

PROTIST NEWS

Meeting Report: Ninth International Conference on Harmful Algal Blooms, Hobart, Tasmania, Australia, February 7–11, 2000

This meeting, organized and hosted by Gustaaf Hallegraeff of the University of Tasmania, was the 9th in a series of conferences that date back to 1974. The first was attended by less than 100 participants representing 4 countries, whereas more than 500 registrants from 50 countries came to Hobart, maintaining the trend of increasing numbers of participants and countries that has continued since the first meeting. That trend reflects the globalization of the harmful algal bloom (HAB) problem over the last several decades (i.e. more toxins, more toxic algal species, more areas affected, more marine resources impacted, higher economic costs, etc.), but it also results from changes in the thematic coverage of these meetings, best evidenced by the progression in conference titles. The first meeting in 1974 was entitled: *Toxic Dinoflagellate Blooms*, which then changed over the years to: *Toxic Dinoflagellates*; *Toxic Marine Phytoplankton*; *Harmful Marine Algal Blooms*; *Harmful and Toxic Algal Blooms*; and in Hobart, *Harmful Algal Blooms*. The initial focus on toxic dinoflagellates and “red tide” blooms has thus shifted over the years, first to include toxic species from classes other than dinoflagellates, then species which are non-toxic but harmful. The environment of interest also shifted - from exclusively marine to include brackish and freshwater systems. Given Australia’s extensive problems with toxic cyanobacteria in its rivers and lakes, the Hobart meeting maintained that diversity and welcomed a significant number of freshwater contributions.

On the logistical front, the Hobart conference was the first HAB meeting with concurrent oral sessions. This may seem like a minor issue, but the HAB community has long argued that one of the more enjoyable and unique aspects of their meetings has been the ease with which scientists from different disciplines interact. With no competing talks to draw them away, chemists sit in on talks on fundamental

biology or taxonomy, and biologists attend chemistry presentations. This could not last forever, as sheer demographics finally forced the schedule to simultaneous sessions in Hobart. The themes of the concurrent sessions were as different as possible, but there were inevitable conflicts. Ironically, freshwater cyanobacteria talks were held opposite marine HAB sessions, defeating in part the purpose of bringing those two scientific communities together. There were, of course, other opportunities to mix, including at the poster sessions which displayed more than 300 papers.

For many, the Hobart meeting marked the “coming of age” of the field. By this I mean that the level of sophistication, the depth of understanding and analysis, the application of new technologies, and the number of disciplines involved in the field have now reached levels indicative of a mature field of science. In the past, certain areas of HAB research showed clear scientific maturity, but many others were in the early stages of development. For example, toxin chemistry has long been a strong area, with world-class chemists using sophisticated instrumentation and analyses, resulting in frequent advances in the identification and characterization of dozens of important algal toxins. On the other hand, progress has been slower in areas such as the molecular biology, genetics, physiology, and even the ecology of many of the HAB species. In Hobart, these and other important topics showed a remarkable level of advancement and sophistication. Here again, we can look to the global expansion in the HAB problem as the explanation - with more outbreaks and more impacts, funding has expanded globally and brought much-needed financial resources and new workers into the field.

The summaries that follow are obviously incomplete given the large number of papers and posters presented in Hobart. My sincere apologies go to those whose work is not mentioned here. The field

truly has grown to the extent that no overview can capture the depth and breadth of the presentations.

Bloom Dynamics and Ecology

One area where increased funding has led to clear advances is in our understanding of bloom dynamics. In particular, the U.S. ECOHAB program has invested substantial financial and personnel resources in large-scale field investigations of the dynamics of several important HAB species. One of these is *Gymnodinium breve*, the dinoflagellate responsible for toxic red tides in the Gulf of Mexico. Led by Karen Steidinger (St. Petersburg, USA), ECOHAB - FLORIDA's 23 investigators were well represented in Hobart. Vargo (St. Petersburg, USA) described how *G. breve* blooms develop in oligotrophic west Florida shelf waters and highlighted the nutritional demands of such high biomass blooms. Neely (St. Petersburg, USA) and Heil (St. Petersburg, USA) both concluded that there was sufficient inorganic phosphorus to sustain these red tides, but not enough dissolved inorganic nitrogen (DON; 0.5 to 2 mmol per day). Merkt (St. Petersburg, USA) suggested that the deficit could be supplied by dissolved organic nitrogen, which was present at concentrations of 4 to 35 μM . Vargo also discussed the importance of the seasonal shelf thermocline and upwelling/downwelling events in the maintenance and movement of blooms. Meteorological and oceanographic events and processes clearly modulate the delivery of established populations to the coast. Upwelling and downwelling was also a component of a provocative presentation by Walsh and Steidinger (St. Petersburg, USA) of the hypothesis that major *G. breve* red tides on the west Florida shelf are fueled by *Trichodesmium* blooms which supply DON to the dinoflagellate. The massive cyanobacteria blooms are in turn dependent upon Saharan dust deposition (with iron as its critical ingredient, required for nitrogen fixation). Over the last 28 years, 22 *G. breve* red tides were preceded by, or co-occurred with, *Trichodesmium* blooms, and most of those occurred at times of major dust deposition.

The ECOHAB-Gulf of Maine project, which focuses on the toxic dinoflagellate *Alexandrium fundyense*, demonstrated the power in combining observations and modeling – the approach that is inherent in ECOHAB projects. That program has been divided into 2 geographic components. The western component, summarized by Anderson (Woods Hole, USA) is examining the bloom initiation process in the Casco Bay region, with the resulting bloom popula-

tion then propagating to the south and west via a coastal current formed by the outflow of a major river system. Cyst abundance and distribution have been mapped throughout the coastal waters of the Gulf, and several prominent "seed beds" are apparent. Efforts to directly measure the flux of germinated cells from these cyst accumulations have not been successful, so investigators are using laboratory-derived germination data and the cyst abundance map to estimate the bloom inoculum. These germinated cells then grow and are advected within a 3-dimensional, coupled physical-biological model of the region. That model suggest that the cyst germination process is not continuous throughout the bloom season, and that cysts in deeper waters may be numerically more important to bloom initiation than those in shallow waters – contrary to the hypothesis formed at the outset of this program.

Studies of the nutritional physiology of *A. fundyense* by Poulton (Woods Hole, USA) suggest that the Gulf of Maine strains of this species may not follow the popular paradigm in which vertically migrating dinoflagellates take up deep nutrients. Mesocosm studies suggest that local strains do not migrate, even when nutrient-starved. This may also explain the low cell concentrations of *A. fundyense* typically observed in western Maine.

Townsend (Bangor, USA) presented results from large field surveys of the eastern Maine region. *Alexandrium* blooms in that area are also linked to a coastal current, but one of a very different character than that in western Maine. Maximum cell densities of *A. fundyense* are found in the offshore waters of the Gulf, not immediately adjacent to the shoreline where toxicity in shellfish is typically reported. The origin of these offshore populations appears to be blooms in the lower Bay of Fundy which are entrained into the eastern Maine coastal current. One interesting aspect of this scenario is that, despite high nutrient concentrations, populations within that coastal current receive insufficient light to develop to substantial size until the highly turbulent waters begin to stratify. Though temporally and spatially separate from the western Maine *Alexandrium* populations, these blooms in eastern Maine may deposit cyst populations near the Penobscot that ultimately seed the western Maine blooms.

Molecular Probes and Genetics

At past conferences, a topic of great interest has been the development of molecular probes for various HAB species. These efforts have been driven by the need to accelerate the cell counting process and

to make species identification more accurate and easier. In the past, presentations have been on laboratory studies and results, whereas at Hobart, several presentations highlighted the use of molecular probes on field populations. For example, Scholin (Moss Landing, USA), used ribosomal RNA probes specific for toxic *Pseudo-nitzschia* to demonstrate the linkage between diatom blooms and the mortalities of sea lions off the coast of California in 1998 (That talk also highlighted the use of the receptor-based assays for algal toxins that are now being used in a number of research programs, providing rapid and accurate toxin detection and measurement). In the sea lion mortality study, as well as the ECOHAB-Gulf of Maine investigations, probes are being used in a whole-cell format, which still requires microscopic observation. Other presentations at Hobart suggest that in the near future, automated or instrument-based detection will soon be possible. A view towards this future was offered by Scholin in his description of a moored instrument which can collect water samples, filter them, lyse the cells, and hybridize the captured nucleic acids to species-specific probes, with results being telemetered to shore. Though still in the developmental stage, prototype instruments exist and are being tested at this time.

Numerous other presentations described the use of oligonucleotide and antibody probes in HAB research. Rhodes (Nelson, New Zealand) described how rDNA probes for *Pseudo-nitzschia* and *Alexandrium* have been incorporated into New Zealand's phytoplankton monitoring program. These are being used in whole-cell and sandwich hybridization formats with great success. Probes for *Heterosigma carterae* are also under investigation in this monitoring context.

In a similar manner, molecular techniques are being used to study the distribution of the fish-killing dinoflagellate *Pfiesteria piscicida* and related organisms. Rublee (Greensboro, USA) described the application of PCR probes and fluorescent in situ hybridization to detect *Pfiesteria piscicida* in U.S. east coast estuarine waters, while Oldach (Baltimore, USA) employed a novel heteroduplex mobility assay to screen field samples for this group of organisms in the Chesapeake Bay. He also described a real-time PCR assay (Taqman) that can be used to quantify that organism. Robledo (Baltimore, USA) described a different PCR assay for *P. piscicida* that had been validated on samples from Chesapeake Bay. A significant hurdle in all of these studies, and one especially critical to *Pfiesteria* studies, is the difficulty encountered by these workers when using their molecular methods on sediment samples,

where matrix effects make it difficult to determine if *Pfiesteria*'s life history stages (cysts, amoebae, or zoospores) are present.

More than a dozen other presentations described the use of molecular methods in the study of HAB species. Some, such as that of Brenner (Bremerhaven, Germany) use probes in combination with other techniques such as flow cytometry and artificial neural networks to distinguish HAB species in natural populations. Some workers are using sequence information to characterize and classify HAB species groups and to fit them into phylogenetic or biogeographic frameworks. Chinain (Papeete, Tahiti) used rRNA genes to accomplish this for the ciguatera dinoflagellate *Gambierdiscus*, identifying four ribotypes that were entirely consistent with morphological classifications. This same approach was used in a global study of the toxic dinoflagellate *Gymnodinium catenatum* by Bolch (Oban, Scotland). Outbreaks of this species in areas with no prior history of shellfish toxicity have been invoked as evidence of recent species dispersal from other regions, presumably via ballast water. Employing cyst morphology, interbreeding, and biochemical and molecular genetic comparisons, Bolch revealed a high level of variability within globally distributed populations. Japanese and Spanish/Portuguese strains resolve into distinct groups that are more closely related to each other than to the Australian *G. catenatum*. Thus the "source" of the *G. catenatum* that suddenly caused shellfish to become toxic in Tasmania in the early 1970's remains unknown. In a similar demonstration of the value of multiple approaches to studies of this type, Haywood (Nelson, New Zealand) described her work on the systematics of several closely related *Gymnodinium*/*Gyrodinium* species. The combined use of morphology, biochemistry, and genetics made it possible for her to describe and differentiate species and strains in what has always been a difficult group to classify. Her overall conclusion is that a suite of HAB species currently assigned to either *Gymnodinium* or *Gyrodinium* are closely related to each other, and not to the type species *Gymnodinium fuscum* or *Gyrodinium spirale*.

Some progress has been made on the identification of genes involved in toxin production, especially with cyanobacteria. Dittmann (Berlin, Germany) described efforts to identify DNA sequences involved in the production of microcystin. The noteworthy aspect of this study was the success achieved in inactivating three genes thought to be required for the synthesis of the microcystin molecule. This was accomplished through genetic transformation and insertional mutation, and led to mutant cells that were

unable to produce microcystin but which could synthesize other small peptides. It was then possible to screen toxic and non-toxic strains for these target genes. This type of genetic manipulation, accomplished here with cyanobacteria, has yet to be successfully applied to dinoflagellates or other toxic groups. Once success is achieved there, progress should be rapid in the study of toxin production and regulation in eukaryotic algae.

Toxin Detection and Measurement

The detection and qualification of algal toxins has always been an important aspect of HAB research and management. In many cases, this has been accomplished through sophisticated instrumentation, and this was again apparent in Hobart. The use of liquid chromatography-mass spectrometry (LC-MS) was described in several papers by Quilliam (Halifax, Canada) or charge-remote fragmentation spectrometry (FAB-MS/MS) by Yasumoto (Tokyo, Japan), who used the latter method to elucidate the structures of 20 ciguatera congeners. The Hobart meeting was noteworthy for the number of contributions describing new, simple assay methods for algal toxins. Many of the new methods rely on antibodies to the toxins, incorporated into ELISA format such as those described by Garthwaite (Hamilton, New Zealand) for the brevetoxins, microcystins, and yessotoxins. Another relatively new class of assays uses specific receptor molecules for individual toxins, providing a sensitive and pharmacologically relevant approach to toxin detection. Jellett (Dartmouth, Canada) described trials of a receptor-based assay for paralytic shellfish poisoning (PSP) toxins that is now commercially available. Over 500 shellfish extracts were tested with the latest version of this test kit, and the results showed good agreement with the official AOAC mouse bioassay. Powell (Charleston, USA) described a high throughput receptor binding assay for PSP toxins that relies on microplate scintillation technology, yielding a turnaround time of four hours for a 96-well plate.

In a fascinating application of fundamental research to a practical problem, Llewellyn (Townsville, Australia) described the use of saxiphilin to detect and quantify PSP toxins. Saxiphilin is a soluble protein that binds to saxitoxin, found in the circulatory fluids of many vertebrates and invertebrates. Animals found to possess saxiphilin include lizards, amphibians, fish, spiders, insects and centipedes. Fundamental research is underway to investigate why so many organisms produce a molecule with such a high affinity for a toxin that many may never

be exposed to. On the practical side, however, the discovery that saxiphilin from a tropical centipede binds to saxitoxin so strongly that the halftime for dissociation is almost two days led to the development of a microplate assay which can be used to specifically detect saxitoxin and its derivatives.

Miscellaneous Topics

Considerable research is being conducted on *Pfiesteria piscicida* and related organisms, some of which are implicated in massive fish mortalities as well as in human illnesses in the southeastern U.S. Burkholder (Raleigh, USA) reviewed the status of current research on this important new organism. Two toxic species have thus far been identified – *P. piscicida* and a newly described species *P. shumwayae* (Glasgow, Raleigh, USA). The ecology and nutritional physiology of these organisms were highlighted, and an update given on the search for their toxins. A water-soluble putative toxin has been partially purified that kills fish and causes prolonged elevation of cytosolic free calcium in pituitary cells. A hydrophobic toxin component has been linked to edema and sloughing of fish epidermal tissue. Human health impacts following contact with water or inhalation of air in areas with actively toxic *P. piscicida* populations have included respiratory, visual, and neurological impairment, the latter described by Grattan (Baltimore, USA).

Bricelj (Halifax, Canada) presented a fascinating study that documented differential responses to PSP toxins by soft shell clams from areas with contrasting histories of toxin exposure. Remarkable differences were observed in burrowing capacity, feeding rate, tissue toxin accumulation, and in vitro blockage of the action potential of isolated nerves exposed to saxitoxin. Clams which had never been exposed to saxitoxin showed great sensitivity, losing their ability to bury themselves and decreasing their feeding rates significantly. Toxicity in these "sensitive" shellfish was significantly lower (up to tenfold) than the "resistant" clams which had prior exposure to the toxins. One can see how mortality would differ in these populations following a toxic bloom, leading to the selection for the resistant genotype, which tends to accumulate more toxin. Such intraspecific differences in sensitivity and toxin accumulation have important ecological and management implications.

Several papers described parasites that infect HAB species. Erard-Le Denn (Plouzané, France) described a parasite that infected toxic *Alexandrium minutum* in Brittany, similar to the newly described

Parvilucifera infectans from Scandinavian waters (Norén, Fiskebackskil, Sweden), which belongs to the Apicomplexan complex and is closely related to *Perkinsus*, an oyster killing protist. The *Alexandrium* parasite only infected dinoflagellates – diatoms and raphidophytes were not affected. The ecological importance of this and other parasites requires further investigation, as does its possible use as a bloom control agent.

Mitigation

Another unique feature of the Hobart meeting was the number of papers on mitigation. Where the preceding conference in 1997 had a single paper on direct bloom intervention or control (with none in the meetings before then), 9 were presented in Hobart. This has always been a controversial topic within the HAB community, where lack of research in the past has been a de facto admission that direct bloom control is not possible or would have unacceptable consequences. There has, however, been very little research on which to base that supposition. The small but significant number of contributions on mitigation at Hobart suggests a change in the attitude of at least some workers. Chemical approaches to bloom control included the use of NaOCl produced by seawater electrolysis (Kim, Pohang, Korea) or ammonium sulfate to destroy *Prymnesium* blooms in brackish water ponds in Israel (Kimor, Haifa, Israel). Several papers (Yu, Qingdao, China and Sengco, Woods Hole, USA) presented data on the use of ordinary clay to flocculate and sediment out HAB species. Removal efficiencies as high as 95% were achieved with loadings as low as 30 ppm with this method, which has already been used on a larger scale to protect fish farms from toxic blooms in Korea and Japan. Biological control was discussed by Kitaguchi (Hiroshima, Japan) who isolated a bacterium capable of killing *Heterocapsa circularisquama*, the dinoflagellate responsible for massive losses to the pearl oyster industry. When the bacterium was added to *H. circularisquama* cultures, the dinoflagellate cells stopped swimming and began to rupture within 24 hours. A related study by Mayali (Charleston, USA) described two bacterial strains that are lethal to *Gymnodinium breve*. Fluorescently labeled rRNA probes developed for both taxa are being employed on both laboratory and field samples to characterize the population dynamics and distribution of these algicidal strains. Progress is also being made towards the identification of the lethal compounds produced by these bacteria. One interesting aspect of these studies is

that algicidal bacteria appear to have close phylogenetic affinities with each other, despite their wide range of target algae, and are quite ubiquitous in the environment.

Other mitigation contributions included papers on ballast water regulations (Paterson, Canberra, Australia) and continuous ballast water exchange during transit (Villac, Rio de Janeiro, Brazil). This method adds ballast water at the top of the tank while simultaneously withdrawing by gravity through the bottom at the same flow rate. This process was very effective in removing 90% or more of the phytoplankton originally in the tank, but cysts and resting spores were not removed as well.

Strategies for mitigating the impacts of HABs on fish farms were reviewed by Rensel (Arlington, USA) who explained that there is no single mitigation method that works for all species or sites. The costs and benefits of strategies such as towing fish net-pens away from blooms, isolating fish from blooms with perimeter skirts, or displacing HAB cells from the fish pens with aeration or airlift pumping were described. The use of clay flocculation on a small scale near fish farms was again highlighted as a promising strategy.

Thomas (the Hague, the Netherlands) described a small and inexpensive instrument called Aquasonic which transmits ultrasonic vibrations through the water, destroying algae. This device can treat large lakes and ponds with a minimum of energy. Over 2000 Aquasonic instruments have been installed in the Netherlands and Belgium to date, mainly in water reservoirs and market gardens.

New Issue

Past HAB conferences have included the first reports of new toxins or toxic outbreaks, and Hobart was no exception. The first toxic bloom ever reported in Kuwait was described by Al Mutairi (Salmiya, Kuwait), resulting in mass fish mortalities caused by an organism similar to *Gymnodinium mikimotoi*. Other papers described a new toxin syndrome called azaspiracid poisoning (AZP) which has led to human intoxication in Europe following the consumption of Irish mussels (James, Cork, Ireland). These intoxications have been attributed to a new family of shellfish toxins called azaspiracids. The etiology of azaspiracid toxicity in shellfish has been studied using LC-MS. Strong evidence has been obtained that these toxins are produced by a dinoflagellate, but the exact species remains unknown. In animal tests, azaspiracid shows pronounced neurotoxic effects and produces severe damage to

the intestine, spleen, and liver tissues. Satake (Sendai, Japan) determined the structure of the azaspiracids and developed a rapid and sensitive LC-MS method to quantify them.

Another relatively new toxin family that had been reported previously but which is now much better characterized is the spirolides. Cembella (Halifax, Canada) described ongoing work to establish the dynamics and source of these "fast acting toxins". *Alexandrium ostenfeldii* is now identified as the causative organism. Richard (Moncton, Canada) described the toxicology and pharmacology of the spirolides. The symptoms in mice injected with this toxin are very different from those associated with other shellfish toxins. Spirolides affect specific receptors in mammalian systems and, at high levels in shellfish, may warrant concern for human consumers of shellfish.

The Future of International HAB Conferences

Over the last several decades, a regular series of HAB conferences has been held, typically biannually, with a steadily increasing number of partici-

pants and a significant broadening of the topics covered under the HAB umbrella. Some of the character of the original meetings has been lost due to this expansion, but increased scientific exchange and cross-fertilization has been a significant benefit. The 10th conference in this series will be held in St. Petersburg, Florida, October 21–25, 2002, and the 11th conference will be in South Africa in early 2005. There will, no doubt, be new toxins and new toxic outbreaks to report, and more information on the nature of this expanding problem. Given the expected advances in technologies for bloom and cell detection, and even bloom mitigation, the scientific basis for successful management of fisheries resources impacted by HABs should be stronger than ever.

Donald M. Anderson

Biology Department
Woods Hole Oceanographic Institution
Woods Hole, MA 02543, USA

fax 1 508 457 2134
e-mail danderson@whoi.edu