Karlodinium veneficum	+	+	+	+	+	+
Pfiesteria shumwayae	+		+	+		+
Pfiesteria piscicida	+	+	+		+	+
Luciella masanensis						
Dictyocha speculum						+

Table 1. List of species for which qPCR assays are available for quantitative (cell abundance) and qualitative (presence/absence) of potentially ichthyotoxic microalgae in this study. '+' = presence of a species in the lagoons used for production of European flounder.

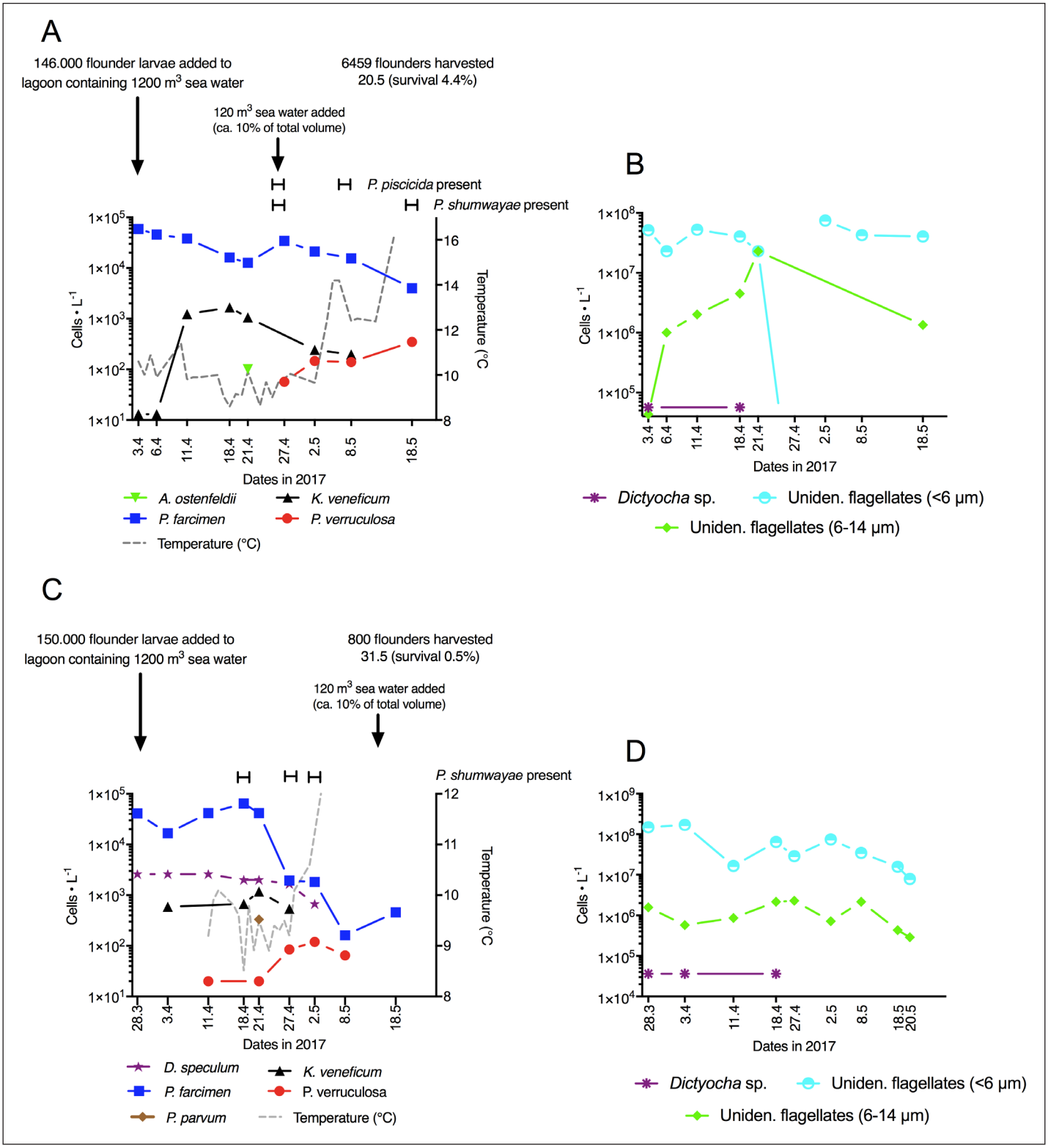

Fig. 2. Comparison of phytoplankton species identification and cell densities (cells L^{-1}) by qPCR (A and C) and light microscopy (target species and groups which potentially could comprise ichthyotoxic organisms) (B and D) in lagoon 1 (A and B) and lagoon 6 (C and D), respectively. The right Y-axis shows the water temperature during the growth period. Presence of Pfiesteria piscicida and P. shumwayae at specific dates are indicated by H. Information on the number of flounder larvae added and the survival rates in percentage at the harvest dates are provided at the top of A and C.

ancy between the qPCR and LM results is obviously quite worrying. There are clearly problems with one or even both methods and it raises many questions over their accuracy. When evaluating phytoplankton data, each inaccuracy associated with sampling, sub-sampling and sample preparation should be taken into account. As each individual step from sampling to counting comes with its own forms of variation, we will critically analyse each method and discuss potential problems and sources of error.

Microscopic analysis

Traditionally light microscopy has been the gold standard for phytoplankton identification due to its relatively low costs and equipment requirements (basically an inverted microscope and a settling chamber). One of the main advantages of LM over qPCR is its ability to identify at least theoretically all organisms present in the sample in contrast to qPCR, which will only target species of interest, e.g. toxic or nuisance organisms. If there were any new species

present in a sample then qPCR would miss those probably due to lack of a developed assay. However, LM does require high levels of taxonomic skills and the precision in identification is only as accurate as the taxonomist allows. Different taxonomists trained in different ways using different identification literature can cause large person-person differences. The ease of identification is also species dependent. For example highly plastic species, or those with a variable life cycle are harder to identify and can often be easily misidentified. The naked form of *Dictyocha speculum* can easily be confused with the rounded cells of *Pseudochattonella*. Some species of the genus *Alexandrium* cannot be identified to species level due their very subtle morphological differences in their thecal plates. When dealing with toxic species, false positives are less problematic but can cause substantial financial losses if they result in the closure of a fishery, but when toxic or problematic organisms are missed completely this could have dire consequences. To reduce confusion, each taxonomist should be provided with a checklist of common species with up to date taxonomic names.

Undertaking frequent inter-comparison exercises, e.g. the ring test or the International Phytoplankton Intercomparison (IPI) exercise, provide feedback on how individual laboratories and taxonomists perform. This forum also provides an opportunity to convene a discussion on nomenclatural changes and new technological advances in monitoring techniques.

As error can be introduced in various different forms, in order to get the most accurate and reproducible results each individual step from: collection, storage, subsampling, homogenisation, filling the chamber, settling and counting strategies all require their own standardised protocols.

All aspects of the protocol need to be considered from the storage containers to the type of fixative used. Many cells e.g. *Pseudochatonella* spp. are sticky and can adhere to plastic walls and as plastic bottles is often preferred over glass especially when transporting samples this can become problematic for accurate cell enumeration. The choice of preservative is important and often the optimal preservation methods are taxa specific. It is not always easy to obtain reliable estimates from fixed material; preservatives can alter the sample in various ways creating a biased measurement. Lugol's iodine [4] has long been the fixative of choice due to its relatively low toxicity and high stability. However, it is known to introduce artefacts such as changes to cell size, a reduction in cell number and in some instances it may fail to preserve certain taxa all together [5-6]. Each alternative fixative comes with its own issues [7].

Settling chambers themselves can be another source of variation. For reliable cell counts, specimens must be completely randomly distributed within the chamber. If cells do not follow a poisson distribution then it will bias the enumeration and any statistical analysis will be affected.

To prevent uneven settling, the samples must be at a constant temperature during the settling period. For a higher chance of getting a well-mixed distribution then samples must first be homogenised. The best way to homogenise a sample is the 'Paul-Schatz' figure of eight rotation method where samples are mixed 100 times in a rhythmic pulsating motion. Even when all precautions are taken, it is still almost impossible for cells to be randomly distributed due to issues such as cell clumping caused by polysaccharide fibrils or inconsistent settling conditions. Due to radial abundance gradients cell abundances at the periphery can be up to 50% lower than at the center, causing a settling bias. Uneven settling will affect the counting strategy. For any counting strategy a predetermined number of units must be observed. The number of units differs depending on the organism and the research objectives. Typically to reach an accuracy of 10% at least 400 cells must be counted [8]. Whole chamber cell counts should be carried out where possible but other counting strategies are often used such as transects and random fields.

qPCR

When carefully designed, with optimisation and validation, qPCR assays are highly accurate and sensitive, but without due care and optimisation, qPCR can be plagued by reproducibility and reliability problems.

The quality of the starting material is one of the key determinants for obtaining reliable and reproducible data. As with microscopy fixatives and storage techniques play a large part in quality of the samples. Fortunately for short term storage Lugol's iodine is the most ideal fixative and the same sample can be used for both microscopic and qPCR analysis [7]. Before amplification DNA must be extracted from the cellular material and commonly commercial extraction kits are applied. To get purified genomic DNA the sample must undergo a number of steps to lyse the cells, remove contaminants and purify the resulting DNA. In cases where the purification step is inefficient the resulting DNA may not be representative of the sample and/or contain compound(s) that will cause assay interference. In these cases the performance of the reaction will be sub-optimal, causing a reduction in the sensitivity and/or amplification efficiency. Inhibition of amplification can occur in different ways. Firstly, when high molecular weight compounds, e.g. humic acids or complex carbohydrates, combine with metal ions to sequester the nucleic acids away from the polymerases and prevent amplification. While some molecules block or inhibit the polymerase or alter the specificity of the primers, inhibitors which block or delay polymerase activity are highly problematic and they can lead to an underestimation of material in the sample or false negatives [9]. Typical approaches to combat inhibition include alternative DNA extraction kits, dilution, specialised polymerases, addition of adjuvants and internal controls.

When designing a qPCR assay it is important to select an appropriate target and to design specific primers with no cross reactivity with other organisms. This study used previously validated species-specific hydrolysis probes in combination with primers to add an extra level of specificity. To ensure accuracy, time is required to optimise the efficiency of the assay and validate it multiple times. This sometimes means altering the constituents of the master-mix used, e.g. nucleotides, magnesium chloride or polymerase concentrations. This is extremely relevant when tackling issues arising from multiplexing assays [9-10].

Once optimised users can still face a number of precision related challenges.

As qPCR measures genetic material rather than viable cells an over estimation of cell numbers can occur due to the inclusion of dead or dying cells. Problems may also occur when targeting multiple copy genes where the organism carries different numbers of the target depending on nutritional status, stress or replication stage. This can lead to an over or under estimation of total cell numbers. Common problems associated with cell number enumeration and copy number do not occur until late exponential-stationary phase meaning that cell numbers can be accurately quantified until this point [11]. One way of potentially overcoming this issue it to use standards created using cells from all parts of the growth curve to produce an 'average' copy number. However, this will decease the overall accuracy of the assay.

As with microscopic analysis qPCR requires standardisation/normalisation for both the laboratory protocols and statistical analysis strategies. To aid this, in 2009 the MIQE guidelines, *Minimum information for publication of Quantitative Real-time PCR Experiments*, were published. These guidelines are designed to 'encourage better experimental practice'. The guidelines establishes a framework for conducting qPCR experiments in the laboratory and are designed to improve experimental workflow and should be followed when designing any qPCR assay [12].

Conclusions

Clearly despite efforts to standardise procedures for both techniques there are still many problems affecting the accuracy and the quality of the results. The comparison of enumeration techniques that was carried out in this study has highlighted the difficulties in obtaining comparative data especially of small-sized, ichthyotoxic microalgae. Enumeration by LM missed many important species, which emphasizes how difficult it is to identify phytoplankton from Lugol's fixed material. We are now moving into the era of 'bio-monitoring 2.0' and with the reduction in costs for meta-barcoding based techniques it is still to be seen if these molecular techniques will eventually replace LM. Yet improvements need to be made across the board for all techniques. The low survival rate of European flounder observed in the 2017 production may be explained by the diverse assemblage of potentially ichthyotoxic microalgae in the lagoons. In previous years the survival rate has been 40-50%.

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The Cawthron Institute Culture Collection of Micro-algae (CICCM)

The CICCM is designated as a "nationally significant database" by the New Zealand government and so receives partial funding for its continued existence. Isolates from 13 classes of micro-algae are maintained either as live cultures or cryopreserved. The approximate 500 isolates include benthic, epiphytic and planktonic marine harmful algal bloom species as well as a collection of freshwater cyanobacteria. Most of the isolates are toxin producers and there are also species that are unique to New Zealand waters. Every isolate in the collection has an associated body of information. The collection underpins research programmes, for example the Safe New Zealand Seafood programme, and is the focus of many student projects both nationally and internationally.

Over the summer of 2017/18 the *Cawthron Foundation* (see box) provided a *Kathleen Curtis* scholarship for an undergraduate student to assist the curators in populating a new CICCM database with the relevant information for each isolate, including site of isolation, culturing tips, molecular and toxin information and associated publications. The scholarship was awarded to Kendall Morman, a biomedical science student at the Auckland University of Technology, New Zealand (Figure 1). To quote Kendall, "It has been a rewarding experience helping this team to construct the CICCM database and I have been fascinated to learn how research involving the collection has increased understanding of marine and freshwater biotoxins in New Zealand."

The database, built using Microsoft's 'Share Point Online' platform, will meet the strategic aim for all collections and databases in Australasia to be easily interrogated. Initially the database will only be accessible 'in-house', but will enable the curators to provide full information with ordered isolates. Online ordering continues via the website: *www.cultures.cawthron.*

The CICCM is distinct from larger collections in the Northern Hemisphere and this has attracted commercial interest in potential micro-algal products. A key user of the collection is the Cawthron Natural Compounds group which aims to make a wider range of toxins available to the global market through a partnership with Sigma-Aldrich to supply algal toxins via their renowned catalogue (see HANews 51 August 2015).

The CICCM will continue to generate economic benefits to New Zealand by underpinning the development of improved and cheaper toxin and molecular tests for the New Zealand shellfish industry and by supporting capability development in all aspects of HABs through provision of cultures to post-graduate students and post-doctoral researchers.

CAWTHRON FOUNDATION is a charitable trust which raises donations, bequests and endowments for public-good science. The Foundation also funds scholarships to support talented emerging scientists and delivers community education programmes: *http://www.cawthn.org.nz/foundation/scholarships/*

Acknowledgements

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Fig. 1. Kendall Morman being welcomed at a powhiri (a Maori welcoming ceremony) led by Harvey Ruru, Cawthron Institute's kaumatua (senior elder member of the group)

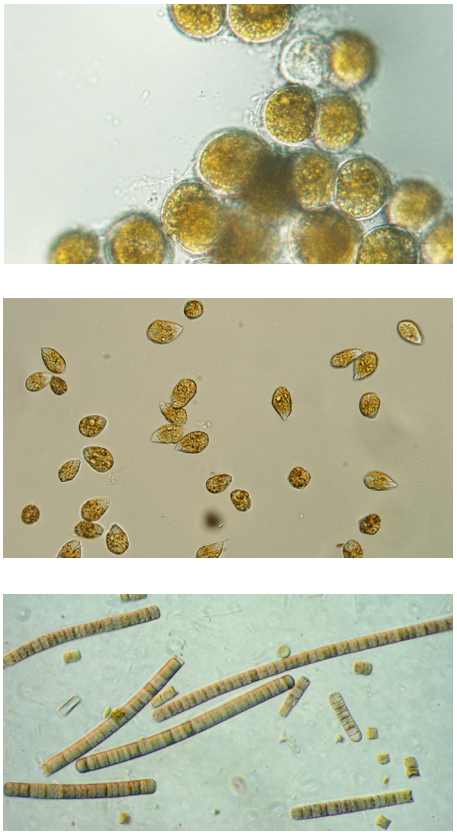

Fig. 2. A selection of cultures held in the CICCM. From top, Vulcanodinium rugosum (non-motile cells), Ostreopsis siamensis, and Phormidium sp

ISSHA's CORNER

The XVIII International Conference on Harmful Algae is approaching! It is time for nominations for achievement awards (Yasumoto Life Time and Patrick Gentien Young Scientist), registration to the conference as a student if you wish to participate in the Maureen Keller Award competition and fundraising for the auction.

Nominations for the 2018 Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award

ISSHA members are invited to submit nominations for the Yasumoto Lifetime Achievement Award and the Patrick Gentien Young Scientist Award. Further information on the appropriate profile of the nominees can be found at *www.issha.org* (click on "Awards").

Any ISSHA member in good standing may submit nominations for either achievement award, which should include a description of the nominee's contribution (not more than one page). Please, make sure that you and your nominee have renewed your memberships for the period 2017-2018.

Nominations should be sent by e-mail to Dr. Marta Estrada (*marta@icm.csic.es*) Chair of the Committee on Achievement Awards. Deadline: May 15, 2018.

Please write "ISSHA Achievement Awards 2018" in the message subject. Nominations will be considered by the ISSHA Council, and the awards will be presented at the 18th ICHA Conference Nantes, France, 21-26 October 2018.

The Maureen Keller Student Award for best student presentations at ICHA

The Maureen Keller Award is given to the outstanding graduate student oral and poster presentation at the ICHA2018. Conference participants are chosen in advance to review students' presentations. The evaluations are processed by the ISSHA Awards Committee and awardees are announced during the ICHA closing ceremony.

The presentations are evaluated based on the following criteria:

- Clearly stated hypothesis or objective
- Scientific merit and originality
- Coherence (consistency between introduction, data, conclusions)
- Intelligibility (selection of important points, brevity, accuracy, and clarity of expression)
- Presentation (appearance, layout, clarity)

Candidates can ask to be considered for these awards during the abstract submission process (*https://www.icha2018.com/abstracts/call-for-abstracts/50*)

ISSHA AUCTION

Donations for the ISSHA Auction 2018

ISSHA auctions have been a tremendous success during past International Conferences on Harmful Algae and are becoming established as a tradition. It is an opportunity for conference participants to bid on a broad range of donated items such as books, signed reprints, photos, jewelry, T-shirts, paintings, algae-related items, liquor, and scientific equipment. The auction items are donated by ISSHA members from around the world. Funds collected during the auction are an important income for ISSHA. ISSHA was able to offer partial travel support to 25 students to attend the Conference in Florianópolis, Brazil (9-15 October 2016).

Please, contact Dr. Wayne Litaker (*wayne.litaker@noaa.gov*) who is responsible for the auction about your donations (including "ISSHA Auction 2018" in the message subject). You may wish to include a picture of your item. A list of items will be presented on the ISSHA website before the conference. Dr. Wayne Litaker (*wayne.litaker@noaa.gov*) can provide a letter template to use to request donations from companies. The address where items may be sent in advance of the meeting will be provided on the conference website.

ISSHA conference

Preparation of the ISSHA conference 21-26/10/2018, Nantes, France (*https://www.icha2018.com*) is progressing well.

Five **plenary speakers** have already confirmed their attendance:

- Prof. Elena Litchman (Univ. Minnesota, Michigan State University, USA)
- Prof. Bill Gerwick (Univ. California San Diego - Skaggs & Scripps, USA)
- Prof. Erik Jeppesen (Aarhus University, NL)
- Dr. Mireille Chinain (Institut Louis Malardé, French Polynesia)
- Prof. Thomas Hartung (John Hopkins University, USA)

Check out the **session topics** here: *https://www.icha2018.com/scientific-program/session-topics/35*

And note the **abstract submission deadline** (15/4/2018)

Please submit abstracts here: *https://www.icha2018.com/abstracts/call-for-abstracts/50*

Forthcoming Events

First announcement of the 11th International Conference on Toxic Cyanobacteria (ICTC)

We are pleased to disseminate the first announcement of the *11th International Conference on Toxic Cyanobacteria* (ICTC) that will be held in Krakow, Poland from May 5 – 10, 2019. The ICTC is a periodic scientific meeting that includes members of the international community and focuses on the science and study of cyanotoxins and toxic cyanobacteria. The theme of the ICTC 11 is: "*Learning from the past to predict the future*". Please mark your calendars and make plans to join us in Krakow during May, 2019! Conference website: *http://ictc11.org/*

Harmful Algal Bloom (HAB) sessions at the upcoming 2018 ASLO summer meeting in Victoria, British Columbia

Ted Talks: The career, contributions, and impact of Theodore J. Smayda

Session SS04 convened by:
Tracy A. Villareal, The University of Texax at Austin (*tracyv@austin.utexas.edu*)
James A. Yoder, WHOI (*jyoder@whoi.edu*)
Edward G. Durbin, Univ. of Rhode Island (*edurbin@uri.edu*)

Ted Smayda's career in marine science spanned 60 years on topics ranging from phytoplankton suspension, ecology, succession, community structure, growth rates, biogeography, and the problems of harmful algae. Through his students and colleagues, his reach extended into phytoplankton-zooplankton interactions and the structuring of planktonic ecosystems. With his passing in the spring of 2017, we invite the community to join us in a celebration of his career with contributions on any aspect of phytoplankton or oceanography that were inspired or affected by his work.
Keywords: Biogeography, Ecosystem, Ecology, Marine, Phytoplankton

There will be another three Harmful Algal Bloom (HAB) related sessions:

- Crossing disciplinary boundaries across the freshwater-marine continuum to advance the understanding of HABs (SS71).
- Cyanobacterial and algal metabolites: occurrence, ecology, prediction, and management (SS07), and
- Cyanobacterial ecology as a basis for their mitigation and control under global change (SS51).

The first session will focus on broad HAB research and management solutions, whereas the second session will focus specifically on cyanobacterial and algal metabolites. The third session will focus on aspects of cyanobacterial ecology in relation to environmental change and bloom management. Session abstracts are located at *https://aslo.org/victoria2018/special-sessions*

If you are a HAB researcher planning on attending the 2018 ASLO summer meeting, please consider submitting an abstract to either session. Abstracts are due 16-Feb-2018; registration can be completed at *https://aslo.org/victoria2018/main*

International Coordination of Research on Harmful Algal Blooms
From GEOHAB to GlobalHAB

International cooperation is fundamental to advance understanding of HAB dynamics and to improve our ability to predict them. Fostering this international cooperation was the mission of GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms), the first worldwide research programme focusing exclusively on harmful marine microalgae. From 1998 to 2013, GEOHAB, under the sponsorship of the Intergovernmental Oceanographic Commission (IOC) of UNESCO and the Scientific Committee on Oceanic Research (SCOR), focused on the physiological, behavioral, and genetic characteristics of harmful microalgal species, and the interactions between physical and other environmental conditions that promote the success of one group of species over another. GEOHAB implementation was based on a multidisciplinary, multi-scale and comparative approach, and stimulated the development of new experimental, observational and modelling tools.

During the 15 years of GEOHAB' activity, the international community has contributed to understanding mechanisms underlying HAB population dynamics within an ecological and oceanographic context. At the end of GEOHAB, advances and existing challenges were summarized in six papers in *Oceanography* (The Official Magazine of the Oceanographic Society) in March 2017, (*http://tos.org/oceanography/issue/volume-30-issue-01*). A GEOHAB synthesis, G*lobal Ecology and Oceanography of Harmful Algal Blooms*, edited by Patricia M. Glibert and co-editors will be published by Springer (*Ecological Studies, Analysis and Synthesis*, Volume 232) in spring 2018.

As GEOHAB ended, the community recognized the benefits ofinternational cooperation and encouraged continuation of a new program built on legacy provided by GEOHAB, but now including freshwater systems, and addressing the effects of HABs on human society now and in a rapidly changing world. Thanks to the invaluable support of IOC and SCOR, the new programme, GlobalHAB, was launched in January 2016. Since then, the GlobalHAB Scientific Steering Committee has been elaborating the Science and Implementation Plan that provides a scientific framework for the integration and coordination of research and expertise of many individual scientists in the study of HABs in different aquatic ecosystems. As in GEOHAB, the direct implication of the international community in the programme is fundamental for GlobalHAB success. Information on the GlobalHAB programme and how to participate can be found at *http://www.globalhab.info*.

Elisa Berdalet, Chair, and the GlobalHAB Scientific Steering Committee

GlobalHAB GOAL AND MISSION

The overall Goal of GlobalHAB is to improve understanding and prediction of HABs in aquatic ecosystems, and management and mitigation of their impacts.

The Mission of GlobalHAB includes the following elements:

- Foster international coordination and cooperative research to address the scientific and societal challenges of HABs, including the environmental, human health and economic impacts, in a rapidly changing world.
- Consolidate linkages with broader scientific fields and other regional and international initiatives relevant to HABs.
- Foster the development and adoption of advanced and cost-effective technologies.
- Promote training, capacity building and communication of HAB research to society.
- Serve as a liaison between the scientific community, stakeholders and policy makers, informing science-based decision-making.

The Themes integrated in GlobalHAB range from small-scale (e.g., cellular) subjects (e.g., biodiversity, adaptive strategies) to studies at ecosystem scale and climate change-related processes.

THE 18TH INTERNATIONAL CONFERENCE
ON HARMFUL ALGAE
FROM ECOSYSTEMS TO SOCIO-ECOSYSTEMS

ICHA 2018
21 – 26 OCTOBER
NANTES, FRANCE

18th International Conference on Harmful Algae
www.icha2018.com

IMPORTANT DEADLINES

Abstract submission deadline: 15 April 2018
Early bird registration: 15 July 2018
Get the 17 ICHA Proceedings at www.issha.org

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Lay-out
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