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# REGIONAL PROGRAM AND SPECIAL SESSION SUMMARIES

# ECOHAB:Florida—A Catalyst for Recent Multi-Agency Studies of the West Florida Shelf

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## Abstract

With state and federal funds, ~175 cruises during March 1998–December 2001 provided stock estimates of *Karenia brevis,* other phytoplankton and zooplankton, nutrients ($NO_3$, $NO_2$, $NH_4$, $PO_4$, $SiO_4$, Fe, DOP, DON), $O_2$, DIC, chlorophyll, phaeopigments, CDOM, optical properties, PON, POC, and POP on the West Florida Shelf. Observed currents, estimated from hydrographic surveys and moored Acoustic Doppler Current Profile arrays, and simulated ones, derived from a 3-dimensional circulation model, are being used to drive ecological models of red tide initiation, maintenance, and fate during 1998–2001 in relation to a prior 45-year time series of *K. brevis* observations. Test cases of small 1979 and 1998 red tides in the numerical models match observed net growth rates and abundance of those *K. brevis* populations, implying that CDOM light-shading, organic nitrogen sources of diazotroph origin within phosphorus-replete coastal waters, selective grazing pressure, and near-bottom onshore transport to frontal regions during fall upwelling periods are all required to elicit naturally occurring large red tides between Tampa Bay and Charlotte Harbor.

## Introduction

In 1844, the first documented account of fish kills and discolored water by a red tide of the unarmored dinoflagellate, *Karenia brevis* (formerly known as *Gymnodinium breve*), off the west coast of Florida was made, published in 1882 by E. Ingersoll. Over the last century, the seasonal duration of red tides off west Florida has varied from none to 18 months, with 70% of the blooms apparently occurring in late summer–fall (Steidinger *et al.,* 1998). The causative agent was not identified until 1948 by C. C. Davis. During the last 30 years, red tides have been observed 29 times within the region between Tampa Bay and Charlotte Harbor (Fig. 1), such that this epicenter of *K. brevis* abundance along the west Florida coast was selected as the study site of the NOAA/EPA project, ECOHAB (Ecology and Oceanography of Harmful Algal Blooms):Florida.

Our ability to predict initiation, maintenance, and dispersal of past red tides on the Florida shelf was severely limited by the lack of a quantitative description, or model, of their population dynamics. When future red tides are observed by our volunteer groups of fishermen, boaters, and charter boat captains, prediction of their landfall and consequent toxic effects will still be impossible without an ability to model successfully both the three-dimensional (3-d), time-dependent flow fields and the growth/loss processes effecting such accumulations of *K. brevis*. Accordingly, the goal of the ECOHAB:Florida project was to make sufficient time series observations of the important physical and biochemical control processes that initiate and terminate blooms of *K. brevis,* to allow construction and validation of these 3-d coupled models.

Such ambitious goals required melding the fiscal and logistical resources of a number of concurrent field projects on the West Florida Shelf (WFS) to achieve a critical mass of investigators and observations. The ECOHAB/Florida project was used as the catalyst for collaboration among the other MMS NEGOM [North Eastern Gulf Of Mexico], the ONR HyCODE [Hyperspectral Coastal Ocean Dynamics Experiment], the ONR FSLE [Florida Shelf Lagrangian Experiment], the NSF DOTGOM [Daughters Of Trichodesmium Gulf Of Mexico], the EPA HABSOS [Harmful Algal BloomS Observing System], and state-supported Florida Marine Research Institute (FMRI)/MOTE/University of South Florida (USF) projects on the WFS. During March 1998–December 2001, 175 cruises (Fig. 1) collected extensive *in situ* WFS data sets on hydrography, turbidity, spectral dependence of absorption, backscatter, water-leaving radiance, light attenuation, Saharan dust, $NO_3$, $NO_2$, $NH_4$, urea, $PO_4$, $SiO_4$, Fe, dissolved organic phosphorus (DOP), dissolved organic nitrogen (DON), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), brevetoxins, chlorophyll, phaeopigment, particulate nitrogen (PN), particulate carbon (PC), particulate phosphate (PP), $\delta^{15}N$ of PN, and counts of dominant phytoplankton and mesozooplankton species in relation to moored arrays of currents, T/S, and bio-optical sensors, aircraft overflights, $SF_6$ dispersion studies, underway sampling of plankton particles and images, multi-beam bathymetric and side-scan sonar surveys of the bottom. These data were used to construct and validate state-of-the art coupled 3-d biophysical models of the circulation, plankton dynamics and bio-optical properties of the WFS (Weisberg and He, 2003; Walsh *et al.,* 2003; Jolliff *et al.,* 2003), which are now being used to guide development of operational models for red tide forecasts.

Collaborating institutions in the State of Florida were FMRI; MOTE Marine Laboratory; USF; Florida State University; Atlantic Oceanographic Meteorological Laboratory; Florida Environmental Research Institute; Florida Institute of Oceanography; and Harbor Branch Oceanographic Institution. Other researchers were located at University of North Carolina; North Carolina State University; National Ocean Service–Beaufort, NC; National Ocean Service–Charleston, SC; University of Southern Mis-

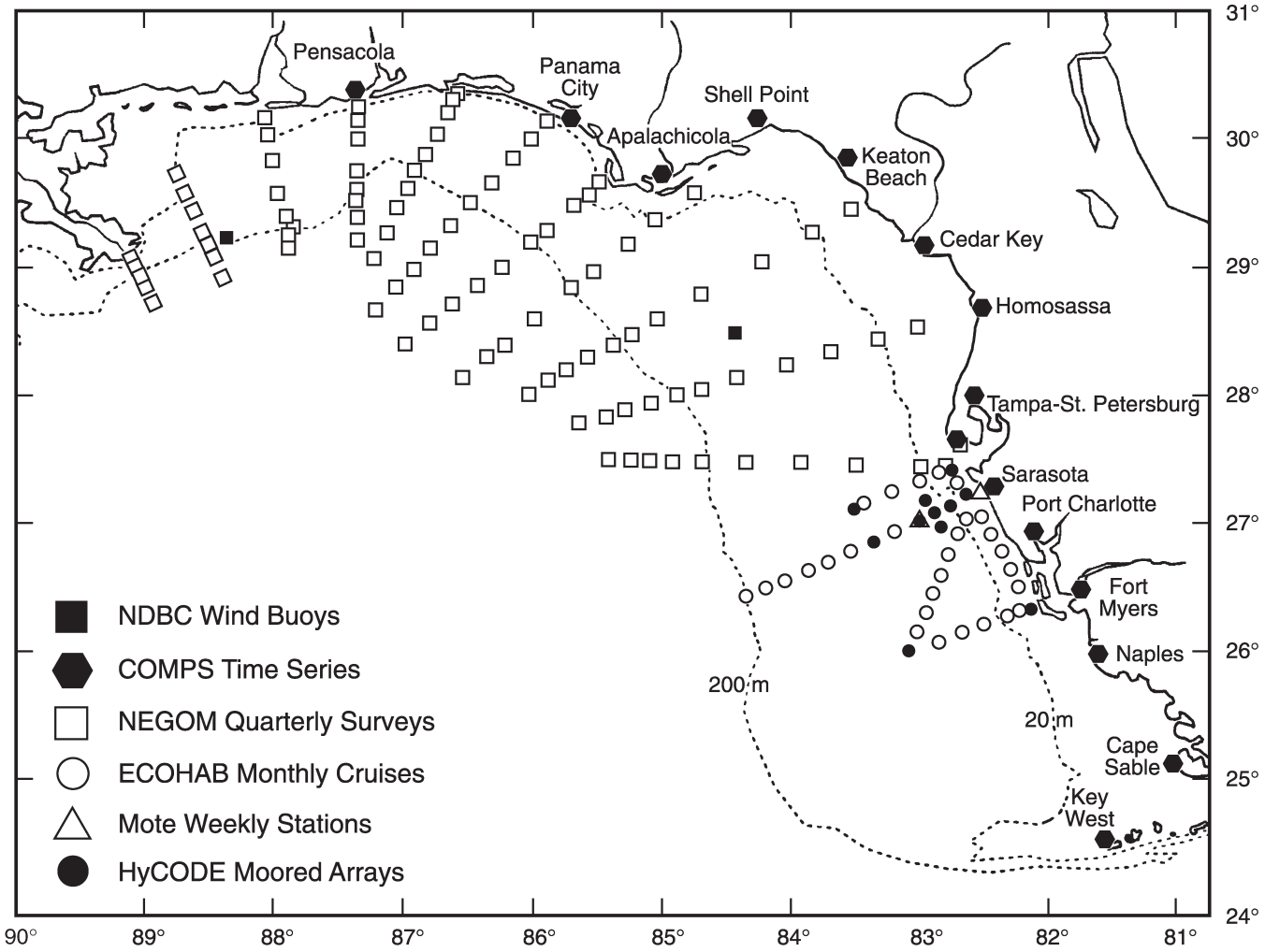


**Figure 1** Station locations of the NEGOM (M), ECOHAB (V), and MOTE (△) surveys of the West Florida Shelf in relation to HyCODE moorings (●), COMPS time series of sea level, and NDBC buoys during June 1998–September 2002. The FSLE tracers during 2000 and 2001 were released within the HyCODE/ECOHAB control volume between St. Petersburg and Fort Myers, FL.

sissippi; Naval Research Laboratory–Washington, DC; Naval Research Laboratory–Stennis Space Center, MS; Texas A&M University; Rutgers University; and Hobbielabs.

## Summary

From some of the field observations, we find the following: 1) As a consequence of stratification, onshore flows of near-bottom water during upwelling are greater— under the same wind forcing—than offshore ones during downwelling, analogous to a flapper valve, such that a preferential landward transport of materials occurs within the near-bottom Ekman layers during the fall (Weisberg *et al.,* 2001). 2) *In situ* populations of *K. brevis* have net growth rates of 0.2–0.3 day$^{-1}$ (Van Dolah and Leighfield, 1999)—not the maximal model and laboratory rates of ~0.8 day$^{-1}$—so red tides must usually be nutrient or light limited, in the absence of grazing pressure. 3) Saharan dust removal of Fe-limitation of *Trichodesmium erythraeum* within local P-replete coastal waters provides DON fuel for red tide initiation (Lenes *et al.,* 2003). 4) Their isotopic signature is not that of nitrate-depleted slope waters (Walsh and Steidinger, 2001), *i.e.,* recycled products of nitrogen-fixation may instead fuel initiation of red tides, while their large N:P ratios imply P-mediated demise (Vargo *et al.,* this Proceedings). 5) Their saturation light intensity is ~100 μE m$^{-2}$ sec$^{-1}$ (Shanley and Vargo, 1993; Millie *et al.,* 1995), compared to surface PAR of >1000 μE m$^{-2}$ sec$^{-1}$ at noon, such that rapid photoadaptation and/or exogenous sun screen is required for both detection of *K. brevis* by satellites and their survival at the sea surface. 6) Dominant copepod herbivores contain more diatoms and non-toxic dinoflagellates in their guts than ambient prey stocks of the WFS, such that usually selective grazing pressure is exerted on phytoplankton competitors of *K. brevis* (Kleppel *et al.,* 1996). 7) Once released, their brevetoxins persist among all components of the food web, providing a cumulative index of red tide duration and impact (Tester *et al.,* 2000).

From the model results, we find the following: 1) Slope water nutrient supplies lead to diatom blooms, not red tides (Walsh *et al.,* 2003). 2) CDOM (colored dissolved

organic matter) of coastal waters removes light-inhibition of *K. brevis* (Jolliff *et al.,* 2003). 3) Shade-adapted *K. brevis* are subsurface innocula of the red tides, entrained onshore within the near-bottom Ekman layers during local upwelling, focused by bathymetry to the north of Tampa Bay (Walsh *et al.,* 2002). 4) Removal of P-limitation of both *T. erythraeum* and *K. brevis* by estuarine outwelling results in concurrent delivery of herbivores, *e.g., Acartia* spp., which may determine bloom onset and decay (Milroy *et al.,* submitted). 5) Building upon the original hypothesis of bloom formation along WFS coastal fronts (Steidinger and Haddad, 1991), *K. brevis* chlorophyll stocks of >10 µg chl $L^{-1}$ on the WFS shelf are probably the result of physical aggregations, allowing co-occurrence and nutrient transfer between poorly grazed, slow-growing populations of *T. erythraeum, K. brevis,* and dying, decaying fish, which serve as an organic source of nutrients for the larger red tides (Walsh *et al.,* submitted).

## Acknowledgements

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# EU-LIFEHAB: Expanding the Discussion on the Life Cycles of Harmful Algae

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## Participants

Donald M. Anderson, Stephen S. Bates, Susan Blackburn, Chris J. S. Bolch, Barrie Dale, Malte Elbrächter, Paul E. Hargraves, Ichiro Imai, Anke Kremp, Jane M. Lewis, Marina Montresor, Louis Peperzak, Christopher A. Scholin, and Carmelo R. Tomas

## Background

The workshop "LIFEHAB: Life history of microalgal species causing harmful blooms," funded by the Fifth Framework Programme (Energy, Environment and Sustainable Development) of the Commission of the European Communities, was held in Calvià (Mallorca, Spain), 24–27 October 2001. Complex and heteromorphic life cycles are part of the adaptive strategies of organisms causing harmful algal blooms (HAB). They can influence the intrinsic potential for growth, persistence and dispersal, allowing the species to occupy different ecological niches. Information about life cycle strategies are very important for understanding bloom dynamics and population structure of HAB species.

The workshop report (Garcés *et al.,* 2002) contains extended abstracts, reports of the discussion groups, tables with summarized information on diatom, dinoflagellate, haptophyte and raphidophyte life cycles and a very comprehensive bibliography. The report is currently available at: http://www.icm.csic.es/bio/projects/lifehab/.

The objectives of LIFEHAB were to

- review current knowledge on the life cycles of phytoplankton organisms, focusing on harmful species;
- identify the role of heteromorphic life cycles in population dynamics;
- define future HAB research directions to fill existing gaps in knowledge;
- debate the most appropriate approaches and methods;
- promote the development of cooperative scientific initiatives.

The aim of the roundtable held during the Xth International Conference on Harmful Algae was to expand the discussion so as to include non-EU scientists who were not involved on the LIFEHAB Workshop.

**S. Bates** noted that although considerable knowledge has been gained on *Pseudo-nitzschia* species, little progress has been made on other diatom genera. Advances in species-specific molecular probe design that allow the detection of different sexual stages in the field and the differentiation of male from female gametangia would be most useful, in order to determine the proportion of these cells in a population. Mating compatibility studies could clarify questions concerning species definitions. Other approaches, such as the use of image analysis or the identification of condensed chloroplasts, could prove effective in tracking changes in size spectra and resting stages. The identification, localization and physiology of possible overwintering stages are other key issues for understanding the population dynamics of *Pseudo-nitzschia* species.

**P. Hargraves** focused on triggering mechanisms for diatom auxospore (size, environment, mating types, pheromones) and resting cell (light, nutrients, temperature) formation. Ecological and physiological studies, as well as monitoring programs, are as good as taxonomic quality allows them to be. The definition of the species/taxonomic units is thus another crucial issue, and the correct approach can be seen as a "three-legged stool" coupling morphology, life cycles, and molecular systematics. Four fields of priority research can be identified in this context: i) relation between life cycle events and interspecific competition at the biochemical level; ii) the role of parasites and pathogens in the control of blooms; iii) the enhancement of toxicity through symbiosis with bacteria; and iv) phylogenetic distribution of toxins, including those affecting organisms other than humans.

**J. Lewis** identified as priority activities: i) investigation of mutation rates in cultures; ii) organization of workshops on dinoflagellate-culturing techniques; iii) development of markers for gametes and viable cysts; iv) investigation of the role of temporary cysts in life cycles; v) search for overwintering stages of species where cysts are not known; and vii) improved methodologies for detailed water-column monitoring and for estimates of *in situ* germination rates.

**S. Blackburn** illustrated examples of crossing matrixes for sexual mating, showing that dinoflagellates have rather complex mating systems, including multiple mating types. She outlined the importance of sexual compatibility among strains, which will ultimately affect cyst production rates and end up in the genetic structure of the population.

**M. Elbrächter** pointed out that little is known about different cell division modalities among dinoflagellates and recalled that in some cases, non-motile stages are involved in asexual reproduction. He also mentioned aspects of dinoflagellate morphology and life cycle traits that have been misinterpreted, as was the case for *Pyrocystis* and *Dissodinium,* the latter with lunate-shaped secondary cysts in which up to 8 asexual planospores are formed, or the asex-

ual pellicle cysts in *Lingulodinium polyedrum.* In his view, more attention should be paid to understanding internal clocks, circannual clocks, social behaviour, communication, and chemical signalling mechanisms.

**L. Peperzak** explained that prymnesiophytes are quite complex because they include motile/non-motile and haplontic/diplontic stages. Molecular probes and flow cytometry are needed to identify species and ploidy levels. The need to develop stage-specific probes and to identify factors influencing life cycle transitions was also mentioned. He speculated on the *quorum sensing* (QS) abilities of microalgae as a way to detect the abundance of the same species by secreting species- and strain-specific competence activators. In bacteria, QS is involved in genetic transformation and sporulation (see Dummy and Williams, 1999). Hypothetically, QS could be involved in syngamy/meiosis and cyst/colony formation in prymnesiophytes. Preliminary tests with *Phaeocystis* indicated that high cell densities induced colony formation. Proving the existence of QS, including among others competence activators, would provide a new perspective in the study of HAB dynamics.

**C. Tomas** and **I. Imai** noted that raphidophytes are naked pleomorphic species not easy to study because they are difficult to preserve without deforming or bursting the cells. Here, the combination of morphology, pigment composition and molecular probes on live and preserved specimens becomes truly a strong argument. While some life cycle information occurs in reports of blooms or cultures, there has been no concerted effort to define the life cycle stages of the different raphidophytes. Given their increasing importance as HAB species, there is a need to re-examine the life cycle phases using a number of techniques now available (gene sequencing, nuclear staining, etc.) as well as traditional ones using clonal cultures. Little is known about processes undertaken in dark and cold bottom waters. Signalling between cells could be through high density or through infochemicals. This kind of communication has not been proven for any HAB species, yet it could be a means for timing life cycle changes in populations capable of forming dense blooms.

**M. Montresor** summarized research priorities for understanding the importance of life cycle events in HAB ecology: a) role of life history stages in bloom dynamics, *e.g.,* when are resting stages produced? how many? how many are viable in the sediments? is there an endogenous control of life-cycle transitions?; b) role of specific life history stages in avoiding predation, preserving genetic diversity and promoting dispersal; c) single species and life-stage distribution through sampling and observational techniques at the appropriate scale (*e.g.,* microlayers, sediment-water interface); d) identification of key areas to be used as "case studies" and the importance of long-term data sets; and e) species-specific models integrating life cycles.

**D. Anderson** illustrated problematic issues related to the study of cyst germination dynamics *in situ.* Different techniques (emergence traps, changes in cyst fluorescence, laboratory incubation of sediments, repeated quantitative cyst enumeration in core samples through time) were used to estimate *in situ* germination rates of *Alexandrium fundyense* in the ECOHAB-Gulf of Maine program, but none proved successful. Ongoing population dynamic studies have therefore relied on large-scale cyst mapping, parameterization of cyst germination rates using laboratory incubations, and incorporation of these data into a coupled physical/biological model. Model runs indicate that light reaching the sediments is surprisingly *not* a crucial factor in germination success. Cysts from shallow waters germinated at nearly the same rate as those in deeper waters. This is because light is rapidly attenuated in bottom sediments, making attenuation due to water depth a minor factor. Layers of sediment above the cysts as thin as several mm may be enough to inhibit germination. Similarly, germination will likely be inhibited by anoxia even a few mm below the sediment surface. In such cases, resuspension by currents or bioturbation may be important in fostering cyst germination. Overall, this presentation highlighted the difficulties that still exist in estimating *in situ* germination rates.

**B. Dale** introduced a geological time perspective into the debate. Changes in time-scales up to 50 years are being related with El Niño-like events, whereas changes in the order of 100-year periods are related to climatic trends. Climate change could be advantageous to cyst-forming species from cold or warm coastal waters, allowing them to better exploit the time shift in seasonal patterns. He criticized the use of the term "harsh environment," which reflects an anthropocentric point of view.

**C. Bolch** celebrated that probes are solving lots of old problems. Twenty out of 90 attendees to this round table are currently using molecular probes in their research. He emphasized the need to present more risky proposals, and to apply innovations in sampling strategies and molecular designs.

**C. Scholin** noted that probes can be applied to intact cells as well as cell homogenates. It is essential that development of these methods remain tightly integrated with traditional microscopy-based species identification techniques. Systems that enable use of molecular probes for near real-time detection of HAB species, *in situ,* are fast becoming a reality. Many approaches that rely on cell-free detection formats offer incredible sensitivity and speed, and can be packaged in very small platforms such as hand-held devices. However, whole cell and cell-free detection methods can yield different answers as to what species are apparently present in a given sample. Working with natural samples, species identifications based on cell-free methods will likely reveal that target organisms are present with greater frequency than those estimates based on intact cells. Key issues to resolve are creating standards for reporting cell presence/abundance using cell-free formats, and documenting the relationship between molecular signatures as seen in intact versus homogenized material.

**A. Kremp** recalled that, in addition to nucleic acids, cell surface molecules are a group of potential target molecules to improve our understanding of life cycle processes.

Studies of *Chlamydomonas* and ciliate sexual reproduction have shown that cell wall molecules, and changes in their structure and composition, largely mediate gamete recognition and fusion. Thus, looking at cell wall proteins or glycoconjugates may help to characterize sexual cells and the physiological processes involved in the sexual reproduction of HAB species. Immunological techniques and proteomic approaches should be explored to identify specific surface molecules and to characterize proteins. Carbohydrates could be targeted with complementary lectins. Fluorescent probes targeting specific cell surface structures can be developed and optimised for detection of life cycle stages in the field, in conjunction with flow cytometry. Recognition and adhesion molecules on the cell surface can also be useful for inhibition experiments to study the function of signalling, recognition and adhesion of gametes.

## Conclusions

From the general discussion, several commonalities emerged for HAB species research priorities. Complementing those outlined by LIFEHAB, research should be focused on the following knowledge gaps:

- Inadequate knowledge of life cycle events for many of the important HAB species. The present state of knowledge does not allow for the formulation of general paradigms.
- Social behaviour, active aggregation mechanisms.
- Signalling (Quorum Sensing), infochemicals, species-specific features.
- Circadian and circannual clocks. Endogenous regulation of life cycle events. Role of photoperiod or photoperiod fluctuations, temperature or temperature fluctuations, in driving life-cycle transitions.

Major gaps related to the ecological role of life cycles were recognized to be caused by methodological and observational constraints in field studies. The limited time-scale of our data sets is also a problem which should be circumvented by a more extended use of sedimentological record, where possible, and by the support to long-term observational programs. Additional suggestions on new approaches, methodologies and research strategies were as follows:

- Couple traditional techniques with advanced/molecular tools, in a bold and creative fashion.
- Complement/substitute observation with adequate models, to be developed.
- Exploit knowledge and experience from different fields (*e.g.,* microbiology, limnology, genetics of non-microalgal organisms).
- Coordinate efforts among scientists and promote cross-validation of methodologies and results.
- Compare the behaviour of a species over its geographic range through international cooperative research.
- Improve the quality of species identification in field studies through the cooperation of classical taxonomists, molecular biologists and ecologists.

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# Summary of the Special Session on Detection and Quantitation of Toxins

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**Abstract**

This special session was organized to offer a forum for presenting and discussing recent advances in sample preparation techniques and analytical methods for identification and quantitation of HAB toxins. The first half of the session consisted of six presentations, including new information on NSP toxins and metabolites in shellfish, validation of LC-MS techniques for multiple toxin analyses (ASP and DSP), application of ELISA for brevetoxins, use of blood cards for collection and storage of samples for biotoxin analyses, improved extraction and clean-up processing techniques, and an LC-ESI-MS-based method for simultaneous determination of algal and cyanobacterial toxins. The second half of the session focused on a multi-laboratory study of methods for determination of brevetoxins and toxin-metabolites in shellfish. This was followed by an open discussion of the sample preparation and analytical methods presented and of the implications for replacing the mouse bioassay.

## Introduction

Harmful algal blooms (HABs) have increased in frequency and intensity worldwide, leading to more frequent outbreaks of seafood-borne illnesses and adverse impacts on natural resources. To meet the demand for more rapid and reliable methods for detection of the causative organisms and the toxins produced, several new techniques and innovative modifications of existing analytical methods are being developed. This special session was organized to provide a forum to present and discuss some of the latest innovations in toxin detection and quantitation. Innovative sample-processing techniques included accelerated solvent extraction (ASE), microwave processing, solid-phase microextraction, immunoaffinity extraction (IAC), capillary electrochromatography and a novel storage technique utilizing blood-card sample storage. New innovations for LC-MS were presented for multi-component analyses of ASP, DSP, NSP and microcycistin toxins as well as for identification of brevetoxin metabolites in shellfish. Modifications in ELISA enabled direct application to water and tissue samples, eliminating the need for solvent extraction steps. The session ended with a discussion of the FDA-coordinated multi-laboratory study comparing five methods for identification and quantitation of brevetoxins in NSP-contaminated oysters, a first step in considering a replacement for the mouse bioassay.

## Summary of Presentations

### *NSP (*Karenia brevis*) Toxins and Metabolites in Oysters, Clams and Whelks*

R. Pierce, M. Henry, R. Dickey, and S. Plakas

Three species of shellfish (clams, oysters and whelks) were collected during and following an intensive *Karenia brevis* bloom that occurred from August to December 2001, in Sarasota Bay Florida, USA. The purpose of this study was to monitor the accumulation of brevetoxins and production of toxin-metabolites in clams and oysters exposed to the same natural harmful algal bloom, to identify the toxins/metabolites responsible for neurotoxic shellfish poisoning (NSP), and to observe trophic transfer of NSP components to whelks feeding on contaminated clams. Both clams and oysters were found to accumulate the proposed toxin-metabolites (*m/z* 1018 and 1034), with little or no measurable parent toxins. The toxin-conjugates were observed in higher concentrations in the oysters relative to the clams during exposure to the bloom; however, both species retained approximately the same level of conjugates after the bloom subsided. Tissue from both clams and oysters exhibited mouse toxicity and retained toxin-metabolites for 6 weeks following the bloom. Trophic transfer of these compounds to whelks was not observed, yet the whelks exhibited low levels of mouse toxicity.

### *Validation of an LC-MS Method to Detect ASP and DSP Toxins in Shellfish*

P. McNabb and P. Holland

An LC-MS method was developed to replace mouse bioassay testing in the comprehensive New Zealand Marine Biotoxin Monitoring Programme. The toxins to be detected and the limits of detection required were considered prior to developing the method so that once fully validated it could be used for routine monitoring in New Zealand. Certified reference materials were obtained from NRC Canada. Results for DA, OA and DTX-1 give confidence of the method validity. Greenshell™ mussel samples which contained DSP, PTX and YTX toxins were used to test a number of performance parameters. A plot of the percentage of contaminated sample against the LCMS response gave highly linear responses except YTX at the highest levels studied (>200 ng/mL). The validation study allowed development of quality-control criteria for determining LODs and acceptable standard recovery levels. In the case of YTX and DA the validation showed that additional laboratory procedures need to be developed to ensure that accurate levels are reported.

Steidinger, K. A., J. H. Landsberg, C. R. Tomas, and G. A. Vargo (Eds.). 2004. Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

### *Competitive ELISA, an Accurate, Quick and Effective Tool to Monitor Brevetoxins in Environmental and Biological Samples*

J. Naar, A. Weidner, and D. Baden

A competitive Enzyme-Linked Immuno-Sorbent Assay (Competitive ELISA) was developed for brevetoxin analysis, based on the activity of goat anti-brevetoxin antibodies following immunization with KLH-PbTx conjugate. A multi-step signal amplification procedure was used to minimize non-specific background noise. Analyses were performed in seawater, mammalian body fluid, and shellfish tissue homogenate without any extraction or purification steps. Because the method is not affected by matrix composition, it eliminates loss and formation of artifacts during sample extraction and processing. Comparisons with the mouse bioassay indicate that it has potential to replace the mouse bioassay for monitoring NSP.

### *Detection and Quantification of Marine Toxin Exposure Using Blood Collection Cards*

M. Bottein Dechraoui, S. Dover, R. Woofter, T. Work, G. Balazs, P. Moeller, and J. Ramsdell

A method was developed to monitor brevetoxin and okadaic acid exposure using blood that was collected, dried and stored on cellulose blood collection cards (0.1 mL blood/spot), widely employed for routine diagnostic testing on newborns. The toxin extraction gave linear response and efficient recovery with low matrix interference for receptor-binding and radioimmunoassay techniques. This technique is being evaluated for additional marine toxins with the expectation that it will provide a method for diagnosing human intoxication.

### *Recent Developments for the Analysis of Algal Toxins*

A.G-Martínez, J.M. Leao, N. Piñeiro, E. Vaquero, F. Davila, P. de la Iglesia, J.A. Rodríguez Vázquez, and J.F. Lawrence

A major source of error in algal toxin analysis is in extracting and processing the sample, leading to unsatisfactory sensitivity, selectivity, efficiency, reliability and accuracy. This work focused on improved methods of sample extraction and clean-up. Improvements included Accelerated Solvent Extraction (ASE), Microwaves Assisted Process (MAP), Solid Phase Microextraction (SPME) and Immunoaffinity (IAC), which not only increase extraction efficiency but also enhance removal of matrix interferences. Innovations in Capillary Electrophoresis (CE) and Capillary Electrochromatography (CEC) for analysis of algal toxins have shown promise for improved clean-up of complex matrices of ASP-contaminated shellfish.

### *An LC-ESI-MS–Based Method for the Simultaneous Determination of Algal and Cyanobacterial Toxins in Phytoplankton from Marine Waters and Lakes Followed by Structure Elucidation of Microcystins*

Jens Dahlmann, Wes R. Budakowski, and Bernd Luckas

A liquid-chromatography (LC)-based method with mass spectrometric (MS) detection was developed for simultaneous determination of various algal and cyanobacterial toxins extracted from phytoplankton occurring world-wide in marine waters and lakes. Phytoplankton biomass was extracted with methanol/water, 50/50, v/v, and the extracts injected directly into the LC-ESI-MS device, providing baseline separations in a single 30-min chromatographic run. The method enables quantification of saxitoxin, anatoxin-A, domoic acid, nodularin, microcystins, okadaic acid and dinophysistoxin-1 with a 0.5 ng limit of detection.

### *Multi-Laboratory Study of Five Methods for the Determination of Brevetoxins In Shellfish Tissue Extracts*

Robert W. Dickey, Steven M. Plakas, Edward L.E. Jester, Kathleen R. El Said, Jan N. Johannessen, Leanne J. Flewelling, Paula Scott, Dan G. Hammond, Frances M. Van Dolah, Tod A. Leighfield, Yasmine Bottein, John S. Ramsdell, Richard H. Pierce, Mike S. Henry, Mark A. Poli, Calvin Walker, Jan Kurtz, Jerome Naar, Daniel G. Baden, Steve M. Musser, Penelope Truman, Timothy P. Hawryluk, Marleen M. Wekell, David Stirling, Michael A. Quilliam, and Jung K. Lee

A thirteen-laboratory comparative study was undertaken to test four methods as alternatives to mouse bioassay for the determination of brevetoxins in shellfish. These include the N2a neuroblastoma cell assay, two variations of the sodium channel receptor binding assay, a competitive ELISA, and LC/MS. From three to five laboratories were enlisted to perform each method. Test samples included brevetoxin-3 standards, brevetoxin-3 spiked shellfish extracts and toxic shellfish extracts derived from a *Karenia brevis* bloom in northwest Florida. Results of this comparison of *in vitro,* instrumental, and mouse bioassay methods show statistically acceptable correlation of mouse bioassay with competitive ELISA and receptor binding assay for the determination of brevetoxins in shellfish. LC/MS performed as well as ELISA on spiked test samples but was inordinately affected by lack of toxin-metabolite standards, uniform instrumental parameters, or both for incurred test samples. Nevertheless, LC/MS proved invaluable for qualitative confirmation of specific brevetoxins and metabolites in shellfish tissues.

## Open Forum Discussion

Workshop participants were receptive to the idea of establishing replacements for mouse bioassay as the standard method for all forms of shellfish toxicity. Much of the discussion focused on the multi-laboratory comparative study of methods for brevetoxins. It was noted that all of the test samples were extracted and processed at a single laboratory, thus eliminating variability derived from sample extraction and clean-up performed at separate labs. It was agreed, however, that the best way to test the analytical methods is to use replicates of the same sample to eliminate inter-lab-

oratory differences in sample processing. Future studies should address variations in results due to differences in inter-laboratory sample processing.

It was observed that the design of the multi-lab study contained within-laboratory and between-laboratory comparative elements for multiple methods, and that perhaps the variability observed was due to lack of adequate controls on the execution of any one method in the different labs. It was suggested that in addition to controlling for variability in sample preparation, that controlling for variability in protocols used in executing the detection methods would help tighten-up the results. An important aspect of the revised ELISA method is that it does not require sample extraction or clean-up, thus eliminating inter-laboratory variability due to sample processing. A second, more refined, comparative study between LC/MS, ELISA and receptor- binding assays was recommended to be undertaken once brevetoxin-metabolite standards become available. Furthermore, because the values reported for incurred test samples, which are known to contain principally brevetoxin metabolites, are expressed in PbTx-3 equivalents, it is not possible to determine from the data how these numbers relate to actual constituent concentrations. Nevertheless, it was surmised that the *in vitro* methods compared favorably with mouse bioassay, providing equivalent if not superior information, and should in fact replace mouse bioassay even in the absence of more definitive quantitative information on the metabolites. The lack of brevetoxin-metabolite standards for LC/MS quantification was recognized as a critical area of need, suggesting that LC/MS would likely perform as well as *in vitro* methods if adequate standards were available, as illustrated by tight correlation found in brevetoxin-3 spiked test samples.

Comments regarding the complexity and expense of alternative methods suggested that the mouse bioassay may still be the best method for many applications. It was noted, however, that mouse bioassays do produce false positive results (*e.g.,* fatty acid content in some shellfish, i.p. vs. p.o administration, toxins that show mouse toxicity but no human toxicity). The challenge is to develop a reliable, uncomplicated, fast and inexpensive method that provides the same level of public health protection as does the mouse bioassay. The consensus was that the ELISA and receptor binding assays are making great progress toward that goal, and that LC-MS will provide essential analytical confirmation once the full complement of NSP-constituents have been identified and standards become available.

# Recent Advances and State of the Technology for HAB Species Detection

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**Abstract**

A roundtable discussion on HAB species detection and quantitation was held on Wednesday, October 23, 2002, from 0800 to 1200 hours. The overall objective of the roundtable discussion was to bring the attendees up to date on recent activities and the state of development of applicable methods for detection and quantitation of harmful algal species. Several short reports from recent meetings relayed the highlights of the technologies and/or approaches covered during those meetings. Attendees with specific recent findings addressed the roundtable with their information. General discussions between all attendees addressed the state of development/test/operation of applicable technology and/or approaches. The discussions were steered toward applied concerns including: the need for certified reference standards in order to establish the intercomparability of various methods; what to do with the data from HAB monitoring/quantification programs; where do these capabilities fit in the bigger picture of forecasting events; how feasible are "early warning systems" based primarily on detecting phytoplankton; when will they be operational?

## Introduction

The economic and public health impacts of harmful algal blooms have generated significant interest in detecting, monitoring and assessing bloom species and toxins. Techniques and technologies for detecting HAB species and toxins are advancing rapidly throughout the world. It is essential that the HAB community worldwide be kept informed about the developing new tools for detection of HABs so there can be adequate preparation for the implementation of programs to apply them.

Most plans for the management and mitigation of HAB impacts require the detection of the harmful algal species as early as possible during the event. Early detection of the HAB organism is also critical for effective research on HABs. Light and electron microscopy are the classical methods for the detection and identification of HAB species. However, they are labor-intensive and relatively slow, making it difficult and expensive to provide regular, synoptic detection and enumeration. It is important to recognize that microscopy is essential to the taxonomic verification of species that is required during the development of other detection techniques.

Based on the current emphasis and activity, it was decided that there should be a special session at HAB X to bring the attendees up to date on recent activities and the state of development of methods for detection and quantitation of harmful algal species.

## Objectives

A roundtable discussion on HAB species detection and quantitation was held on Wednesday, October 23, 2002, from 0800 to 1200 hours. It was organized to include reports on recent meetings, discussions of recent activities and findings and general discussions on the state of development, test and operation of HAB detection and quantitation techniques. The discussions were directed toward applied concerns including: where do these capabilities fit in the bigger picture of forecasting events; how can these techniques and technologies be implemented in a cost effective, timely manner; how feasible are early warning systems; what to do with data from HAB monitoring/quantification programs; when will they be operational; who will produce them?

## Results and Discussion

This special session began with summaries of recent HAB species detection workshops. The workshop titled "Molecular Probe Technology for the Detection of Harmful Algae" was held in Galway, Ireland, May 20–24, 2002. There were approximately 50 participants from around the world. General findings included the need for more personnel and funds to develop assays and a need for wider availability of developed assays. The proceedings of this workshop are available through the GeneProbes.org Web site. The "Analysis of Single Cells in the Marine Phytoplankton (ASCMAP)" workshop was held in Bremerhaven, Germany, April 15–21, 2002. Approximately 90 participants attended. The workshop focused on combining three phytoplankton analysis technologies: flow cytometry, artificial neural networks and molecular probes. A report (Groben and Medlin, 2002) is available through the Urban and Fisher Verlag website. The "Biosensors for Harmful Algal Blooms" workshop was held in Solomons, United States, March 20–22, 2002. That workshop involved approximately 50 participants including representatives from the HAB research community, the resource management community, and the test and instrument manufacturing community. It was designed to bring those communities together to identify needs from all perspectives and develop mechanisms to connect those communities to enhance the development and transfer of new technologies. A report on the workshop is available through the Alliance for Coastal Technologies (ACT) Web site.

Discussions then turned to specific detection technologies including gene probes, flow cytometry, pigment signature, airborne and satellite remote sensing and optical characteristics. It was agreed that it is essential to the development of detection technologies to have reliable and accessible material standards, be they cultures, genes or

pigments. Another general concern for all new detection techniques is how to transfer basic decision criteria to the new technologies. For example, how do cell counts equate to gene probe intensity or gyroxanthin-diester concentration? Extensive side-by-side trials is one way of building a transfer formula.

The loss of skilled scientists with expertise in basic taxonomic identification was stated as a major concern because all of the detection technologies require validation based on accurate, morphologically-based identification of species. Retirement of the existing cadre of taxonomic specialists could potentially disrupt the advancement of detection technologies (as well as many other fields) by limiting the ability to verify the species being used in technique development. The IOC conducts taxonomy workshops as do some individual research institutions, but academic curricula are not receiving enough emphasis and support. There remains a critical need for academically trained algal taxonomists that can not be filled by non-specialists trained in short-courses workshops.

It was pointed out that most of the discussions were directed toward applications in marine systems with little mention of the need for HAB detection capabilities in freshwater systems. Freshwater applications are not excluded; most of the technologies being applied to marine systems are applicable to freshwater also. Species-specific molecular probes must be developed for the important freshwater HAB species and those species must be optically characterized, but the marine techniques should be applicable. Satellite remote sensing is somewhat problematic for freshwater systems because of the spatial scales and water color. Satellite remote sensing of phytoplankton blooms has been successful in large freshwater lakes.

The possibility of establishing centers for HAB detection was discussed. Their purpose would be to coordinate and facilitate applications of new technologies for HAB detection. These centers could house the technology experts and the specialized facilities that are too costly to establish everywhere. They should be established with a permanent core of staff scientists and a flexible visiting expertise. The centers could establish standard procedural guidelines, conduct comparative pilot studies and assessments, determine specific technologies for specific taxa, foster government-academic institution-private industry collaborations, and serve as distribution centers for supplies, equipment and information. It was suggested that the IOC might be able to serve as a clearinghouse for technology transfer. The IOC's current role is in the dissemination of information and conducting surveys of need.

Gene probe techniques have been developing worldwide, but not with a common basis. The field is at a level of development that certified reference standards need to be developed and made available to laboratories in order to establish the intercomparability of results obtained by different methods. It was suggested that it is time to establish an international workgroup that could seek funding specifically to develop standards. One reason that standards have not been a focus in the past is that much of the development of gene probe technology has been done at the research level and there is low publication potential in work on standards. It would be beneficial if HAB stakeholders would voice their encouragement for the development of standards. There are cases where some standards have been established, such as through the IOC, and standard materials may be available, such as frozen clone libraries, but a comprehensive, international standards agreement is lacking. There are several pragmatic obstacles to the establishment of international standards, including the resistance to technology transfer and the current focus on many monitoring programs on toxins.

There are currently two general approaches to remote sensing of HABs. The direct approach seeks to observe a characteristic of ocean color that can be attributed to the target species and is dependent on the dominance of the biomass by that species. The correlative approach makes a linkage between an observed condition and the presence or absence of the target species based on knowledge of the particular ecosystem. Within those two approaches, there are active and passive sensor systems. Active systems include laser-stimulated fluorescence, which is currently done from aircraft. Passive sensors sense the natural or background light signal coming from the water. Applications of both of these approaches and both sensor types are experiencing some success with HAB detection, but many interfering environmental conditions still limit their reliability.

In the field of optical detection, there are approaches that use either color or shape to discriminate species. With the availability of high-speed data processing, the use of flow cytometry linked to micrographic imagery is being applied with encouraging results. Fluorescence-based techniques range widely in their discrimination capability. Single wavelength excitation and emission systems are able to detect the presence of algae. Multi-wavelength excitation and emission techniques can discriminate algal groups and potentially genera. Species discrimination based on light absorbance characteristics has been applied to *Karenia brevis* in the Gulf of Mexico. This technique is currently being adapted to *in situ* platforms in the Gulf. Though it is not strictly an optical detection technique, the use of diagnostic photopigments to detect the presence and intensity of HABs is developing where distinct pigments are characteristic of bloom species. Though variability in physiological condition confounds the quantitation based on pigments alone, the technique is a good "first approximation" approach. Additionally, the photopigments can provide some measure of physiological state and may help parameterize forecast models.

Operational HAB detection is in various stages around the world. For example, New Zealand has an operational detection program as does Norway, but the United States is just recently implementing monitoring and detection programs at the federal level. In the U.S., the states have responsibility for protecting the public health but generally not the resources to fully execute that responsibility. It may

be possible to link the HAB public health concerns to protection from bioterrorism and incorporate some of the technological developments available to the military. The incorporation of issues of freshwater HABs could open more avenues of support for technique development. There are sectors of the technology community that are not aware of the needs for HAB detection. In the U.S., it may be possible to increase the involvement and activity of technology developers through the Small-Business Innovative Research (SBIR) programs.

Two upcoming HAB detection workshops were announced. These include the Real-time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Blooms Workshop (HABWATCH), Villefranche, France, June 2003, and HABTECH2003, Nelson, New Zealand, November 2003.

## Acknowledgements

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# Harmful Dinoflagellate Species in Space and Time and the Value of Morphospecies

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**Abstract**

Harmful dinoflagellates are the main HAB organisms briefly discussed, taxonomically, biogeographically and palaeologically. It is argued that morphospecies are still the most useful formal taxonomic units for HAB studies, accepting that considerable genetic variation can be expected within them. Depending on interest, formal intraspecific categories can be recognized, as well as multiple mating groups, ecotypes, variants in toxicity, luminescence, etc. The global distributions of such morphospecies are remarkably wide and bihemispheric, but predictably limited by proximity to land (neriticism) and temperature boundaries, with currents having major dispersal influence. A few examples of true endemism are given. Regional ribotypes can be recognized which may be useful for historic distributional reconstructions. The need for appreciating the huge time-scales over which the group and its species have existed is stressed. The dinoflagellate lineage has existed for more than 800 million years and most of its thecate groups apparently underwent explosive radiation in the Mesozoic. On a shorter time-scale, annual varving, in some anoxic basins dating back for 10,000 years, may provide more fine scale information.

## Introduction

Mephistopheles to the student:

*"Was diese Wissenschaft betrifft*
*Es ist so schwer, den falschen Weg zu meiden,*
*Es liegt in ihr so viel verborgnes Gift…"*

—Goethe, "Faust" Part 1 (1808)

Roughly translated, the quote above reads: "When it comes to this field of knowledge, it is so difficult to avoid the wrong roads, and so much hidden poison lies around..." Although this seems apt advice for a student of devilish red tides, Mephistopheles, given his character, was referring to theology.

The Xth HAB conference comes on the 40th anniversary of my first publications in this field. At that time, a handful of Harmful Algal Bloom (HAB) papers appeared per year. The growth of interest and knowledge since then has been phenomenal in the past decade or so. Gradually, the oft-made assertion that there are probably no coastlines in the world without some form of HAB (Taylor, 1989, 2001) is being validated. Even countries with very short coastlines, like Brunei or Singapore, cannot escape their impact. Monaco may seem to be a HAB-free haven, but with *Alexandrium taylori* making a nuisance of itself at many Mediterranean beaches, can Monaco escape?

It was a massive marine fauna mortality (fish, crabs, limpets, sea cucumbers etc.) near Cape Town that first involved me in HAB studies (Grindley and Taylor, 1962) and dinoflagellate taxonomy (Taylor, 1962). I had no sooner arrived in British Columbia than there were illnesses and a fatality due to Paralytic Shellfish Poisoning (PSP) caused by a bloom of *Alexandrium acatenella* (Prakash and Taylor, 1966), a problematic species that seemed halfway between *A. catenella* and *A. tamarense.* This coast is chronically toxic, with high saxitoxin levels co-occurring every year, and interannual variability on a five- to seven-year cycle (Gaines and Taylor, 1985; Taylor and Harrison, 2002). This periodicity is similar to PSP evident in the western Pacific due to *Pyrodinium bahamense* (Azanza and Taylor, 2001). The 1965 Canadian incident was the first time in PSP history that a human fatality, the toxic shellfish and the culprit organism were all present simultaneously. Shortly thereafter, a bloom of *A. catenella* was found to be infected by the then-obscure dinoflagellate parasite, *Amoebophrya ceratii,* first raising the possibility of biological control (Taylor, 1968). Subsequently, multiyear blooms of the raphidophyte *Heterosigma* that killed farmed salmon led to recognition of predictive environmental indices for the Strait of Georgia (Taylor and Haigh, 1992). A sabbatical in Phuket, Thailand, provided detailed knowledge of tropical, phycophilic ("seaweed-loving," Taylor, 1987b) dinoflagellates which were later shown to be associated with ciguatera in Hawaii (Taylor, 1979b), the Marshall Islands and the eastern Caribbean (Taylor, 1985). As elsewhere, new sources of harm are still being discovered in British Columbia waters (Taylor and Harrison, 2002), even though human impact, other than aquaculture, is minor (Taylor and Horner, 1994). The goal of this paper is to provide a personal perspective on some current HAB issues, focusing in particular on harmful dinoflagellate species in terms of their taxonomy, global distributions and time.

## Dinoflagellate HAB Species

Because of their harmful potential, HAB species have received a great deal more sophisticated and detailed attention to cell biology than most phytoplankton species, and dinoflagellates are the group most heavily involved. Sound taxonomy in all its dimensions is obviously critical to all aspects of their study. This requires the full application of all the tools currently available to characterize the units of biological study, including a knowledge of both the classical morphological basis on which the taxonomy of present species rests and the genetic basis of the phenotype. The range of expertise that is needed is now so wide, from classical to molecular, that a single scientist cannot be expert in all these aspects. The formation of "taxonomic teams," such as those currently working on the *Pfiesteria* problem

or redescription of former gymnodinioids, may be the answer, but it is the classical end of the spectrum that is weakening. The early literature needs to be interpreted in a modern context, not ignored.

The expected degree of conservatism and variability in the characters under study is essential to the interpretation of the data. Because most HAB species have been studied over many years, often in dense blooms or cultures, the morphology of literally thousands of individuals of each species has been observed closely with light and/or electron microscopy. As a result, there is very thorough knowledge of the expected conservativeness of size, shape and surface structure in various life cycle stages of populations of individuals from many localities. The taxonomic value of dinoflagellate thecal and cyst morphology has been described by many, such as Taylor (1979a, 1979b, 1985,1987a), Balech (1980), Evitt (1985), Fensome *et al.* (1993) and Steidinger and Tangen (1997). We know that tabulation is a highly reliable indication of identity and relationships, generally confirmed by molecular sequencing at higher and species levels, with the exception of several "species complexes" (see below). Gonyaulacoids group coherently in molecular trees, *e.g.,* Saldarriaga *et al.* (2001), although some anomalies are found in SSU trees, such as the morphologically improbable disjunct biphyly of prorocentroids. With the use of a plate-homology model, Taylor (1979a) first pointed out that most saxitoxin-producing dinoflagellates have a closely similar tabulation, being classified later as goniodominean gonyaualcoids (Fensome *et al.,* 1993), these grouping in molecular trees as predicted, e.g.; Montresor *et al.,* (2004). Hypothecal patterns are less variable than epithecal, but are not totally invariant in cultures, *e.g.,* in *Alexandrium tamarense* (Taylor, 1975). Sulcal plates are the most conservative of all (Balech, 1980) but difficult to observe. The most variable area in peridinioids is the left latero-dorsal area, especially the anterior intercalary plates (Fensome *et al.,* 1993). The tabulational distinction of *Pfiesteria piscicida* from *P. shumwayae* rests on a very small anterior intercalary shape difference (triangular versus quadrangular, respectively) and an extra precingular plate. It is apparently supported by toxicity and genetic distinctions.

A few definitions and explanations might be helpful for non-specialists. Morphospecies are morphologically defined species, *i.e.,* the usually described species within a genus, named using a Latin binomial. Morphostasis is the surprising conservativism of form in some taxa, particularly protists, over long periods of geological time. At present there are roughly 1.7 million named species (Tudge, 2000) and this is considered by most biodiversity specialists to be a great underestimate of the species currently existent. Sournia (1995) provided a figure of 5,000 named living marine phytoplankton species, to which should be added several thousand more benthic species (especially diatoms) and, in the case of HABs, some microzooplankton species. In dinoflagellates there are more than 2,000 named extant species with over 2500 fossil species (Taylor, 1987b; Fensome *et al.,* 1993). Known HAB species comprise less than 75 of these (Hallegraeff, 1995). The current Intergovernmental Oceanographic Commission (IOC) list (Moestrup *et al.,* 2002) should be consulted for the most recent catalogue of actual or potential harmful species.

It should be noted that there are multiple species concepts (see Taylor, 1992a; Medlin *et al.,* 1995; Gallagher, 1998 for a discussion of those applicable to HAB species) and even debate as to whether a single concept can be applied to all organisms (Wilson, 1999). The best known, the Biological Species Concept (BSC), in which sexual isolation is a key criterion, was developed from the study of animals and plants. It is inapplicable to prokaryotes and inadequate for recognizing protist taxa. In protists it seems likely that each morphospecies contains multiple (probably many) discrete mating groups with variable degrees of compatibility and reproductive success. The BSC was strictly applied to two widespread ciliate morphospecies of the genera *Paramecium* and *Tetrahymena* (reviewed by Nanney, 1999), resulting not only in the creation of multiple new Latin binomials for cryptic "sibling species" (sexually compatible strains) in each that are virtually indistinguishable morphologically (although molecular techniques can also be used), but also the scrapping of the original species names. A similar picture emerged when populations of the dinoflagellate *Crypthecodinium cohnii* from around the world were studied in terms of mating compatibility and allozyme characterization by Beam and Himes (1987). They recognized more than 20 types that could be considered as "sibling species." It is probable that these species only differ from others in the degree and manner in which they have been studied. If applied rigidly to HAB species, a taxonomic nightmare would result. Each would end up subdivided into numerous, morphologically indistinguishable "species," each with its own Latin name. Furthermore, mating compatibilities and survival success between isolates within some well-studied HAB dinoflagellates are much more complex than "yes" or "no," *e.g.,* Destombe and Cembella (1990). The morphospecies is also the only unit that allows for comparisons with the rich fossil/sedimentary record. As a result of these and other considerations, I have argued in favor of retaining the morphospecies as the most useful named unit in HAB studies (Taylor, 1992a,b) as long as it is applied in the *expectation* of genetic diversity.

Infraspecific taxa can be formally recognized. Zoologists use the category *subspecies* formally, but this usually requires evidence of geographic isolation. Botanists have long used variety (*var.*) and form (*f.*). In dinoflagellates the former has been used for small but constant differences in morphology, the latter for variations considered to be environmental or life-cycle regulated (see Taylor, 1976; 1987a). Zoologically trained taxonomists, *e.g.,* Kofoid, Abé, and Balech, use a new Latin species name for even the smallest constant morphological variant, such as the presence of a pore or pore type. Any overlap will negate species recognition. Botanists (Hustedt, Schiller etc.) have more options, even if variably defined. Since specialists of both types work on dinofla-

gellates, inconsistencies can arise from this simple but subtle bias. Steidinger *et al.* (1980) made the Indo-Pacific taxon *compressum* of *Pyrodinium bahamense,* formerly a distinct species, a variety, because although nearly all the cells of populations of the variety in S.E. Asia were readily distinguished from the Atlantic populations by chain formation and accompanying morphological differences (more flattened cells, suppression of spines within chains), plus a difference in pore and cell surface appearance, a few single cells that were ambiguous could be found. Balech did not agree with their separation but, as a user of zoological conventions, could not formally use infraspecific taxa other than subspecies. In addition to these formal taxa there are various informal categories, such as "strain," which are essential in experimental studies. The distinction between the *Pyrodinium* varieties is being further studied morphologically, genetically and toxicologically (Steidinger, pers. comm.).

At the other end of the species spectrum is the "species complex." This is a cluster of closely similar, but usually distinguishable morphospecies, which cluster very closely or even intermesh using other characters. This has been in use in dinoflagellate taxonomy since the1980s. The classic example is the "*tamarense* species complex" in the genus *Alexandrium* (formerly *Protogonyaulax;* Cembella and Taylor, 1985; Cembella *et al.,* 1987). This has included the named species *A. tamarense, A. catenella, A. acatenella* and *A. fundyense* and, of course, their synonyms, *e.g., A. excavata.* Taylor (1984) noted that in the same culture medium, when growing slowly, the first two could become so similar that only the presence or absence of the tiny ventral pore on the former could be used to distinguish them, and yet in the field they had distinct distributions. The chain-former predominates along the outer coast of British Columbia, Washington State and into Puget Sound, whereas the *tamarense* morphotype prevails in the estuarine waters of the Strait of Georgia (its type locality is the Tamar estuary, Plymouth, U.K.). *A. fundyense* occurs on the North American east coast where its distinction from *A. tamarense* rests solely on the same ventral pore character (Balech, 1995). It has sometimes been considered synonymous with *A. tamarense* (Taylor and Fukuyo, 1998), but it could equally well be considered a non-chain-forming form *A. catenella.* Allozyme analysis not only revealed more than four types, but cladistically they clustered in interleaving, non-exclusive groups. Sako *et al.* (1989) obtained similar results with Japanese populations and found that *A. catenella* strains were sexually compatible with Japanese *A. tamarense.* Anderson *et al.* (1994) found the same with *A. fundyense* and *A. tamarense.* The BSC would presumably "sink/lump" them into one species, but information may be lost as a consequence.

Molecular sequence analysis, particularly of nuclear ribosomal RNA genes (small subunit (SSU), large subunit (LSU) and the associated internal transcribed spacer (ITS) regions), provided the opportunity to make quantitative genome comparisons on a finer scale, using the sequence whose conservativeness or variability was appropriate to examine the taxon level in question. These, in turn, provided the opportunity to construct gene probes as taxonomic tools for optical discrimination based on ribotype features, *e.g.,* Scholin *et al.* (1994). Indeed, it was tempting to apply a quantitative molecular test to the recognition of taxonomic rank, such as species (Medlin *et al.,* 1995). Lacking much else to go on, microbiologists have used such measures to estimate "species" richness in natural samples. Unfortunately, while it is empirically true that morphospecies often do differ by roughly similar sequence divergences, there is no sound theoretical basis for doing so. Not only does it seem that "young" (recently diverging) and "old" (earlier diverging) species in the same genus are theoretically possible, but molecular clock rate variability, *i.e.,* rapidly changing sequences in some closely related taxa, are well known (Ayala, 2000). Examples of extraordinary morphostasis are a common feature in the dinoflagellate fossil record; see, *e.g.,* Goodman (1987).

How fine a level should one discriminate to? Like everything else in science, the simple answer is to discriminate to the level required to answer the question you are asking. When in doubt, go deeper, *i.e.,* be a "splitter" in taxonomy, not a "lumper." The reason for this is that data from oversplit categories can always be combined, but insufficiently distinguished categories may not be possible to separate later. In HAB studies, genus level identification is usually relatively useless.

## HAB Dinoflagellate Biogeography

The biogeography of marine protists (unicellular eukaryotes, including microalgae and microheterotrophs) is critical to debates and claims about human intervention in the distribution of harmful species, such as spread through ballast water or live shellfish. Also, it is important to know what might be expected in unexplored, or inadequately explored coasts around the world. There currently appear to be two extreme views regarding such distributions. The first is implicit in claims that new records of well-known HAB species, in localities from which they have not been previously recorded, are largely due to human introductions rather than discovery resulting from increased regional study, *i.e.,* that endemism is common. The other asserts that, due to their small size, protists are so easily dispersed that they can be found almost anywhere in soils, freshwater, or the marine environment: a global cosmopolitanism in which there are essentially no recognizable biogeographies (Finlay, 2002).

When I began learning microplankton taxonomy in Cape Town in the early 1960s, I was able to identify more than 99% of the species of diatoms, dinoflagellates, tintinnids, etc., using European monographs, such as Rabenhorst's Kryptogamenflora, Cupp's Diatoms of the West Coast of North America and other northern hemisphere texts. The few unidentifiable taxa were usually new to science. As a consequence, the fact that the whole temperate microplankton community is virtually identical in the northern and south-

ern hemispheres was quite evident. It came as a surprise to learn that this was not known to many northern hemisphere scientists. Equally so, taxonomic publications dealing with the tropical Atlantic or Pacific work equally well for the Indian Ocean in which I was working (Taylor, 1976). Apparent cases of endemism (restriction to only one geographic region) were rare, disregarding species that had only been seen once or twice. Southeast Asian waters seem to support some endemic dinoflagellates, such as *Ceratium dens, Dinophysis miles* var. *schroeteri* (Taylor, 1987b) and the "green *Noctiluca*" caused by symbiosis with the intravacuolar microflagellate *Pedinomonas noctilucae.* This is the only type of *N. scintillans* found in Indo-Pacific waters from the Bay of Bengal/Andaman Sea to the South China Sea and extending south of New Guinea. Its precise boundaries are still unclear.

Most interestingly, the presumed non-toxic *Pyrodinium bahamense* var. *bahamense* is still restricted to the tropical Atlantic, with the toxic, chain-forming var. *compressum* just across the Isthmus of Panama. The Panama landbridge lifted only 3 to 13 Ma (mega-anna = million years ago) (Haug and Tiedemann, 1998). Given its narrowness, it is surprising that eastward aerial distribution has not contaminated this separation (the canal has a freshwater barrier, Gatun Lake, within it). The accidentally produced Salton Sea in California, initially freshwater, does have marine species in its now hypersaline waters, and it is twice as far from the Pacific as the Isthmus of Panama is at its narrowest distance, but some marine species were deliberately or accidentally introduced by humans. It now has blooms of the fish-killing raphidophyte *Chattonella marina* (Tiffany *et al.,* 2001).

A general rule in protists seems to be, perhaps unsurprisingly, that those living in the harshest or most disjunct environments are the most widely cosmopolitan, with soil, intertidal and deep sea communities, including vents, most cosmopolitan, either due to greater tolerance or better survival during dispersal.

## Time

A clear understanding of the magnitude of the time element is also essential to obtaining a better perspective when considering the events in question. From a personal viewpoint, it seems that many of the recently published postulated changes in HAB phenomena (introductions etc.) have been viewed on time scales that are too short. The presence of some types of robust cysts in sediments of less than 100 years can be used to support or refute very recent introductions, such as *Pyrodinium* in Manila Bay, but more delicate types, such as those of *Alexandrium,* usually do not preserve. However, in anoxic varved sediments, such as those in Saanich Inlet in British Columbia, it has been claimed that there is documentation of the latter genus for at least seven thousand years (Mudie *et al.,* 2002). It should be noted, however, that dissolution of calcitic elements in calcareous cysts, such as those of *Scrippsiella,* can leave an inner layer which resembles the cyst of *Alexandrium* (J. Lewis, pers. comm.). It should also be realized that much of the radiation of gonyaulacoid and peridinioid dinoflagellates apparently took place much earlier, in the Mesozoic, particularly before 100 Ma in the Jurassic to mid-Cretaceous (Fensome *et al.,* 1996). It is in the Cretaceous that the goniodominean tabulation typical of *Pyrodinium* and *Alexandrium* (essentially identical to each other) first appears, although the cyst ornamentation is different.

Dinoflagellates are one of three main groups of the Alveolata. Judging from SSU rDNA molecular clock estimates, admittedly contentious but carefully corrected, the dinoflagellate lineage diverged from their closest sister group, the apicomplexans (malaria parasites and their relatives), more than 900 Ma, and from the next closest group, the ciliates, more than 1300 Ma (Escalante and Ayala, 1996, using an average rate of $0.85 \times 10^{-8}$ substitutions per site per year). On its own, this evidence might produce strongly raised eyebrows, but there is strong paleo-geochemical evidence (dinosterane) supporting a dinoflagellate presence in the Proterozoic and some Paleozoic spiny cysts known as acanthomorph acritarchs are probably dinoflagellates (Fensome *et al.,* 1999 and references therein). It should also be remembered that metazoans evolved roughly during this same period in the late Proterozoic. A time-reasonable hypothesis of the paleodistribution of *Alexandrium,* combining fossil with molecularly-based dates, has been proposed by Montresor *et al.,* (2004). It supports a tropical origin of the genus, with the *tamarense* complex being later, not earlier, than 45 Ma.

The purpose of the above is to emphasize the huge periods of time available for dinoflagellates to achieve the general latitudinally cosmopolitan, bihemispherical distributions seen today (Taylor, 1987b) and also for exposed animals to adapt to the toxic species in the regions where they occur.

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# AWARDS

# XHAB2002/ISSHA Yasumoto Lifetime Achievement Award 2002

# GRETHE RYTTER HASLE

Øjvind Moestrup presenting Grethe Hasle a wood carving of *Pseudo-nitzschia multiseries.*

Grethe Hasle was unable to attend the awards ceremony at the Xth International Conference on Harmful Algae, St. Pete Beach, Florida, 25 October 2002. Nevertheless, during the awards ceremony Gustaaf Hallegraeff presented a talk celebrating her achievements. Then on 9 September 2003, a special celebration took place for her at the University of Oslo, Norway. About 60 people took part in the celebrations. Apart from those from the University of Oslo, there were also attendees from Denmark, Sweden and other institutions in Norway. Professor Øjvind Moestrup presented Professor Hasle with a unique wood carving of *Pseudo-nitzschia multiseries* frustule, carved by Haruyoshi Takayama, on behalf of ISSHA.

Professor Grethe Rytter Hasle comes from a long tradition of leaders in Marine Botany at the University of Oslo, Norway, including famous names such as Gran, Braarud and Gaarder. She was born in the small village of Horten, at the mouth of the Oslofjord, where she grew up with a father who was a sea captain; her late husband was a navy officer. Her academic teacher, Prof. Trygve Braarud, further promoted her interest in marine and freshwater biology. Grethe Hasle started work at the University of Oslo as a Research Assistant, and her first published work dates from 1950, with studies on the vertical migration of phototactic dinoflagellates and the reliability of single observations in phytoplankton surveys; her subsequent work was on the taxonomy of coccolithophorids and ciliates. In 1961, she was appointed as a Lecturer in Marine Botany, and in 1969 she was awarded her PhD for a dissertation entitled "An analysis of phytoplankton of the Southern Pacific Ocean: abundance, composition and distribution during the 'Brategg' Expedition."

Stimulated by a study visit to "diatom guru" Dr. Friedrich Hustedt in Bremen, her true calling in life turned to diatom taxonomy, ranging from pennate (*Nitzschia, Fragilariopsis*) to centric (*Thalassiosira, Cymatosira*) genera, and eventually covering samples from every corner of the globe. As a pioneer in the use of combined light, scanning and transmission electron microscopy, she became an internationally renowned expert on diatom microarchitecture, describing what was finally named strutted and labiate processes in the diatom family Thalassiosiraceae. Grethe Hasle was nominated as a Full Professor in Marine Botany at the University of Oslo in 1977. The lasting quality of her work is well illustrated by her 1965 publication in "Skrifter utgitt av Det Norske Videnskaps-Akademi," on the group *Pseudo-nitzschia* within the diatom genus *Nitzschia.* She paid careful attention to details of the number of striae and fibulae, rows of poroids on the striae membrane, the presence/absence of a central interspace, as well as to diatom type material. This ground-breaking work, which was well ahead of its time, includes a description of *Nitzschia pungens* forma *multiseries* (now *Pseudo-nitzschia multiseries;* type locality Drøbak; as distinct from *P. pungens*), which in 1987–88 was found to be responsible for the production of domoic acid, causing Amnesic Shellfish Poisoning in Prince Edward Island, Canada. Ever since, she has been painstakingly assisting workers from all over the world, coming to grips with the bedazzlingly difficult taxonomy of this domoic-acid-producing genus, culminating in her 2002 review in "Harmful Algae" on the global distribution of toxigenic *Pseudo-nitzschia* species. A dedicated website on *Pseudo-nitzschia* is being maintained (an honor not bestowed on any other diatom!).

Responding to a recommendation of the SCOR Working Group 33 on Phytoplankton Methods (which also led to the 1978 Sournia Phytoplankton Manual), starting in 1976, Prof. Hasle was instrumental in organizing UNESCO phytoplankton training courses for experienced participants, where she taught the diatom portion. The 1976 course was offered at the field station of the University of Oslo and the next two at Drøbak; later they moved to the Stazione Zoologica Anton Dohrn in Napoli, where she continued her teaching. Her UNESCO diatom lecture notes were published in 1996 as part of an authoritative 300-page chapter (co-authored with Erik Syvertsen) in "Identifying Marine Diatoms and Dinoflagellates"; an updated chapter on the taxonomy of harmful diatoms (co-authored with Greta Fryxell) will appear in the 2003 "Manual on Harmful Marine Microalgae."

Prof. Hasle is a hard-working, worthy recipient of the Yasumoto Lifetime Achievement Award, who admirably has continued her prolific publication output well after retirement. The HAB scientific community gives her our most heart-felt congratulations!

—Gustaaf Hallegraeff

# XHAB2002/ISSHA Yasumoto Lifetime Achievement Award 2002

# THEODORE J. SMAYDA

Takeshi Yasumoto presenting Ted Smayda a wood carving of *Heterosigma,* carved by Haruyoshi Takayama.

Theodore John Smayda is the co-recipient of the XHAB 2002/ISSHA Yasumoto Lifetime Achievement Award. As a major contributor to the ecology of harmful marine phytoplankton, he has stimulated students, researchers and fellow faculty alike. His recent interpretation of the harmful algal bloom paradigm serves as a focus for delving into basic ecological principles governing blooms in the sea.

As a young graduate student at URI's Narragansett Marine Laboratory, Ted discovered that marine phytoplankton was his passion. With the completion of his Masters Degree, for which he received the Phi Sigma Award, Ted was admitted to a graduate program at Yale University to work under Gordon Riley. Ted also received a Fulbright Fellowship for a year-long stay at the University of Oslo, Norway. In Professor Trygve Braarud's laboratory, he absorbed all he could from the world's leading authority on phytoplankton ecology. This one-year stay extended into four, culminating in his becoming a candidate for the Dr. of Philosophy degree. He received Woods Hole Oceanographic Associates and Crown Princess Martha (Scandinavian-American Foundation) Fellowships to continue work on his dissertation studies dealing with the Phytoplankton of the Gulf of Panama. Professor Braarud, along with his colleagues and students, made a dynamic team that molded Ted into the unique scientist that he is today.

Ted returned from Oslo to URI's newly formed Graduate School of Oceanography, where he today is an active research faculty member. His research themes at GSO include seminal works on phytoplankton suspension, species succession in marine environments, and population dynamics related to diatom and harmful algal blooms. Armed with the skills of knowing the marine species, an enviable knowledge of the international literature, and a constantly inquisitive mind, Ted continues to delve into driving forces regulating phytoplankton blooms. His early emphasis on the importance of life cycles, nutrients, and eutrophication in driving the bloom phenomena on a global basis were quickly adopted by others and presented or reiterated in their publications. In this regard, he has been a trend-setter of ideas that stimulated many others to explore further.

Among Ted's talents are his uncommon abilities to synthesize disparate observations, ideas, and concepts into coherent insights. With the benefit of a classical education, a strong background in Latin, native fluency in Russian, studies of German and Norwegian, and familiarity with Latin languages, there are few barriers to his access to the world's scientific literature. Ted studied with tutors prior to spending three months in Japan, where he plied phytoplankton literature normally not available to others.

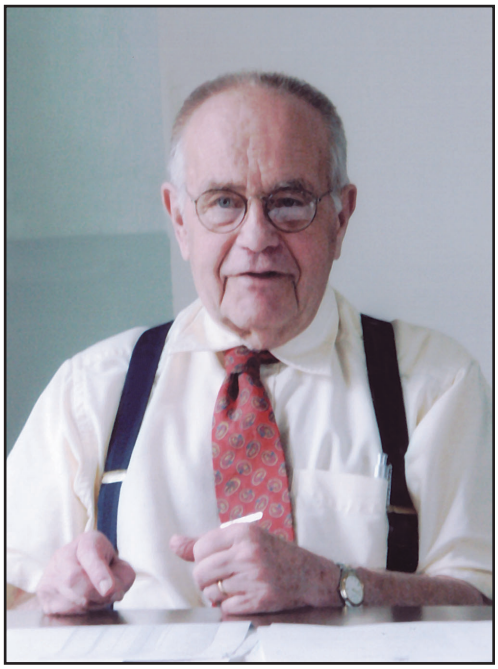
Among the many honors Ted received were his election into the Norwegian Academy of Science and Letters, the Phycological Society Award of Excellence, and faculty vote to receive URI's Scholarly Achievement Award. As an international scientist and educator, he has participated in numerous courses abroad, including the Advanced Phytoplankton Ecology course (Finland), the IOC International HAB Course in Copenhagen, and Ecology of Harmful Algal Blooms Course at the Bigelow Laboratory for Ocean Sciences. In 1991, Ted co-organized the Vth International Conference on Toxic Marine Phytoplankton and co-edited the symposium volume "Toxic Phytoplankton Blooms in the Sea." To date, Ted has taught graduate courses and seminars, served on numerous student and faculty committees, mentored 32 MS and PhD students, and published more than 130 publications in refereed journals and several book chapters. He remains very active with graduate students and continues to be a prolific author. He currently works on grant-funded research synthesizing the long-term (38 years) weekly ecological observations on the phytoplankton of Narragansett Bay. With his backlog of manuscripts in preparation, we can expect to hear his words for many years to come.

As a multifaceted individual, Ted's love for science and language expands into a keen interest in literature, a passion for poetry and art, and Japanese woodblock prints. He enjoys the company of colleagues and new acquaintances alike and makes every effort to have new visitors feel welcome. Given all of this, Ted Smayda is truly deserving of the Lifetime Achievement Award. It is fitting that Ted and the co-recipient, Grethe Hasle, are both scions of the world-famous "Oslo Phytoplankton School" and its master, Trygve Braarud. Both actively contribute to understanding Harmful Algal Blooms, giving us new insights with every work.

—Carmelo R. Tomas

# XHAB2002 Award for Service to Harmful Algal Bloom Science

## JOHN W. HURST, JR.



One cannot think about shellfish safety or red tide anywhere in the world without hearing the name John Hurst. Born in Bozeman, Montana, John received a degree in Biology (Botany) from Montana State College in 1949 and traveled east where he began employment at what was then the State of Maine Department of Sea and Shore Fisheries on January 15, 1951. John immediately became involved with shellfish work at what was in those days known as "the hatchery" working on problems related to quahogs, soft-shell clams, depuration, and even lobster disease and mortality.

When shellfish poisoning due to *Gonyaulax tamarensis* (now *Alexandrium tamarense*) made its debut in New Brunswick in the late 1950s, tests in waters Downeast in Maine also showed the presence of PSP. John was tapped to begin a monitoring program for PSP in Maine waters. In 1958, he began the State of Maine's PSP monitoring program. The program being used in Maine today is a testament to his years of experience and knowledge of this highly unpredictable public health issue. It is often cited by the US Food and Drug Administration as one of the best PSP monitoring programs in the world. The extensive shellfish toxin monitoring program in use today serves as the gold standard for other developing programs. The monitoring program is one of the most extensive and comprehensive in the world, and assays are run on some 4000 shellfish samples annually—that's over a half million mice in 50 years! John is especially proud of the fact that there have been no cases of PSP in the State of Maine as a result of commercially harvested shellfish during his tenure.

John has served twice as president of the Northeast Shellfish Sanitation Association and has received many awards, most notably the US Food and Drug Administration Commissioner's Special Citation, Maine State Scientist of the Year, and citations from the Canadian Food Inspection Agency, from the Maine legislature, and most recently from the Governor of Maine. On January 18, 2002, John recorded 50 years of employment with the State of Maine. I had the great fortune to work with John Hurst for over 10 years, and it was he who introduced me to the wonders of toxic dinoflagellates. We had many lively and stimulating conversations about the possible impacts of PSP toxins on shellfish, and my new line of work was in place. I still consult him regularly. John is a walking gold mine of information, and his phone rings constantly with calls from scientists, fishermen, public health officials from other regions (both state and federal), and concerned citizens. He treats them all the same. While he may at times appear gruff, he has a deep and unshakable concern for both public safety and the shellfish industry and a genuine desire to help people. He is highly respected by scientists and serves as a walking encyclopedia for researchers. I have seen John work tirelessly, sometimes for up to 18 hours a day for 10 days straight in the summer, not only to ensure public health safety, but also to help the shellfish diggers maintain their livelihood by recommending closures of shellfish harvest areas. John continues to pursue knowledge of new toxins as well as the old stand-by (PSP) and stays in close contact with his network of scientists and friends in neighboring states and foreign countries.

In 1953, John married Nancy Snowman and has four children and grandchildren. Recently dubbed "the grandfather of red tide" by a local newspaper, John received the David Wallace Award for outstanding contributions to industry, one of the National Shellfisheries Association's two highest honors.

John has been a constant source of data and experience and has shared his knowledge freely with scientists and managers alike from all corners of the globe for over five decades. The shellfish and HAB communities owe much to John Hurst.

—Sandra E. Shumway

# XHAB2002/ISSHA Maureen Keller Best Student Presentation Awards

The quality of student presentations, both oral and poster, was exceptional. The selection committee, chaired by Drs. Cynthia Heil and Sandra Vargo, chose two recipients this year. One was from the United States, the other from Germany. Both were truly deserving and were awarded wood carvings made by Professor Takayama of Japan.

Dr. Heil presenting the carvings to Deeds and Dahlmann.

**JONATHAN DEEDS**
Marine, Estuarine and Environmental Sciences Graduate Program (MEES), University of Maryland, College Park, Maryland, and Center of Marine Biotechnology (COMB), Baltimore Maryland, USA.

### ABSTRACT Toxins from *Karlodinium micrum*—A Cosmopolitan, Ichthyotoxic Dinoflagellate

Jonathan R. Deeds, Jeffrey L.C. Wright, Allen R. Place

*Karlodinium micrum* (formerly *Gyrodinium*/*Gymnodinium galatheanum*) is a 10–15 µm, non-thecate, mixotrophic dinoflagellate that has been observed at a range of salinity and temperatures, often dominating the nano-plankton assemblage. *K. micrum* is distributed worldwide, but in the USA has often been confused with similarly sized gymnodinioids such as *Gyrodinium estuariale* and *Pfiesteria* sp. In recent years, high *K. micrum* densities have increasingly been associated with fish mortalities, especially in estuarine aquaculture facilities, but to date no toxic substances had yet been identified. We have recently isolated polar lipid-like compounds with hemolytic, cytotoxic, and ichthyotoxic properties from several *K. micrum* isolates, which may help to explain the adverse effects observed associated with high numbers of this organism. Thus far, we have been able to isolate these compounds from clonal cultures from Maryland and South Carolina, as well as from water samples collected during a South Carolina non-aquaculture related fish kill in which high *K. micrum* densities were present (68,000 cells/mL). Preliminary spectroscopic data for the major toxin from our Chesapeake Bay isolate (CCMP 1974) indicates that it is a large complex molecule possessing many structural features typical of certain dinoflagellate metabolites. Progress on the chemical as well as the toxicological characterization of these compounds will be discussed.

**JENS DAHLMANN**
University of Jena, Faculty of Biology and Pharmacy, Department of Food Chemistry, Jena, Germany

### ABSTRACT An LC-MS-Based Method for Simultaneous Determination of HAB Toxins Coupled with Structure Elucidation of Microcystins

Jens Dahlmann, Bernd Luckas

An LC-MS method was established for simultaneous determination of various algal and cyanobacterial toxins. This so-called "multitox method" enables the quantification of saxitoxin, nodularin, microcystins, domoic acid, anatoxin-A, okadaic acid, and DTX-1 within one 30 min-chromatographic run using a simple gradient elution with acetonitrile and 0.01 M trifluoroacetic acid containing 0.01% heptafluorobutyric acid. This efficient, robust, and reproducible LC-MS method is suitable for use on board of a research vessel. The method allows rapid phytoplankton analyses without exhaustive sample preparation. In addition, the applied chromatographic conditions enabled isolation and identification of unknown substances suspected to be "new" microcystins (cyclic peptides). A split system allowed a 80% fraction of the eluate to be collected by an automated fraction collector. The fractions were subjected to microwave assisted hydrolysis followed by derivatisation of the resulting amino acids with the "advanced" chiral Marfey's reagent Nalpha-(2,4-dinitro-5-fluorophenyl)-L-valinamide (L-FDVA). Derivatisation with L-FDVA converts the amino acids from the microcystins into UV active diastereomers. This allows separation of D- and L-amino acids as diastereomers on a nonchiral C-18 column. In addition, L-FDVA derivatives of amino acids can be detected by both simple UV and by more selective mass spectrometric devices.

# INDICES

# Author Index

Senior authors' names are **bold**.

# Subject Index

The Subject Index is alphabetical and cross-referenced where possible with an emphasis on HAB species for categorizing and subcategorizing. Page references are to the first page of a paper and may not be the exact page for that particular subject.