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Symposium Abstracts

ABSTRACTS OF ORAL PRESENTATIONS

HAB IMPACTS ALONG THE WASHINGTON COAST – STAKEHOLDER OUTREACH

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The two most important shellfish harvest activities that occur along the Pacific Coast of Washington State are the recreational Pacific razor clam (*Siliqua patula*) and commercial Dungeness crab (*Cancer magister*) fisheries. However, stakeholders in these two major fisheries have found their activities disrupted several times over the last decade by sudden increases in domoic acid levels in these shellfish, as a result of harmful algal blooms (HABs). Convincing stakeholders that HAB events truly pose a threat to human health has been a major challenge for the state fishery and health managers.

Stakeholders include the thousands of recreational fishers who sometimes travel long distances to participate in the extremely popular razor clam fishery; the hundreds of business owners who greatly benefit from the money spent by clam diggers that stay overnight or pass through Washington's small coastal communities; and the many tribal fishers who harvest razor clams for both commercial and subsistence purposes. They also include the 200 licensed Dungeness crab fishermen whose livelihood depends on this highly valued commercial product, and the owners of the crab processing and distributing facilities and their hundreds of employees.

Realizing that having accurate information allows these stakeholders to make correct decisions, WDFW has taken steps to provide information in a variety of forms, including: (1) the agency website to post general domoic acid levels in shellfish in both tabular and graphical formats; (2) annual public meetings held in various communities around the state each fall; and (3) a series of news and press releases. Also WDFW has facilitated domoic acid discussions between commercial Dungeness crab fishers, processors, state fishery managers and human health managers during the annual Tri-State Dungeness Crab Committee process with representatives from Washington, Oregon and California.

Since the summer of 2000, Washington State has been the recipient of a grant from NOAA Centers for Coastal Ocean Science Monitoring and Event Response for Harmful Algal Blooms (MERHAB) Program. This funding has allowed WDFW shellfish managers to set up a plankton-monitoring program to augment standard clam testing. A federally funded state-employed technician regularly collects and analyzes plankton samples from waters adjacent to productive razor clam beaches and Dungeness crab grounds. The data received from this monitoring program has given managers notice of pending HAB problems allowing WDFW to provide all affected stakeholders time to adjust their activities and avoid the serious disruptions that have occurred in past years. Washington State's MERHAB grant has also allowed WDFW to be a part of the larger collaborative effort of several state, tribal, federal and private partners under the umbrella of the Olympic Region Harmful Algal Bloom (ORHAB), a project that brings together the expertise of multiple partners to help look at additional ways of monitoring for HAB events.

The ORHAB project has been able to provide even more detailed information on the HAB events that can result in fishery closures. ORHAB has used a variety of outreach tools to disseminate this information ranging from maintaining a web site with details about the project, to sending a quarterly newsletter to stakeholders, to nurturing close ties to legislative representatives, both state and federal, who are regularly approached by constituents looking for solutions to HAB-related problems. ORHAB played a key role during the 2003 state legislative session where, though Washington State was facing decreasing tax revenues, lawmakers passed a bill that created a surcharge on all recreational shellfish licenses. The monies generated by this surcharge will help fund a state-supported plankton monitoring program intended to sustain the ORHAB effort once federal funding expires.

EFFECTS OF INHALED FLORIDA RED TIDE BREVETOXINS: AN INTERDISCIPLINARY STUDY IN OCEANS AND HUMAN HEALTH

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Florida red tides occur in the Gulf of Mexico and result from blooms of the marine dinoflagellate *Karenia brevis*. *K. brevis* produces highly potent polyether toxins known as brevetoxins that activate voltage sensitive sodium channels. The brevetoxins are associated with massive fish kills, marine mammal poisoning and human health problems. The severity of human health effects varies annually and temporally in coastal regions. Explanations for the variable toxicity include meteorological, biochemical and strain toxicity differences. Nine natural toxins have been identified and their structures are based on two different polyether backbones, brevetoxin-a (eg. PbTx-1) and brevetoxin-b (eg. PbTx-2). A number of smaller polyether compounds, the first of which is known as brevenal, also have been identified. The present study sought to examine the complex relationship between brevetoxin and brevenal concentration in coastal ocean water as measured by liquid chromatography-coupled mass spectrometry, the concentration and composition of toxin which becomes airborne as measured on particle filters, and the human health effects in occupationally-exposed individuals and recreational beachgoers. Six significant results have been achieved recently: (1) the relative concentrations of brevetoxin and brevenal vary over 10-fold in water and air depending on bloom stage. This is offered as one additional explanation for the low potency in blooms with high toxin concentrations, or for the high potency demonstrated in blooms with lower toxin concentrations. The non-toxic but competitive polyether antagonist brevenal reduces lethality in fishes at nM concentrations, prevents or subdues the bronchoconstrictor activity caused by inhaled brevetoxin at fM concentrations, and prevents the binding of toxin to site 5 on sodium channels at nM-□M concentrations; (2) the intratracheal LD_{50} for brevetoxin PbTx-3 in animals is 10 □g/kg, fully 25-fold more potent than previously demonstrated. The half-maximal bronchoconstriction concentration in air is 1 picogram/liter; (3) the particle size distribution of brevetoxin particles in air (a mix of salt and toxin) is 10 microns, indicating it will deposit primarily in the upper airways. A few nanograms of toxin per cubic meter of air is sufficient to elicit symptoms in people on the beach; (4) therapeutically, the effects of brevetoxin-induced bronchoconstriction can be relieved by histamine H1 antagonist diphenhydramine, or naphthoyl-brevetoxin derivatives; (5) brevetoxin exposure results in altered immune response by inflammatory cells (neutrophils) and also may cause decreased viability of macrophages in exposed individuals. This, when coupled to a decreased tracheal mucous velocity caused by toxin, can lead to increased contact time of toxin with pulmonary tissue as well as increase the likelihood of secondary pulmonary infections; and, (6) in collaboration with the CDC and FL Dept of Health, investigators have begun to evaluate potential biomarkers for assessing exposure. The concentration of toxin in air that causes human respiratory discomfort is orders of magnitude lower than the analytical capability of current measuring devices.

LONG-TERM PATTERNS OF NARRAGANSETT BAY PHYTOPLANKTON DRIVEN BY DECADAL SHIFTS IN PHYTOPLANKTON HABITAT

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A 38-year (1959 to 1996) time series of weekly observations of Narragansett Bay phytoplankton was analyzed to evaluate the interactions of climate, physical, chemical and biological variables on the selection and succession of HAB and benign red tide phytoplankton. The phytoplankton community was diatom dominated from 1959 until 1978 and from 1990 until 1996. During the intervening period of the 1980s (1979 to 1989) diatom abundance declined and flagellate abundance increased such that flagellate abundance rivaled that of diatoms. Abundance of most of the 17 HAB and nuisance species recorded in the time series occurred in the 1980s, implying flagellate-favorable modification of the phytoplanktonic habitat during that period. Peak abundance of HAB and nuisance species including *Dinophysis acuminata, Heterocapsa triquetra*, *Heterocapsa rotundata*, *Prorocentrum minimum*, *Prorocentrum micans* as well as summer blooms of the brown tide pelagophyte *Aureococcus anophagefferens* occurred during the 1980s.

De-trended and de-seasonalized timeseries of phytoplankton habitat variables show several potential mechanisms driving the 1980s flagellate increase. Estimates of flushing time (t) derived from salinity and riverflow observations indicate that the early 1980s were characterized by elevated flushing time, and a relative increase in the tidal component of estuarine circulation. Coincident with the elevated flushing time was an increase in nitrate concentration, which peaked at a level two-fold the long-term mean level in the 1980s. Combined with a long-term decline in phosphorus, the 1980s peak in dinoflagellate abundance coincided with a peak in DIN:DIP ratio.

While flagellate abundance was on the increase in the 1980s, diatom abundance, driven by a ca. 40% post-1980 decline in *Skeletonema costatum* abundance, was declining. Fluctuations in *Skeletonema* abundance and bloom pattern can be partially explained by variation in a large-scale proxy indicator of winter weather patterns, the North Atlantic Oscillation Index (NAOI). In Narragansett Bay, and other temperate estuaries, competition for the summer phytoplankton niche is usually between a diatom (typically *Skeletonema*) and one or more small flagellates (*Prorocentrum minimum, Heterosigma akashiwo*). The 1980s increase in flagellate abundance occurred during a decline in diatom abundance suggesting a release of competitive exclusion for the 'open' summer phytoplankton niche that favored flagellates over diatoms in the 1980s.

THE CYST TRANSCRIPTOME: COMPREHENSIVE ANALYSIS OF GENE EXPRESSION PATTERNS IN *Pfiesteria piscicida* LONG-TERM RESTING CYSTS

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Following a bloom, the toxic dinoflagellate, *Pfiesteria piscicida*, encysts and quickly leaves the water column. The cysts are then poised to respond to conditions that directly motivate excystment into toxic or non-toxic swimming zoospores. The cellular responses to environmental conditions that trigger life stage transitions in *Pfiesteria* are currently unknown. It can be assumed, however, that the load of viable *Pfiesteria* cysts in the sediment at any given time may directly determine the dynamics and success of a future bloom. In addition, repeated minor blooms in a restricted area over many seasons could charge the sediment for a more significant bloom several seasons later. Using molecular techniques, we can establish the organism's distribution in sediments and thus its potential for future blooms. DNA-based techniques, however, are not a reliable indication of cyst viability. Because gene transcripts are more labile than DNA, the presence of specific transcripts may be used as a proxy for cyst viability. Using Serial Analysis of Gene Expression (SAGE), we prepared gene expression libraries of nontoxic zoospore and long-term resting cyst life stages of *P. piscicida*. This technique provides a catalog of gene transcripts that is both comprehensive and quantitative. By comparing the SAGE libraries, we are able to identify transcripts that are specific to each life stage.

Here, we present our investigation of gene expression patterns in long-term resting cysts of *Pfiesteria piscicida*. The SAGE library provides us with an unprecedented opportunity to examine cellular activities and mechanisms involved in the formation, maintenance and termination of the cyst life stage in response to environmental conditions. In addition, several transcripts have been identified that may be used as molecular markers for cyst viability in sediment samples. When combined with DNA-based techniques, these markers will allow us to assess both the distribution and viability of *Pfiesteria* cysts in sediment samples.

TOXIC MODE OF ACTION OF KmTx 2, A NEW FISH-KILLING TOXIN FROM *Karlodinium micrum* (DINOPHYCEAE)

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Blooms of the estuarine dinoflagellate *Karlodinium micrum* have been associated with fish kills worldwide since the 1950's. However, in US mid-Atlantic states prior to the late 1990's, *K. micrum* was often misidentified as other similarly sized gymnodinoid dinoflagellates, such as *Gyrodinium estuariale* and *Pfiesteria* spp. For several years, our goal has been to establish the potential ichthyotoxicity of *K. micrum* in the United States.

To be presented will be a detailed description of the cytotoxic mode of action of KmTx 2, a newly described fish-killing toxin from *K. micrum*. KmTx 2 was first isolated during a fish kill in South Carolina, USA, and has subsequently been identified in US Atlantic coast isolates from North Carolina to Florida. A similar compound (KmTx 2-like) has recently been isolated from water samples collected during a fish kill in Western Australia associated with a large, persistent, bloom of *K. micrum*.

KmTx 2 is toxic to all mammalian cell types tested, including epithelial cells, neurons, fibroblasts, cardiac myocytes, and lymphocytes. Whole-cell voltage-clamp and single-cell microfluorimetry studies revealed that cytotoxicity occurs through permeabilization of the plasma membrane to cation fluxes, which results in osmotic cell lysis. This study also reveals mechanisms that underlie the historical association between *K. micrum* blooms and fish kills in the marine environment: KmTx 2 is lethal to zebrafish (*Danio rerio*) at environmentally relevant concentrations, while sub-lethal doses severely damage gill epithelia. In addition, KmTx 2 is toxic to representative fungal and dinoflagellate species, but is not toxic to *K. micrum* itself. Membrane sterol composition appears to be critical in determining both cellular susceptibility to KmTx 2 toxicity, and immunity of *K. micrum* from the membrane disrupting properties of its own toxins.

In US Atlantic coastal states, *K. micrum* co-occurs with, and has been confused with, the ichthyocidal dinoflagellate *Pfiesteria piscicida*, but has been reported as non-toxic under ecologically realistic conditions. This study, in conjunction with recent work from our laboratory, proves that *K. micrum* is far from being benign, and is in fact highly toxic. This work confirms the association between high densities of this organism and fish kills that have been observed worldwide for decades.

THE DEVELOPMENT AND APPLICATION OF A QUANTITATIVE PCR ASSAY FOR *Alexandrium fundyense* FROM THE GULF OF MAINE

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Toxic dinoflagellates of the genus *Alexandrium* are responsible for seasonal harmful algal blooms (HAB) in New England coastal waters and other temperate environments. Members of this genus produce saxitoxin that can result in paralytic shellfish poisoning (PSP) along the coastlines of the US, Canada, and many other countries. Even low cell densities of *Alexandrium fundyense* can result in PSP with serious illness, or death, if humans or other consumers ingest sufficient contaminated shellfish. Observations of *A. fundyense* and PSP outbreaks in the northeastern US indicate that populations accumulate in a variety of oceanographic habitats and there is considerable interest in the development and application of monitoring tools that will allow better prediction and study of toxic, but low density populations, in these environments.

To expand the tools available for *A. fundyense* detection and monitoring we developed a PCR-based assay that is specific, quantitative, and appears to be useful for high throughput screening of *A. fundyense* cell density. Primers were designed to amplify a 174bp region of the large subunit ribosomal RNA gene (LSU). These primers specifically amplify target *A. fundyense* strains and not *A. ostenfeldii*, *A. andersonii*, or toxic dinoflagellates from other genera that we tested. With this primer set we used SYBR green to quantitatively amplify the LSU product from surface field samples during a June 2003 cruise in the Gulf of Maine. Cell density was calculated by comparing the amplification in field samples against a standard curve built from an *A. fundyense* Gulf of Maine isolate in culture. In the field samples *A. fundyense* abundance, as calculated with the PCR-based assay, was in the same range as the cell density calculated with other methods. Cloning and sequencing of the amplification product from several stations revealed it to be from *A. fundyense*.

With this PCR-based approach to assessing *A. fundyense* cell density we are able to screen 26 samples in triplicate at one time. We believe this method shows great promise for continued research and monitoring efforts that need estimates of cell number.

PRIMARY PRODUCTIVITY BY THE TOXIC FLORIDA RED-TIDE DINOFLAGELLATE, *Karenia brevis*: EVALUATION OF A BIO-OPTICAL MODEL WITH LABORATORY AND FIELD DATA

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The Florida red-tide dinoflagellate, *Karenia brevis*, frequently forms large blooms in the coastal waters of the Southeastern USA and throughout the Gulf of Mexico, and can contribute significantly to the annual production of these areas. Accordingly, understanding the primary productivity of this key HAB species is integral to understanding the community-level processes of these water bodies. Previous work has established oxygenic and fluorometric photosynthesis versus irradiance (P/E) parameters, photoprotective capabilities, pigmentation dynamics, absorption characteristics, carbon acquisition rates, and the spectral irradiance of the ambient light field in both laboratory and *in situ* bloom conditions. However, to date there have been no attempts to collect all of these disparate pieces of data into a coherent bio-optical model of *K. brevis* productivity. We will present a wavelength resolved model that is parameterized from laboratory-/field-derived data and validated against both simulated *in situ* and *in situ* field measurements. Results from model optimization routines will be discussed.

PUBLIC OUTREACH MATERIALS REGARDING HARMFUL ALGAL BLOOMS AND THEIR POSSIBLE EFFECTS ON HUMAN HEALTH

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The potential interactions between marine and freshwater harmful algal blooms (HABs) and humans are increasing. Humans are exposed through food, drinking water, and recreational and occupational water use to an increasing number of organisms and their toxins. Nevertheless, the amount of clinical and epidemiologic research concerning acute and chronic human health effects from the HAB organisms and their toxins is relatively sparse. At the same time, the public is increasingly aware of and interested in the potential dangers associated with exposure to HABs. Public health authorities and researchers must respond to these public health concerns. The development of appropriate educational and outreach materials based on limited scientific databases is the challenge of informing the public concerning the possible human health effects of HAB organisms and their toxins, and their prevention. Educational materials developed by a group of researchers and public health personnel for general HABs, Florida Red Tide Toxins (brevetoxins), Cyanobacteria, and Ciguatera are discussed, as well as methods for their dissemination.

SEAGRASS AS A ROUTE OF BREVETOXIN EXPOSURE IN THE 2002 RED TIDE-RELATED MANATEE MORTALITY

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Brevetoxins produced by *Karenia brevis*, the Florida red tide dinoflagellate, have previously been associated with mass mortalities of manatees in Florida. In 1982, three weeks after the dissipation of a red tide bloom, 39 manatees died and others exhibited debilitation and uncoordinated motility. Incidental ingestion of toxic filter feeding tunicates attached to seagrass was believed to be the primary source of brevetoxin. A red tide bloom in 1996 resulted in fewer sick animals but 149 deaths. In this case, manatees were dying in areas with high concentrations of red tide cells and environmental conditions conducive to aerosolization of brevetoxin. These events suggest that manatees can be exposed to brevetoxins through both ingestion and inhalation routes.

In mid-February of 2002, high levels of *K. brevis* were present in southwest Florida. In mid-March, three weeks after cell counts had dropped to low levels, manatee deaths greatly increased along the coasts of Sarasota, Charlotte, Lee, and Collier counties. With no active red tide and a noted absence of pulmonary lesions like those seen in 1996, ingestion was the hypothesized route of primary exposure. The 2002 mortality event ended in early May.

Guided by the location of initial manatee carcass recoveries, we selected four sites and one control in Charlotte County to determine if there was residual brevetoxin remaining in the system. From March 28 through August 15, 2002, water, sediment and seagrass samples were collected every two weeks and analyzed for brevetoxins using a competitive enzyme-linked immunosorbent assay (ELISA). From each site, one set of seagrass samples was analyzed whole. A second set was scraped for epiphytes and detritus. The scraped seagrass was then rinsed vigorously with tap water and separated into blades, sheaths, and rhizomes/roots.

Karenia brevis cells were either absent or present at very low levels (max. 2,670 cells/L) for the entire sampling period. Brevetoxin concentrations in the water column were typically below 1 mg/L and toxin in sediments averaged 12-17 ng/g dry wt. All grass components consistently tested positive for brevetoxins, but the highest concentrations were detected in the grass scrapings (up to 3,130 ng/g dry wt.). Based on the dry weight of each component, the contribution of the scrapings was 46-97% of the total toxin in the seagrass, with a median value of 89%. A subset of grass and scrapings was also analyzed by LC-MS and PbTx-3 was confirmed at an average ratio of 30% of the total brevetoxin measured by ELISA. Limpets were abundant in the epiphytic community, and samples of these limpets also tested positive for brevetoxin. Seagrasses continued to test positive for brevetoxins up until August 1, 2002.

These results confirm the stability of brevetoxins in the environment in the absence of an ongoing *K. brevis* bloom, and demonstrate the chronic risk of manatees to brevetoxins through an indirect exposure route. That physical and biological concentration of brevetoxins on and in seagrass can occur post-red tide bloom was unconfirmed in previous manatee mortality events. Based on necropsy findings and brevetoxin analyses of the urine and tissues of the 2002 carcasses, a total of 34 manatee deaths were attributed to brevetoxicosis. This is the first time that a significant number of manatee deaths have been positively related to the ingestion of brevetoxin via seagrass and associated epiphytes subsequent to a red tide event.

VIRUSES AS POTENTIAL REGULATORS OF REGIONAL BROWN TIDE BLOOMS CAUSED BY THE ALGA, *Aureococcus anophagefferens* AND THE ASSESSMENT OF BROWN TIDE BLOOMS AND RELATED ENVIRONMENTAL FACTORS IN COASTAL WATERS OF NEW JERSEY (2000-2002)

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The objective of the first study was to determine whether viral-like particles (VLPs) infected and lysed natural populations of *A. anophagefferens* in coastal bays of New Jersey and in New York in 2002 with the same frequency as in 1999-2000 and especially, at the termination of the bloom. The results indicated that intracellular VLPs continued to infect *A. anophagefferens* throughout the bloom event. Similar to previous years, the percentage of visibly infected cells was higher at the beginning of the bloom than during the height of the bloom. For the first time, our results confirmed that the highest percentage (60%) of VLP-infected cells occurred at the termination of the brown tide bloom in New Jersey in 2002. The intracellular VLPs in natural populations of *A. anophagefferens* were consistent in size and shape (approximately 140 nm in diameter) and comparable to those in previous studies. Concentrated viral isolates, prepared from waters during brown tide blooms in New York and New Jersey in 2002, infected healthy laboratory *A. anophagefferens* cultures in vitro. The viral isolates associated with the highest laboratory viral activity (lysis positive) were concentrated from water samples having the highest viral and bacteria concentrations. Moreover, the intracellular viruses in these virally infected laboratory cultures of *A. anophagefferens* were similar in size and shape to those found in natural populations. The successful isolation of an *A. anophagefferens* specific virus from a brown tide bloom in the field, the similarity of ultrastructure of VLPs infecting both natural populations and laboratory infected cultures, and the pattern of VLP infection during bloom activity in combination with the observed high percentage of VLP-infected cells during bloom termination, supports the hypothesis that viruses may be a major source of mortality for brown tide blooms in regional coastal bays of New Jersey and New York.

The second study was conducted in Barnegat Bay-Little Egg Harbor (BB/LEH), New Jersey (USA) (2000-2002) by the New Jersey Department of Environmental Protection, Division of Science Research and Technology (DSRT), in cooperation with several partners, to assess the spatial and temporal extent of *A. anophagefferens* blooms and associated environmental factors and analyze the potential risk of these algal blooms to submerged aquatic vegetation (SAV) communities. Water samples were collected by boat and helicopter at coastal stations from 2000-2002. *A. anophagefferens* were enumerated and associated environmental factors were measured. *A. anophagefferens* abundances were classified using the Brown Tide Bloom Index and mapped, along with sampled parameters, to their geo-referenced location using the ArcView GIS. To determine the possible risk that brown tide blooms pose to the BB/LEH submerged aquatic vegetation (SAV) communities, the GIS was used to determine the spatial coincidence between locations of high *A. anophagefferens* abundances or duration and the mapped location of seagrass habitat. The results indicated that the highest *A. anophagefferens* abundances ($>10^6$ cells ml^{-1}), including Category 3 blooms ($\geq$ 200,000 cells ml^{-1}) and Category 2 blooms ($\geq$ 35,000 to $\leq$ 200,000 cells ml^{-1}), recurred during each of the three years of sampling and covered significant geographic areas of the estuary, especially in

Little Egg Harbor. While Category 3 blooms were generally associated with warmer water temperatures (> 16^{o}C) and higher salinity (> 25-26 ppt), these factors were not sufficient alone to explain the timing or distribution of *A. anophagefferens* blooms. There was no significant relationship between brown tide abundances and dissolved organic nitrogen measured in 2002 but this was consistent with other studies. However, there was a significant difference in Secchi disk depth between the three bloom categories. Extended drought conditions, with corresponding low freshwater inputs and elevated bay water salinities, occurring during this time were conducive to blooms. *A. anophagefferens* abundances were well above the reported threshold levels that have been reported for negative impacts on shellfish. For the first time, it was shown that 35% of the SAV habitat located in Barnegat Bay/Little Egg Harbor was categorized as having a high frequency of Category 2 or 3 blooms for all three years.

CULTIVATION AND CHARACTERIZATION OF AMOEBOID PROTISTS IMPLICATED IN THE PUTATIVE LIFE CYCLE OF *Pfiesteria piscicida* AND RELATED DINOFLAGELLATES

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This study was undertaken to assess whether or not amoebae, common in mesohaline environments, are stages in the life cycles of *Pfiesteria piscicida*, and *Pfiesteria*-like dinoflagellates. Primary isolations were made from water and sediment samples from five tributaries of the Chesapeake Bay. Enrichment cultivation methods used for the isolation of dinoflagellates and amoebae were performed in parallel. Recovered protists that were morphologically indistinguishable from putative life cycle stages of *Pfiesteria piscicida*, and *Pfiesteria*-like dinoflagellates were studied in xenic and clonal cultivation systems. Cultures of amoebae cloned from laboratory aquaria where fish mortality had been attributed to *P. piscicida* and *Pfiesteria*-like dinoflagellates were determined to belong to the genera *Korotnevella* and *Vannella*. Species of these genera were also recovered from environmental samples with *Pfiesteria*-like dinoflagellates, as were species of at least six other genera of gymnamoebae. Morphologically, all could be confused with putative life cycle stages of *P. piscicida* or *Pfiesteria*-like dinoflagellates. Based upon environmental sampling, cultivation methodologies, long-term observations of established cultures and light and electron microscopy, ubiquitous gymnamoebae of mesohaline environments are not related to *P. piscicida* or *Pfiesteria*-like dinoflagellates that co-occur within the natural environment. The phylogenetic analyses of the SSU rRNA gene data corroborate our conclusions based upon morphological data using key informative features. The SSU rRNA gene has now been sequenced from representatives of many of the major genera of mesohaline gymnamoebae and it is clear that these amoebae are not remotely related to Pp/PLD or other dinoflagellates (Amaral-Zettler et al., 2000; Peglar et al., 2003). Based upon our combined morphological and gene analyses, the assertion that many mesohaline amoebae are stages in the life cycle of dinoflagellates is clearly erroneous. This conclusion is further supported by other work involving laboratory aquaria, where all putative forms of *Pfiesteria* are present during fish-kills, but the amoebae are unrelated to the dinoflagellates (Litaker et al., 2002). Hence we find no support for the ambush predator hypothesis as a model explaining fish death in the natural environment.

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DIRECT UPTAKE OF INORGANIC AND ORGANIC NITROGEN BY *Pfiesteria piscicida AND P. shumwayae*

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Although *Pfiesteria piscicida* and *P. shumwayae* are heterotrophic dinoflagellates, and obtain the bulk of their nutrition via grazing, they can take up nutrients directly. The capacity for nitrogen uptake, nitrogen nutritional preferences, and the extent to which these vary with genus and physiological state, has been the subject of debate, and are important in modeling nitrogen flow and population dynamics. Our objective in this study was to compare the rates and kinetics of uptake of different forms of nitrogen, both inorganic and organic, by both different strains of *P. piscicida* and *P. shumwayae* and cells in different physiological states. All strains under all growth conditions were capable of direct uptake of nitrogen.

Of the nitrogenous substrates investigated (nitrate, ammonium, urea, glycine, and glutamic acid), the highest absolute uptake rates for all cultures were found for ammonium and glutamic acid. Relative to ambient nitrogen availability, all forms of organic nitrogen (urea and the amino acids) were consistently preferred over inorganic nitrogen forms both at near ambient levels and at levels sufficient for uptake saturation. When different cultures were compared, these preferences were most pronounced for both *P. piscicida* and *P. shumwayae* recently removed from active fish killing conditions compared to those that had been maintained on algal food long-term.

The kinetics of uptake differed by substrate, but general patterns were consistent across cultures. The lowest half saturation concentrations (e.g. highest affinity) for uptake were found for glycine and glutamic acid. Uptake of urea did not appear to saturate over the concentration range measured (up to 25 mg at N l^{-1}) and uptake kinetics for this substrate were generally linear rather than hyperbolic.

In regions where *Pfiesteria* spp. are found, such as upper estuaries and aquaculture systems, nutrient enrichment – including organic enrichment - is common. For example, in several of the tributaries of Chesapeake Bay where these species are commonly found, urea concentrations can exceed 10 mg at N l^{-1}. Such high levels result from agricultural runoff of fertilizers and manures. The high uptake rates of, and preferences for, organic forms of nitrogen documented in this study suggest that direct uptake of nitrogen by *Pfiesteria* spp. in nutrient enriched waters may be more significant than previously thought.

COLLABORATIVE, INTERNATIONAL PROGRAMS ON HABS

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For decades HABs have been studied around the globe, but the comparative studies on the underlying reasons for these blooms, and the means to mitigate them when they do occur, have not been undertaken. It is now well recognized and accepted that our understanding of the population dynamics of organisms, their impacts, and the potential management implications, is dependent on working within a global arena. Although HAB impacts may be local, solutions may be found in distant locales. Several efforts have been initiated to bring a multidisciplinary, collaborative approach to the study of HABs.

The Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) Programme was initiated under the auspices of the Scientific Committee on Oceanic Research (SCOR) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO to address the need for broad-based advancement in the understanding of HABs. The mission of GEOHAB is to "foster international cooperative research on HABs in ecosystem types sharing common features, comparing the key species involved and the oceanographic processes that influence their population dynamics." GEOHAB is not a source of research funding; rather, research will be supported by national funding agencies that must respond to national scientific priorities utilizing nationally based facilities, resources and expertise. GEOHAB will, however, coordinate and build on related national, regional and international efforts in HAB research within an ecological and oceanographic context, much like other large-scale ocean research projects, such as the Joint Global Ocean Flux Study (JGOFS) and the Global Ocean Ecosystem Dynamics (GLOBEC) project. The GEOHAB Scientific Steering Committee is currently planning the implementation of four Core Research Projects, representing ecosystem types in which HABs are recurrent phenomena, including (1) HABs in Upwelling Regions, (2) HABs in Semi-confined Eutrophic Zones and Estuaries, (3) HABs in Fjords and Coastal Embayments and (4) HABs in Stratified Regions. The initial Core Research Projects will encourage involvement of the scientific community and international Open Science Meetings are being held on each of these four topics to encourage wide participation.

A significant new program for joint research in Europe and the US is currently underway through financial support from the European Commission (E.C.) and the U.S. National Science Foundation (NSF). This collaborative EU-US Programme on HABs builds on a joint workshop that was held in September 2002 in Italy. The outcome of that workshop was a planning document which outlines the opportunities provided by this program. The first call for proposals was announced this fall, and proposals are currently under review. The rationale for comparison of similar harmful algal events and taxa across environmental and species is compelling. The global research community, as well as HAB management community will benefit from these collaborations.

IMPACTS OF VIRUSES ISOLATED FROM NEW YORK WATERS ON GROWTH OF THE BROWN TIDE ALGA, *Aureococcus anophagefferens*: A FIELD AND LABORATORY ASSESSMENT

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Viruses may be an important source of mortality for brown tides caused by the picoplanktonic pelagophyte *Aureococcus anophagefferens*, particularly since other biological mortality mechanisms such as benthic and pelagic grazing are disrupted during intense blooms. During 2002, we used cross flow filtration techniques to concentrate and isolate high molecular weight (HMW: 30 kDa – 0.2 µm) material from New York estuaries which commonly host blooms. Since concentrated HMW material contains elevated densities of viruses, this material was used to conduct field-based viral enrichment experiments and to isolate *A. anophagefferens*-specific lytic viruses. During 48 hr bottled, field experiments, we found that enriching background levels of viruses episodically enhanced net growth rates of *A. anophagefferens* compared to control treatments. This result suggests that activity within total viral community (bacteriophage, cyanophage, algal viruses) may indirectly enhance brown tide growth during blooms by regenerating dissolved organic matter or by altering the composition of microbial assemblages. Screening of HMW viral concentrates isolated from two estuaries during 2002 indicated that viruses able to lyse *A. anophagefferens* cultures were present on nine dates within both bays, only one of which hosted a brown tide. Results from pulsed field gel electrophoresis (PFGE) used to compare the viroplankton communities collected from these sites before and after serial passage through axenic *A. anophagefferens* cultures will be presented. The nine viral isolates have been serially propagated through laboratory cultures of *A. anophagefferens* weekly since the summer of 2002. Ultrastructural analysis of *A. anophagefferens* cultures infected with isolated viruses revealed the presence of intracellular viral capsids similar to those found previously in field populations. The viral isolates are chloroform sensitive, and hence likely have lipid associated with their protein coat. The viruses also appear to be species specific, as they do not lyse 10 other classes of algae and are only able to lyse *A. anophagefferens* within the pelagophyte class (three other pelagophytes screened including the Texas brown tide, *Aureoumbra lagunensis*). Isolated viruses are able to lyse clonal cultures of brown tide originating from multiple NY and NJ estuaries, as well as axenic cultures of *A. anophagefferens*. The ability of propagated viruses to lyse only a portion of clonal brown tide cultures isolated from the same date and location (Great South Bay, NY; 6 May 1998) suggests that there may be a greater degree of clonal diversity within brown tide field populations than has previously been hypothesized. The experimental additions of laboratory propagated viruses to NY bloom waters demonstrated that some, but not all, of the viral isolates were capable of significantly reducing the abundance of *A. anophagefferens* during five-day, field bottle experiments. This result suggests lab propagated, *A. anophagefferens*-specific viruses may be a viable biological control agent for brown tides. Finally, preliminary experiments indicate that viral lysis of *A. anophagefferens* is delayed in the dark, suggesting these viruses may partly depend on host photosynthetic pathways to replicate. If confirmed, this finding would suggest that the low light conditions which prevail during peak abundances of dense brown tide blooms may reduce the ability of viruses to replicate and cause mortality in *A. anophagefferens*.

RELATIVE CONTRIBUTION OF TOXIN AND MICROPREDATION TO ICTHYOTOXICITY OF TWO STRAINS OF *Pfiesteria shumwayae*

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Controversy exists regarding the mechanisms by which *Pfiesteria shumwayae* kills fish. Several studies have implicated a *Pfiesteria*-associated exotoxin in fish mortality while other studies indicate that direct attack of dinoflagellates on fish (micropredation) and not exotoxin is responsible. Many microorganisms, including dinoflagellates, exhibit variability in toxin expression and/or composition among strains of the same species. Accordingly, we examined the ichthyotoxicity of two strains of *P. shumwayae* (CAAE 101272 and CCMP 2089) in a bioassay system that was designed to expose test fish to *P. shumwayae* cultures both with and without direct contact between fish and dinoflagellate cells. Cell free supernatants from both strains were also tested for toxicity. CAAE 101272 has previously been associated with toxin production while CCMP 2089 has been reported to kill by micropredation alone.

Juvenile tilapia (*O. nilauticus*) were exposed to cultures of each *P. shumwayae* strain in 10 liter aquaria. Controls consisted of tanks that were not inoculated with *Pfiesteria* cultures. In each experiment four fish were placed directly into test tanks allowing direct contact between the dinoflagellates and the fish. Four additional fish were placed into aerated containers constructed from tissue culture flasks and polycarbonate membrane (3 μm). These containers were placed into the same tank as the non-contained fish. Fish were exposed for 48 h. Dead fish were tallied after 24 and 48h. Dinoflagellates were counted inside and outside of the containers and water quality parameters were monitored. In separate experiments cultures from highly toxic aquaria (killing fish in ca. 4 hr) were centrifuged (9,635xg 20 min) and fish were exposed to the resultant dinoflagellate-free supernatants.

The results indicate that direct contact between *P. shumwayae* and fish generally enhances fish mortality with both strains. Time to death was quicker and percent of fish killed was higher outside of the containers except in two of 22 trails. Mortality of fish exposed to CAAE 101272 cultures but protected from direct contact with *Pfiesteria* cells ranged from 0 to 100% of exposed fish in individual trials. In contrast only one contained fish (of 36) exposed to CCMP 2089 cultures died. No deaths were observed in control tanks inside or outside of the containers. Supernatants obtained from cultures in highly toxic condition (killing all fish in ca. 4 hr.) killed fish when obtained from cultures of CAAE 101272 but not when obtained from CCMP 2089.

These results suggest that production of *Pfiesteria*-associated toxin varies among strains of *P. shumwayae* and with time in cultures of toxin-producing strains. The toxin(s) can kill fish when present at sufficiently high concentration but dissolved toxin production is apparently not required for fish mortality. The dominant and most consistent mechanism of fish mortality in these cultures required direct contact between fish and *Pfiesteria* cells in both of the strains we examined.

RADIOLABELLED SAXITOXIN FOR RECEPTOR ASSAYS: DEVELOPMENT OF IMPROVED LABELING STRATEGIES

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Due to their response spectrum to the various saxitoxins, assays based on the sodium channel receptor site provide the most reliable estimate of paralytic shellfish poison toxicity in seafood samples. Receptor binding assays, using appropriately labeled toxin, offer the most practical option to the mouse bioassay, but the appropriate labeling of the toxin presents a challenge. At present, the employment of the receptor binding assay is constrained by uncertainties in the availability of exchange labeled saxitoxin, which has been the basis of previous work. Current efforts are focused on both improving the technique for exchange labeling, to assure a reliable supply, and on developing alternative labeled toxins.

ASSESSING THE BIOAVAILABILITY AND UPTAKE OF DISSOLVED HUMIC SUBSTANCES BY THE HAB SPECIES *Karenia brevis* USING RADIOISOTOPIC TECHNIQUES

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Blooms of some HAB dinoflagellates species initiate in coastal regions characterized by high concentrations of colored dissolved organic material (CDOM), suggesting that these compounds are potentially bioavailable and may serve as both a carbon (C) and/or nitrogen (N) source. Measuring the direct uptake of dissolved humic substances by phytoplankton has been confounded by the polymictic nature of these compounds. The bioavailability of humic compounds to the HAB dinoflagellate *Karenia brevis* was addressed via two radioisotopic techniques: measurement of the uptake of ^{125}I-labelled humic acids extracted from the Peace River, a river implicated in supporting *K. brevis* blooms on the west Florida shelf, and measurement of the uptake of laboratory synthesized 'model' humic compounds which have been labeled in either the carbon or nitrogen moieties with ^{14}C during their synthesis. *Karenia brevis* took up a ^{125}I-labeled Peace River humic acid fraction at rates ranging from 1.0 - 2.2 pg cell^{-1} hr^{-1}, with the highest uptake rates observed in darkness. A similar range of uptake rates were observed with ^{125}I-labelled IHSS standard Suwannee River humic acids. Comparatively, *Dunaliella tertiolecta* and *Skeletonema costatum* took up the same labeled fractions at rates of 0.5 – 1.0 and 0.01 – 0.02 pg cell^{-1} hr^{-1} respectively. Stoichiometric calculations utilizing measured humic acid uptake rates and N content and a typical *K. brevis* bloom concentration suggest that the maximum amount of N available to a moderate *K. brevis* bloom from humic sources on the west Florida shelf is approximately 0.04 μM N L^{-1} d^{-1}. Assuming average growth rates and reported N:Chl ratios, the same bloom would have a N demand of 1.0 μM N L^{-1} d^{-1}. This suggests that, although humic fractions of estuarine CDOM are actively taken up by *K. brevis*, they are not a significant source of N to *K. brevis* blooms.

NITROGEN UPTAKE BY THE RAPHIDOPHYTE *Heterosigma akashiwo*: A LABORATORY AND FIELD STUDY

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The nitrogen uptake capabilities of the potentially harmful raphidophyte *Heterosigma akashiwo* Hada (Sournia) were examined in unialgal laboratory cultures (strain CCMP 1912), and natural populations using the ^{15}N-tracer technique. The effect of various nitrogen substrates (nitrate, ammonium and urea) on the exponential growth rate of cultures at saturating and sub-saturating photosynthetic photon flux densities (PPFDs) were examined.

Maximum specific uptake rates (V_{max}) for unialgal cultures grown at 15ºC and saturating PPFD (110 $\mu E \cdot m^{-2} \cdot s^{-1}$) were 18.0, 28.0 and $2.89 \cdot 10^{-3} \cdot h^{-1}$ for NO_3^-, NH_4^+ and urea, respectively. The traditional measure of nutrient affinity - the half saturation constants (K_s) were similar for NO_3^- and NH_4^+ (1.47 and 1.44 $\mu mol\ N \cdot L^{-1}$), but lower for urea (0.42 $\mu mol\ N \cdot L^{-1}$). Whereas the α parameter ($\alpha = V_{max}/K_s$), which is considered a more robust indicator for substrate affinity when substrate concentrations are low ($< K_s$) were 12.2, 19.4 and $6.88 \cdot 10^{-3} \cdot h^{-1}/(\mu mol\ N \cdot L^{-1})$ for NO_3^-, NH_4^+ and urea, respectively. These results suggest that at both saturating and sub-saturating N concentrations, N preference would follow the order: ammonium > nitrate > urea. A dense *H akashiwo* bloom ($> 1 \cdot 10^8$ cells L^{-1}) in June 2002, positively identified using both molecular and microscopic techniques in Richardson Bay (western San Francisco Bay, CA), was supported primarily by NO_3^-, although it appears that both NH_4^+ and urea were utilized first or simultaneously with NO_3^-. The ratio of nitrate uptake to total N (nitrate + ammonium + urea) uptake was substantially different inside and outside of the *H. akashiwo* bloom patches in SF Bay. The percent NO_3^- uptake [% NO_3^- uptake = (NO_3^- uptake/total N uptake) x 100], averaged 74 for the bloom population, but only 29% for natural assemblages outside of the bloom patches. However, the relative utilization of urea remained constant and averaged 12 and 11% for the phytoplankton assemblages inside and outside of the bloom. A strain of *H. akashiwo* was isolated by sequential dilution from samples collected later that summer, accepted into the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), and is available as (CCMP 2274).

Trends in growth rates were observed in the unialgal batch cultures. At saturating PPFD (110 $\mu E \cdot m^{-2} \cdot s^{-1}$), the growth rate of *H. akashiwo* was slightly greater for cells grown on NH_4^+ (0.89 d^{-1}) compared to cells grown on NO_3^- and urea, which had identical growth rates (0.82 d^{-1}). At sub-saturating PPFD (40 $\mu E \cdot m^{-2} \cdot s^{-1}$), both urea- and NH_4^+-grown cells grew faster than NO_3^- grown cells (0.61, 0.57 and 0.46 d^{-1}, respectively). However, growth rates within each PPFD were not statistically different (n = 3 for each substrate/light combination). Increasing the number of replicates may result in a statistically lower growth rate for NO_3^- at low PPFD when compared to NH_4^+ and urea.

CROSS-FRONTAL ENTRAINMENT OF PLANKTON INTO A BOUYANT PLUME: THE FROG TONGUE MECHANISM

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A mechanism for the cross-frontal entrainment of plankton by a buoyant plume influenced by wind stress is described and tested using an idealized numerical model. Under the right circumstances, plankton may enter a buoyant plume during an upwelling wind stress, then be transported shoreward during a subsequent downwelling wind stress. In order for the plankton to enter the plume, they must swim upward at a velocity (w_p) bounded by

$$H_{plume} / T < w_p < k / H_{mix}$$

where H_{plume} is the thickness of the buoyant plume, H_{mix} is the thickness of the upper oceanic mixed layer ($H_{mix} > H_{plume}$), k is the magnitude of vertical mixing within the mixed layer, and T is the time between upwelling and downwelling events. In words, this equation states that the plankton must swim slow enough so that they are evenly distributed through the mixed layer, so that the buoyant plume may override the plankton patch during upwelling. Once the plume has overridden the patch, in order to enter the plume, the plankton must swim fast enough to be able enter the plume in the time while it is over them. These two bounds on the swimming rate suggest that, given various physical parameters, there may be a range of swimming speed that will maximize entrainment into a plume. Numerical experiments corroborate the feasibility of the proposed mechanisms and associated scaling.

PATTERNS AND VARIABILITY OF WATER PROPERTIES IN 2003 IN THE ECOHAB PNW REGION

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Time series of domoic acid in razor clams on Washington beaches are consistent with intermittent toxic events that primarily follow storms and onshore transport over the shelf. A new program, ECOHAB Pacific Northwest (PNW), has begun to address the origin of these events by studying the physiology, toxicology, ecology and oceanography of toxic *Pseudo-nitzschia* species off the Pacific coast of Washington (WA) and British Columbia. Over the course of five years we expect to determine the physical/biological/chemical factors that appear to make the Juan de Fuca eddy region more viable for growth and sustenance of toxic *Pseudo-nitzschia* than the nearshore WA upwelling zone; to determine the combination of environmental factors that regulate the production, accumulation, and/or release of domoic acid (DA) from *Pseudo-nitzschia* cells in the field; and to determine possible transport pathways between DA initiation sites and shellfish beds on the nearby coast. June and September cruises took place in 2003. On each cruise, the strategy was to intersperse large-scale surveys of water properties, including nutrients, fluorescence, species and particulate domoic acid, with laboratory studies following water masses from the eddy and also from the coastal upwelling region.

During the June cruise, the Juan de Fuca eddy was persistent throughout the cruise, although its location changed with time. The robust nature of the eddy and of the coastal front was confirmed with surface drifters, whose tracks were very similar to those released much later in the upwelling season and in different years. The transport pathway from the eddy region to the inner shelf and to the shelf from Washington and even to California was confirmed by surface drifters deployed in June in the eddy and even in the strait—those drifters escaped the eddy and traveled south southeast at speeds of 15-20 miles per day. However, during a downwelling event drifters in the water at that time moved onshore toward the WA coast, with one drifter moving to within 7 miles of the beach. It then traveled north about 20 miles before turning south again after upwelling resumed. Surface fluorescence and satellite imagery during upwelling showed two regions of high values—one southeast of Barkley Sound, the other, adjacent to the Washington coast in the upwelling zone. A region of lower fluorescence emanated from the strait appearing to "wrap around" higher chlorophyll water. Macro nutrients were as high at the mouth of the strait as in the upwelling region near the WA coast. These and other property patterns from both cruises will be discussed in terms of the physical forcing. Seasonal changes in water property structure as deduced from moored sensors in the eddy, in the strait and off the WA coast will also be discussed.

USE OF THE RAPID LATERAL FLOW IMMUNOCHROMATOGRAPHIC TESTS FOR PSP AND ASP IN PHYTOPLANKTON APPLICATIONS

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The Jellett Rapid Tests have been used in many countries for detection of PSP and ASP toxins in shellfish tissue. These data have been published (Jellett et al. 2002, Toxicon 40: 1407-1425). Recently the Rapid Tests have been applied to the detection of PSP and ASP toxins in phytoplankton from culture and in seawater. Approximately 20 types of toxigenic dinoflagellates (*Alexandrium* spp., *Gymnodinium catenatum, Pyrodinium bahamense* var. *compressum*) in culture were positive in the Rapid Test for PSP and in the HPLC, while 7 strains of non-toxigenic *Alexandrium* and several strains of co-occurring non-toxigenic dinoflagellates were negative. Similarly, ASP toxins in *Pseudonitzschia* spp. cultures were detected using the Rapid Test for ASP.

Semi-quantitation using serial dilutions of the samples showed similar results to HPLC quantitation. Phytoplankton were prepared by collection of the cells using filtration and then transfer of the cell material into 0.1N acetic acid solution. The limit of detection was about 2ng toxin of ASP or PSP toxin in the sample. The Rapid Tests were then used to detect PSP and ASP in the field. A toxigenic bloom of *Pseudonitzschia seriata* was detected using field sampling over one summer in Nova Scotia and toxin was confirmed using LC-MS. Net tows containing *Pseudonitzschia* and/or *Alexandrium* spp. from Nova Scotia, the Gulf of Maine and coastal Washington State, USA, were also found to be positive in both the Rapid Tests and by analytical methods. Samples found to be negative using analytical chemistry were also negative in the Rapid Tests. Toxin per cell determines how many cells can be detected in the Rapid Tests, but it was possible to detect as few as 50 cells of a toxic strain of *Alexandrium* sp. (AL18B).

CELL-CONCENTRATION DEPENDENCE IN NET GROWTH OF *Alexandrium monilatum*

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The red tide dinoflagellate, *Alexandrium monilatum*, was experimentally inoculated over a range of initial cell concentrations into seawater containing a natural plankton community. In the presence of potential predators and competitors, positive net growth of the dinoflagellate population did not occur unless its initial cell concentration exceeded a threshold of 300-400 cells ml^{-1}. However, in filtered seawater, growth of the dinoflagellate was unrelated to cell concentration. The relationship between cell concentration and growth in whole seawater appeared to be due to inhibition of predation as initial cell concentration increased. A suite of hypothetical interactions between *A. monilatum* and a generalized predator were modeled and fit to the experimental data. To fit the experimental data with realistic parameter values, models had to include: a realistic carrying capacity for *A. monilatum*, mortality of *A. monilatum* that was related to contact probability with the predator, and growth of the predator that was independent of *A. monilatum* concentration (prey switching). This modeling exercise demonstrated that the unusual cell-concentration-dependence of *A. monilatum* net growth could be described by simple models with straightforward predator-prey interactions. The models constrained the potential ways in which the dinoflagellate and predator could interact and provided parameter values for growth and mortality rates of this hypothetical predator.

It is novel to suggest that dinoflagellate populations must exceed a cell concentration threshold to escape predation. By implication, red-tide initiation may have to be preceded by aggregation of dinoflagellate cells to levels exceeding such a threshold for positive net growth. Our results suggest that within such aggregations, mortality of the dinoflagellate would be reduced, allowing positive net growth and further accumulation of cells.

CHARACTERISTICS OF THE *Karenia brevis* DIEL VERTICAL MIGRATION BASED ON LABORATORY OBSERVATIONS

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Several years of laboratory observations on the cell division, physiology, biochemistry and behavior of *Karenia brevis* are combined to provide alternate diel vertical migration scenarios based on unequal and equal daughter cell formation. These scenarios form the basis of the programmed behaviors that are used to direct field-deployed *K. brevis* population mimics. Pertinent observations include: 1) cell division occurs throughout the occupied water column; 2) the strength of the daylight surface aggregation increases after a large cell division the previous night; 3) repeated (2-3 times) isolation of the noon surface aggregate, that occurs after a large cell division the previous night, to inoculate new water columns eventually yields a quantized cell division in a *K. brevis* population; 4) under the tested experimental conditions, approximately half the population in a water column aggregates at the surface by noon after a quantized cell division the previous night; 5) cells in the post-quantized, noon, surface aggregate can contain much less lipid and chlorophyll and somewhat less protein and carbohydrate than cells remaining deeper in the water column; 6) at night, cell sub-populations at all water depths return to the same average physiological potential and to the same average biochemical content; 7) some clones of *K. brevis* exhibit a nutrient-related chemotaxis that increases with nutrient deprivation and during the daylight part of a diel cycle; and, 8) cells require three days between divisions under standard laboratory growth conditions. Both alternate scenarios incorporate cell differences related to cell age (time since the last cell division) to explain observations 1-3. The alternate scenarios diverge in the explanation of observations 4-8 depending on whether cell division yields unequal or equal daughter cells. In the former case, initial cell composition strongly influences subsequent water column position, while in the latter case, initial water column position and conditions strongly influence subsequent water column position. Since water column position determines environmental exposure and physiological rates, cell growth follows complex patterns both within and between the alternate scenarios. Though present evidence tends to support many aspects of the equal daughter cell scenario, the exact nature of the daughter cell formation remains uncertain. A recently developed physical-biological model examines the influence of selected *K. brevis* behavioral patterns on bloom dynamics in a water column characterized by three-dimensional shear.

MAPPING *Karenia brevis* BLOOMS UTILIZING AUTOMATED OPTICAL DISCRIMINATION

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In the waters off western Florida, blooms of the toxic dinoflagellate *Karenia brevis (=Gymnodinium breve)* (Davis) have occurred nearly annually (K. Steidinger, pers. comm.) and the geographic extent of the blooms appears to have increased in recent years. When undetected, these blooms resulted in contamination of commercial seafood production, disruption of recreational fishing enterprises, and despondent tourists. Economic impact studies showed a loss to Florida of nearly 20 million dollars from a bloom in 1971 and more than 15 million dollars from a 1973-74 bloom. There is a critical need to detect and track these toxic algal blooms over ecologically relevant spatial/temporal scales. To do this requires long-term monitoring efforts which can delineate the frequency and distribution of both phytoplankton and HABs.

Satellite and airborne remote sensing are beginning to show promise for the detection and tracking surface expressions of these HABs, but subsurface events remain problematic (Stumpf and Culver, pers. comm.). An approach has been developed to detect *Karenia brevis* blooms through analysis of absorbance spectra, measured by *in situ* instrumentation. The classification technique relies on fourth-derivative analysis of particle absorbance spectra. When applied to natural, mixed populations of phytoplankton in the eastern Gulf of Mexico the fourth-derivative discrimination technique showed a significant, linear relationship between the derivative spectrum-based similarity index and the fraction of chlorophyll biomass contributed by *K. brevis*.

This presentation will be a compilation of results from four ECOHAB-Florida cruises and five local surveys utilizing this approach over the past six years. These results demonstrate the utility of the technique to mapping the distribution of *Karenia brevis* bloom patches over the central west coast of Florida. New modes of deployment that are underway, including fixed moorings and autonomous underwater vehicles, will be presented and results discussed. This technique, applied in these deployment modes, in conjunction with new molecular-based species-detection technology form the core of an automated detection and tracking network being developed along the west coast of Florida.

APPLICATION OF CULTURED NEURONAL NETWORKS FOR IDENTIFICATION OF TOXIC ALGAE

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On-line, near-real time detection systems for harmful algal species and their toxins is a rapidly emerging field aimed at forecasting bloom development, persistence, and toxicity as well as providing data to facilitate rapid and more effective responses to harmful algal blooms. Recently the Naval Research Laboratory demonstrated that the neuron-based biosensor, which relies on the use of cultured neuronal networks grown over microelectrode arrays (MEA) is capable of detecting the concentration-dependent alterations in extracellular action potentials or spikes from the network upon introduction of several purified marine toxins to recording media. We have now sought to determine whether the neuronal networks could detect these toxins directly in the seawater growth medium of the toxic algae.

Spinal cord and frontal cortex tissue were prepared from embryonic mice and cells were seeded onto 64 electrode MEAs. All recordings were performed at 37˚ C with media/toxin perfusions at flow rate of 0.75 ml/min.

Our results demonstrated that neuronal networks could be used for analysis of algal samples lysed directly in the seawater growth medium of the toxic algae *Alexandrium fundyense* and *Karenia brevis*. The MEA responded to signals characteristic of each toxin class from these cultures, but not from non-toxic isolates of the same algal genus. This successful trial provided evidence that the prototype MEA has the capacity to detect toxins associated with cells of toxic algal species and has the potential for monitoring toxin levels during harmful algal blooms.

HARMFUL ALGAL BLOOMS IN FLORIDA's MARINE WATERS: THE NEW MILLENIUM

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Florida's marine and estuarine waters continue to experience new and sustained impacts from HAB events. Since early 2002, with more than 20 cases of saxitoxin (STX) food poisoning caused by the consumption of toxic southern puffer fish (*Sphoeroides nephelus*) recreationally harvested from the Indian River Lagoon (IRL) (Quilliam et al. 2002), STXs were confirmed in *Pyrodinium bahamense* for the first time in the U.S. (Landsberg et al. 2002). In the IRL, southern puffer fish remain highly toxic, while trace or below regulatory levels of STXs have been confirmed in a wide variety of fish and invertebrates including commercially harvested hard clams *Mercenaria* sp. Non-harvestable small razor clams, *Ensis minor*, with up to 4300 µg STX eq./100g tissue, are of interest for their potential risk as toxic prey (Abbott et al., this conference). In 2001, preceding the puffer fish toxicity outbreak by six months, a significant chronic mortality of more than 30 dolphins (M. Stolen, Hubbs-Sea World, pers.comm.) occurred in the general area of the IRL where high STX concentrations have now been found. An unusual mortality of seven manatees in the IRL in late 2001/early 2002 (E. Haubold, FWC, pers.comm.) was of concern and potentially significant in that STXs were confirmed in manatee stomach contents containing toxic tubeworms. To what extent STXs may chronically affect marine mammal health in the IRL is unknown. Florida now has all major groups of HABs with a potential to affect public health, cause economic losses, and impact ecological resources. Historically, *Karenia brevis* red tides have caused significant threats to the public from Neurotoxic Shellfish Poisoning or from aerosolized brevetoxins. In spring 2002 and 2003, an unusual time of year for blooms to occur, more than 100 endangered manatees died from brevetoxicosis in southwest Florida. In 2003, possibly for the first time, more than eight domestic dogs roaming on the beach were reportedly affected by exposure to brevetoxins during a highly concentrated red tide event. Urine samples repeatedly sampled from two dogs, independently submitted to two veterinary clinics, tested positive for Pbtx-2 eq. by ELISA; one dog for at least three weeks post exposure (Flewelling, unpubl. data). Although not verified and only visually confirmed in one case, dogs were considered exposed to brevetoxins via consumption of dead fish or from the ingestion of highly toxic foam at the seawater/beach interface. In spring 2002, a mortality of more than 20 lesser scaup *Aythya affinis* was also attributed to brevetoxicosis with toxin concentrations of almost 16,000 ng/g PbTx-2 eq. by ELISA in the gastrointestinal contents with less toxin present in the lungs, liver, and kidney (Flewelling, unpubl. data). These incidents demonstrate the persistent and expanding nature of brevetoxin impacts as well as signaling possible effects from other *Karenia* species now known in Florida's waters (Haywood and Steidinger, unpubl.data). Recent blooms of toxic *Pseudo-nitzschia pseudodelicatissima* along Florida's west coast signal potential concerns for Amnesic Shellfish Poisoning outbreaks. Ciguatera continues to be attributed to benthic *Gambierdiscus toxicus* along Florida's reef tract, but a potential role for *Prorocentrum* or *Ostreopsis* toxins in this food poisoning complex has not yet been explored. Eleven ichthyotoxic species, including *Alexandrium monilatum*, *Gymnodinium pulchellum*, *Karenia brevis*, *Karenia mikimotoi*, and *Chattonella* sp., have varying impacts statewide. Low and essentially benign concentrations of *Pfiesteria piscicida* and *P. shumwayae* were monitored in less than 4% of sites statewide with no evidence thus far for *Pfiesteria*-associated fish kills. *Trichodesmium* is present in high concentrations along Florida's coasts and has been implicated as a nutrient source for *Karenia* red tides, while *Lyngbya majuscula* causes frequent bloom mats in inshore bay areas. Continued networking and surveillance activities through the Florida HAB Task Force established in 1997 have ensured in-state investigations and monitoring for potential HAB events. Management plans need continuous reappraisal to address the changing scope and impacts associated with HABs in Florida's waters.

PHYSIOLOGY AND ECOLOGY OF MACROALGAL BLOOMS (GREEN TIDES) ON CORAL REEFS OFF NORTHERN PALM BEACH COUNTY, FLORIDA (USA)

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Since 1990 coral reefs in 20 to 50 m depths off northern Palm Beach County have experienced an unprecedented succession of macroalgal blooms involving the genera *Codium* and *Caulerpa*. Previous work has established that: 1) blooms of *Codium isthmocladum,* which initially expanded on these reefs in the early 1990's, are seasonal and grow from late spring through fall with peak biomass in late summer, 2) blooms of *Caulerpa verticillata* and *Caulerpa racemosa*, which appeared in 1997, and the non-native *Caulerpa brachypus* (Pacific native) that invaded the reefs in 2001, are now competing with the seasonal blooms of *C. isthmocladum*, and 3) blooms of both *C. isthmocladum* and *Caulerpa* spp. appear to be supported by nitrogen derived from land-based sewage discharges. Our research in 2003 involved comparative studies of seasonal growth patterns, fluorescence yield, tissue biochemistry, and the potential for herbivores to control these "Green Tides". Analysis of quarterly digital underwater video transects from our two monitoring stations at the Princess Anne (PA) and North Colonel's Ledge (NCL) indicated that, unlike *C. isthmocladum*, some *Caulerpa* spp. are capable of year-round growth. In January/February 2003 when reef temperatures were ~ 20 ^{o}C, biotic cover at both stations was dominated by *C. verticillata* (58 % at NCL, 62 % at PA) with relatively low cover of *C. racemosa* (9.1 % at NCL, 5% at PA) and *C. brachypus* (1 % at NCL, 19 % at PA). Following increased water temperatures to > 20 ^{o}C in late-February, the invasive *C. brachypus* developed extensive blooms in early March coincident with extreme low tides and increased concentrations of ammonium in the water column. By late April, *C. brachypus* was the dominant alga at both sites, accounting for 63 % cover at NCL and 72 % cover at PA. The molar C:N, C:P, and N:P ratios of *Caulerpa* spp. in the winter sampling averaged 16.8, 487, and 32.5 at NCL and 14.2, 504, and 35.6 at PA. Stable nitrogen isotope values (d ^{15}N) of *Caulerpa* spp. during winter averaged 5.56 o/oo at NCL and 5.18 o/oo at PA, values characteristic of enrichment with sewage nitrogen. Measurements of fluorescence yield (Fv/Fm) in *Caulerpa racemosa*, *C. mexicana*, and *C. prolifera* consistently approached the maximum value for PS II activity (a value of 0.8); in comparison, high but less consistent values were observed in *Codium isthmocladum*, *Caulerpa verticillata*, and *C. brachypus,* particularly in July 2003 when upwelling resulted in temperatures as low as 14 ^{o}C at NCL. Comparative studies of fluorescence yield in macroalgae from oligotrophic Bahamian waters showed lower values of Fv/Fm, suggesting that the high fluorescent yield values of bloom species on northern Palm Beach County's reefs are indicative of eutrophic conditions. *In situ* bioassays of bloom species palatability to resident icthyofauna indicated that although *C. brachypus* is highly palatable to grazing fish, its high net productivity after grazing losses allows it to be a persistent and often dominant component of the reef biota. Other bloom species, including *C. isthmocladum*, *C. racemosa*, *C. mexicana*, and *C. prolifera*, are generally not preferred diets for grazing icthyofauna.

ECOLOGY OF *Pfiesteria* spp. AND RAPHIDOPHYTE BLOOMS IN SOUTH CAROLINA

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Initial HAB research efforts in South Carolina targeted tidal creeks and open estuaries. Overall, *Pfiesteria piscicida* and *P. shumwayae* were concluded to be widespread in these systems, but never observed in high (potentially toxic) abundances. From more recent efforts (after spring 2001) targeting brackish stormwater detention ponds, we now know that "blooms" of *Pfiesteria* spp. can occur in these lagoonal systems, in some cases associated with fish kills. Also, positive results from fish mortality bioassays have been demonstrated. Based on real-time PCR, *P. piscicida* is prevalent in the sediments of these ponds (~60% of ponds sampled and 47% of samples taken from adjoining tidal creeks). Assuming that these are cysts or amoebae, these results have implications for *Pfiesteria* dispersion through exchange with adjacent tidal creeks and sediment dredging.

Brackish pond sampling has also revealed the near-ubiquitous occurrence of raphidophyte blooms by four species, *Heterosigma akashiwo, Chattonella subsalsa, Fibrocapsa japonica,* and *C. verruculosa.* Axenic cultures of local isolates of the former three species have been obtained. Through distributional associations with environmental conditions and bioassays with field and culture material, we are testing the following attributes of raphidophytes as explanation for the prevalence of blooms in brackish lagoonal ponds: a high mixotrophic ability, resistance to herbicides, high metal (e.g. Fe) uptake capabilities, and antipredator strategies. In addition, we present documentation of an anomalous April 2003 *H. akashiwo* bloom that extended from Bulls Bay estuary to 5 miles offshore (Fig. 1), and was associated with a fish kill and physiological stressful effects on shellfish. We present data on *Pfiesteria* and raphidophyte distribution and association with environmental variables that suggest that these numerous hypereutrophic, low-flow lagoons are favorable environments for proliferation of these HABs.

Fig. 1. *Heterosigma akashiwo* bloom in nearshore waters just outside of Bulls Bay (29 April 2003).

USE OF ITS-SPECIFIC POLYMERASE CHAIN REACTION (PCR) ASSAYS TO IDENTIFY PHYTOPLANKTON SPECIES IN COMPLEX NATURAL ASSEMBLAGES

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Pfiesteria piscicida, *Karenia brevis* and other potentially harmful microalgae frequently co-occur with morphologically similar species that cannot be distinguished using light microscopy. The inability to distinguish harmful from non-harmful species can make accurate assessment of public health threats based solely on light microscopy difficult. Over the past ten years molecular assays have been developed that allow unambiguous detection of harmful species in natural assemblages. This study specifically evaluated whether unique sequences in the ribosomal internal transcribed spacer regions (ITS1 and ITS2) could be used to develop PCR assays capable of detecting *Pfiesteria piscicida* and related co-occurring species (Litaker et al., 2003). The ITS regions were selected because they are more variable than the flanking small subunit (SSU) or large subunit (LSU) ribosomal RNA genes and more likely to contain species-specific sequences. Sequencing of the ITS regions revealed unique oligonucleotide primer binding sites for *Pfiesteria piscicida*, *Pfiesteria shumwayae*, Florida "Lucy" species, two cryptoperidiniopsoid species, "H/V14" and "PLO21," and the estuarine mixotroph, *Karlodinium micrum*. These unique primer sites were used to develop species-specific PCR assays with a minimum sensitivity of 100 cells in a 100 mL sample (1 cell mL^{-1}). The assays were then used to successfully detect *P. piscicida* and related PLOs in field samples.

Each PCR assay included positive, negative, blank extraction, and spiked DNA controls. The positive controls consisted of amplifying a known amount of purified target DNA. This control ensured that the PCR reagents were properly assembled and that the DNA *Taq* polymerase was functional. The negative control reactions lacked DNA and served to identify if reagent stocks had been contaminated with target DNA, or if cross-contamination of target DNA had occurred during reaction preparation. The blank DNA extraction controls consisted of processing unused filters using the standard DNA extraction procedure. This allowed identification of cross-contamination during the DNA isolation step. The spiked controls involved adding the same quantity of target DNA as used for the positive control reactions to a random subset of extracted DNA reactions. Reduction of PCR amplification in the spiked control reactions relative to the positive control reactions indicated PCR inhibition. The most common causes of PCR inhibition include the carry over of cations, carbohydrates or phenolics (humic compounds) that adversely affect the function and specificity of DNA polymerases. Modification of the PCR reaction pH by compounds extracted with the DNA can also inhibit PCR reactions or cause production of nonspecific amplification products. Omission of these crucial controls can lead to misinterpretation of the field PCR results. Additional details of assay development including field sample collection, DNA purification, primer design, and PCR optimization of will be discussed.

References:

R. W. Litaker, M. W. Vandersea, S. R. Kibler, K. S. Reece, N. A. Stokes, K. Steidinger, D. F. Millie, B. J. Bendis, R. M. Pigg, and P. A. Tester. 2003. Identification of *Pfiesteria piscicida* (Dinophyceae) and *Pfiesteria*-like organisms using ITS-specific PCR assays. Journal of Phycology 39: 754-761.

MICROPREDATORY BEHAVIOR AND PATHOGENICITY IN *Pfiesteria*-LIKE DINOFLAGELLATES

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Pfiesteria piscicida and *Pfiesteria shumwayae*, members of the "toxic *Pfiesteria* complex" (TPC), are reported to secrete potent exotoxins in response to the presence of fish or fish excreta. These toxins are believed to be responsible for fish pathology and mortality as well as human illness. Recently, however, *P. shumwayae* was demonstrated to cause fish mortality through the process of micropredatory feeding upon the epidermal tissues of fish rather than through the action of a toxin. Other morphologically similar dinoflagellate species frequently occur in the same environment as *Pfiesteria* spp. Many of these organisms are heterotrophic and, like *Pfiesteria* spp., feed upon algal prey through a peduncle that attaches to the prey cell and extracts the cell contents into a food vacuole. We hypothesize that these other heterotrophic, penduncle-feeding *Pfiesteria*-like dinoflagellates may also have the ability to cause pathology to fish in a manner similar to what has been demonstrated for *P. shumwaye*. Using a larval fish bioassay we tested the ability of several species of heterotrophic dinoflagellates to cause pathology and mortality in larval cyprinodontid fishes. Additionally, we used membrane insert studies to evaluate the effects of direct vs. indirect contact. Results indicate that all species tested exhibited some degree of micropredatory attack behavior and several species caused fish pathology or mortality. None of the species tested caused pathology or mortality when not in direct contact with fish. The results of these studies indicate that, under controlled laboratory conditions, *P. shumwayae* is not unique in its ability to cause fish pathology or mortality as a result of micropredatory feeding upon fish epidermal tissues.

NUMERICAL MODELING OF THE JUAN DE FUCA EDDY

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The counterclockwise cold eddy off the Strait of Juan de Fuca has been implicated as an initiation site for toxic *Pseudo-nitzschia* cells. A diagnostic finite element circulation model was developed for this region and the northern Washington shelf. When forced with summertime temperature and salinity measurements, tides and typical summer winds, the model circulation shows strong retention in the eddy region and model drifter trajectories compare well with true drifters released in 2001 through 2003. Both model and true drifter trajectories show that surface waters tend to leave the eddy to flow southeastward along the Washington shelf. During storms onshore Ekman surface flow moves drifter pathways closer to the coast suggesting that phytoplankton from the surface waters of the Juan de Fuca eddy could impact the Washington coast.

Initialization of a prognostic numerical model (ROMS) is also underway. This model will both provide flow fields for a biological model and be used for process studies looking at the dynamics of the eddy itself. This model will also be used to further examine the transport of HABs to the coast, working towards a forecasting ability when forced by the UW MM5 atmospheric model.

MEDIATION OF BENTHIC-PELAGIC COUPLING BY MICROPHYTOBENTHOS: AN ENERGY- AND MATERIAL-BASED MODEL FOR INITIATION OF BLOOMS OF *Aureococcus anophagefferens*

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Prior research has demonstrated that growth of the brown Tide pelagophyte, *Aureococcus anophagefferens,* is stimulated by organic nutrients. However, even under nutrient-replete conditions, it also has a constitutively low maximum growth rate and growth becomes light-saturated at a low irradiance when compared to other estuarine species. This is so when grown with either nitrate or urea as the sole nitrogen source. It is therefore unlikely to out-compete the other species, except at low light, regardless of the availability of organic nutrients. Further, its high Chl*a*-specific absorption cross-section, large PSU size and very low ratio of photoprotective to photosynthetic carotenoids make it susceptible to photoinhibition. We argue that it is most likely to bloom in shallow waters when high turbidity provides a refuge from sustained exposure to bright light.

The light environment in many shallow bays is dominated by the dynamics of benthic resuspension. This is mediated by the presence of microphytobenthos (MPB), which stabilize the sediment matrix and resist transfer of material into the overlying water. The MPB also modulate benthic fluxes of nutrients and are capable of altering both the magnitude and the direction of flux. Photosynthesis and growth of the MPB is frequently correlated with the intensity of light penetration to the sediment surface. We present a model of bloom initiation in response to benthic coupling that is based on these three observations. The exchange is modulated by the MPB through one of two positive feedback cycles.

1) In one cycle, a dense assemblage of MPB reduces sediment-water exchange, maintaining high water clarity and low nutrient efflux. Increased water clarity sustains high growth rates of the MPB, reinforcing the condition. The high water clarity and low nutrient levels do not favor growth of *Aureococcus*. This condition will prevail only if shear stress at the sediment surface is relatively low.

2) In the other cycle, an initial resuspension event reduces water clarity. The consequent reduction in growth rate of the MPB causes a reduction in their density as grazing continues. This results in a reduction in their ability to stabilize the sediment matrix, leading to continued resuspension and a further reduction in water clarity. The increased turbidity and release of organic material as the MPB are grazed create the conditions that favor growth of *Aureococcus.* This condition will occur only if shear stress at the sediment surface is high enough to erode the initial population.

We present a suite of data in support of the model: the physiological responses of cells in culture; observations of the dynamics of resuspension under controlled conditions in a microcosm; and field observations from Quantuck and Peconic Bays, Long Island, NY. Our field data were collected before and after a moderate-sized bloom and in a non-bloom year. A comparison of bi-weekly cell counts collected from Quantuck Bay during the bloom by the Sussex County Department of Health Services with National Weather Service wind data recorded at an adjacent site indicate that the bloom occurred after a 2-day period of prolonged wind stress. This is the condition in which the transition from a low to high probability of bloom development is predicted by the model (i.e. erosion of high MPB density by increased shear stress).

MECHANISMS REGULATING THE LARGE-SCALE SEASONAL DEVELOPMENT OF *Alexandrium fundyense* BLOOMS IN THE GULF OF MAINE (U.S.A)

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Observations of *Alexandrium fundyense* in the Gulf of Maine indicate three salient characteristics of the vegetative cell distributions: (1) patterns of abundance are gulf-wide in geographic scope, (2) their main features occur in association with the Maine Coastal Current, and (3) the center of mass of the distribution shifts upstream from west to east during the growing season from April to August. The mechanisms underlying these aspects are investigated using coupled physical-biological simulations that represent the population dynamics of *A. fundyense* within the seasonal mean climatological flow. A model that includes germination, growth, mortality, and nutrient limitation is qualitatively consistent with the observations. Germination from resting cysts appears to be a key aspect of the population dynamics that confines the cell distribution near the coastal margin, as simulations based on a uniform initial inoculum of vegetative cells across the Gulf of Maine produces blooms that are broader in geographic extent than is observed. In general, cells germinated from the major cyst beds (in the Bay of Fundy and offshore of Penobscot and Casco Bays) are advected in the alongshore direction from east to west in the ambient coastal current. Growth of the vegetative cells is limited primarily by temperature from April through June throughout the gulf, whereas nutrient limitation occurs in July and August in the western gulf. Thus the seasonal shift in the center of mass of cells from west to east can be explained by changing growth conditions: growth is more rapid in the western gulf early in the season due to warmer temperatures, whereas growth is more rapid in the eastern gulf later in the season due to severe nutrient limitation in the western gulf during that time period. A simple model of encystment based on nutrient limitation predicts deposition of new cysts in the vicinity of the observed cyst bed offshore of Casco and Penobscot Bays, suggesting a pathway of re-seeding the bed from cells advected downstream in the coastal current. Seasonal spinup of a retentive gyre at the mouth of the Bay of Fundy would tend to favor re-seeding that cyst bed from local populations.

CELLULAR STRESS RESPONSES OF *Karenia brevis* TO HEAT AND OXIDATIVE STRESSES: IDENTIFICATION AND RESPONSE CHARACTERIZATION OF STRESS PROTEINS AND ANTIOXIDANT ENZYMES

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The environmental conditions under which *Karenia brevis*, the Florida red tide dinoflagellate, often exists and thrives are remarkably variable and seemingly non-ideal for bloom growth and maintenance. The primary line of defense against any factor inducing stress is at the biochemical level, underlying all effects at higher organizational levels; however, little is known about the cellular mechanisms by which *K. brevis* adapts to adverse and/or changing environmental conditions. The induction of stress proteins (Hsps) and/or antioxidant enzymes and measurements of photosynthetic efficiency (F_V/F_M) are commonly used indicators of cellular stress. To date, no published studies have identified any stress proteins or antioxidant enzymes in *K. brevis*. The current study identifies Hsp 60, chloroplast small heat shock protein (chlshsp), mitochondrial small heat shock protein (mitoshsp), manganese superoxide dismutase (Mn SOD), and iron superoxide dismutase (Fe SOD) in laboratory cultures of the Wilson isolate of *K. brevis*. These 5 proteins represent 2 superfamilies of stress proteins, chaperones (Hsp 60) and low molecular weight (LMW) Hsps (chlshsp and mitoshsp), and 1 superfamily of antioxidant enzymes, superoxide dismutases (Fe SOD and Mn SOD). *K. brevis* shows differential induction of a subset of these proteins in response to different stressors: mitoshsp is induced by heat stress whereas the chlshsp is oxidatively induced. Furthermore, the chlshsp responds differentially to various sources of oxidative stress (hydrogen peroxide, lead, or increased light). Light stress results in changes in F_V/F_M at levels that do not result in induction of chloroplast-specific stress proteins. In contrast, stressors that do not selectively target the photosynthetic machinery (all stressors excluding increased light) result in the induction of the stress proteins and antioxidant enzymes that are not paralleled by a decrease in F_V/F_M, suggesting that expression of these proteins represents a more immediate stress response and thus a more sensitive indicator of general cellular stress in *K. brevis*. These results provide, for the first time, evidence of stress proteins and antioxidant enzymes functioning in the adaptive mechanisms of *K. brevis* and, with further research, may provide a sensitive indicator of bloom health.

TOXICITY OF THE CHAIN FORMING DINOFLAGELLATE, *Alexandrium monilatum* ISOLATED FROM THE GULF OF MEXICO WITH PRELIMINARY STRUCTURAL DETERMINATION OF A NOVEL NON-POLAR TOXIN

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Cultures of *Alexandrium monilatum*, isolated from a red tide off Mississippi, were examined for the possible production of bioactive compounds. The strain, AM01, was grown in batch culture until mid-log growth phase and harvested. The resulting cell mass was extracted using an elutropic series of increasing polarity using 5 different solvents. Each extract was tested for activity using different live animal and cell based assays. Two distinctive toxic fractions were observed; a polar soluble fraction and a non-polar soluble fraction. The polar fraction was tested for saxitoxin-like activity using the STX receptor binding assay. This assay was negative therefore this species of *Alexandrium* does not produce saxitoxin or any saxitixon-like compound.

Structural determination of the icthyotoxic non-polar fraction is currently underway. This fraction was purified using a several step process using TLC and HPLC. Mass spectral analysis using both LC-MS and MALDI -MS of this purified compound yielded a molecular ion of 790 amu. Proton and carbon NMR structural analysis demonstrate a macrolide-like compound with four exo-cyclic double bonds. This compound has a nominal molecular formula of $C_{47}H_{98}O_8$. This new toxic compound is compared to known toxins produced by other species of *Alexandrium*.

DOES NITROGEN REGENERATION FROM THE N_2 FIXING CYANOBACTERIA *Trichodesmium* spp. FUEL *Karenia brevis* BLOOMS IN THE GULF OF MEXICO?

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Blooms of the toxic dinoflagellate, *Karenia brevis,* occur in the oligotrophic waters of the eastern Gulf of Mexico where known nitrogen (N) sources are insufficient to support observed biomass accumulations. Large *K. brevis* blooms frequently co-occur or occur subsequent to blooms of the N_2 fixing cyanobacteria, *Trichodesmium* spp. *Trichodesmium* alleviate N limitation where they occur by using atmospheric N_2. Much of the recently fixed N_2 is regenerated as NH_4^+ and dissolved organic N (DON). This regenerated N is then available to support the growth of other cells. We hypothesized that N regenerated from N_2 fixation provides the N necessary to support blooms of *K. brevis* in the Gulf of Mexico, and have conducted a combination of field and laboratory investigations to demonstrate a viable nutritional link and to quantitatively assess the role of *Trichodesmium* in providing N to support the growth of *K. brevis*. Preliminary results demonstrated that *Trichodesmium* fix N_2 at high rates with more than 50% of this new N released as NH_4^+ and DON, that *K. brevis* has a high affinity for reduced N sources and can extracellularly degrade some organic compounds. In addition to these indirect lines of evidence, we have conducted a number of studies to establish direct links between *Trichodesmium* and *K. brevis* production. In the field, stable isotopes were used to trace the uptake of $^{15}N_2$, its regeneration into dissolved N and its subsequent uptake into *K. brevis* biomass. Dialysis bags containing *Trichodesmium* were suspended in gas-tight incubation bottles containing *K. brevis* and $^{15}N_2$ enriched water. We observed that regenerated ^{15}N label (as NH_4^+ and DON) passed through the dialysis bag and was taken up by *K. brevis*. In the laboratory, we have established continuous cultures of *Trichodesmium*, grown them at 3 different growth rates and determined that N released from *Trichodesmium* cultures could fuel *K. brevis* growth by directing the outflow from continuous cultures of *Trichodesmium* into recently isolated cultures of *K. brevis* growing on medium without added N. With these experiments we demonstrate that N released from *Trichodesmium* can support the growth of *K. brevis*.

OBSERVATIONS ON THE SEXUAL LIFE CYCLES OF *Pfiesteria piscicida* AND CRYPTOPERIDINIOPSOIDS (DINOPHYCEAE)

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The occurrence of meiosis is fundamentally important in defining the life history of organisms with a sexual cycle. Video photography and photographic series were used to document sexual life cycle events in *Pfiesteria piscicida* as well as closely related cryptoperidiniopsoid dinoflagellates. Individual pairs of fusing gametes were isolated, and their development was followed in single-drop microcultures with cryptomonad prey. The observed patterns of zygote development and postzygotic divisions were similar in these related taxa. Isolated, motile gamete pairs each typically fused within 30 minutes to produce a rapidly swimming planozygote with a typical dinoflagellate shape and two trailing flagella. Planozygotes grew in size as they fed repeatedly on cryptomonads. In < 12 hours in most cases, each planozygote formed a transparent-walled nonmotile cell (cyst) with a single nucleus. The prominent chromosome movements of nuclear cyclosis occurred in the nucleus of the mature zygote. Nuclear cyclosis is believed to coincide with the homologous chromosome pairing of meiotic prophase in dinoflagellate zygotes, prior to meiotic division. In *P. piscicida* it was determined that nuclear cyclosis occurred in either the mature planozygote or in the zygotic cyst formed by it. A single cell division occurred in the *P. piscicida* zygotic cyst, and two offspring were produced that emerged from the cyst as biflagellated cells. In the cryptoperidiniopsoids, nuclear cyclosis occurred in the zygotic cyst formed by the planozygote. Following nuclear cyclosis, a single cell division occurred and two biflagellated offspring were produced. In both taxa, the two flagellated offspring formed by division of the zygote were motile and fed on cryptomonads before each forming a cyst. A single cell division in these cysts produced two biflagellated offspring that also fed before encysting for further reproduction. This pattern of zygote development and postzygotic divisions was confirmed in examples ($n > 9$) from different isolates of each taxon. Thus it was determined that in both *P. piscicida* and cryptoperidiniopsoids: 1) planozygotes were phagotrophic before encysting for cell division; 2) no lengthy dormancy occurred in zygotic cysts under these conditions; 3) nuclear cyclosis occurred in the zygote nucleus prior to nuclear division; and 4) the two divisions that followed zygote formation were spatially and temporally uncoordinated, unlike the pair of consecutive divisions that occur in the conventional pattern of meiosis. These results are discussed and compared to the sexual cycles of other dinoflagellates. Meiosis has only rarely been demonstrated in dinoflagellates, and has not yet been proven in *P. piscicida* or cryptoperidiniopsoids. The methods and results of this study provide new information toward clarifying the sexual cycle, including meiosis, in these dinoflagellates. Recent discrepancies in the literature regarding the sexual and asexual reproductive cycles in *P. piscicida* are also addressed.

HARMFUL ALGAL TOXINS AND STEROLS: NOT SO STRANGE BEDFELLOWS?

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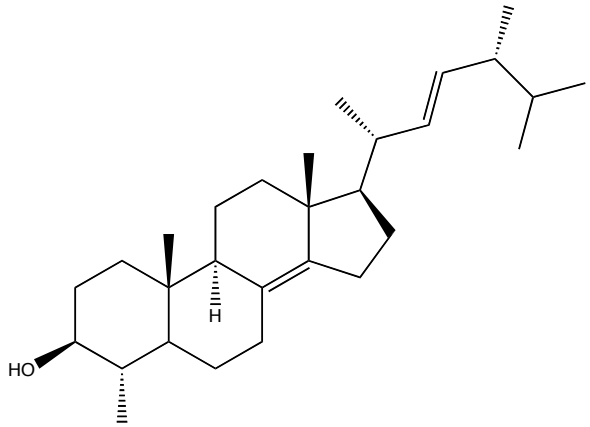
Recently, the unique sterols of *Karenia brevis* (Davis) Hansen & Moestrup were characterized by Giner et al., (J. Phycol. 39, 315-319, 2003) with the two predominant sterols being (24*R*)-4a-methy-5a-ergosta-8(14),22-dienol and (24*R*)-4a-methy-27-nor-5a-ergosta-8(14),22-dienol, called **gymnodinosterol** and **brevesterol**, respectively. These same two sterols were shown by Leblond and Chapman (J. Phycol., 38, 670-682, 2002) to also be the dominant sterols in *Karenia mikimotoi* and *Karlodinium micrum.*



Gymnodinosterol
(24S)-4α-methyl-5α-ergosta-8(14), 22-diene-3β-ol

Each of these groups produce polyketide toxins (brevetoxins, gymnocins, and a newly descried suite of toxins putatively called karlotoxins) which function by insertion into biological membranes. Our recent work on the mode of action of karlotoxins has found that sterol type effects toxin activity, with cholesterol being the most effective. Purified **gymnodinosterol** from *K. micrum* appears to be least effective in effecting karlotoxin activity. This finding is consistent with our observation that karlotoxins have little effect on *K. micrum* yet cause death or growth retardation in cells with cholesterol and ergosterol.

Two of the unique structural characters of these sterols are the 4-a methyl group and the 8,14 double bond in the C ring. These same characters are found in the major sterol, 4a-methyl-24-methylene-cholesta-8(14)-3b-ol, (amphisterol) of all species tested of the dinoflagellate genus *Amphidinium,* including *A. klebsii* which is known to produce a series of polyhydroxy-polyene compounds named **amphidinols**. Amphidinols exhibit a variety of biological actions such as antifungal, hemolytic, cytotoxic and icthyotoxic activites, identical to the activities we have found with karlotoxins. Moreover, this bioactivity is enhanced when cholesterol is present in the membrane.

Giner et al., (J. Phycol. 39, 315-319, 2003) proposed a possible function for these unusual sterols whereby the structural modifications render the sterols non-nutritious to marine invertebrates, reducing predation and thereby enhancing the ability of the dinoflagellates to form blooms. We would like to suggest one additional function. **The presence of these sterols in the dinoflagellate renders them impervious to the membrane disrupting effects of their own toxins.**

We will present our findings on toxin binding to artificial surfaces containing different sterols using surface plasmon resonance as a direct measure of interaction.

MESOZOOPLANKTON GRAZING OF MICROZOOPLANKTON: THE AFFECT OF FOOD WEB DYNAMICS ON THE POTENTIAL FOR HARMFUL ALGAL BLOOM FORMATION

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The impact of mesozooplankton grazing on protozoan microplankton and the subsequent impact on the phytoplankton biomass was measured in laboratory experiments. The calaniod copepod *Acartia* spp. was fed natural assemblages of microzooplankton collected from the Patuxent River, a tributary of the Chesapeake Bay, during mid and late April of 2002 and 2003. The percentage of ciliates lost to total *Acartia* spp. community grazing per day (g) was much greater in 2003 (43.5 and 26.2%) than in 2002 (0.69 and 1.7%). This corresponded with preliminary data showing an increased heterotrophic dinoflagellate concentration in April of 2003 above that of 2002, which suggests top-down control of ciliates as a factor influencing heterotrophic dinoflagellate biomass. *Acartia* spp. abundance was 1 to 2 fold greater in April of 2003 (2.3 - 2.7 L^{-1}) than in 2002 (0.04 – 0.15 L^{-1}). However, the differences in g were not a function merely of increased copepod abundance as the percentage of *Acartia* spp. body carbon ingested, as a function of ciliate biomass, was slightly greater during 2003. These results suggest that top-down control of microzooplankton communities (specifically herbivorous ciliates), resulting from increased copepod abundances and greater grazing rates, may be an important factor affecting the formation of algal blooms in the Chesapeake Bay.

EFFECTIVENESS OF CLAY FLOCCULATION FOR *Heterosigma akashiwo* BLOOM REMOVAL AND BENTHIC EFFECT STUDIES IN PUGET SOUND, WASHINGTON

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A *Heterosigma akashiwo* bloom was treated with phosphatic clay dispersed inside replicated, open-ended, floating mesocosms in East Sound, Orcas Island. Separately, bottom sediments rich with invertebrate infauna near a fish farm site at Cypress Island were exposed to the same area-rate of clay loading inside replicated cylindrical enclosures that were removed after the clay settled.

Methods: On 27 June 2003, after counts with a field microscope and visual observations confirmed an ongoing bloom, six cylindrical mesocosms of 1m diameter by 2.6 m deep were deployed in inner East Sound. Water temperature ranged from 17˚C at 0.1 m to 14.4 ˚C at 4 m on this hot, calm and sunny day, while salinity was near 28.3 psu at all depths. Clay loading was 300 g/m^2 (dry weight) of surface area, equivalent to 0.12 g/L inside each mesocosm, although cells may have not been equally distributed in the mesocosms. Clay was dispersed in each mesocosm over a 5 minute period with a small bilge pump. Water sampling was conducted with a pipe sampler. A Hydrolab 4a multiprobe with standard probes, a SCUFA fluorometer and Wetlabs Inc. shuttered turbidity probe were used for vertical profiling. Cell counts were performed with live samples the same day, using wet mounts of 0.1 ml volumes. Benthic treatments were conducted in 11 m depth (MLLW), 24 m downstream of a large commercial Atlantic salmon net pen farm. Despite the close proximity, the bottom was rich with clams, crab, snails, worms and demersal fish. Weighted, ABS plastic pipe hoops of 0.8 m diameter were randomly placed on the bottom in the test area prior to the treatment. Baseline grab samples were collected and then cylindrical concrete form tubes of 0.75 m diameter were placed inside of each hoop. The clay slurry was then directed from the surface through plastic tubing, into the concrete form tubes. After a settling period of 30 minutes the concrete form tubes were removed, leaving only the ABS pipe ring to mark the treatment area for subsequent diver-assisted grab sampling. Results available to date indicate that:

- Removal efficiency of *H. akashiwo* averaged 84% of the monospecific bloom in East Sound, comparable to or slightly less than prior studies of several other microflagellates in Puget Sound (Rensel and Anderson, in press, HABX proceedings). Removal was better than expected for the pre-treatment density of 2.8 x 10^6 cells/L compared to laboratory studies by Sengco et al. (2001, Mar Ecol Prog Ser).
- Time for cell removal was rapid, with these post treatment data reported for one hour after treatment.
- No significant differences were observed in DIN, PO_4 or *in vivo* or laboratory extracted chlorophyll *a* or phaeophytin before versus after clay treatment.
- Maximum turbidity was initially 150 to 190 NTU near the surface of the treated mesocosms, declining to <20 NTU within an hour. Subsurface values were consistently much less, as seen in prior experiments.
- Live cell counts were deemed useful to evaluate removal efficiency, due to the paucity of other species and the ease of identifying *H. akashiwo* in live versus preserved samples.
- I*n vivo* chlorophyll *a* sensors were not useful to monitor the cell removal, as other studies have shown East Sound to have significant CDOM (colored dissolved organic matter) content and our own observations have shown large surface areas of reddish water, often with very few live microalgal cells.
- Clay deposited on the bottom was rapidly re-suspended after removal of the concrete form tubes, which would be expected at typical, high-energy fish farm sites in the Pacific Northwest.

Benthic samples were collected for infauna and other types of analysis before and after treatment. Samples are being processed and results will be presented at this meeting.

AN INITIAL ASSESSMENT OF GYROXANTHIN-RADIOLABELING AS A TOOL FOR DETERMINATION OF *Karenia brevis* CARBON-SPECIFIC GROWTH RATES *IN SITU*

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Determination of *in situ* growth rates of HAB-forming species is critical to an accurate description of bloom dynamics but there are currently few reliable methods of directly determining growth rates on natural populations. We are examining the use of radiolabeling of the biomarker pigment gyroxanthin to determine growth rates of *Karenia brevis*. We compared photopigment-radiolabeling derived rates to those determined by time-course measurements of cell numbers and chl *a*.

Batch cultures of *Karenia brevis* (Texas clone SP3) were grown at 2 light levels (80 and 130 mmol m^{-2} s^{-1}) with nitrate, ammonium, or urea (20 mM-N) as the N source. At 130 mmol m^{-2} s^{-1}, chl-based growth rates were 0.31 ± 0.02 d^{-1} on nitrate, 0.28 ±0.01 d^{-1} on ammonium and 0.26±0.05 d^{-1} on urea; there was no significant difference between growth rates on the three forms of N. Cells grew significantly more slowly at 80 mmol m^{-2} s^{-1} irradiance. Rates were 0.25 ± 0.03 d^{-1}, 0.18 ± 0.01 d^{-1} and 0.19 ± 0.02 d^{-1} for ammonium, nitrate, and urea cultures, respectively. Again, there was no significant difference between the nutrient treatments. A significant light x nutrient interaction was observed in that the light level had a significant effect on the growth rate of cells grown on nitrate.

Photopigment (Chl *a*)-based growth rates measured on batch cultures in exponential growth were not significantly different from the time series (chl a) growth rate measurements when the calculation method of Goericke & Welschmeyer (1992) was used. Gyroxanthin-based rates were more difficult to discern, because gyroxanthin concentrations per cell were low and growth rates were slow, hence it was difficult to get a good radiolabeled gyroxanthin signal (above background). When gyroxanthin rates could be calculated (in the fastest growing cultures) they agreed well with the chl a based rates (both radiolabeling and time course approaches). Future experiments will examine differences between growth rates of cells in semi-continuous culture (~balanced growth) and rates determined by the photopigment radiolabeling approach.

References:

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GENE EXPRESSION PROFILING IN MOUSE BRAIN AFTER DOMOIC ACID EXPOSURE

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Domoic acid (DA) is a rigid analog of the neurotransmitter glutamate and a potent agonist of the kainate subtype of the glutamate receptor. Persistent activation of these receptor subtypes results in rapid excitotoxicity, which leads to calcium dependent cell death and neuronal lesions in areas of the brain where glutaminergic pathways are heavily concentrated. To better understand the mechanisms and pathways involved in response to toxic levels of DA in mammals, we have employed microarrays to study global gene expression in the mouse brain. Adult ICR mice were subjected to an IP, LD50 dose (4mg/kg) of DA while controls received PBS. Mice were sacrificed and immediately dissected at 30, 60 and 240 minute time points. Agilent catalog 20K feature mouse oligo arrays were run in triplicate for each time point, comparing a different experimental mouse against a pooled control for each time point, totaling nine arrays. Using Acuity v3.1 software, the arrays were filtered for quality based on signal intensity and signal to noise ratio. The remaining features from the replicate arrays were averaged and used for K-means and hierarchical clustering. Approximately 2-2.5% of all the genes represented on the array showed a significant change in expression compared to controls. Using selected genes, array expression data was additionally verified through RT-PCR. Fos, a calcium dependent immediate early response gene, was one of the highest up-regulated genes at both 30 and 60 minutes, which is consistent with previously published data. Both Fos and Jun-B, which dimerize to form an AP-1 transcription factor, followed a similar expression pattern of up regulation at the 30 and 60 minute time points, and a decrease down to basal levels at 240 minutes. Some other expected up regulated genes included pain and inflammatory pathway elements such as COX-2. Two genes involved in regulating gene transcription that were found to be down-regulated were transcription factor 7-like 2 and Janus kinase 3 (JAK3), although these genes are not known to work together. Down regulated genes comprised less than 40% of all differential expressors while up regulated genes contributed to over 60% of all differential expressors. K-means and hierarchical clustering produced groups of genes with similar expression patterns that may work in conjunction with each other.

Understanding the coordinated expression and interaction of clustered genes will most likely allow a better understanding of the mechanisms of neurotoxicity and neuroprotection.

BIOLOGICAL AND PHYLOGENETIC CHARACTERIZATION OF *Amoebophrya* sp. ex *Alexandrium tamarense*

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Amoebophrya ceratii is a parasitic dinoflagellate that infects several dinoflagellate genera. The first report of infections in *Alexandrium tamarense* was made by Jacobson (1987), although cultures were not established at the time. In May 2003, infected *Alexandrium tamarense* were again observed during a bloom in Salt Pond, Eastham, MA. Water samples were collected and filtered through a 20-mm sieve, enriched with f/2-Si medium, and kept at 15ºC. Five to ten infected *Alexandrium* cells were isolated by microcapillary technique under a fluorescence microscope. The cells were then placed in each well of a 96-well plate containing f/2-Si medium as well as uninfected *Alexandrium tamarense* isolated from the same location. The plates were maintained at 15ºC. Free-living zoospores were observed after 1 to 2 days, but no vermiforms were found. Late-stage infections were seen after 4 to 5 days in several wells. These were then transferred to new wells with uninfected hosts. Basic features of the parasite and its life cycle were documented using video fluorescence microscopy (*in vivo*). Phylogenetic analysis and other biological characterizations are underway and will be presented at the symposium.

Culture experiments revealed that this parasite can infect several *Alexandrium* isolates from Salt Pond, as well as isolates from the Gulf of Maine (GTCA28, GTCA29), Alaska (PW06), Northern Europe (BAHME184), South Africa (SA2), Hong Kong (HK, HK1), Japan (OFO41) and Australia (ACPPO9). Surprisingly, this parasite from *Alexandrium tamarense* was also capable of infecting other dinoflagellate species including *Prorocentrum minimum* (CCMP1329), *Prorocentrum micans* (CCMP21), *Scrippsiella trochoidea* (SA2 Scripp) and *Heterocapsa triquetra* (Het), all of which are known previously to harbor *Amoebophrya* infections themselves. However, the infection was quickly lost in *P. micans*. The parasite did not infect the athecate dinoflagellates, *Akashiwo sanguinea* (GSBL) and *Gymnodinium instriatum* (GIAL177). Recent work suggests that *Amoebophrya ceratii* consists of a species complex with a large degree of host specificity (Coats et al. 1996; Coats and Park, 2002). However, these findings suggest that certain isolates of *Amoebophrya* may have a wider host range than previously found. Further studies planned with this isolate may provide new insights into this issue.

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PATTERNS OF MARINE MAMMAL STRANDINGS AND TOXIC *Pseudo-nitzschia*: A MULTI-YEAR PERSPECTIVE FROM CENTRAL CALIFORNIA

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Pseudo-nitzschia abundance, including that of the domoic acid (DA) producing *P. australis* and *P. multiseries*, has been tracked in Monterey Bay, central California for 3 years. Several blooms (i.e. periods with cells $\geq 5 \times 10^4$/l) of toxic species have occurred each year with DA levels high enough that planktivorous anchovy and sardines, if present, could become unfit for human consumption (i.e. reach $\geq$20 ppm DA). During this multi-year period, beach strandings of fish-eating marine mammals, particularly the California sea lion (*Zalophus californianus*), also have been recorded and their behavior sometimes has indicated intoxication by DA. Several dramatic events in Monterey Bay have already demonstrated the vulnerability of marine bird and mammal populations, especially the California sea lion, to DA toxins after consumption of planktivorous fish. Reports of such mass strandings of sea lions may be unusual developments or they may represent serendipitous recordings of more frequent but less commonly reported contamination of pelagic predators.

In this presentation, we provide the first multi-annual data set demonstrating the relationship between DA-producing microalgae and DA-related marine mammal strandings. The relationship is not expected to be a simple one, since both the usual vector of the toxin, i.e. plankton-feeding fish, as well as the marine mammals that feed on them have wide-ranging distributions and are not typically year round residents in the study area. For instance, toxic *Pseudo-nitzschia* could be abundant in the region, but only minimal numbers of planktivorous fish present at the time, resulting in the switch of local mammal diets away from contaminated vectors to species in food webs based on non-toxic primary producers. Similarly, there could be both toxic *Pseudo-nitzschia* and contaminated vectors in the region, but marine mammals may be absent from the bay or, if present, avoiding the heavily contaminated species in favor of alternate prey. Here we present data showing the multiyear cycle of toxic *Pseudo-nitzschia* species in the Monterey Bay region and the co-incident levels of DA in the plankton. We then show the regional pattern of stranded marine mammals whose behavior and/or post mortem findings suggest DA intoxication. We review possible explanations for matches and mismatches of toxic blooms with mammal deaths. These results will also show the extent to which DA is affecting marine mammals along the California coast, a phenomenon known in marine mammal rescue centers but only occasionally reported to the community of harmful algal bloom researchers.

AN INDIVIDUAL-BASED MODEL OF THE TOXIC ALGAE SPECIES *Pseudo-nitzschia multiseries*

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In 1987 an outbreak of a previously unobserved disease occurred in Canada and was traced back to the toxin domoic acid produced by the diatom *Pseudo-nitzschia multiseries*. Since then, fisheries closures due to domoic acid have occurred worldwide. *Pseudo-nitzschia* species produce domoic acid under nutrient stress, including low silicon or phosphorus under high nitrogen conditions. However, it is still unclear what conditions cause the dangerously high levels that have sometimes been observed.

We present an individual-based algae model detailing the physiology of an algal cell with a focus on nutrient and energy flows to delineate the causes of domoic acid production. The model has been adapted to the specific problem of *Pseudo-nitzschia multiseries* by including silicon dynamics, a frustule component, domoic acid production, and sexual reproduction. The individual model is incorporated into a population model using McKendrick-von Foerster partial differential equation.

The model is compared to experimental data from chemostat and batch experiments on two separate strains of *Pseudo-nitzschia multiseries*. The differences in parameter values required to fit each experiment reveal differences in the physiology of the two strains, specifically in nutrient uptake, photosynthetic rate and the level of toxin production possible. Simulations using the calibrated model show that silicon limitation must be concurrent with an abundance of nitrogen for domoic acid production to be high. Using this modeling approach allows us to peer into the internal nutrient pools of the cell to invstigate which nutrients are limiting to each process, revealing interesting internal nutrient dynamics.

While the focus of this work is on *Pseudo-nitzschia multiseries,* the model would be easily adaptable to other toxic algae species.

LONG-TERM BLOOM BEHAVIOR OF *Prorocentrum* SPECIES IN NARRAGANSETT BAY

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The annual bloom dynamics of *Prorocentrum micans, P. minimum, P. scutellum* and *P. triestinum* have been followed over a 38-year period in Narragansett Bay based on weekly sampling. Arrayed along their mean annual abundance: *P. triestinum* > *P. minimum* > *P. scutellum*> *P. micans*. Significant interannual and seasonal bloom patterns were observed. Annual mean abundance of *P. minimum* increased between 1959 to 1982; there has been a long-term decline in *P. triestinum* since 1966; *P. scutellum* has become rare since 1983, while mean abundance of *P. micans* has been more or less invariant over the four decades of sampling. While the bloom duration varied, the maximum mean weekly abundance occurred between weeks 23 to 25 for *P. micans, P. minimum* and *P. triestinum,* and week 30 for *P. scutellum*. Within these patterns, there is considerable interannual variability in whether *Prorocentrum* will bloom and, if selected for, which of the four species will bloom. There appears to be a repetitive sequence of high annual *Prorocentrum* abundance followed by a year of lower abundance. This variability in *Prorocentrum* bloom behavior is partly linked to whether there is a *Heterosigma akashiwo* bloom, an event influenced by a variety of factors, including grazer structure and growth of the important diatom *Skeletonema costatum.* Some aspects of this "open niche" and the influence of the long-term regime shift that has occurred in Narragansett Bay from a diatom to flagellate dominated system on *Prorocentrum* bloom behavior are discussed.

The long-term relationships between *Heterosigma akashiwo* and *Prorocentrum* blooms, and between the latter and *Skeletonema costatum* are discussed. The associations between long-term changes in climate, proxied as the North Atlantic Oscillation Index, the Groundwater Index and Palmer Drought Index, and habitat conditions of nutrients, their ratios and flushing intensity on *Prorocentrum* bloom behavior are also considered.

HARMFUL ALGAL BLOOM SCIENCE AND STANDARDS: ONE PERSPECTIVE

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There are scientific standards available as codes of ethics, codes of conduct, or requirements. Each of these has been developed to address specific issues, for example, intellectual property rights and proprietary authorship. In addition to this literature, there are articles, reports, and discussions on release and sharing of data, observations, techniques, and ideas. Institutes, governments and academics have contributed to discussions involving the following points: competition versus cooperation, how much data access to provide and when, how to resolve professional conflict problems, and other science policy issues. One particularly relevant discussion has been "Should publicly funded research be in the public domain"? There are even scenario-based videos that present the various topics, including misconduct, and give handling options.

Some of the early efforts in establishing science policy promoted openness and sharing of ideas and data as a mechanism to advance science. Today, as harmful algal bloom scientists, we are also being asked to share, collaborate or partner so the scientific community can have a more comprehensive picture of bloom dynamics, species identification and distribution, toxins and toxicity, pathways of toxin transfer, types of toxic events, etc. To this end, new analytical tools and predictive models are being developed so they can be used in management options.

Multiple component studies can require the expertise of a few to many scientists. In this cooperative, and at the same time competitive, environment the issue of intellectual property rights and authorship can arise. "How do you protect your data and ideas and still share?" becomes a paramount question.

There are arguments for "openness" versus limited or no access to data and scientists have followed both paths. Sharing can be initiated as scientific progress is made or it can be at the time of publication. This is essentially a decision of the data creator(s). Today, the amount of electronic data being generated has led to web-based data portals for shared information and data retrieval, and in some cases data management. The implications of the internet for not only data sharing but for data and scientific article dissemination is probably understated. There will obviously have to be standards for these databases, including their metadata, and for internet publications. How can the Harmful Algal Bloom scientific community be involved in evaluating standards and setting standards if necessary?

More collaborative projects to capitalize on expertise and resources can often result in multiple authors on publications. Some journals are responding with "standards of responsible authorship", for example, restricting authorship only to those contributing to the following: original concept, design, experimentation, testing, analysis, and write-up or some other aspect detailed in their instructions to authors. In these examples, a letter of submittal has to be signed by all authors indicating that they accept responsibility for the content of the manuscript. For a young harmful algal bloom scientist in today's competitive yet cooperative environment, there may be more effort needed to gain recognition and be successful in grant funding. Hopefully, openness and sharing will still be part of our fiber.

EVALUATING HYPOTHESES FOR THE INITIATION AND DEVELOPMENT OF *Alexandrium fundyense* BLOOMS IN THE WESTERN GULF OF MAINE USING A COUPLED PHYSICAL-BIOLOGICAL MODEL

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A coupled physical/biological model and observations are used to investigate the factors governing the initiation and development of an *Alexandrium fundyense* bloom in the western Gulf of Maine during the spring of 1993. The physical circulation is modeled with a 3D primitive equation model forced by climatological elevation fields and observed winds, irradiance, and river outflow. This is coupled with a biological model constructed from laboratory and field data that estimates the germination and growth rates of *A. fundyense* as a function of environmental conditions. Four biological model structures of increasing complexity are considered, with each structure representing a hypothesis for factors controlling bloom initiation and development. The model/data fit is optimized over the uncertainty in the parameters to which the model is most sensitive. The significance of changes in the model/data fit between structures is quantified using a maximum likelihood ratio test. Biological models incorporating a strong dependence of growth on dissolved inorganic nitrogen (DIN) produce results that are significantly better (>90% confidence) at matching observations than those that do not. The optimal simulation generally reproduces mean regional cell levels in time and space, but considerable misfits remain at individual points. Diagnosis of the best-fit model solution suggests that cysts germinating from sediments at greater than 50 meters depth account for the majority of cells within the study area. However, cells germinating from cysts in shallow waters (< 50m), and the inflow of cells from the eastern Gulf of Maine can make significant contributions to the cell budget, particularly late in the spring and inshore. The growth of *A. fundyense* is strongly limited by low water temperatures until mid-May and by low levels of DIN afterward. These alternating limitations prevent growth from dramatically enhancing the initial cyst-driven source. This is consistent with previous work (Franks and Anderson, 1992) stating that patterns in cell abundance and toxicity in the region are consistent with the along-shore advection of established populations of *A. fundyense*.

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MICROZOOPLANKTON GRAZING AND THE POPULATION DYNAMICS OF HABS

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Harmful algal blooms (HAB) are evidence of uncoupling of predator:prey dynamics. Why does this happen? Microzooplankton grazing is usually tightly coupled to phytoplankton growth in the sea. Are the interactions between HAB species and microzooplankton an exception to grazing control? Three types of interactions between HABs and microzooplankton can be distinguished. Type I interactions involve HAB species that are an appropriate size for most microzooplankton but are not grazed. Blooms of these species tend to persist and disrupt planktonic food webs. Examples are brown tide, and toxic blooms of *Chrysochromulina spp.* and *Prymnesium parvum* (Gobler et al., 2002; Jakobsen et al., 2001; John, 2002; Tillman, 2003). Type II interactions involve relatively large cell size HAB species that have specialized microzooplankton grazers. Grazing often contributes to the decline of these blooms. In some cases, micro- and mesozooplankton can serve as vectors for toxin transfer to higher trophic levels. Examples are *Chattonella antiqua, Heterosigma akashiwo* and some of the large cell size HAB dinoflagellates (Nakamura et al., 1992; Jeong et al., 2003; Matsuyama et al., 1999). Type III interactions involve small and medium cell-size HAB species that are susceptible to grazing when at relatively low densities mixed with other phytoplankton species. However, at bloom densities, these species often are not grazed or have a detrimental affect on grazer populations. Many HAB dinoflagellates appear to have this type of interaction with grazers (Johnson et al., 2003; Hansen, 1995; Kamiyama, 2000; Stoecker and Gustafson, 2002; Rosetta and McManus, 2002). In type III interactions, microzooplankton grazing has important role in populations dynamics. Initiation of blooms may be suppressed or regulated by microzooplankton grazing. However, microzooplankton grazing may have little effect on dense blooms of these algae.

"Windows" of low grazing pressure that coincide with appropriate algal growth conditions are necessary for Type III blooms. Experimental studies of three small dinoflagellates (*Pfiesteria piscicida*, *Prorocentrum minimum* and *Karlodinium micrum)* that are harmful or nuisance species in the Chesapeake Bay region have shown that potential microzooplankton grazing is usually greater than potential growth rate, but that temporal and spatial "windows" of low grazing pressure occur (Johnson et al., 2003; Stoecker et al., 2000; Stoecker and Gustafson, 2002). Hypotheses for "window" formation include top down control of microzooplankton by mesozooplankton after the spring diatom bloom, freshets that flush-out resident microzooplankton populations or create conditions unsuitable for their rapid growth, and alterations in microzooplankton species composition due to eutrophication and/or pollution.

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DETECTION AND MONITORING OF HARMFUL ALGAL BLOOMS WITH SATELLITE

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Since the late 1970's, remote sensing with both ocean color and temperature has been found to have the potential to detect and monitor harmful algal blooms.[1-4] With the launch of the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) in September 1997, interest was renewed in the use of ocean color imagery. A monitoring program in the Gulf of Mexico began in September 1999 with imagery constituting a key part of that program.[5]

The efforts on *Karenia brevis* blooms in the Gulf of Mexico have revealed several potential capabilities for imagery as a part of a detection and monitoring program. While spectral methods have been presumed to be preferential, they can be problematic. *Karenia* spp. are dinoflagellates and many of the other common blooms are diatoms, which often have quite similar pigment suites.[6] In fact, many HAB species are either dinoflagellates or diatoms. Several solutions are being used for the Gulf of Mexico. The nature of these solutions may allow new options for exploring HABs in other environments.

1) Ecological detection. The primary detection method involves an ecological solution. *K. brevis* usually dominates the biomass from late summer through winter. As a result, significant new blooms are usually *Karenia*. New blooms are identified from imagery as an increase in chlorophyll-a concentration of > 1 g L^{-1}. This method is effective over 80% of the time[7], and can apply in turbid water, where most optical methods would fail.
2) Ecological discrimination. While this method is effective in Florida, resuspension events in Texas frequently lead to the appearance of new blooms. The resuspension appears to introduce benthic phytoplankton to the water quality, given the impression of a new bloom. The chlorophyll associated with resuspension can be estimated and eliminated, this allows for the potential of discriminating the presence of ambient blooms in the water column, from the resuspended "blooms". [8]
3) Optical techniques. Differences in backscatter between phytoplankton may allow discrimination that is not possible from absorption. *K. brevis* appears to have less backscatter than diatoms, allowing for discrimination.[9] The solution allows for potential separation of blooms in optically simple water.
4) Ecological associations. Blooms of *K. brevis* appear to start and intensify under upwelling favorable winds.[5] This association indicates that coastal blooms appearing under these conditions are more likely to be *K. brevis*.

These methods broaden the potential use of remote sensing and have counterparts with HABs in other regions.

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DOMOIC ACID AND *Pseudo-nitzschia* OFF THE WASHINGTON COAST: THE FIRST SEASON OF ECOHAB PACIFIC NORTHWEST CRUISES

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Pseudo-nitzschia spp. are frequently observed over the continental shelf off Washington, near the Washington coast and in the Juan de Fuca eddy between Washington and British Columbia. Measurements made during cruises and beach sampling of seawater and shellfish in 1997 and 1998 were consistent with the possibility that domoic acid (DA) from the Juan de Fuca eddy appears to move southward in prolonged upwelling events and then onshore during the first major storm of the fall season, resulting in elevated concentrations of DA in razor clams on coastal beaches. Such events result in immediate closure of coastal clamming beaches, often for the entire season (or longer, due to the slow depuration of DA from razor clam tissue). During our first two ECOHAB cruises, an objective was to determine: (1) the distribution of *Pseudo-nitzschia* spp. in the eddy and coastal upwelling regimes, (2) the production of DA and release by *Pseudo-nitzschia* in response to environmental conditions, and (3) the potential impact of macronutrient availability on toxin production. We measured both particulate and dissolved levels of DA by receptor binding assay and enzyme linked immunosorbent assay near the Juan de Fuca eddy and off the Washington coast to determine the spatial distribution of toxin during the early (June) and late (September) upwelling seasons. Low but measurable levels of particulate DA were measured in the eddy, off the central coast near Kalaloch beach, Washington State, and off Barkley Sound, British Columbia, Canada. In early June particulate DA measured less than 30 pM in the eddy region, but increased to a maximum of 500 pM in surface waters after a storm in mid-June. Depth profiles of particulate DA to 50 m indicated that the highest levels were concentrated in the upper 10 m of the water column and at frontal zones near the edge of the eddy. Shipboard analysis of DA allowed a responsive sampling strategy to be used for onboard nutrient manipulation and grazing studies.

IMPACT OF ZOOPLANKTON GRAZING ON *Alexandrium* spp. BLOOMS IN THE OFFSHORE GULF OF MAINE

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Zooplankton grazing was investigated in 22 shipboard experiments during natural blooms of *Alexandrium* spp. (*A. fundyense* and *A. ostenfeldii*) in the offshore Gulf of Maine in spring and/or summer of 1998, 2000, and 2001. Grazing studies were done in conjunction with studies of accumulation of *Alexandrium* toxins in the zooplankton, as part of the ECOHAB-Gulf of Maine regional program. Several species of copepods, marine cladocerans, and appendicularians were allowed to graze upon natural phytoplankton assemblages at natural abundances, and ambient temperatures (14-17^{o}C). Grazing was measured by quantitative microscopic analyses of disappearance of cells in experimental, compared to initial and control suspensions, preserved in Utermöhl's solution. Thus, we were able to examine grazing upon *Alexandrium* in comparison to grazing on all other co-occurring phytoplankton taxa. Even during *Alexandrium* "blooms," this dinoflagellate was a minor component of the overall phytoplankton assemblage, present at stations where grazing experiments were conducted at low levels of 0.12-5.11 cells ml^{-1}, or 0.03-3.9% of total phytoplankton cells present. Phytoplankton assemblages were dominated by athecate microflagellates (3-6 µm diameter), and secondarily by diatoms and non-toxic dinoflagellates. Microflagellates were present at abundances of 159.62-793.93 cells ml^{-1}, accounting for 60.6-95.6% of total cells. Ingestion of *Alexandrium* spp. and microflagellates accounted for up to 3.2% and 35.6-98.2% of total grazing, respectively. Grazing was significantly non-selective, with *Alexandrium* spp. and microflagellates being ingested in similar proportions to their availability in food assemblages. There were no apparent adverse effects on grazers during incubations of 18-24 hours, and grazer survival was 100%. Multiplication of daily experimental rates of ingestion of *Alexandrium* spp. per grazer by *in situ* abundances of grazers used in experiments permitted estimations of the proportions of the *in situ* populations of *Alexandrium* removed daily by experimental grazers. Experimental grazers removed a mean of 5.79% of *Alexandrium* spp. cells (range = 0-117%), but 18 of 22 experiments (81.8%) exhibited experimental grazer impacts of < 1% of *in situ* populations of *Alexandrium* spp.. The few instances where grazing impact was high were more due to high abundances of experimental grazers than to high ingestion rates of individual grazers, or unusually low abundances of *Alexandrium* spp. These estimates are undoubtedly conservative, in that they do not account for additional potential grazing by abundant zooplankters such as copepod nauplii and the copepod *Oithona similis* that were not used as experimental grazers. However, concurrent studies of toxin accumulation in smaller zooplankton size fractions that were dominated by such small zooplankters revealed low levels of toxin accumulation, suggesting that they do not appreciably ingest *Alexandrium* spp.. We conclude that due to its low *in situ* abundance in the offshore Gulf of Maine, *Alexandrium* appears to be an unimportant component of the diets of its grazers. Thus, the antipredation effects of high concentrations of *Alexandrium* on some grazers that have been reported from laboratory studies probably occur in nature only rarely, due to low *in situ* abundances of *Alexandrium* spp., and dilution of adverse effects of *Alexandrium* toxins on their grazers by ingestion of other co-occurring food sources. Further, the impact of zooplankton grazing on *Alexandrium* spp. populations in the Gulf of Maine appears to be minimal, except in rare cases where abundances of grazers are unusually high.

AN ALGICIDAL BACTERIUM ACTIVE AGAINST *Karenia brevis*: INVOLVEMENT OF A DISSOLVED COMPOUND

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Cytophaga sp. (strain 41-DBG2) is a bacterium isolated previously in our laboratory and found to be algicidal against the brevetoxin producing dinoflagellate, *Karenia brevis*. In fact, many algicidal bacteria identified by other investigators also belong to the Cytophaga-Flavobacterium-Bacteroides (CFB) group. Algicidal activity may be induced either via direct attack of a target alga by the bacterium or indirect attack through release of a dissolved substance. Recent investigations have been aimed at obtaining insights as to the nature of 41-DBG2's killing activity. Results of experiments involving the co-culture of *K. brevis* (isolate C2) with strain 41-DBG2 indicate that this bacterium produces a soluble, heat-sensitive, algicidal compound with a molecular weight between 0.5 and 300 kDa (thereby eliminating viral-mediated activity) and capable of killing various algal species. Dilution of filtrate (<0.22 μm) from the above co-culture with filtrate from a co-culture of *K. brevis* (isolate C2) and the non-algicidal bacterium, *Cytophaga latercula*, illustrate a concentration-dependent killing by the dissolved 41-DBG2 algicidal agent upon re-inoculation with *K. brevis* (isolate C2) (Figure 1).

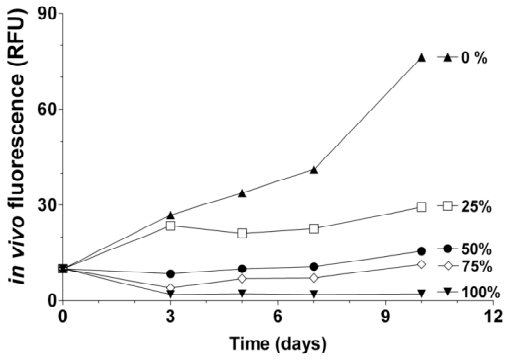

Figure 1. Growth of *Karenia brevis* (isolate C2) in diluted filtrates from *K. brevis*/41-DBG2-killed co-culture. Killed co-cultures were filtered (<0.22 μm), re-amended with *f/2* nutrients, and then diluted (100, 75, 50, 25%) with co-culture filtrates of *K. brevis*/*Cytophaga latercula* (non-algicidal). Negative controls (0%) were co-culture filtrates of *K. brevis*/*C. latercula* only, which showed normal growth of the re-inoculated *K. brevis* cells.

Algal taxa susceptible to lysis by the 41-DBG2 algicidal agent include the dinoflagellates *K. brevis* (strains C2 and Wilson), *K. mikimotoi*, *Gymnodinium simplex*, and the diatom *Skeletonema costatum*. Non-susceptible or resistant algal strains include selected *K. brevis* strains (NOAA-1, C5), the dinoflagellate *Akashiwo sanguinea*, the diatoms *Thalassiosira weissflogii* (CCMP 1336) and *Chaetoceros neogracile* (CCMP 1318), and the raphidophyte *Heterosigma akashiwo* (CCMP 1870). In the case of *K. brevis* isolates, work in our laboratory has demonstrated that differences in susceptibility reflect the ability of the resident bacterial assemblage to confer resistance by some yet to be determined mechanism.

Current work is aimed at using bioassay-guided fractionation (HPLC) of dialyzed culture filtrates to isolate and identify the 41-DBG2 algicidal agent. Subsequently, mode of action studies will be undertaken in order to better characterize this potentially novel compound.

CIGUATERA FISH POISONING ASSOCIATED WITH OIL PRODUCTION PLATFORMS ALONG THE TEXAS COAST

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Ciguatera Fish Poisoning (CFP) is the most common form of HAB intoxication known, and accounts for > 90% of the medical costs associated with HAB toxins in the U.S. The causative organism, *Gambierdiscus toxicus*, is endemic in tropical waters throughout the world, and is part of a benthic dinoflagellate assemblage that produces a variety of water- and lipid-soluble toxins. Polyether toxins produced by *G. toxicus* are transformed and biomagnified up the food web and accumulate as ciguatoxin (and its derivatives) in upper level predators such as barracuda, grouper, and jacks. Other toxins in the benthic dinoflagellate assemblage may accumulate as well and are suspected to play a secondary role in producing the diverse variety of symptoms that are typical of ciguatera.

Ciguatera-causing dinoflagellates are typically associated with coral reef ecosystems but have been noted in pelagic *Sargassum* communities as well. The Texas coast has no coral reef systems except for the Flower Gardens National Marine Sanctuary well offshore and is generally considered a low risk area. However, oil production platforms are common along the coast and extend out past the edge of the continental shelf. The only reported cases of ciguatera come from barracuda caught at these rigs. The toxin burden in the barracuda population and presence/absence of *G. toxicus* remains unknown. This is potentially a serious public health risk since barracuda are commonly eaten in Texas, and few physicians are familiar with ciguatera symptoms.

In order to provide baseline data for assessing the distribution of both toxic fish and *G. toxicus*, we conducted a fish toxicity survey and examined potential dinoflagellate substrates. Barracudas collected from oil production platforms were examined for ciguatoxins using the receptor binding assay and cytotoxicity assay. Substrate (mixed algae, barnacles and other benthic fouling organisms) were collected from platforms and examined for *G. toxicus*. In addition, *Sargassum* was collected as well when present. As part of the fish donation process, fishers volunteered information on fish consumption and related symptomology that permitted documentation of probable ciguatera cases in the past.

At the time of this writing, over 100 barracuda (0.8-21.3 kg) have been collected from over a dozen sites including the Flower Gardens National Marine Sanctuary. Initial assays indicate the presence of toxic fish from the rigs and from the Flower Gardens. *G. toxicus* and *Prorocentrum lima* have been identified from platform substrate. Two additional incidents of CFP have been found in the past 10 years, with one case involving multiple poisonings by relatively small fish (approximately 1 m, about 9-10 kg weight). Ciguatera appears to be endemic to Texas coast, although at this time we cannot determine if the toxins are incorporated locally, or are being transported via fish migrations.

PATHOGENICITY OF *Pfiesteria piscicida* AND *P. shumwayae* TO FISH: DOSE RESPONSE STUDIES

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Pfiesteria piscicida and *P. shumwayae*, members of the toxic *Pfiesteria* complex (TPC), are reported to secrete potent exotoxins responsible for fish pathology and mortality as well as human illness in mid-Atlantic estuaries. We recently demonstrated that *P. shumwayae* causes it's adverse fish health effects through the process of micropredatory feeding on the epidermis rather than by secretion of a potent exotoxin, as previously claimed. We have observed a similar mechanism of fish killing in *P. piscicida* and several related lightly armored heterotrophs. However, the biological, "toxicological" and ecological determinants that modulate pathogenicity of these organisms are not well understood and urgently require clarification. Using our new larval fish bioassay, we therefore conducted dose response studies with *Pfiesteria* spp. to clarify issues affecting time-to-fish-death, with particular focus on the role of zoospore density. Cell density was observed to be a critical determinant of time-to-death, with higher initial zoospore concentrations resulting in more rapid time to fish-death for all cultures tested. Toxigenicity (e.g., exotoxin secretion) was not a determinant of fish pathogenicity for any species or strain tested, however, ability to kill fish varied tremendously among the two species.

INCREASED MAGNITUDE AND GEOGRAPHICAL SPREAD OF HARMFUL ALGAL BLOOMS IN PUGET SOUND, WASHINGTON STATE, USA

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Puget Sound, Washington, is a complex, deep fjord with a long history of paralytic shellfish poisoning (PSP) in its northern basins. We have examined the general trends for PSP in Puget Sound using forty-five years of data collected by the Washington State Department of Health (WDOH). Although the dataset has certain limitations, including the lack of consistency in number of samples and collection sites, we conclude that the approximately ten-fold increase in maximal levels of paralytic shellfish toxins over the last four decades is not due to increased sample frequency. Since 1978, 'historically' unaffected areas within southern Puget Sound have experienced more frequent and intense outbreaks of PSP indicating a southward spread of toxigenic algae over the past four decades. By 1988, the first shellfish harvest closures occurred in the southern areas of Puget Sound. A combination of factors may have contributed to this geographical spread including: 1) increased urbanization and population, 2) the movement of *Alexandrium* cells and/or cysts past sills from northern Puget Sound into the central and southern basins. Although greater numbers of closures have been observed over time, the percentage of closures relative to the total sites monitored has decreased in all but south Puget Sound. Rigorous monitoring by WDOH has resulted in a greater number of open shellfish harvesting sites in the Puget Sound region where the risk for PSP is high.

IRON LIMITATION OF NATURAL PHYTOPLANKTON ASSEMBLAGES ASSOCIATED WITH THE PACIFIC NORTHWEST ECOHAB *Pseudo-nitzschia* BLOOMS

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Community bioassay experiments were performed during the first two cruises of the Pacific Northwest ECOHAB project – a study designed to investigate environmental factors that influence the formation and domoic acid content of *Pseudo-nitzschia* spp. that periodically bloom in a local large scale eddy (Juan de Fuca eddy) and coastal upwelling regions along the Washington and Oregon coasts. Surface waters were collected from regions of the Juan de Fuca eddy and micronutrient availability regulated by iron and copper enrichment, or the addition of metal chelators (desferal) during on-deck incubation experiments. Growth responses were determined from the increase in whole community chlorophyll concentrations, and the concomitant drawdown of macronutrients. The resulting impact of the supplements on the community composition was recorded through microscopic observations, flow cytometry, the productivity of phytoplankton (^{14}C-uptake) and heterotrophic bacteria (^{3}H-leucine) and concentration of dissolved and particulate domoic acid.

The findings from the initial ECOHAB cruise (June 2003) showed that the planktonic community was iron-stressed despite being closely associated with water influenced by coastal discharge. The effect of iron additions was minimized with the addition of the iron chelator, desferal. These bioassay experiments will be repeated during the second cruise in September 2003 to determine if there are seasonal differences in the iron nutrition in of this region, and the resultant effect of micronutrient manipulations on domoic acid production.

ABSTRACTS OF POSTERS

TRANSFER OF SAXITOXINS WITHIN THE INDIAN RIVER LAGOON, FLORIDA FOOD WEB

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Since January 2002, 23 cases of Puffer Fish Poisoning (PFP) were reported in four states due to saxitoxin (Quilliam et al., 2002) traced back to southern puffer fish (*Sphoeroides nephelus*) originating from the northern Indian River Lagoon (IRL) on Florida's east coast (Bodager 2002). Saxitoxin was previously unknown in Florida marine waters (Landsberg et al., 2002). Because puffer fish were involved in PFP we have now routinely screened > 400 southern, 40 checkered (*S. testudineus*), and 40 bandtail puffer fish (*S. dorsalis*) for saxitoxins (STXs) statewide using the Ridascreen® STX ELISA kits (Usleber et al., 1991). Since April 2002 selected biota from the IRL have also been tested for STX distribution and prevalence within the food web. The geographical hot spot for STXs in the IRL is in the north from Titusville south to Melbourne. Approximately 18.1% of samples (n = 791) were below the detection limit of 1 µg STXeq/100g tissue, 27.4% were below 80µg STXeq/100g tissue, while the majority of samples, 54.5% contained moderate to high levels of STX in a range of tissues. Except for southern puffer fish, the muscle, skin, and mucus of which contain up to 5865.5 µg STXeq/100g tissue, STXs are present but below regulatory levels in the muscle of checkered puffer fish, Atlantic spadefish (*Chaetodipterus faber*), striped burrfish (*Chilomycterus schoepfi*), and porcupine fish (*Diodon hystrix*). Recreationally prized fish such as sheepshead (*Archosargus probatocephalus*), Gulf flounder (*Paralichthys albigutta*), southern kingfish (*Menticirrhus americanus*) and spotted sea trout (*Cynoscion nebulosus*), contained up to 35.9 µg STXeq/100g tissue in the skin and mucus. Commercially significant species such as blue crabs (*Callinectus sapidus*) had a maximum of 11.1 µg STXeq/100g tissue in the hepatopancreas and whole hard clams (*Mercenaria* sp.) a maximum of 17.2 µg STXeq/100g tissue. A maximum of 116.5 µg STXeq/100g tissue was found in polychaetes (*Glycera dibranchiata*), 14.2 µg STXeq/100g tissue in gastropods (*Urosalpinx cinerea)*, 1.1 µg STXeq/100g tissue in brittle stars (*Ophiothrix spiculata*), and up to 4301.2µg STXeq/100g tissue in small non-harvestable whole razor clams (*Ensis minor*). With the exception of puffer fish, concentrations do not presently pose significant threats to public health but they indicate the significant transfer of STXs within the IRL food web. Both natural bloom samples and clonal isolates of *Pyrodinium bahamense* from the IRL have tested positive for STXs (Landsberg et al., 2002). On Florida's west coast, where *P. bahamense* blooms are less frequent and likely reach less toxic biomass, STX concentrations are markedly lower in biota than those in the IRL. By comparison, STX concentrations in southern puffer tissues from Florida's west coast were no higher than 2735.5 µg STXeq/100g tissue in the skin and mucus when compared with up to 10111.8 µg STXeq/100g tissue in the gut contents of southern puffers from the IRL. In addition, STX levels in west coast sheepshead and flounder are normally below our detectable limit. We still have to confirm the major transfer route of STXs from *P. bahamense*, the most likely source of STX, into puffer fish. Small benthic filter-feeding bivalves, a significant component of the southern puffer fish diet, are likely vectors for STX transfer and will be tested for toxicity in the upcoming months. Comparison of analytical methods is also required.

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ECOHAB-PNW AND ORHAB: A COLLABORATION TO UNDERSTAND THE DYNAMICS OF *Pseudo-nitzschia* BLOOMS ON THE COAST OF WASHINGTON STATE

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The Ecology and Oceanography of Harmful Algal Blooms-Pacific Northwest (ECOHAB-PNW) project and the Olympic Region Harmful Algal Bloom (ORHAB) partnership, have formed a strong synergy that will allow researchers and managers to better understand the dynamics of domoic acid-producing *Pseudo-nitzschia* blooms that frequently occur on the coast of Washington State. ECOHAB-PNW will study the physiology, toxicology, ecology and oceanography of toxic *Pseudo-nitzschia* species off the Pacific Northwest coast, a region in which both macronutrient supply and current patterns are primarily controlled by seasonal coastal upwelling processes. Recent studies suggest that the seasonal Juan de Fuca eddy, a nutrient rich retentive feature off the Washington coast serves as a "bioreactor" for the growth of phytoplankton, including diatoms of the genus *Pseudo-nitzschia*. The ORHAB partnership was formed in June 1999, in response to seemingly random closures of the shellfisheries due to outbreaks of marine biotoxins. It became clear that in order to manage these outbreaks there was a need to better understand underlying dynamics of these disruptive HAB events. The goal of ORHAB is to develop a cost-effective monitoring program for HABs that will be taken over by state managers and tribes at the end of five years (2000-2005). Together, these two projects aim to understand the environmental conditions that initiate and maintain blooms of harmful species, develop a sampling program and models for the prediction and mitigation of HABs, and test and implement new technologies. ECOHAB-PNW is funded by NSF and NOAA under the ECOHAB program while the ORHAB partnership is funded by the NOAA National Center for Coastal Ocean Sciences (NCCOS) under the Monitoring and Event Response to Harmful Algal Blooms (MERHAB) program.

DETERMINING THE FATE OF SAXITOXIN IN CENTRAL CALIFORNIA MARINE FISHERIES

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Saxitoxin, a phycotoxin responsible for the affliction Paralytic Shellfish Poisoning (PSP), occurs along the western coast of North America, yet in California the ecological fate of the toxin is virtually unknown. A goal of the present study is to understand the dynamics of toxin accumulation in potential vector species and its consequences with respect to benthic and pelagic food webs, particularly in several commercially important fish (e.g. anchovies, sardines, rockfish and flatfish). The principle objective of this research is to identify to what degree, if at all, saxitoxins contaminate commercial fisheries in central California. Specific aims are: (1) Evaluate the toxicity of anchovies, sardines, rockfish and flatfish when saxitoxin producing dinoflagellates are present, (2) Determine whether saxitoxin is bioaccumulated in fish tissues, (3) Test whether saxitoxin is detectable in fish tissue in the absence of toxic dinoflagellates in the water. Preliminary information, as available, will be presented on results to date. Fish and water samples collected along the central California coast (Monterey Bay, Santa Cruz, and San Mateo counties) will be analyzed for PSP toxins. Several individuals of each fish species will be pooled and toxin concentrations determined by the standard mouse bioassay and receptor binding assay. The presence of the toxin producing alga will be monitored using species specific molecular probes (whole cell and sandwich hybridization). By examining the relationship between toxic algae and potentially contaminated fish this research will contribute to our understanding of harmful algal bloom ecology and determine if there is need for a PSP monitoring program in California commercial fisheries.

MITIGATION OF HARMFUL ALGAL BLOOMS WITH CLAY: A TEST OF THE EFFECTS OF REPEATED APPLICATIONS ON JUVENILE SOFT-SHELL CLAMS, *Mya arenaria*

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An apparent global increase in harmful algal blooms (HABs) has prompted research on control and mitigation strategies to reduce ecological and economic impacts. Inert clays have been used to effectively remove dinoflagellate bloom species from the water column at Asian fish farms and their use is being tested experimentally in the USA. Potential repercussions revolve around the increasing flux of suspended particles to the bottom in the case of a depositional flow environment, where animals such as juvenile suspension-feeding bivalves could suffocate and/or starve due to burial. The objectives of this study were to determine the effects of a sedimented layer of phosphatic clay (IMC-P), resulting from single vs. repeated clay applications, on survival and growth of a commercially important, infaunal, suspension-feeding bivalve, the soft-shell clam *Mya arenaria.* The rationale for this experiment is that repeated clay application is likely to be required over the duration of a HAB for effective mitigation under field conditions. Previous work on the hard clam, *Mercenaria mercenaria*, showed no significant growth inhibition by sedimented clay (Archambault et al., submitted) but only tested the effects of a single clay application.

Juvenile *M. arenaria* held in sand were subjected to the following parallel experimental treatments during two sequential trials: a) single addition of clay [1 g dry weight (DW) l^{-1} of clay] to a bloom of a representative non-toxic dinoflagellate species (*Heterocapsa triquetra*), b) multiple (10) clay-*H. triquetra* additions (0.25 g DW l^{-1}) at 2-day intervals over 3 wks after an initial application of 1 g DW l^{-1}, c) a control with no clay addition.

Experiments were conducted in 3 recirculating raceways, where low flow (<2 cms^{-1}) allowed complete sedimentation of clay, thus simulating a low-energy field scenario. Clams suffered no mortalities, and were able to rapidly regain contact with the overlying water column and resume feeding even when subjected to repeated clay applications. However, preliminary analysis indicates that multiple clay applications resulted in a significant reduction in *Mya* tissue growth rates, ranging from 8 to 20% relative to controls. The single clay treatment showed only a small reduction in tissue growth rate (2% in trial 1) or even an increase in growth (10% in trial 2) compared to the respective controls. In both clay treatments, 93% of the suspended clay at 1 g DW l^{-1} was removed from the water column within 20 minutes. During subsequent clay applications of 0.25 g DW l^{-1} in the multiple clay treatment, IMC-P removal ranged between 50-63% within 20 minutes. The depth of the deposited clay layer averaged 0.7 cm with the single application, and attained 3 cm with multiple clay applications by the end of the experiment. Repeated clay delivery and bottom organic enrichment (due to dinoflagellate sedimentation at the time of clay application) did not result in sediment hypoxia. Although *M. arenaria* grows well in fine-grained sediments in its natural habitat, this study indicates that under an extreme-case scenario of 10 repeated clay deposition events, small but significant growth inhibition may occur in this species. The number of applications required for effective mitigation remains to be established under varying field conditions. However, fewer clay applications (~2-3) than the maximum number tested here will likely be considered in bloom treatment efforts in the U.S. and worldwide, due to economic and logistical considerations. Our future work will investigate effects of sedimented clay/dinoflagellates on other potentially vulnerable epifaunal suspension-feeders (e.g. scallops) and on deposit-feeders, which may benefit from bottom organic enrichment.

NITROGENOUS PREFERENCE OF TOXIC *Pseudo-nitzschia* SPP. FROM ENRICHMENT EXPERIMENTS CONDUCTED IN IRON REPLETE AND IRON DEPLETE REGIONS IN CENTRAL CALIFORNIA

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The occurrence of harmful algal blooms appears to be increasing in frequency and intensity in recent years (Hallegraeff, 1993). This is especially evident along the central California coast where domoic acid poisoning caused by the diatom *Pseudo-nitzschia*, is of paramount concern to the ecosystem.

Although there have been many laboratory studies conducted, the physiological mechanism(s) that initiates the production of domoic acid in the field is currently unknown. These studies have suggested that silicate and iron limitation, as well as copper toxicity, can induce toxin production (Kudela et al., 2002, Maldonado et al., 2002). Despite many studies on toxin production, very few have evaluated the nutritional preference of *Pseudo-nitzschia*, particularly nitrogen sources.

A nutrient enrichment experiment was conducted off the coast of California from two different shelf regions to evaluate nutritional preference due to coastal runoff (or upwelling). A series of grow out experiments were designed to determine if there was a differential response to inorganic ammonium, nitrate or organic urea. Four separate carboys were used to add 50 μmol of NO_3 , 10 μmol NH_4 , 10 μmol urea, one with no addition (control) and grown in a deckboard incubator. There was very low rainfall at the time leading to oceanic conditions (low nutrient levels and an unstratified water column). The first grow out experiment was conducted off of the coast of Big Sur, a region characterized by high nutrient, low chlorophyll and low iron concentrations (Bruland et al., 2001). There was no growth regardless of the nutrient additions and very little biomass in the water initially, suggesting iron limitation. The second grow out experiment was conducted at the mouth of the San Francisco Bay (Bolinas Bay) and had very high initial biomass. Surprisingly, *Pseudo-nitzschia australis* bloomed regardless of the nutrient type added, and growth rates did not indicate a strong preference of nitrogen source. Light limitation and nutrient limitation coexisted as likely indicated by the increase in biomass for the control. The results of this experiment will be discussed in detail particularly, domoic acid concentration (overall and per cell), growth rates and changes in biomass, chlorophyll per cell, biogenic silica as well as community composition.

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LINKAGES BETWEEN BIOCHEMICAL FLUXES AND MIGRATION BEHAVIOR IN POPULATIONS OF THE RED TIDE DINOFLAGELLATE, *Karenia brevis*

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Prior laboratory studies and modeling have explored the possibility that the migratory behavior of the red tide dinoflagellate, *Karenia brevis*, is influenced by cellular biochemical fluxes. To determine whether there were systematic differences in biochemical fluxes in different stages of migration in natural populations, we measured the incorporation of photosynthetically fixed inorganic ^{14}C into major subcellular end products of *K. brevis* during a bloom event in Florida coastal waters in 2001. Samples were incubated in simulated *in situ* conditions on board ship, and determinations were made of ^{14}C-incorporation into low molecular weight materials (LMW), lipid, carbohydrate+nucleic acids, and protein. Measurements were also made of incorporation of ^{14}C into the nitrogen transport amino acids, glutamine and glutamate. Carbon flux showed systematically higher proportions in carbohydrate+nucleic acids and lower proportions in protein in surface samples compared to that in deep samples. Nutrient enriched samples exhibited enhanced protein incorporation in both surface and deep populations and decreased incorporation into carbohydrate +nucleic acids. Therefore the ratio of protein/ carbohydrate+nucleic acids appeared to provide an index of nutrient status. The ratio of carbon flux into glutamine relative to glutamate in nutrient enriched samples increased as much as 3.8-7.4 times. The enhanced carbon flux into glutamine in response to nutrient addition is consistent with a GS-GOGAT pathway of amino acid assimilation. Our results suggest that the patterns of biochemical fluxes differ among different migrating subpopulations, but that the patterns are sensitive to nutrient status.

SOLUBILIZATION OF A DOMOIC ACID BINDING SITE FROM PACIFIC RAZOR CLAM

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The Pacific razor clam, *Siliqua patula*, is known to retain domoic acid, a water-soluble glutamate receptor agonist produced by diatoms of the genus *Pseudo-nitzschia*, for periods up to one year. The mechanism by which razor clams tolerate high levels of the toxin, domoic acid, in their system while still retaining normal nerve function is unknown. In our study, a domoic acid binding site was solubilized from razor clam siphon using a combination of Triton X-100 and digitonin. In binding experiments using [^{3}H]kainic acid, the solubilized tissue showed a single high affinity binding site similar to the membrane-bound site. Competition experiments showed that the rank order potency for competitive ligands in displacing [^{3}H]kainate binding from the membrane-bound receptors was quisqualate>ibotenate>iodowillardiine =AMPA= fluorowillardiine >domoate>kainate>L-glutamate. At high micromolar concentrations, NBQX, NMDA and ATPA showed little or no ability to displace [^{3}H]kainate. Dissociation of [^{3}H]kainate was monophasic with a rate constant of 0.09 min^{-1}. The association rate constant was 2.1 x 10^7 $M^{-1}min^{-1}$. The kinetically derived K_D value was 4.3 nM. In contrast, competition experiments using [^{3}H]glutamate showed nanomolar affinities to L-glutamate and AMPA, but relative insensitivity to both kainic acid and domoic acid. These results suggest that razor clam siphon contains at least two subtypes of non-NMDA glutamate receptors, one that is highly sensitive and another that is insensitive to kainic acid and domoic acid.

MICROBIAL INTERACTIONS ASSOCIATED WITH THE TOXIC DINOFLAGELLATE *Karenia brevis*

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Harmful blooms of the toxic dinoflagellate, *Karenia brevis*, occur annually in the Gulf of Mexico along the west Florida shelf. Recent investigations suggest that algicidal bacteria may play a key role in regulating *K. brevis* blooms. Our laboratory has isolated three bacterial strains algicidal to *K. brevis*, two of which have been identified and characterized previously (Doucette et al., 1999). A third strain (S03) is currently being described and appears to require direct contact with algal target cells in order to induce killing. Efforts using both classical and molecular techniques are also directed at assessing bacterial composition and succession within samples of the natural microbial assemblage collected during a 2001 *K. brevis* bloom, with an emphasis on algicidal taxa. Our research has shown that all three algicidal bacteria are able to kill *K. brevis* isolate C2, yet other *K. brevis* isolates, also originating from west Florida shelf waters, are resistant (NOAA-1 and C5). Based on earlier findings (Mayali and Doucette, 2002), we hypothesize that antagonistic, bacteria-bacteria interactions within these latter cultures confer this apparent resistance to algicidal attack and work is underway to characterize these interactions as well as isolate and identify the bacteria involved. Results from preliminary experiments indicate that a dissolved substance is not associated with this antagonistic activity. Describing details and the nature of these interactions may help to better elucidate the role of algicidal, and corresponding antagonistic, bacteria in regulating the growth of harmful species such as *K. brevis*. Such information is also essential for the critical evaluation of bacteria as a potential component of more comprehensive management strategies for these toxic bloom events.

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COMPETITION FOR ORGANIC RESOURCES: BACTERIA VERSUS *Aureococcus anophagefferens*

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Blooms of the brown tide pelagophyte, *Aureococcus anophagefferens*, occur seasonally along the eastern seaboard of the USA, where high concentrations of dissolved organic nitrogen (DON) relative to dissolved inorganic N (DIN) are thought to fuel their growth. High dissolved organic C (DOC)/DON ratios are also thought to contribute to bloom formation. In addition to providing N, dissolved organic matter (DOM) can also provide carbon to cells and previous work has illustrated that *A. anophagefferens* assimilates both N and C from amino acids and dipeptides. While this may be advantageous for out-competing other phytoplankters when cell densities are high (e.g., 10^6 cells/ml) and self-shading becomes significant, there are new competitive challenges since bacteria are thought to be the primary consumers of DOM in nature. During 2002 and 2003, we have experienced intense brown tide blooms in Chincoteague Bay, MD and VA (2003 only). *A. anophagefferens* cell densities exceeded 1 million cells/ml during these blooms. During the 2002 bloom, we observed that photosynthetically-mediated uptake of bicarbonate and organic C uptake during the daylight was insufficient to meet the cellular C demand based on N uptake (C:N uptake ratio of 0.87). Therefore, during 2003, we examined inorganic and organic C and N uptake over diel cycles to see whether nighttime uptake of organic C could significantly reduce this imbalance. We compare uptake in the two size fractions, in order to correct for the bacterial contribution to organic C uptake. Preliminary results suggest that during the day, bacteria are responsible for most of the organic C uptake while *A. anophagefferens* primarily takes up bicarbonate. By contrast, during the night, uptake of organic C by the *A. anophagefferens* size fraction is substantial. These results suggest that photosynthesis during the daylight and DOM uptake at night combine to meet the C demands of *A. anophagefferens* in nature.

CAN NATURAL SELECTION FOR RESISTANCE TO PARALYTIC SHELLFISH POISONING TOXINS IN SOFTSHELL CLAM, *Mya arenaria*, POPULATIONS OCCUR DURING EARLY LIFE HISTORY STAGES?

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We have previously documented significant differences in the prevalence of individuals resistant to paralytic shellfish poisoning (PSP) toxins, measured by burrowing incapacitation and *in vitro* nerve resistance to saxitoxin (STX), among populations of juvenile (~30-40 mm in shell length) softshell clams, *Mya arenaria*, on both Atlantic and Pacific coasts of North America. Site-specific differences in the percentage of resistant clams generally correlate with the history of exposure to PSP, with highly resistant populations occurring in regions recurrently affected by red tides.

The main goal of this study is to test the hypothesis that natural selection for resistance to PSP toxins could potentially occur during early life history stages of *M. arenaria*. Progeny from a naïve, sensitive population from the Lawrencetown River estuary, southeastern Nova Scotia (NS) was used as a laboratory test population. Clam veliger larvae fed a non-toxic algal suspension spiked with a high-toxicity *Alexandrium tamarense* strain (PR18b, 29 to 80 µg STX equivalents $cell^{-1}$, mean diameter = 35 µm) for 1 wk showed no difference in survival or growth rate relative to non-toxified controls. This indicates that ingestion of toxic cells is required for adverse effects of PSP toxins to occur, as *Alexandrium* cells are too large for ingestion by bivalve larvae. In contrast, paralysis and significant mortalities of small juveniles occurred within 4 h of exposure to toxic *Alexandrium* cells, resulting in 95% cumulative mortalities of 4 mm juveniles (spat) following only one week of exposure. Mortalities are attributed to anoxia of the pallial cavity resulting from toxin-induced muscular paralysis and reduced irrigation of the pallial cavity. This rapid mortality of early post-settlement stages contrasts with previous findings for large (35-42 mm) juveniles from the same source population, in which mortalities varied considerably between experiments and were only initiated after 8-10 days of toxin exposure. Summer blooms of *A. tamarense* in the Bay of Fundy coincide with the main period of spawning and larval development of *M. arenaria* and also with the occurrence of small, year-2 spat. This study demonstrates that in post-settlement stages lethal effects of PSP toxins and thus strong selection of more resistant individuals could potentially occur within a few days of exposure to a highly toxic *Alexandrium* bloom. We are currently testing progeny (larvae and spat) from a resistant population in Lepreau Basin, Bay of Fundy for comparison.

MULTIVARIATE ANALYSIS OF HAB ORGANISM OCCURENCE ON A CROSS-SHELF TRANSECT IN LOUISIANA

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The Louisiana Shelf is a highly eutrophic system impacted by the outflow of the Mississippi and Atchafalaya Rivers. Several HAB organisms, and many other potentially harmful organisms are commonly found at certain times and under certain conditions on the Louisiana shelf. Increases in nutrients and nutrient pulses have been linked to HAB events in many places including the northern Gulf of Mexico, exemplified by increases in *Pseudo-nitzschia* spp. concurrent with increases in nutrients to the Louisiana shelf since the 1950's. Also, the current ratio of Si:N approximates 1 in the Mississippi River, which creates the possibility for Si to become limiting on the shelf, which is potentially a more favorable condition for non-siliceous flagellates, some of which could be harmful.

HAB organisms are a threat to coastal Louisiana and it is important to understand what conditions and environmental variables influence their distribution along the Louisiana Shelf. A long time series from 1990-1998 of phytoplankton counts and environmental sampling were taken monthly along a cross-shelf transect influenced by the outflow of the Mississippi River. Counts of certain HAB or potential HAB species or groups from surface samples were investigated along with environmental variables utilizing canonical correlation analysis. Organisms included in the analysis were: *Alexandrium monilatum, Ceratium tripos, Dinophysis caudata, Dinophysis ovum/acuminata, Gymnodinium sanguineum, Heterocapsa triquetra, Heterosigma akashiwo, Karenia brevis, Katodinium* spp., *Lingulodinium polyedrum, Mesodinium rubrum, Prorocentrum micans, P. compressum, P. gracile, P. mexicanum, P. minimum, P. scutellum, Pseudo-nitzschia* spp., and *Scrippsiella* spp. The environmental variables used in the analysis were: station (to describe inshore vs. offshore), season, salinity, temperature, dissolved oxygen, nitrate, phosphate, and silicate.

Initial results of canonical correlation analysis of data from 1990-1998 highlighted several relationships between some species and environmental variables. *Pseudo-nitzschia* spp. and *Dinophysis ovum/acuminata* were positively related with spring, high NO_3, and high O_2, conditions and negatively related with summer and high temperatures. *Alexandrium monilatum* and *Pseudo-nitzschia* spp. were positively related with fall, hi salinity, and low SiO_3 conditions and negatively with summer. *Prorocentrum compressum* was positively related to summer, high SiO3, and low salinity and negatively with fall. *Dinophysis caudata, Prorocentrum micans, P. compressum,* and *Pseudo-nitzschia* spp. were positively related to spring and lower salinity. While this analysis does not perfectly describe the conditions for all of the potentially harmful species on the Louisiana shelf, it does give insight into the controlling factors of the distribution for some of the more commonly occurring HAB or potential HAB organisms.

A BUOY-BASED IMAGING SYSTEM FOR CONTINUOUS MONITORING OF THE TOXIC DINOFLAGELLATE *Karenia brevis* IN THE GULF OF MEXICO

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A new optical detection system, the submersible FlowCAM (FluidImaging Technologies, Inc.) has been modified to optimize detection of *Karenia brevis*, the toxic dinoflagellate responsible for fish kills in the Gulf of Mexico. We are developing a buoy-based *in situ* continuous monitoring system capable of detecting *K. brevis* in real-time. Preliminary laboratory tests to validate automatic counting and to optimize data retrieval of captured images of *Karenia brevis* will be presented. Ultimately, the FlowCAM will be tested in conjunction with the existing Texas Automated Buoy System (TABS) and modeling program as a tool for early warning of harmful algal bloom events in the Gulf of Mexico. Continuous measurements of cell abundance, nutrient and oxygen concentrations, temperature, salinity and currents will be linked directly to the existing TABS web site for real-time display along with phytoplankton images.

THE ROLE OF ZOOPLANKTON GRAZERS IN THE BLOOM DYNAMICS OF THE TOXIGENIC DINOFLAGELLATES *Alexandrium* SPP. IN THE GULF OF MAINE

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Increasing incidence of harmful algal blooms threatens public health and economic activities (fisheries and aquaculture) in coastal environments. In the Gulf of Maine region of the northeastern United States, toxigenic dinoflagellates of the genus *Alexandrium* may form moderately dense aggregations in surface waters during spring, summer and fall of each year. Blooms of *Alexandrium* spp. are the source of the potent neurotoxins responsible for paralytic shellfish poisoning (PSP) in New England. We investigated the role of zooplankton grazers in the bloom dynamics of *Alexandrium* spp. in different regions of the Gulf of Maine. In the near-shore environment of the western Gulf of Maine, moderate blooms (<3000 cells/l) of *Alexandrium* spp. occurred and it was a minor component of the phytoplankton assemblage. Here, the copepod *Acartia hudsonica*, the most important grazer, fed non-selectively and had a significant grazing impact on the *Alexandrium* population. In contrast, in the Grand Manan Basin in the Bay of Fundy, *Alexandrium* spp. concentrations were much greater (>50,000/l) and it was a dominant component of the bloom assemblage. In this region, the copepod *Calanus finmarchicus*, the dominant grazer, had reduced grazing rates and did not appear to have a significant impact on the *Alexandrium* population. In both instances, despite low toxin retention efficiencies, toxin accumulation in zooplankton was significant and posed risks to higher trophic levels.

SUBLETHAL EFFECTS OF THE TOXIC DINOFLAGELLATE *Karenia brevis* ON COPEPOD BEHAVIOR

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Karenia brevis blooms are most frequently found off the west coast of Florida, to a lesser extent elsewhere in the Gulf of Mexico, and on one occasion, as far north as North Carolina. Increasing attention is being paid to the interactions between copepods and harmful algal species, with copepods as a potential factor in bloom dynamics through grazing or avoiding toxic cells and/or selecting for non-toxic cells. While toxin accumulation in copepods is generally noted during blooms and has been seen under some laboratory conditions, depuration is generally rapid, but sometimes incomplete. However, vectorial transfer of toxins through copepods to higher trophic levels has been demonstrated both in the field and in laboratory studies. What is not generally appreciated is the effect of toxic phytoplankton on the suite of behavioral defenses that copepods routinely use to avoid predation (e.g., diel vertical migration and escape responses). During sublethal exposure to HAB species, vectorial toxin transfer could be magnified if copepods were more vulnerable to predation. Given the important role that copepods play in marine food webs, aspects of this scenario warrant study. To this end, we examined feeding, mortality and behavior of copepods at ecologically relevant concentrations of dissolved brevetoxins (PbTx-2) and *K. brevis* cells.

At the highest *Karenia brevis* concentrations offered, *Temora turbinata* displayed similar grazing rates on *K. brevis* and the control dinoflagellate. In mortality experiments, dissolved purified brevetoxin (PbTx-2) did not affect survival in either *T. turbinata* or *Centropages hamatus*. In contrast, mortality in both species increased in a dose-dependent manner upon exposure to whole *K. brevis* cells. For *C. hamatus*, the LD_{50} for mortality was lower at $2.4x10^5$ cells L^{-1} (95% CI = $5.2x10^4 - 5.6x10^5$ cells L^{-1}), whereas the *T. turbinata* LD_{50} was $7.1x10^6$ cells L^{-1} (95% CI = $2.6x10^6 - 7.3x10^7$ cells L^{-1}). Further experiments with *T. turbinata* suggest that adverse sublethal behavioral effects are apparent after 24 hr exposure to either dissolved purified brevetoxin (PbTx-2) or whole *K. brevis* cells. Swimming behavior (rate of change in direction, net-to-gross displacement ratio) was negatively affected at a dissolved brevetoxin concentration of $2x10^{-5}$ g L^{-1}. A cell concentration of $5x10^6$ cells L^{-1} was required to negatively alter swimming behavior (swimming speed, rate of change in direction). Photobehavior was affected in a dose-dependent manner, with loss of photosensitivity beginning at a dissolved brevetoxin concentration of $2x10^{-6}$ g L^{-1}. Cell concentrations as low as $1x10^5$ cells L^{-1} resulted in loss of photosensitivity, but severe behavioral alteration was not apparent until $5x10^6$ cells L^{-1}. As both swimming behavior and photobehavior are involved in predator avoidance, their alteration upon exposure to sublethal concentrations of dissolved brevetoxins and *K. brevis* cells may mean that exposure of this copepod species to blooms could result in increased predation risk. Future studies will investigate *C. hamatus* behavior upon exposure to sublethal levels of dissolved brevetoxin and whole cells. Similar experiments will also be done with *Acartia tonsa*, a species known to be relatively insensitive to *K. brevis.*

DETERMINATION OF OPTIMAL BIOTIN-LABELED PNA CAPTURE PROBE TO STREPT-AVIDIN BINDING RATIOS

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Identification of harmful algal species directly from environmental samples is a rapidly growing field. Water quality managers, researchers and public health officials require timely and accurate detection of harmful species in a cost effective manner. Therefore, there is a need for improved methods capable of rapid on-site analysis. Emerging techniques utilized peptide nucleic acid (PNA) instead of DNA as probes. PNAs are DNA mimics that bind to RNA or DNA targets as do DNA, but they possess unique hybridization qualities demonstrating enhanced sensitivity and broad hybridization conditions (Nielsen & Egholm 1999).

These experiments are a first step in incorporating PNAs to the standard sandwich hybridization assay (SHA) currently in use for harmful algae (Scholin et al 1996). In the SHA a biotin-labeled capture probe is bound to a solid support through a streptavidin (SA) -biotin linkage. This solid support is then cycled through wells containing target molecules, signal probe, washes and color development reagents. This set of experiments was designed to optimize one of the initial steps in the SHA, that of SA binding to the biotin-labeled capture probe. Early work found an optimal SA to biotin-labeled probe ratio was 1.2:1, with a standard probe concentration of 400ngml^{-1} and a streptavidin concentration of 2.5µg ml^{-1}. To determine the optimal biotin-labeled probe to SA ratio for biotin-labeled PNA probes two sets of experiments were designed. The first kept the probe concentration constant (400µg ml^{-1}) and varied the SA concentration (1.25µg ml^{-1}, 2.5µg ml^{-1}, 5µg ml^{-1}, and 10µg ml^{-1}) (Fig 1) and the other kept the SA concentration constant (2.5µg ml^{-1}) while changing the probe concentration (1.5µg ml^{-1}, 1µg ml^{-1}, 0.5µg ml^{-1}, .0.25µg ml^{-1}) (Fig 2). The target molecule was large subunit ribosomal RNA extracted from pre-frozen (-80^{o} C) *Alexandrium tamarense* cells (isolate NWFSC 405). Target cell concentration in these experiments was 1000 cells per reaction, signal probe concentrations were kept at 300ng ml^{-1} and reactions were run in triplicate.

These data show that the optimal concentration of SA for 400ng ml^{-1} capture probe is 2.5µg ml^{-1} and the optimal capture probe concentration for 2.5µg ml^{-1} SA is 500ng ml^{-1} (or 0.5µg ml^{-1}). This ratio is similar to that found for SA binding to a DNA based biotin-labeled capture probe of 1.2:1.

Fig.1 Vary SA

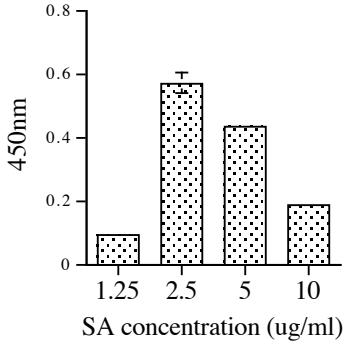

Fig. 2 Vary Probe

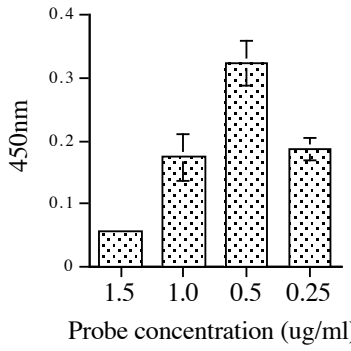

Table 1 PNA probe sequence 5' to 3'

Capture probe	GTG CAA CAC TCC CAC C
Signal probe	GTC CTT TTC ATA TTT CCC

Figure 1 and 2.
Both SA (fig 1) and capture probe (fig 2) concentrations were varied in a standard SHA format using frozen *Alexandrium* cells as target.

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Scholin, C.A., Buck, K.R., Britschgi, T., Cangelosi, G., Chavez, F.P. 1996. Identification of *Pseudo-nitzschia australis* (Bacillariophyceae) using rRNA-targeted probes in whole cell and sandwich hybridization formats. Phycologia 35, 190-197.

SEAPORT: IMPROVING BIOTOXIN MANAGEMENT THROUGH CITIZEN INVOLVEMENT

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Monitoring for marine biotoxins is made more challenging by the patchy and ephemeral distribution of the phytoplankters that produce them. The cost of sampling at adequate temporal and spatial density, coupled with the intrinsic limits of toxicity testing (both cost and time delay), place a significant burden on agencies responsible for seafood safety. Employing networks of field observers, primarily volunteers equipped with portable microscopes, to give advance warning and help focus toxicity testing effort can significantly improve the effectiveness and reduce the cost of marine biotoxin management programs.

BENTHIC RESPONSE TO APPLICATION OF PHOSPHATIC CLAY FOR THE REMOVAL OF *Karenia brevis* FROM THE WATER COLUMN, SARASOTA BAY, FLORIDA

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Public perception is that red tide blooms on the west Florida coast are becoming both more frequent and more persistent in duration. Whether or not this is true remains to be established. The blooms are caused by the toxic dinoflagellate, *Karenia brevis*. Release of brevetoxins as a result of normal cell growth and divisions as well as cell breakage may reach levels that are toxic to both fish and invertebrates at sufficiently high cell concentrations. It is certain that red tide blooms have an economic impact due to losses in tourism and fishing revenues. Public concern over the effects of red tide is resulting in pressure to develop control measures.

Clay suspensions are used in South Korea to precipitate harmful algae from the water column when blooms occur in the vicinity of shellfish aquaculture areas. The characteristics of the clay suspension are such that algal cells adhere to the particulates and settle to the bottom.

The present study was undertaken as one component in examining the utility of phosphatic clay to precipitate *K. brevis* (as well as other plankton) from the water column, and to focus on the fate and impacts of the settled clay-dinoflagelate-plankton floc on the benthos. Potential impacts include: change in the surface sediment grain-size distribution, release of brevetoxins at the benthic boundary layer, and the increase of organic matter at the benthic boundary layer. A small-scale pilot study was conducted in May of 2003 utilizing controlled phosphatic clay applications in 5 enclosures (clay-pens) over natural substrate in Sarasota Bay at a depth of ~ 3.7 m. The area consisted of a gently sloping bottom of clean (i.e. low silt/clay fraction and low organics), medium to fine quartz sand, with some shell material. The low silt/clay content was considered to be advantageous in terms of detecting any added clay. The clay-pens consisted of a circular plastic coated hog wire mesh (diameter ~3.0 ft.) lined with fiberglass window screen, and a thin walled rigid fiberglass tube that was temporarily placed within the screen enclosure to confine the clay floc so that all or the bulk of the material would settle on the intended target benthos. Clay slurry (~10% solids in seawater, = 0.5 kg dry wt) was applied to five clay-pens. Five identical pens were left untreated as controls. After settlement the rigid liner was removed, with the screened enclosure remaining to limit currents and prevent disturbance from large mobile epifauna and bottom feeding fishes. Benthic fauna were samples four days prior to clay application and again 15 days after application. In addition, a mix of *Mercenaria mercenaria* juveniles (obtained as aquaculture seed stock) and wild mollusks were placed into each clay-pen just prior to treatment. Temperature, salinity, pH, and dissolved oxygen were monitored for treatment, control, and ambient conditions. Tidal effects on these parameters were observed but there were no treatment effects. The clay floc settled rapidly and there was no difference in turbidity between control and treatment clay-pens one hour after application. After settlement the clay floc was clearly visible as a fine surface layer on the bottom. A one-way ANOVA comparing the faunal abundance of control and treatment clay-pens showed no significant difference in numbers of organisms collected. However, there was a significant difference between pre- and post-treatment organism abundance for both control and treatment site fauna indicating a significant effect, possibly due to disturbance during clay-pen installation and sampling. The results of the survival of *M. mercenaria* juveniles indicate the possibility of a treatment effect. The overall survival of *Mercenaria* within the control pens was 47% while the survival within the treatment pens was 15%. All of the treated pens exhibited a lower number surviving treatment than their control counterparts. The effect was significant (n=5, p=0.045, non-parametric t-test). There were complications, however, due to portunid crab predation within the clay-pens.

PIN THE PART ON THE DINOFLAGELLATE: A HANDS ON LEARNING ACTIVITY

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Marine science education often uses animals with high student appeal, such as manatees, sea turtles, whales and dolphins, to introduce science concepts. The challenge in teaching microalgae concepts is to make the activity equally appealing and engaging to students. The activity created used an enlarged diagram of a marine dinoflagellate, *Karenia brevis*, to introduce anatomy and function concepts. The activity was simple in design, requiring only a large color diagram of the organism (laminated for durability) and hook and loop fasteners for placement of parts in the appropriate area. Because of its simplicity in design, the activity is inexpensive and can be easily reproduced. Written key words were also created and laminated to reinforce vocabulary and spelling.

This activity was found to reach a wide age range from elementary school students to high school students. The facilitator could adjust the prompts to assist the students in the proper labeling based on age. At the elementary level, basic anatomy concepts such as “nucleus” and “flagella” could be introduced. At the middle school level, labeling of the “chloroplasts” could lead to discussion of photosynthesis and the food web. At the high school level, discussion could be focused on primary productivity and harmful algal blooms (HABs). The concepts taught in this activity easily address many key points in the National Science Education standards, such as the structure and function of living systems and the diversity and adaptations of organisms.

This activity could easily be adapted to numerous algal organisms in both the marine and freshwater environments.

LONG-DISTANCE AND LOCAL-SCALE TRANSPORT OF *Aureococcus* BY SHIPS AND SMALL BOATS IN FRESH AND MARINE WATERS

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It is well established that cyst-forming phytoplankton species are transported in ships' ballast tanks. However, there is increasing evidence that other phytoplankton species, ones which do not encyst, are capable of surviving ballast transit. These species have alternative modes of nutrition (hetero- or mixotrophy) or are able to survive long-term darkness, or both. In our studies of NOBOB (No-Ballast-On-Board) vessels arriving to the Great Lakes, we tested for the harmful algal bloom (HAB) species *Aureococcus anophagefferens* (brown tide) in residual (i.e., unpumpable) ballast water using polymerase chain reaction based methods.

The brown tide organism was detected in 10 out of 20 residual water samples (50%) following transit from foreign ports. Not only this, it was detected after 10 days of ballast-tank confinement during a vessel transit in the Great Lakes (Port of Hamilton, Lake Ontario; Ports of Windsor and Detroit, Lake Erie; Port of Burns Harbor, Lake Michigan). This result is significant, given the large disparity between the salinity tolerance of *Aureococcus* (>22 ppt; Cosper et al., 1989) and the low salinity of the residual ballast water (approximately 2 ppt).

We also investigated the potential for smaller recreational vessels to transport and distribute *Aureococcus*. During the summer of 2002, 11 boats were sampled at boat ramps as they emerged from the Delaware Inland Bays and Maryland Coastal Bays. Brown tide was detected in the bilge water at the bottom of small trailered boats in 8 out of 10 cases, as well as the one live well sample we collected. Work is currently underway to quantitatively assess *Aureococcus* abundance and metabolic activity in these samples. However from our results to date, it appears that commercial ships and small recreational boats are significant vectors for long-distance transport and local-scale dispersal of *Aureococcus*.

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DEVELOPMENT OF LSU rRNA PROBES FOR *Cochlodinium* FROM KOREA AND THE WEST COAST OF NORTH AMERICA

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Blooms of the dinoflagellate, *Cochlodinium polykrikoides*, were first recorded off of the Korean coast in September 1982. Since that time, recurring events have resulted in both wild and farmed fish kills, culminating in the government's designation of *C. polykrikoides* blooms as natural disasters in 1990. The economic impacts associated with these blooms reached a peak in 1995, when widespread fish mortalities resulted in losses estimated at USD $95 million. More recently, blooms of *Cochlodinium* sp. have been observed along the west coast of North America in British Columbia, Canada, with losses to the farmed salmon industry of approximately CDN $2 million reported in 1999. The appearance of this harmful algal species in Canadian waters suggests there is a reasonable likelihood that *C. polykrikoides* will ultimately affect aquaculture interests in the adjacent U.S. Pacific northwest, an area already under pressure from other fish-killing algae (e.g., *Heterosigma akashiwo*). Early detection of bloom formation, along with attempts to circumvent losses by relocating fish cages or premature harvesting, are critical aims of the aquaculture industry throughout the world's coastal waters. To this end, and with particular emphasis on the fish killer, *C. polykrikoides*, our laboratory is developing a species–specific probe in order to facilitate early detection of this taxon's presence and thereby support efforts by the aquaculture industry to mitigate the devastating effects of this harmful algal species.

We have sequenced the D1-D3 variable regions of the large-subunit ribosomal RNA gene (LSU rDNA) from five Korean isolates of *C. polykrikoides* (2002, 2002-1, PP-3, PP-6, and *Cochlodinium*), originating from various locations along the Korean coast during three different years, and sequencing of a North American *Cochlodinium* isolate is currently underway. We detected no sequence differences among the Korean isolates, and a consensus sequence was compiled for comparison to previously published data for *C. polykrikoides* (Genbank #AF067861) and to additional members of the Gymnodiniaceae. These data were then used to identify potential target sequences unique to *C. polykrikoides*. Several oligonucleotide probes specific for *C. polykrikoides* LSU-rRNA, ranging in length from 18-22 bases, have been designed and are being evaluated for their ability to access the target sequence using whole cell hybridization, as well as for their lack of cross-reactivity with other species. Ongoing work includes the design of a genus level probe to be used along with the species-specific probe in a sandwich hybridization assay for the automated field-based detection of *C. polykrikoides*. This assay technique has the potential to rapidly detect low levels of *C. polykrikoides* and thus provide early warning of impending bloom activity. Moreover, we anticipate that this assay will facilitate a more efficient and effective response to any increasing frequency of *Cochlodinium* blooms in N. American, and specifically U.S., waters.

TOXICITY OF *Prorocentrum lima* AND THE POTENTIAL FOR DIARRHETIC SHELLFISH POISONING ALONG THE NEW ENGLAND COAST ON THE UNITED STATES

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Following the occurrence of several unexplained incidents of shellfish-related gastroenteritis, field studies were conducted to determine if diarrhetic shellfish poisoning (DSP) toxins were present in the coastal waters of New England states. Previous studies have found the toxic dinoflagellate, *Prorocentrum lima*, is widespread in New England coastal waters. The abundance and seasonality of this toxin producer was followed within the planktonic and epibiotic community. Samples were collected bimonthly at eight sites from Rhode Island, New Hampshire, and Maine**.** In an effort to evaluate the potential for diarrhetic toxins to contaminate shellfish resources, the digestive glands of wild and cultured shellfish were collected.

The epiphytic samples and digestive glands were analyzed for potential okadaic-acid activity using the fluorometric protein phosphatase inhibition assay. Samples positive for protein phosphatase activity were analyzed for okadaic acid and related congeners using the ADAM-HPLC method. Epiphytic samples showed a seasonal trend in both the population of *P. lima* and total toxicity, with increase cell number and toxin content during summer months. Analysis of these samples showed the production of both dinophysis toxin-1 and dinophysis toxin-2. Like previous studies, no okadaic acid was detected. Cultures of *P. lima* isolated from the sample area showed a similar toxin profile with a predominate production of dinophysis toxin-1 and little okadaic acid production. The digestive glands did display very low protein phosphatase activity. Although the presence of DSP-type toxins in shellfish digestive glands indicate uptake, the levels are well below the maximum accepted concentration.

ASSESSMENT OF BIO-OPTICAL MODELS DURING TWO EAST COAST ALGAL BLOOMS: CELL SIZE DEPENDENCE

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Recently there has been increased interest in the remote detection of harmful algal blooms (HABs), given that they are often associated with significant changes in water color. Questions have arisen, however, regarding the applicability of established optical models to extreme algal blooms, as algorithms are generally developed and tested during conditions of low to moderate chlorophyll (Chl) concentrations and polydispersed particle distributions, conditions atypical of blooms. We assessed the application of bio-optical models during two intense algal blooms along the east coast of the US: 1) a bloom of the dinoflagellate *Prorocentrum micans* in West Boothbay Harbor, Maine and 2) a brown tide of the pelagophyte *Aureococcus anophagefferens* in Quantuck Bay, New York. In particular, we investigated: 1) a spectral chlorophyll-specific absorption (a*) model based upon Chl concentration (Bricaud et al. 1995), 2) an inherent optical property (IOP) inversion model to estimate particle size distributions from particulate beam attenuation (Boss et al. 2001), and 3) an apparent optical property (AOP) reflectance inversion model to estimate phytoplankton absorption spectra (Roesler and Perry 1995).

In situ conditions during each bloom were characterized by high Chl concentrations (up to 494 and 40 $\mu g\ L^{-1}$, respectively) and particle size ranges that did not display the Junge-like distributions typically encountered in the ocean. Particle area and volume distributions exhibited a modal diameter of 23 μm and 2 μm during the *P. micans* and *A. anophagefferens* blooms, respectively. Even with extremely high Chl, measured a* coefficients indicated algal photoacclimation, yet measurements of Chl alone were not sufficient to model spectral phytoplankton absorption for either algal species. The IOP model was robust despite violation of key assumptions regarding the particle size distributions. However, limitations exist for the interpretation of particle size slopes predicted from spectral attenuation slopes. For a large cell such as *P. micans*, the inversion model accurately predicts the size distribution of non-bloom particles. However, for blooms of small cells like *A. anophagefferens*, the model accurately estimates the entire particle size distribution including the bloom-forming cells. This is because the beam attenuation is preferentially sensitive to small particles. The reflectance inversion model was robust, predicting phytoplankton absorption to within 10% of the measured values for each bloom. Success of certain optical parameters and relationships during blooms suggests that some existing optical models may be used for detecting/studying algal blooms, although for some models the size of the cells causing the bloom may have to be considered for accurate interpretation. From continued studies during blooms, regional-specific and/or species-specific sets of appropriate optical techniques can be determined thereby improving our capabilities for optical HAB detection and the development of early warning systems.

A SURVEY OF PELAGOPHYTE STEROLS AND A STEROL PALEOCHRONOLOGY OF THE BROWN TIDE ALGA *Aureococcus anophagefferens* IN LONG ISLAND WATERS

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The rare marine sterol 24-propylidenecholesterol is produced by algae of the class Pelagophyceae. While the E-isomer of this sterol has been found in other algae in this class, the Z-isomer was considered specific to *Aureococcus anophagefferens*. We analyzed the sterols of 44 algae representing all strains of *Aureococcus* and all of the Pelagophyte algae that are cultured at Bigelow Lab (CCMP). The chemotaxonomic categories compare well with rRNA based taxonomic analysis. The results show that all strains of *Aureococcus* contain both the E- and Z-isomers in ratios ranging between 1.3 and 4.2 with an average of 3.2. Seawater samples taken during a brown tide bloom on Great South Bay contained large amounts of 24-propylidenecholesterol as a 4:1 mixture of the E- and Z-isomers. Analysis of surface sediments from the same location showed a 2:1 ratio of the isomers. A dated sediment core was obtained from Peconic Bay and was submitted to sterol analysis. A 2:1 ratio of the E and Z isomers of 24-propylidenecholesterol was clearly evident in the top six 2 cm sections of the core, representing the past 120 yrs. These data strongly suggest that *Aureococcus* has been present in Long Island waters for at least a century.

DOMOIC ACID IN THE BENTHIC FOOD WEB OF MONTEREY BAY, CALIFORNIA

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Phytoplankton cell flocculation and sinking is an important food source for benthic communities. If the flocculate is composed of harmful algal bloom (HAB) species like *Pseudo-nitzschia australis*, a producer of domoic acid (DA), the flocculate transfer could represent an important flux of phycotoxins into benthic food webs. Here we test the general hypothesis that high levels of DA should be detectable in benthic organisms during blooms of *P. australis* ($\geq 10^4$ cells L^{-1}). To test for trophic transfer and retention of DA in the benthic food web we sampled eight benthic species comprising four feeding types: filter feeders (*Emerita analoga* and *Urechis caupo*); predators (*Citharicthys sordidus*); scavengers (*Nassarius fossatus* and *Pagurus samuelis*); and deposit feeders (*Callianassa californiensis*, *Dendraster excentricus*, and *Olivella biplicata*). Sampling occurred before, during, and after blooms of *P. australis*, in Monterey Bay, CA during 2000 and 2001. Domoic acid was detected in all species, with depuration of DA burdens occurring over variable time scales. Maximum DA levels detected in *N. fossatus* (673 ppm), *E. analoga* (278 ppm), *C. sordidus* (514 ppm), *C. californiensis* (144 ppm), *P. samuelis* (55 ppm), *D. excentricus* (13 ppm), and *O. biplicata* (2 ppm) coincided with *P. australis* blooms. These high concentrations of DA could have deleterious effects on higher-level consumers (marine birds, sea lions, and the endangered California Sea Otter) known to prey upon these benthic species, and for which there are recent documented accounts of injury and death attributed to DA ingestion.

NUTRIENT EFFECTS ON *Pfiesteria* SPP. GROWTH RATES

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Nutrient enrichment associated with cultural eutrophication is believed to be a primary factor in regulating environmental abundances of *Pfiesteria* spp. Laboratory experiments which quantify the effect of nutrient enrichment on *Pfiesteria* spp. growth rates are complicated by a.) nutrient utilization and subsequent growth stimulation of algal prey, which in turn support enhanced *Pfiesteria* spp. growth and b.) alteration of nutrient utilization and subsequent growth as a result of kleptoplastidy by *Pfiesteria* spp. Utilizing a procedure in which algal prey are incapable of nutrient utilization and growth, we investigated the direct growth response of *Pfiesteria* spp. to enrichment with both inorganic and organic nutrients. The effect of kleptoplastidy on the growth response to various nutrient enrichments by *Pfiesteria* spp. was also evaluated. The results of this study, which may have significant implications for observations of *Pfiesteria* spp. abundance in the natural environment, are reported.

MONITORING NUTRITIONAL PHYSIOLOGY IN HARMFUL ALGAE: ASSESSMENTS OF ENZYMATIC ACTIVITY AS A MEASURE OF ORGANIC NUTRIENT HYDROLYSIS

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Dissolved organic forms of nitrogen (DON), such as amino sugars, and phosphorus (DOP), such as phosphomonoesters, can influence growth, bloom dynamics, and toxin production in harmful algae. DON and DOP may be difficult nutrient pools to exploit and often have to be hydrolyzed before they can be taken up or used by the cell for growth. We can assess potential DON and DOP utilization by harmful algal species through measures of enzymatic activity. Specifically, our study is focused on two major enzymes, acetylglucosaminidase and alkaline phosphatase. Acetylglucosaminidase is used to break down amino sugars and alkaline phosphatase is typically able to hydrolyze a variety of phosphomonoesters.

Classical enzyme assays, such as the bulk assay for alkaline phosphatase, have been used to investigate DOP hydrolysis. However, these assays are not able to determine the enzymatic activity of specific phytoplankton species and are confounded by the fairly ubiquitous nature of these enzymes in many phytoplankton and heterotrophic bacteria which may regulate their activity in subtly different manners. Consequently, we have targeted our study to those enzymes where cell-specific assays may be possible. Here we utilize two enzyme substrates from Molecular Probes Inc. that are soluble and colorless when unreacted, but insoluble and fluorescent when reacted. Thus, they can precipitate and fluorescently tag individual phytoplankton cells that have specific enzyme activities. This technology is called enzyme labeled fluorescence (ELF).

We are screening harmful algae for acetylglucosaminidase and alkaline phosphatase activity. Preliminary data from members of the genus *Alexandrium* indicates that *A. catenella* expresses acetylglucosaminidase activity when grown on a variety of nitrogen sources. In related work with other harmful species, such as the dinoflagellate *Gymnodinium catenatum,* we have identified alkaline phosphatase activity when grown under phosphate deplete conditions. With these initial screenings, it appears that we can detect both acetylglucosaminidase and alkaline phosphatase activity using ELF substrates. With the use of ELF we are hopeful that field populations can be screened for these activities, thus indicating potential DON and DOP utilization in specific harmful species.

STUDY OF THE IMMUNE RESPONSES OF THE BIVALVES *Crassostrea virginica* AND *Argopecten irradians irradians* EXPOSED TO NATURAL AND ARTIFICIAL BLOOMS OF THE DINOFLAGELLATE, *Prorocentrum minimum*

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Under controlled laboratory conditions, juvenile oysters and scallops were exposed to bloom concentrations of a cultured dinoflagellate, *Prorocentrum minimum,* with demonstrated lethal and sub-lethal pathological effects upon these bivalves. Immune status of mollusks was assessed, using flow-cytometric hemocyte analyses for hematological characteristics and several hemocyte functions, periodically during 7 days of continuous exposure. For both oysters and scallops, *P. minimum* exposure had a significant effect upon immune profile, and this effect was dependent upon duration of exposure.

In a second experiment, oysters, *Crassostrea virginica*, from two populations, one from a coastal pond experiencing repeated dinoflagellate blooms ('native'), and the other from another site where blooms have not been observed ('non-native'), were analyzed for cellular immune-system profiles before and during natural and simulated (by adding cultured algae to natural plankton) blooms of the dinoflagellate *P. minimum*. For both populations of oysters, *P. minimum* exposure had a significant effect upon immune profile. Moreover, natural and artificial blooms of *P. minimum* triggered a similar immune response in the oysters, indicating that the artificial bloom was a good simulation of a natural bloom. We believe these are the first reports of immune-system effects of harmful algae upon grazing bivalve mollusks.

STATE OF DELAWARE HAB SURVEILLANCE MONITORING PROGRAM

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The Delaware Department of Natural Resources and Environmental Control initiated a HAB Surveillance Monitoring Program as an expansion of the existing *Pfiesteria* Surveillance Monitoring Program, which started in May 1998. This State Program is truly a partnership effort between experts in academia and state and federal natural resource agencies from Maryland to Florida. They assist Delaware in conducting analyses not available within the State and in confirming HAB species identifications.

The Program is dynamic. Spatial and temporal monitoring changes are made to reflect newly acquired knowledge from local and regional monitoring efforts as well as from research programs. New technologies and detection tools are added to the program as needs arise to document existing environmental conditions. Recently, the State developed a Standard Operating Procedure for the use of a commercially available biotoxin kit.

The Program now consists of both an estuarine and a freshwater component. In the Delaware Inland Bays, we monitor for HABs using light microscopy and DNA molecular probes. Up to 45 water-based environmental variables are recorded per collection. Sediment sampling is conducted in the spring and fall to locate potential HAB "seed banks". Continuous monitoring of salinity, temperature, and DO exists at 8 sites within the three waterbodies. Water sample collections are performed by both State agency scientists and citizen monitors, who also serve as the "eyes and ears" for the State in detecting episodic HAB events.

The freshwater surveillance monitoring effort is directed toward obtaining information on bluegreen algae blooms in ponds/lakes historically known to exhibit bloom conditions. We test selected lakes for microcystin, a toxin associated with *Anabaena* and *Microcystis*, using both a field application kit and a more quantitative laboratory analysis.

PHYLOGENETIC DIVERSITY OF BACTERIA ASSOCIATED WITH *Alexandrium* spp. AND OTHER PHYTOPLANKTON FROM THE GULF OF MAINE

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Previous work relying on the measurement of bulk community parameters has indicated a close link between bacteria and phytoplankton dynamics in marine environments. However, little is known about how these communities interact at a species-composition level or whether there are specific associations between bacteria and phytoplankton. Studies of the interactions between several harmful algal bloom (HAB) species and associated bacteria have suggested that these interactions can be specific and may be important controlling factors for HAB events. The objective of the current study was to determine whether there are specific interactions between diverse phytoplankton and the bacteria that are closely associated with them. Our analysis included representatives of the major taxonomic groups of phytoplankton in the Gulf of Maine (GOM), as well as several strains of the toxic dinoflagellates, *Alexandrium* spp., from the GOM and elsewhere. We determined the molecular phylogenetic diversity of bacterial assemblages associated with xenic, uni-algal phytoplankton isolates that were chosen to (1) represent a broad taxonomic range; (2) represent a broad geographic range for *Alexandrium* spp. isolates; (3) grow under similar cultivation conditions; (4) have a minimal length of time since the original isolation; and (5) have been isolated from a vegetative cell (not from a cyst). DNA was extracted from xenic cultures using a FastDNA Spin Kit and used as template in PCR amplification of 16S rDNA fragments with a primer set that targets most *Bacteria*. The PCR products were analyzed using denaturing gradient gel electrophoresis (DGGE), and resolved DGGE bands were recovered from the gel and either sequenced directly or cloned into *E. coli* and then sequenced. DGGE analysis revealed little similarity among bacterial assemblages from different phytoplankton cultures, with only a few bacteria associated with more than one phytoplankton culture. However, sequence analysis indicated that similar bacterial phylogenetic groups were dominant across distantly related phytoplankton taxa. In particular, the *Rhodobacter* and *Cytophaga-Flavobacterium-Bacteroides* groups were important members of assemblages in most phytoplankton cultures. These groups are known to include important colonizers of marine particulates and bacteria that have both positive and negative interactions with phytoplankton. Taken together, these results indicate that specific bacteria-phytoplankton interactions may exist, but that they are not the result of coevolutionary relationships that would be expected to occur in tight symbioses.

DEVELOPMENT AND DISSEMINATION OF A FREQUENTLY ASKED QUESTION (FAQ) CARD ABOUT THE FLORIDA RED TIDE, *Karenia brevis*

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The Florida coastline is well known for onshore *Karenia brevis* blooms. The State experiences onshore red tides annually which significantly affects the quality of life for residents and tourists alike. Solutions to Avoid Red Tide or START, a grassroots citizen's organization's mission is to support programs that control and/or mitigate the harmful effects of red tide. Misinformation about harmful algal blooms is common, particularly in a tourist area where people may be experiencing red tide for the first time. Although the media frequently reports the conditions of red tide blooms, informal surveys reveal that tourists do not routinely watch local news or read the local newspapers. They are on vacation. Therefore, a need was identified to develop a Travel and Leisure Project to minimize economic impacts of red tide on Florida businesses by providing accurate and factual information in a user-friendly format. The Red Tide Alliance, consisting of START, the grassroots citizen's group, Mote Marine Laboratory, a private non-for profit research laboratory, and the Florida Marine Research Institute collaborated and funded a Frequently Asked Questions or FAQ card about red tide that merchants such as hotels, motels, restaurants, and visitor bureaus could distribute. The Florida Chamber of Commerce, Visit Florida, the Hotel and Motel Association, Florida Restaurant Association, and the Florida Beach and Shore Association were some of the stakeholders who participated as focus groups to provide feedback on the cards during design and development and also obtain their support for the project. Along with FAQ's, the card provides the Marine and Freshwater Toxin Hotline number as well as the red tide/fish kill toll free number. START mailed a supply of FAQ cards to all Florida Coastal Chambers of Commerce and Florida Visitor and Convention Bureaus. START also mailed cards to businesses and communities at their request for distribution. The FAQ cards have been extremely popular and to date, START has printed 200,000 cards and received requests for dissemination of over 120,000 cards. In addition to the production of the FAQ card, a web site, www.RedTideOnline.com was established to electronically provide information. In addition to providing information, another goal of the website was to function as a portal to other red tide and HAB web sites, the Alliance partner websites as well as others. The website has received as many as 75,000 hits per month during an onshore bloom. This project may be used as a model to other communities regarding dissemination of accurate information regarding harmful algal blooms.

DEVELOPMENT OF MOLECULAR AND BIOCHEMICAL SIGNATURES FOR THE DETECTION OF TOXIN PRODUCTION IN *Pseudo-nitzschia* SPP. UNDER NUTRIENT STRESS

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Pseudo-nitzschia spp. are known to vary domoic acid (DA) production as a function of multiple nutrients, including nitrogen, phosphorous, silicate, iron, copper, and possibly lithium. Recent evidence from laboratory and field experiments also demonstrates that nutrient stress impacts photosynthetic performance as diagnosed by physiological parameters such as variable fluorescence. The detection of of *Pseudo-nitzschia* in the field has also been greatly improved by the application of molecular methods such as rRNA probes. Despite the large body of information on DA production and the occurrence of DA poisoning events in the field, we still have a very poor understanding of event- specific triggers for DA production in the field, or the ecological basis for its production; this has thus far hampered our ability to predict or detect early onset of toxin production.

We are beginning to characterize gene expression patterns associated with DA accumulation in *Pseudo-nitzschia*, in order to identify targets for development of molecular probe assays of active toxin production by field populations of *Pseudo-nitzschia.* Two complementary approaches are being pursued: analysis of subtracted cDNA libraries enriched for transcripts differentially expressed under a variety of DA-producing culture conditions, and RT-PCR based cloning of specific gene products whose hypothesized association with DA biosynthesis is supported by biochemical evidence (e.g. proline metabolism). Because silicate limitation provides a reversible trigger for DA production, cDNA pools enriched for transcripts expressed during Si-limited growth provide molecular targets for Si-metabolism (acquisition, storage), cell cycle regulation (growth restriction) and DA biosynthesis. We also hypothesize that DA provides increased trace metal buffering capacity in toxin-accumulating *Pseudo-nitzschia.* Therefore, biochemical signatures related to metal stress may provide another mechanism for early identification of toxigenesis.

Chemostat runs using Si-limited *P. australis* isolated from Monterey Bay, California, have revealed several genes and biochemical pathways that may provide viable candidates for development of molecular signatures. *P. australis* specific primer-pairs have been developed for a subset of genes encoding structural (*fcp*, *s19*) and metabolic (*pck*, *pdh*) products. RT-QPCR assays of RNA pools isolated from both culture and field samples suggest that *pck* may provide a critical constitutive expression marker for normalization of RT assays. Our assessment of these preliminary results based on Si-limited cDNA subtractions, QPCR and RT-PCR of specific targets, and biochemical signatures of macro- and micronutrient limited cultures provide an encouraging foundation for development field-applicable molecular and biochemical signatures for toxin production in *Pseudo-nitzschia.*

A MESOCOSM STUDY EXAMINING THE INFLUENCE OF NUTRIENTS ON *Alexandrium tamarense/fundyense* TOXIN CONCENTRATION AND COMPOSITION

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Laboratory and field studies exploring the influence of varying nutrient regimes on *Alexandrium tamarense/fundyense* have revealed that they can have dramatic effects on toxin content and composition. Therefore, we hypothesize that certain "generic" toxin composition profiles, or ratios of the various saxitoxin derivatives, may be indicators of a cell's nutrient status.

As part of a larger study to investigate the impact of a number of different environmental parameters on toxin content and composition, a field-deployed mescosm study was conducted along the shoreline of Salt Pond, Eastham, MA in the spring of 2003. The primary objective of this study was to determine if nutrient variability would result in discernable patterns in toxin composition in both natural and cultured *Alexandrium* sp. populations.

Two, eight-foot diameter, fiberglass pools, each containing six, 230-liter, polyethylene tanks were used to house the experiment. Salt Pond water was pumped into the 2 pools at a rate of 170 liters per minute to regulate the tanks to ambient water temperature. At the beginning of the *Alexandrium* bloom, nine of the 230-liter tanks were filled with 64 µm-filtered pond water (to remove grazers) while the other 3 tanks were filled with 20 µm-filtered water (to remove *Alexandrium* and grazers). Three of the 64 µm-filtered tanks were enriched with f/20 levels of nutrients (nutrient replete), while 3 were nitrogen limited and 3 were phosphorus limited. The 20 µm-filtered tanks were inoculated with the *A. tamarense* culture ATSPG5-1 (a clonal Salt Pond isolate) at an initial density of 1000 cells per liter. One of these tanks was nutrient replete, one nitrogen deplete and one phosphorus deplete. During the course of the month-long study, samples were collected from each tank at least twice daily for chlorophyll content, cell density, cell volume and life cycle status, cellular nutrient status, saxitoxin content, dissolved and particulate nutrients. A pond sample taken through the pool cooling water discharge line was also collected and was assessed for the same parameters as the tanks.

Results of HPLC analysis of saxitoxin, as well as analysis of the other ecological parameters, will be presented and evaluated to identify generic trends in cellular toxin content and composition in relation to nutrient availability.

COPPER INFLUENCE ON THE PRODUCTION OF DOMOIC ACID IN TOXIC *Pseudo-nitzschia* spp. IN MONTEREY BAY, CA: A FIELD STUDY

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Recent laboratory experiments have indicated that the production of domoic acid (DA) in *Pseudo-nitzschia* spp. may be linked to limiting [Fe] or excess [Cu] and [Li]. Because California coastal waters have experienced significant increases in dissolved [Cu] since 1977 a field sampling effort was designed to establish whether elevated concentrations of labile copper (Cu') correlated with the presence of elevated [DA] in *situ*.

Surface net plankton tows and water samples were collected twice weekly, from three sites (SB, WB, MB) using trace metal clean techniques along a 3 kilometer transect in Monterey Bay, CA (N 35°35'20", W 121°53'10" to N 35°37'17", W 121°53'50") from Mar 28 – July 03, 2001. Evaluation of total *Pseudo-nitzschia* spp. abundance patterns along the sampling transect indicated that the 97 day sampling program overlapped several bloom events. Furthermore, variability in cellular and water column DA burdens indicated the presence of *Pseudo-nitzschia* assemblages with different toxicity and hence physiological status. Scanning electron micrographs of samples from peak toxic (April 24, 2001) and non toxic (June 04, 2001) assemblages from each site, revealed that the sampling period encompassed two distinct communities of varied species composition. A toxic community, consisting primarily of *Pseudo-nitzschia australis* occurred during the period from Mar 28- May 22 (POP 1) and a non toxic community dominated by *Pseudo-nitzschia fraudulenta* and *Pseudo-nitzschia heimii* occurred from May 25 – July 03, 2001 (POP2). Although average cell yields for POP1 were two orders of magnitude less than POP2 mean particulate [DA] for all three sites of POP1 were at least an order of magnitude greater than sites of POP2. Furthermore, while [Cu'] exhibited only a 10.7% increase from POP1 to POP2, the mean [Cu_{total}] increased by 52.3%.

Time-series, cross correlation analysis of [Cu'] to the total fraction of DA_{total} [(particulate DA + dissolved DA) 10^{-6} cells] for POP 1, yielded highly significant correlations ($P < 0.005$) at all three sites ($r = 0.87$, $r = 0.82$ and $r = 0.79$ respectively) with no significant correlations existing between DA_{total} and [Cu'] for POP2. These results indicate that a significant relationship between [DA_{total}] and [Cu'] exists only when potentially toxic species of *Pseudo-nitzschia* are present.

LIPID COMPOSITION OF *Karenia brevis* BLOOMS IN THE GULF OF MEXICO

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In the Gulf of Mexico, recurring *Karenia brevis* blooms lead to significant health and economic impacts. *K. brevis* is one member of a small group of dinoflagellates, related morphologically and by DNA-based phylogenetic analysis, that synthesize the carotenoid, gyroxanthin diester, in place of the more widely distributed peridinin. While this novel photopigment has been proposed as a biomarker, especially for remote-sensing imaging technologies, to detect the emergence of *K. brevis* blooms, other chemicals such as sterols and triglycerides, respectively, with potential to report the distribution and physiological condition of *K. brevis* are required. Recent work from our laboratories characterizing the lipids of dinoflagellates has confirmed that *K. brevis*, together with those its close relatives, *Karenia mikimotoi* and *Karlodinium micrum*, lacking peridinin, possesses a relatively simple sterol profile comprised of two unusual primary 4-methyl sterols, (24*S*)-4a-methyl-5a-ergosta-8(14),22-dien-3b-ol (ED) and its 27-*nor* derivative (NED). An October 1999 *K. brevis* bloom in the waters of the northwest Gulf of Mexico provided an opportunity to examine the usefulness of these sterols and other lipids as indicators of *K. brevis* in phytoplankton communities. Lipid extracts of filtered bloom samples, fractionated to separate free and esterified sterols, were examined by GC/MS of trimethylsilyl ether derivatives. ED and NED were the major sterols found in all bloom samples. Fatty acids found in lipid fractions containing membrane phospholipids, chloroplast-associated glycolipids, and storage triglycerides, respectively, differed significantly. The glycolipid fraction was found to contain octadecapentaenoic acid [18:5(n-3)], a fatty acid commonly associated with dinoflagellates. The phospholipid fraction was found to contain small amounts of the recently described highly-unsaturated fatty acids, octacosaoctaenoic acid [28:8(n-3)] and octacosaheptaenoic acid [28:8(n-6)]. Fatty acids from the triglyceride fraction were more abundant than those associated with glycolipids and phospholipids. These results were found to closely resemble cultured *K. brevis*. They will be compared to a more recent Fall 2002 bloom.

MORPHOLOGICAL ABNORMALITIES AND SENSORIMOTOR DEFICITS CAUSED BY ALGAL TOXIN EXPOSURE IN LARVAL ZEBRAFISH

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The dietary uptake of dinoflagellate-produced neurotoxins, commonly called paralytic shellfish poisoning (PSP) toxins, is known to cause acute fish kills. However, little is known about the effects of dissolved phase exposure and the potential sublethal effects of this route of exposure on early developmental stages of fish. Toxin exposure during early development is of particular concern because the embryos and larvae of some marine fish species may be unable to actively avoid the dissolved toxins that algal cells release into the water column during harmful algal blooms. Here we use the zebrafish (*Danio rerio*) as a model experimental system to explore the sublethal effects of a dissolved PSP toxin, saxitoxin (STX), on early development in fish, including sensorimotor function, morphology, and long-term growth and survival. Aqueous phase exposures of 229 ± 7 µg STX equiv. L^{-1} caused reductions in sensorimotor function as early as 48 hours postfertilization (hpf) and paralysis in all larvae by 4 days postfertilization (dpf). Rohon-Beard mechanosensory neurons appeared to be more sensitive to STX than dorsal root ganglion neurons at this dose. Additionally, exposures of 481 ± 40 µg STX equiv. L^{-1} resulted in severe edema of the eye, pericardium, and yolk sac in all exposed larvae by 6 dpf. The onset of paralysis in STX-exposed larvae was stage-specific with older larvae (6 dpf) becoming paralyzed more quickly (5 h) than younger larvae. When transferred to clean water, larvae recovered from the morphological and sensorimotor effects of STX. Thus, the sublethal effects of the toxin on larval morphology and behavior are reversible. However, zebrafish exposed to STX transiently during larval development (from 2 to 4 dpf) had significantly reduced growth and survival at 18 and 30 days of age. Collectively, these data show that 1) dissolved phase STX is bioavailable to fish embryos and larvae, 2) the toxin is a paralytic with potencies that are stage-specific for fish larvae, and 3) short-term toxin exposure can have long lasting consequences for the survival of exposed fish. Dissolved algal toxins may therefore have important sublethal effects on at-risk species of marine fish.

ANALYSIS OF EXPRESSED SEQUENCE TAGS (ESTs) FROM THE DINOFLAGELLATE *Karenia brevis*

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Sequencing of cDNA libraries to generate expressed sequence tags (ESTs) is an effective means of gene discovery. Our objective was to sequence cDNA clones from *Karenia brevis* cells and to identify putative genes for future genome-wide functional analysis studies.

Dinoflagellate genomes vary widely in a species specific manner, but in general are characterized as having a large DNA content, up to 40 times that of the human; however, little is known about complexity. The *K. brevis* haploid genome contains approximately 100 pg/cell or ~1 x 10^{11} base pairs. Although the total number of distinct genes is unknown for any dinoflagellate genome, approximately 6000 genes have been reported for other protists. Thus to gain insight into the genetic information carried by *K. brevis*, a cDNA library was constructed from cells in logarithmic growth phase and 8000 5′-end sequence tags were established. Total RNA (1.1 mg) was isolated from exponentially growing *K. brevis* cells using Qiagen RNeasy columns and the cDNA library was constructed from mRNA in the lZapII expression vector. For EST analysis, *E. coli* host strain XL1-Blue MRF' was infected with the lambda phage and *in vivo* excision of the pBluescript SK(-) phagemid from the lZAP II vector was performed with the ExAssist helper phage. The excised phagemid was then transformed in *E. coli* SOLR strain and plated on LB-ampicillin agar. Individual colonies were grown and purification of pBluescript DNA was performed with QIAprep Miniprep Kits using a QIAvac 96 Top Plate system (Qiagen, Valencia, CA). Sequencing was performed using the universal T3 and/or T7 primers. Nucleotide sequences obtained were then compared to non-redundant GenBank sequence database using the basic local alignment search tool program (BLAST) in its version for nucleotides (BLASTN) and aminoacids (BLASTX).

To expedite the screening process, we used an implemented EST pipeline to automate the high throughput EST data analysis process. This integrated pipeline enabled large batch submission of sequences and automated procedures included sequence phredding/phrapping, quality control (i.e. elimination of short sequences, empty vectors and those containing too many unreadable bases), NCBI BLAST submission and redundancy calculations. Preliminary results for a total of 1392 5′-end sequence tags indicated that 74% were similar to registered sequences when compared to the GenBank sequence database. Classification of these homolgs into functional categories revealed that 11% are involved in metabolism, 5% transcription, 2% cell growth, 5% defense, 3% communication, 6% transport, 4% energy, 3% protein synthesis, 10% membrane/structural, and 13% DNA/nuclear. The remaining sequences were defined as novel ESTs. This, as well as future sequence information, serves as a powerful source for genome-wide functional analyses of *K. brevis* and investigations into the pathways that control the growth of harmful algae.

STRONG DEPENDENCE OF *Pfiesteria piscicida* GROWTH ON THE ABUNDANCE OF PREY

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Growth dependence of the heterotrophic dinoflagellate *Pfiesteria piscicida* on its prey was investigated using batch and cyclostat cultures and the cryptomonad *Rhodomonas* sp. as prey. Extremely rapid grazing was observed in video recordings. Predator-prey dynamics were investigated using batch cultures and various feeding schemes, ranging from adding a small amount of the prey alga *Rhodomonas* sp. to a large population of *P. piscicida* to adding a small amount of *P. piscicida* to dense *Rhodomonas* sp. cultures. In most cases, *Rhodomonas* sp. was depleted within a day, and this was accompanied by an increase in *P. piscicida*. The magnitude of *P. piscicida* increase was dependent on the initial prey concentration and prey/grazer. Only in cases where few *P. piscicida* were added to *Rhodomonas* sp. cultures, i.e. under very high initial prey/predator ratio (50:1 or higher), was *Rhodomonas* depleted more slowly (up to 5 days). On the contrary, *P. piscicida* populations did not increase when the initial *Rhodomonas* to *Pfiesteria* ratio was very low (<1). In a 2-stage cyclostat with hourly supply of prey, growth of *P. piscicida* increased with grazing rate. Grazing by *P. piscicida* caused *Rhodomonas* to decline exponentially in both the batch and the cyclostat cultures. The dependence of *P. piscicida* growth on *Rhodomonas* sp. was further verified with cell size measurements and flow cytometric analysis of the cell cycle. In the starved culture, cell size and S-phase cells decreased markedly, with most *P. piscicida* cells apparently arrested at the G1 and the G2 phases. Re-supply of *Rhodomonas* sp. released the cell cycle arrest of *P. piscicida* and led to a rapid increase in the number of S-phase cells as well as cell size. Regression analysis revealed a significant positive correlation between *P. piscicida* yield and *Rhodomonas* concentration. The maximum and the 5-day-averaged growth rates also exhibited a Michaelis-Menton functional response to *Rhodomonas* sp. concentration and prey/predator ratio. Our results demonstrate that *P. piscicida* is a voracious grazer with its population being regulated directly by the availability of prey algae, and thus should be considered an obligate heterotroph.

THE ASSOCIATION OF ALGICIDAL BACTERIA AND RAPHIDOPHYTE BLOOMS IN SOUTH CAROLINA BRACKISH STORMWATER DETENTION PONDS

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Over the past 3 years, raphidophyte blooms have been documented with notable frequency in brackish stormwater detention ponds along the South Carolina coastal zone. During bloom events in 2002 and 2003, we investigated temporal fluctuations of algicidal bacteria against raphidophyte species (*Heterosigma akashiwo* and *Chattonella subsalsa*) abundance using the microplate most probable number (MPN) method. A total of 168 strains of algicidal bacteria have been isolated from raphidophyte blooms. In 2002, an increase in *C. subsalsa* algicidal bacteria from 2 MPN ml^{-1} to 103 MPN ml^{-1} was noted in response to a bloom of *C. subsalsa* (2.5 X 10^3 cells ml^{-1}). Subsequently, the abundance of *C. subsalsa* decreased to 14 cells ml^{-1}. A second bloom of *C. subsalsa* followed (1.1 X 10^3 cells ml^{-1}) during which *C. subsalsa* algicidal bacteria increased from 52 MPN ml^{-1} to 131 MPN ml^{-1}. A similar response was noted with *H. akashiwo* algicidal bacteria where abundance estimates increased from 18 MPN ml^{-1} to 97 MPN ml^{-1} associated with a decrease in *H. akashiwo* abundance from 1.6 X 10^3 cells ml^{-1} to 373 cells ml^{-1}. High population densities of *H. akashiwo* algicidal bacteria (> 100 MPN ml^{-1}) were noted in several other Kiawah Island pond samples associated with raphidophyte bloom events. In the summer of 2003, *C. subsalsa* and *H. akashiwo* were noted at low levels (< 5 cells ml^{-1}) and the numbers of algicidal bacteria targeting these two flagellates also appeared at low densities (< 2.2 MPN ml^{-1}) in the same ponds. In addition, bioassay experiments often indicated a stimulatory effect of antibiotic addition on raphidophyte growth (e.g. Fig. 1). These results suggest that algicidal bacteria may play an important role in the initiation and termination of raphidophyte blooms in brackish stormwater detention ponds.

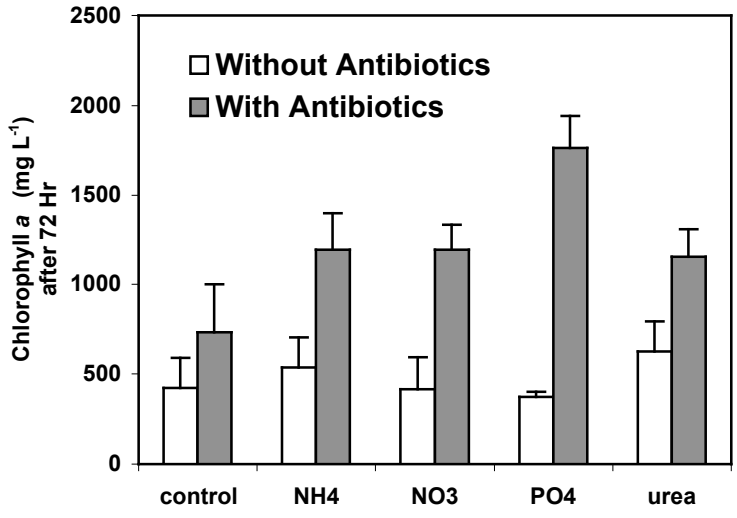

Figure 1. Bioassay results using water collected from *Chattonella subsalsa* bloom.
Comparison of 72-hr chlorophyll in nutrient treatments without (white) and with (gray) antibiotics.

A MOLECULAR APPROACH SPECIFIC FOR THE DETECTION OF *Prorocentrum lima* IN NEW ENGLAND COASTAL WATERS

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In the eastern US and Canada, the source of DSP toxins in shellfish is closely linked to the presence of the epibenthic/epiphytic dinoflagellate *Prorocentrum lima*. Although *P. lima* is generally easy to recognize under light microscopy, its presence within the epibiota of filamentous seaweeds complicates sampling and renders quantification difficult and very time-consuming. In an attempt to accelerate sample processing, a molecular approach using two *P. lima*-specific oligonucleotide probes is currently under investigation with New England coastal populations.

The two fluorescent probes, originally developed at AWI, are based on target sequences of the small subunit 18S and the large subunit 28S rRNA. Probe reactivity and specificity, first determined by DNA dot blot hybridization (digoxygenin system), have been verified in a whole-cell hybridization format with fluorescence microscopy using *P. lima* strains from Rhode Island and Maine coastal waters and dinoflagellates likely to co-occur with *P. lima*. Each probe satisfactorily reacts with the target organisms from U.S. northeast coastal waters. However non-specific probe-binding to cell surfaces is particularly problematic with one strain of *P. mexicanum*, an occasionally co-occurring species. Enumeration of *P. lima* in natural as well as *P. lima*- enriched field samples yields comparable cell concentrations, whether a traditional method based on microscopy or the developed molecular approach is used. The two methods of processing field samples will be compared using data on cell concentration within the epibiota, time expended to process the samples, difficulty of application and expenses.

LOSS OF TOXICITY AND CHARACTERIZATION OF ASSOCIATED BACTERIA IN A SINGLE CLONE OF *Alexandrium lusitanicum*

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Here we examine a specific case whereby a culture of *A. lusitanicum* from Obidos Lagoon, Portugal appears to have lost the ability to produce saxitoxins. Two subcultures of this isolate were established and maintained as separate isolates. Three independent toxin analysis methods (mouse bioassay, HPLC and mouse neuroblastoma receptor binding assay) show that while one of the variants maintains the same levels of toxicity and the toxin profile as the initial culture, the other no longer produces detectable toxin levels. Through morphological analysis and sequencing of three domains of the ribosomal gene the two cultures proved to be identical.

Several explanations can be suggested for the loss of the ability to produce saxitoxins, including mutations in one or more genes, or the change in associated bacterial flora due to continued antibiotic treatment of one of the subcultures. The hypothesis that bacteria living in close association with the dinoflagellate cells are directly or indirectly involved with toxin production is currently under investigation. The total bacterial assemblage (culturable and non-culturable) of both toxic and non-toxic subculture was identified using two different molecular methods. Denaturing gel electrophoresis and a clone library were carried out using 16s rDNA amplified with universal primers from total dinoflagellate DNA. Results of both methods show that the associated bacteria differed significantly between the toxic and the non-toxic dinoflagellate culture. The identity of associated bacteria was obtained through sequence comparison with entries in GeneBank. These results and new data from ongoing studies of bacterial involvement in toxin production will be discussed.

ULTRASENSTIVE DETECTION OF BLOOD DOMOIC ACID BY COMPETITIVE ELISA OF BLOOD COLLECTION CARDS

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Measurement of blood toxin levels provides the optimal means to biomonitor exposure of humans and animals to marine toxins, as blood is in equilibrium with all body tissues and serves to transport toxin to target organs. Blood collection cards provide an excellent means for collection, storage and solid phase extraction of various endogenous and nonendogenous substances in blood, and have proven effective to measure brevetoxins in both laboratory and environmentally exposed animals. Domoic acid is another marine toxin that requires frequent detection, particularly in marine mammals; however, domoic acid is cleared rapidly ($t_{1/2}$ < 1hr) from blood and is usually not detectable much longer than two hours after exposure in experimental animals. Accordingly, ultrasensitive detection methods are necessary for biomonitoring exposure to domoic acid. We report here the use of a direct competitive enzyme linked immunosorbant assay (cELISA) with extract from blood collection cards. This cELISA format was sensitive to picogram levels of toxin and unaffected by the presence of blood extract (ED_{50} =23.1 and 26.1 pg/ml, ED_{80} = 13.7 and 13.4 pg/ml; blood extract *vs* no extract, respectively). We next tested blood from ICR mice treated intraperitoneal with a nonlethal dose (2 mg/kg) of domoic acid. Blood was collected by cardiac puncture at 0.5, 1, 2, 4, and 24 hours, then dried and stored on blood collection cards. Domoic acid was detected at all time points and rapidly decreased from 27 to 0.97 ng/ml. The ability to detect domoic acid a full day after exposure should enable blood collection cards to serve as a useful method to biomonitor both marine mammals and humans for domoic acid exposure.

BEHAVIOR AND INTERNAL CELLULAR STATE OF *Karenia brevis* UNDER VARIOUS LIGHT AND TEMPERATURE CONDITIONS

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Karenia brevis is a photosynthetic dinoflagellate responsible for many HAB events in the Gulf of Mexico. Behavior is an integral part of the life history of *K. brevis.* The species undergoes a diel vertical migration where cells typically aggregate at the surface during the day and spread throughout the water column at night. Swimming characteristics, combined with physical factors, dictate the distance a cell can move in the water column, influencing not only environmental exposure (light, nutrients) but also influencing horizontal movement as the cell's relative position in the water column can expose it to varying flow regimes. Three clones of *K. brevis* - Manasota, Apalachicola, and Jacksonville - were examined under a range of light intensities and temperatures that correspond to the viable range of the organism. These clones were chosen because they were isolated from different geographic areas on Florida's panhandle, west coast and east coast, and because work by Schaeffer et al. (2002) suggested these clones have different photosynthetic capabilities. Cultures were grown under standardized conditions and then incubated in a radial photosynthetron for six or twelve hours under constant and changing light. Subsamples taken from each light level were 1) videotaped and analyzed with Expert Vision Motion Analysis package for swimming characteristics, 2) analyzed on the Pulsed-Amplitude-Modulated-Fluorometer for production, and 3) stained and evaluated on the flow cytometer for liposome content. Swimming speeds at 22°C for all three clones demonstrate an unusual response where speeds initially decrease with increasing light (50-300 umol quanta/m2/s), but then increase at intermediate light (631-1126 umol quanta/m2/s) before diminishing at high light (>1300 umol quanta/m2/s). While all three clones showed the same trend, Apalachicola tended to swim the fastest at all light levels, whereas Jacksonville tended to swim the slowest. Production decreased at lower temperatures. Results indicate an increase in liposome content with increasing light. This type of comparative data provides insights into the resource allocation within the cells by examining their swimming capabilities with respect to their photosynthetic capabilities. Observations detailing aggregation patterns among *K. brevis* clones in laboratory cultures have shown distinct surface patterns in the three clones examined. Further work will investigate intraspecific differences in swimming speed and liposome and chloroplast distribution, and what this might mean for the patterns described.

References:

Schaeffer, B., Kamykowski, D., Milligan, E., and McKay, L. (2002) Xth International Conference on HAB – *in review.*

APPLICATION OF A SPECIES-SPECIFIC *Karenia brevis* LSU rRNA PROBE ALONG THE TEXAS GULF COAST, USA

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Blooms of the brevetoxin producing dinoflagellate, *Karenia brevis*, occur in coastal waters throughout much of the Gulf of Mexico and are responsible for marine mammal mortalities and fish kills, as well as severe socio-economic impacts in this region. Methods for the detection of *K. brevis* cells and discrimination from morphologically-similar species in mixed natural communities are necessary for both monitoring and research purposes. We have recently developed a species-specific LSU rRNA oligonucleotide probe that distinguishes *K. brevis* from the morphologically similar and often co-occurring dinoflagellate, *K. mikimotoi*, in laboratory cultures using a whole-cell hybridization approach. This probe has also been successfully applied to preserved field samples from the west Florida shelf, showing strong labeling of cells identified microscopically as *K. brevis*. One of our present aims is to test the suitability of the *K. brevis* probe for use in other coastal areas of the Gulf of Mexico (e.g., Texas), including its application for discriminating against frequently observed morphological variants of *K. brevis* that may actually represent a different taxon or taxa.

As part of this study, the *K. brevis*-specific probe (Kbprobe-7) was applied to four "*K. brevis*-like" cultures isolated from Corpus Christi Bay, TX during the winter of 2002 (B1, C9, C18, C15) and one isolate from Nueces Bay, TX (NBK) sent in the blind by the Texas A&M laboratory to NOAA, Charleston. Prior to shipping, cultures were fixed using a modified saline ethanol fixative (Miller and Scholin, 2000) with 10% formalin added. A culture known to be *K. brevis* (NOAA-1) was fixed simultaneously at NOAA, Charleston in the same way to serve as a positive control for both the probe and the fixation protocol. Kbprobe-7 was applied to all cells using a whole cell hybridization/FISH protocol. Only one of the isolates tested (NBK), in addition to the *K. brevis* positive control, yielded a positive signal, whereas the remaining four Corpus Christi Bay isolates were clearly negative. Positive and negative control probes applied to each isolate gave the expected results. Sequence data from the nuclear ribosomal ITS region made available by the Texas A&M laboratory following the probe experiment indicated that the four negative isolates were, in fact, *K. mikimotoi*-like, while the single positive isolate was *K. brevis* and thus consistent with all probe results. Sequencing of the LSU rDNA is now underway to provide supporting phylogenetic data. Our findings represent the first successful application of probe Kbprobe-7 for distinguishing *K. brevis* from closely related dinoflagellates in Texas coastal waters, and also confirm the effectiveness of our fixation protocol. These results also indicate that use of this probe has a strong potential for incorporation into existing and planned efforts to monitor the occurrence of *K. brevis* in this region.

References:

Miller, P.E. and Scholin, C.A., 2000. On detection of *Pseudo-nitzschia* (Bacillariophyceae) species using whole cell hybridization: sample fixation and stability. J. Phycol. 36: 238-250.

ISOLATION AND STRUCTURAL INFORMATION ON A WATER SOLUBLE TOXIN DERIVED FROM *Pfiesteria piscicida*

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The structure and function of marine biotoxins are inextricably linked. To measure, characterize or chemically modify the activity of a particular toxin, its molecular structure must be determined unambiguously. Bioassay guided extraction and fractionation schemes have yielded discrete water soluble toxic fractions from *Pfiesteria piscicida*. Small sample sizes, molecular degradation processes and a consistent loss of activity over the time period required for purification and structural elucidation have required the use of innovative chromatographic methods coupled to large scale culturing techniques in our efforts to complete structural analysis as quickly as possible. We have been able, using rapid novel purification methods, to provide partial MS and NMR data on active fractions prior to molecular degradation.

Though obtained on microgram quantities from purified fractions, the data obtained to date has provided interesting structural information, providing clues to the identity of functional groups associated with the toxin(s). Identification of these functional groups in turn provides us opportunities to chemically stabilize the toxin(s) allowing the design of preparative scale isolation and purification schemes. Up to date 13C, 1H NMR as well as MS data are reported.

EXPERIMENTAL BIOACCUMULATION OF ICHTHYOTOXIC BREVETOXINS IN HEALTHY FISH

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Brevetoxins and ciguatoxins are potent neurotoxins produced respectively by *Karenia* spp. and *Gambierdiscus* spp. These two kinds of marine toxins present a similar chemical nature (trans-fused polyether rings) resulting inactivation of the voltage sensitive sodium channel by a specific interaction of the toxins with the site 5 of the α-subunit of the sodium channel. Ciguatoxins are transferred up the food chain to carnivorous fish usually without any signs of toxicity to contaminated fish. However, it is well known that fish are very susceptible to brevetoxins during blooms of *Karenia brevis*. During such events, brevetoxins are present in the water resulting in massive fish kills. Fish can potentially be exposed to brevetoxins by absorption of soluble toxin across the gills, by ingestion of *K. brevis* cells, or by transfer of brevetoxins through the food web. By feeding two species of coastal fish: Croakers (*Micropogonius undulatus*) and Pinfish *(Lagodon rhomboides)* with highly contaminated shellfish (total brevetoxin: 1.8 μg/g of shellfish tissue) collected in Florida, we demonstrated bioaccumulation of brevetoxins in tissue of the fish without any signs of toxicity or disease in the fish. Up to 4.2 μg of total brevetoxins per gram of fish tissue were found to be associated with the stomach and the intestine but significant amounts were also present in the muscles and skin of the exposed fish. The total amount of toxins in the body of some fish (up to 96 μg) was several magnitudes higher than what is known to kill fish when toxins are present in seawater (LD_{50} 4 hours: 6 ng/ml)

The toxin profile in the fish organs was similar to the profile in the clams used in the feeding experiments. In these experiments, fish were fed toxic clams for 2 consecutive weeks and then fed non-toxic clams for another 2 weeks. During the 4 weeks of the experiments, brevetoxins exposed fish were as healthy as the control fish fed only with non-toxic clams. When exposed to both toxic and non-toxic clams, fish could not discriminate between the two, and were feeding on all shellfish. These results strongly suggest that fish have the potential to accumulate non lethal concentrations of toxins in the wild after a red tide, when toxins are no longer present in the water but are concentrated in shellfish. This hypothesis was partially indicated but not confirmed by the detection of high concentration of brevetoxins by ELISA in tissues of dead striped burrfish (*Chilomycterus schoepfi*) collected in Florida after a recent red tide. Several days to a few weeks after a local red tide, a mortality of striped burrfish occurred in southwest Florida. Internal organs including liver, gills, and muscle as well as intestinal content, were found to contain high concentrations of brevetoxins (up to approximately 6500 ng/g PbTx-2 equivalents by ELISA), and fish had been feeding on small bivalves. Although this mortality indicated that fish were exposed to lethal concentrations of brevetoxins, the presence of brevetoxins (but as yet uncharacterized for potentially non-toxic metabolites) in the muscle of these fish does not necessarily indicate any potential risk to human consumers. Thus far we have no evidence for the accumulation and persistence of high concentrations of brevetoxins in the muscle of healthy fish during or after natural bloom events. Additionally, routine sampling of a range of fish species with different feeding habits indicates no or minimal accumulation of brevetoxins in the muscle, while mouse bioassays to date have not indicated the presence of toxic metabolites in the muscle. To ascertain to what extent these experimental data can be extrapolated to natural field situations and their potential as a human health risk needs further study. It must be determined 1) the condition of accumulation of brevetoxin in fish in the wild, 2) the level of contamination in healthy fish and 3) the toxicity of the fish after red tide.

Heterosigma akashiwo IN CENTRAL CALIFORNIA WATERS

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Brevetoxicosis has been reported to be responsible for the deaths of common murres (*Uria aalge*) in Monterey Bay, California (Jessup et al, 1998). However, there are no published studies on brevetoxin-producing species in Central California coastal waters. This study establishes baseline information suggesting the possible presence of *Heterosigma akashiwo*, a brevetoxin-producing species, based on water samples collected from the Santa Cruz pier in Monterey Bay (on the open coast) and the Berkeley pier in San Francisco Bay. Here we document the presence of *Heterosigma akashiwo* in seawater samples collected in 2001-2002, using two species-specific methods based on cell homogenates preparations. First, rRNA targeted probes from a "sandwich hybridization assay" (Scholin et al. J. Phycol. 35:1356 [1999]) were used to provide semi-quantitative data showing the intermittent presence of the species during a seventeen month period in Monterey Bay. Samples that showed the highest responses were then subjected to further analysis to confirm species identification, using polymerase chain reaction (PCR) amplification of nuclear internal transcribed spacer (ITS regions) (Connell: Phycologia 41:15 [2002]): these included samples from Monterey Bay and one from a "red tide" event in San Francisco Bay. In contrast, water samples from the sites that were fixed for microscopic analyses and taxonomic identification of species yielded equivocal results. Basically it was extremely difficult to recognize the species after fixation, even in the samples that showed the highest abundance of *Heterosigma akashiwo* by cell homogenate methods. The results suggest that *Heterosigma akashiwo*, though present, may not be sufficiently common along the open California coast to cause outbreaks of the frequency and severity documented in some other coastal environments. Furthermore, the microscopic identification of the species represents such a challenge that outbreaks of *Heterosigma akashiwo*, should they occur, would be difficult to recognize using standard microscopic techniques. The molecular methods, as documented here, thus may be preferred method of detecting the species for such morphologically difficult and fragile species.

TEMPORAL AND SPATIAL VARIABILITY IN THE CHARACTERISTICS OF *Alexandrium fundyense* BLOOMS IN THE COASTAL ZONE OF THE BAY OF FUNDY

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The abundance of *Alexandrium fundyense* has been monitored at four locations in the Bay of Fundy (eastern Canada) at seasonally varying weekly to monthly intervals since 1987. The date at which Alexandrium first appears in samples varied from day of year 105 to 179. This corresponds with a range of 74 days and a standard deviation of 16 days. The mean and median date of the first appearance of *Alexandrium fundyense* varied by only a few days between stations. The mean (median) days of first appearance estimated were 135 (132), 128 (130), 128 (129) and 128 (133) for sampling stations 3, 15, 16 and 17 respectively. The overall mean (median) date of first appearance was day 136 (134). The null hypothesis that the date of first appearance varied randomly (alpha 0.05) could not be rejected (alpha=0.05) by a two-sided runs test. The dates of the maximum concentration of *Alexandrium* cells vary by about 30 days between stations and years. The maximum cell counts occur earliest at the inshore estuarine station (day 172-175) and latest at the offshore station (day 197-203). The day of maximum cell counts at the other two stations is 188 and 194. The standard deviation in the date of maximum cell count ranges from 15 to 28 days. The time series of dates visually suggest the possibility of a low frequency trend suggesting an earlier date of maximum cell count in the more recent years. However, a non-parametric runs test is unable to reject the null hypothesis that the variation in the date of maximum concentration is random. The annual maximum concentration of *Alexandrium fundyense* varies by about three orders of magnitude and there is a range of about one order of magnitude in the station mean concentrations. The mean concentrations form a gradient from inshore to offshore with the mean and median cell concentrations being least in the inshore estuarine station and greatest in the offshore stations. Although the time series may appear to have a low frequency trend suggesting a lower maximum concentration in the more recent years, a non-parametric runs test is unable to reject the null hypothesis that each series is a random series of values. The duration of *Alexandrium* blooms ranged from 42 to 205 days. The mean (median) duration of the bloom was 114 (112) days. The mean bloom duration is 20-30 days shorter at inshore estuarine stations than at the other stations. The temporal character of the *Alexandrium* bloom also varies interannually with some blooms consisting of 1 to 3 pulses per year.

CHANGES IN THE COMPOSITION OF BREVETOXINS FOLLOWING CLAY FLOCCULATION FROM SEAWATER MEDIA

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Harmful algal blooms (HABs) occur worldwide affecting marine life and public health through seafood-borne illnesses and exposure to toxin-containing marine aerosol. This study was undertaken to investigate the fate of brevetoxins (from the toxic dinoflagellate, *Karenia brevis*) removed from seawater culture media with settled phosphatic clay. Concentrations of the most abundant brevetoxins were monitored in association with both clay and those remaining in the water column over a 14-day period. This study was conducted with lysed (ruptured) *K. brevis* cells to ensure that all of the toxins were extra-cellular, available for adsorption to the clay particles. The experimental design included three treatments: 1) lysed control cultures of *K. brevis* with no clay added, to observe brevetoxin degradation in the presence of ruptured cellular debris, 2) clay added to lysed cultures to observe brevetoxin degradation in association with clay particles that have settled to the bottom, and 3) clay plus natural sediment added to lysed culture to assess the role of natural microflora on toxin degradation. Laboratory cultures of 5 x 10^6 cells/L of *K. brevis* were obtained from the Mote Marine Laboratory red tide culture facility. Flocculation experiments were performed using phosphatic clay (IMC-P2) (International Mining Corporation [IMC], Bartow, FL) following the work of Sengco et al. (2001). The cells were lysed with ultra-sonication and 500mL of the lysed culture was added to 1-L beakers. Natural sediment was obtained from Sarasota Bay in an area frequently experiencing red-tide blooms. Triplicate 500-mL samples from the original culture were analyzed to determine the initial brevetoxin content. Thereafter, triplicate sets of beakers from each treatment were recovered for toxin analyses at intervals of 2, 5, 9 and 14 days. Toxin analysis was performed by LC-MS using standard brevetoxins obtained from Dan Baden at UNC, Wilmington. In the original culture, the most abundant toxins were: PbTx-2 (11µg/L), PbTx-3 (1.5 µg/L) and a trace of PbTx-1 (<0.03 µg/L). In the lysed culture with no clay or sediment, PbTx-2 was totally degraded by day 14 and PbTx-3 remained at about the original concentration. In association with settled clay, PbTx-2 diminished to 2 µg/L by day 9 and then remained at that level through day 14. In the presence of natural sediment with clay, PbTx-2 diminished more rapidly to 1 µg/L by day 9 and to 0.5 µg/L at day 14, while the PbTx-3 concentration remained about the same throughout the 14-day study.

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Sengco, M.R., A. Li, K. Tugend, D. Kulis, and D.M. Anderson. 2001. Removal of red- and brown-tide cells using clay flocculation. I. Laboratory culture experiments with *Gymnodinium breve* and *Aureococcus anophagefferens*. Mar. Ecol. Prog. Ser. 210: 41-53.

PROTEOLYTIC ACTIVITY DURING DINOFLAGELLATE BLOOMS IN THE CHOPTANK RIVER, MARYLAND (U.S.A.)

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The leucine aminopeptidase (LAP) assay is a measure of extracellular exopeptidase activity. We measured the LAP activity using the artificial substrate L-leucine **7**-amido-4-methyl-coumarin (Leu-AMC) during dinoflagellate blooms in spring 2003 in the Choptank River, a tributary of the Chesapeake Bay. In March and April the blooms were dominated by *Heterocapsa rotundatum* which reached high densities ($88x10^3$ cells ml^{-1} in March and $67x10^3$ cells ml^{-1} in April). *Prorocentrum minimum* and *Karlodinium micrum* were dominant during the blooms that occurred in May.

LAP activity ranged from 1.9 to 7.5 mmoles AMC l^{-1} h^{-1} with 2 to 34% of the total activity associated with the >2 mm fraction. The percentage of LAP activity associated with the >2 mm fraction was positively correlated with dinoflagellate concentration (Fig.1), but we did not find any significant correlation between the density of dinoflagellates and the activity in the <2 mm fraction. LAP activity and ammonium concentration were inversely correlated.

LAP activity in seawater is usually ascribed to bacterial activity, but, during blooms, dinoflagellates could be an important source of proteolytic activity. Leucine aminopeptidase degrades polypeptides, releasing amino acids which may be directly taken up by dinoflagellates or oxidized to ammonium which can be assimilated. This proteolytic activity may play a role in nutrition of mixotrophic dinoflagellates and enhance carbon flow through the microbial loop.

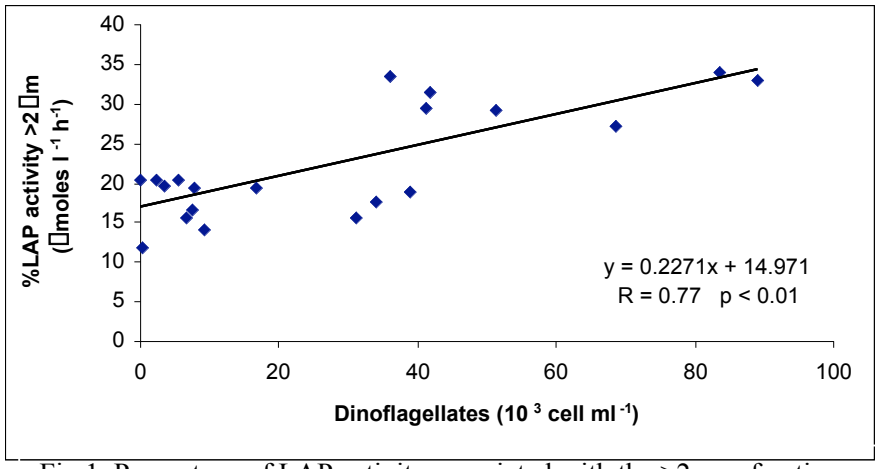

Fig.1. Percentage of LAP activity associated with the >2 mm fraction and dinoflagellate density in the Choptank River, spring 2003.

ALTERED FOS EXPRESSION AS A BIOMARKER OF NEURONAL STRESS ASSOCIATED WITH HARMFUL ALGAL BLOOM EXPOSURE IN MUMMICHOG, *Fundulus heteroclitus*

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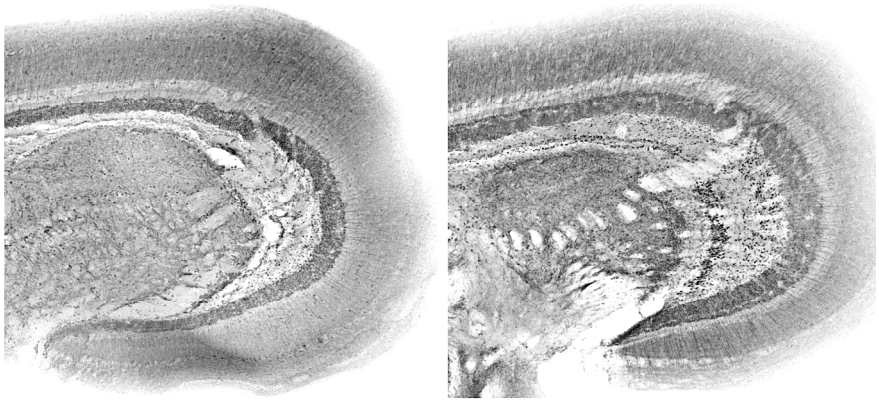
Behavioral changes in organisms can result from complex alterations at the biochemical and physiological levels of organization. The goal of this work was to develop and test the utility of Fos expression using immunocytochemistry as a biomarker of stress exposure in mummichog, *Fundulus heteroclitus*. c-Fos, an immediate early gene, and its protein product Fos, are induced in neurons as a result of neuronal stimulation. This study examined alterations in the brains of mummichog exposed to different harmful algal bloom (HAB) stressors including brevetoxin, domoic acid and *Pfiesteria shumwayae*. Brains of exposed fish were removed, sectioned and stained, and neurons expressing Fos were quantified. HAB-exposed fish brains showed increased neuronal Fos labeling compared to control fish brains. A dose response relationship was observed in *P. shumwayae* exposed fish, with increased labeling in fish exposed to higher dinoflagellate cell counts. Areas of the brain with increased labeling included optic lobes, midbrain and portions of the medulla. Alterations in swimming and respiratory behaviors were observed during all HAB exposures and may be associated with the increased regional neuronal activity. Alterations in Fos labeling as a biomarker of exposure may link quantifiable changes in fish swimming behavior associated with HAB exposure to changes in brain activity. General alterations in brain activity, as well as knowledge of specific, stress-activated regions within the brain, can provide valuable insights into the neural control of fish behavior as well as sublethal effects of chemical and physical HAB stressors.



Optic lobes of typical control (left) and *Pfiesteria*-exposed (right) mummichog brains. Brains of *Pfiesteria*-exposed animals reveal a higher density of dark, focal neuronal c-fos staining.

THE SOUTH CAROLINA PHYTOPLANKTON MONITORING NETWORK: THE USE OF VOLUNTEERS TO MONITOR PHYTOPLANKTON

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The South Carolina Phytoplankton Monitoring Network (SCPMN) is a NOAA sponsored community outreach program developed to increase awareness of harmful algae to constituent groups and directly involve volunteers in coastal stewardship. SCPMN commenced in January 2001 with three school groups monitoring waters in Charleston County. In the past two and a half years, this program has expanded to 28 middle and high schools, 12 environmental citizens organizations, and 4 state parks in six coastal counties. Volunteer monitoring groups are trained in phytoplankton sampling techniques and identification methodologies.

In the monitoring network's first year and a half of existence, volunteers observed three species of potentially toxic algae including *Pseudo-nitzschia* spp., *Dinophysis caudata*, and *Prorocentrum lima*. For all three species, cell concentrations were lower than the threshold for possible human health problems. In addition, volunteer groups have reported blooms of nontoxic species including *Akashiwo sanguinea* and *Synechoccus*. These observations made by volunteer groups will be used to develop a species list and serve as a preliminary investigation of HABs in South Carolina waters.

PHOTOOXIDATION AND PHOTOINHIBITION IN THE RED TIDE DINOFLAGELLATE *Karenia brevis*

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Laboratory studies have identified the internal biochemical status of the cell as a determinant for the growth, reproduction, and possibly migratory behavior of *Karenia brevis*. The internal biochemical status of the cell is directly influenced by the cell's capability to photosynthesize. Understanding mechanisms that affect photosynthesis in *Karenia brevis* is crucial in identifying the organism's ability to use resources in local waters and to proliferate. Previous work included a quantized population in which all cells divided at night. At dawn, the same biochemical composition was found throughout the mesocosm, but by noon surface low lipid and subsurface high lipid subpopulations developed. This biochemical differentiation was thought to result from differential diel vertical migration by daughters with different resource allocations from the parent cell. An alternative hypothesis described the biochemical differentiation as a response to a light gradient. Here, photooxidation and photoinhibition mechanisms are thought to be primarily responsible for the cellular biochemical decreases observed at the surface while optimal production is thought to be responsible for cellular biochemical increases at depth. To investigate this alternative hypothesis cultures were grown in a 225L mesocosm to allow diel vertical migration for three days during 12 hour light/dark cycles. Prior to lights on (day 1) and in mid-afternoon (day 3), samples were removed from the mesocosm and incubated in a radial photosynthetron. Aliquots from the mesocosm and the photosynthetron were subsequently collected to determine pulsed amplitude modulate fluorometer (PAM-FL) electron transport, yield, chlorophyll, and lipid content for populations with (mesocosm) and without (photosynthetron) behavior. All lipid samples were analyzed by thin layer chromatography with a flame ionization detector Iatroscan using a multiple step separation technique. This technique focused on triacylglycerol storage lipids, 1,3 diacylglycerol, 1,2 diacylglycerol intermediates, and finally monogalactosyldiglyceride and digalactosyldiglyceride chloroplast membrane lipids which are likely targets for oxidation. PAM-FL measurements were compared to lipid content within the cells at different depths and times during the diel cycle.

DEVELOPMENT OF THE "SECOND GENERATION" ENVIRONMENTAL SAMPLE PROCESSOR (2G ESP) FOR REMOTE DETECTION OF HARMFUL ALGAE AND TOXINS THEY PRODUCE

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Development of instruments that enable long term, unattended, *in situ*, application of molecular probes with real-time telemetry of results will offer an exceptionally novel approach for detecting HAB species, genes they harbor and express and toxins they may produce. In a step toward reaching this goal, scientists and engineers at the Monterey Bay Aquarium Research Institute (MBARI) have developed a prototype instrument known as the Environmental Sample Processor (ESP; http://www.mbari.org/microbial/ESP). The "first generation" (1G) ESP was designed to collect discrete water samples remotely, subsurface, concentrate microorganisms and automates application of DNA (or other) molecular probes to enable identification and quantification of particular species captured. The instrument transmits results of DNA probe array assays in real-time via radio modem to a shore based location for processing and interpretation. In addition, the 1G ESP can archive discrete samples for nucleic acid, microscopic and toxin analyses for validating real-time data from the probe arrays as well as facilitating other analyses in the laboratory (such as construction of gene libraries).

The 1G ESP has worked successfully on several, limited test deployments with extensive support by the original science/engineering design team. Development of a "second generation" (2G) ESP has begun, including adding an analytical capability for detection of domoic acid. The overall goal in designing the 2G ESP is to make it much more robust and user friendly relative to the 1G prototype, and to produce a system that can be successfully deployed for a variety of applications on a routine basis by a team of trained technicians. A major effort is being made to reduce the size, complexity and power consumption of the instrument, and to take advantage of microfluidic-scale detection technologies. There will also be an effort to provide space and appropriate electrical, software and fluid connectivity to support the addition of future sample and analytical 'modules', separate devices that can be used to extend the range of sample collection and analyze detection options of the core 2G ESP. The first 2G ESP is scheduled to begin operation mid-2004. This presentation focuses on progress made to date designing and developing the 2G hardware, and emphasizes development of DNA probe array and domoic acid detection technologies.

GERMINATION OF DINOFLAGELLATE RESTING CYSTS FOLLOWING DEPOSIT-FEEDER GUT PASSAGE AND PELLET ENCAPSULATION

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As dinoflagellate cysts may remain viable in marine sediments for months to years, they may pass through the guts of deposit feeders many times before conditions become favorable for germination. Little is known, however, about how dinoflagellate cysts are affected by deposit-feeder digestion, fecal pellet formation, and translocation within the sediment column by bioturbation. To answer the question whether gut passage leads to cyst mortality, we fed cysts of the dinoflagellate *Scrippsiella lachrymosa* to three species of deposit feeders, *Capitella* sp., *Streblospio benedicti*, and *Polydora cornuta*. These species differ in the extent to which they form fecal pellets. To examine the effects of longer gut-passage times, we incubated cysts in *Arenicola marina* digestive fluid for up to 24 h. We then monitored the cysts to determine rates of germination. We found that cysts were remarkably resistant to digestion by deposit feeders and that they were capable of germinating even within the robust fecal pellets of *Capitella*. Although burial due to bioturbation by deposit feeders might reduce cyst germination in the field, we expect that gut passage and pelletization does not result in substantial mortality of dinoflagellate resting stages.

SHELLFISH AS VECTORS FOR INTRODUCTION OF HARMFUL ALGAE, WITH EMPHASIS ON THE TOXIC DINOFLAGELLATES *Karenia brevis* AND *Alexandrium monilatum*

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We summarize research on harmful algal species that have been shown to pass intact through the digestive tract of shellfish, with emphasis on *Karenia brevis* and *Alexandrium monilatum* which form toxic blooms in the Gulf of Mexico. Successfully invading species are difficult or impossible to eradicate; therefore, potential vectors of such species should be carefully examined in efforts to minimize bioinvasive species dispersal. Introduction of HAB species via cargo ship ballast water has received considerable attention in recent years, but other potential modes of transfer mostly have been ignored. One obvious vector for the possible transfer of HAB species is molluscan shellfish (e.g. oysters, clams, mussels). Some harmful dinoflagellates survive ingestion, gut passage, and egestion by bivalve molluscs. Dinoflagellates potentially may be introduced into new areas through molluscan feces if cells are able to survive passage through the digestive tract, thus rendering molluscs prime vectors for transfer of harmful algal species. Viable cells within the feces can also influence the recurrence and duration of toxic blooms. If live dinoflagellate cells or cysts are transported into areas via shellfish and their feces, the cells may be able populate or re-populate the site. We microscopically examined fecal material from shellfish species *Crassostrea virginica* (eastern oyster), *Mercenaria mercenaria* (northern quahog), *Argopecten irradians* (bay scallop) and *Perna viridis* (green-lipped mussel) after exposure to harmful or potentially harmful algae to determine whether cells remained viable after passage through the digestive tract. Feces produced by the shellfish were gently removed and washed several times with 0.22-µm filtered seawater to remove free dinoflagellate cells and debris. The viability of the cells within the feces was determined by inoculating rinsed, intact fecal strands (n = 5) into flasks containing sterile growth media and into flasks containing 0.22-µm filtered natural seawater. Dinoflagellate cell densities were quantified at 7-day intervals to assess whether the cells within the feces had been viable. If cysts were detected within the feces, they were removed using a micromanipulator (100 cysts per replicate; n = 5). The cysts were placed into conductive media for growth, and into 0.22-µm filtered natural seawater. Excystment and subsequent zoospore motility were observed at 1-hour intervals over 24 hours. A viability stain was also used on washed feces and extracted cells and cysts from fecal material as a second test for cell viability. Cells and/or cysts of toxic strains of *Pfiesteria piscicida* and *Pfiesteria shumwayae* were structurally intact and viable after passage through the digestive tract of eastern oysters, northern quahogs, bay scallops, and/or green-lipped mussels. Cells of *Karenia brevis, Alexandrium monilatum, Karenia mikimotoi* and *Prorocentrum minimum* were also structurally intact, and additional tests of viability are ongoing. Overall, the data indicate that molluscs are potential vectors for the dispersal of toxic dinoflagellates, and could aid in increasing the geographical range of toxic species.

BIOINFORMATIC APPROACHES TO THE STUDY OF BIOCHEMICAL PROCESSES IN HAB SPECIES: PATHWAY ANALYSIS OF COVARIATION IN PROLINE METABOLISM WITH GROWTH CONDITIONS MODULATING DOMOIC ACID PRODUCTION BY *Pseudo-nitzschia multiseries*

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A variety of HAB species are being subject to modern genomic analysis with the goals of identifying gene products and regulatory networks functioning in toxin production as well as to provide markers for querying the metabolic status of these organisms in the field. While these technologies provide a wealth of data, their interpretation beyond cataloging presence/absence of metabolic functions represents a considerable challenge due to the complexities of genetic and biochemical networks. Computational approaches are needed to bridge these interpretive gaps with the added benefit that *in silico* analysis of metabolism based on hypothesis driven model runs can help identify critical targets for, and aid interpretation of on-going gene expression studies. Biochemical Systems Theory (BST) is one of several analytical frameworks that enable co-analysis of disparate genomic and metabolic data sets and is based on approximation of kinetic rate laws with multivariate power-low functions (Savageau, 1976; Voit and Radivoyevitch, 2000). Here, using BST we describe the development of a generalized mass action (GMA) model of proline metabolism in *P. multiseries* in order to characterize the dynamics of pathway intermediates under growth conditions supporting different levels of domoic acid (DA) biosynthesis.

In general, continuous culture experiments with phytoplankton offer tractable systems for computational analysis of metabolism. Metabolite profiling of the quasi-steady-state conditions obtained permit modeling of associated biochemical pathways using GMA systems and related approaches. As a prototype analysis we first defined a generic, but detailed pathway model of proline metabolism, incorporating both the glutamate and arginine/ornithine pathways to proline biosynthesis, and populated the catalytic steps with estimates of enzyme kinetic parameters derived from the BRENDA database (http://brenda.bc.uni-koeln.de/). The pathway model was subsequently re-coded as a GMA system based on analysis of intracellular free amino acid (FAA) pools sampled from continuous cultures of *P. multiseries* grown in Si-limiting (Si/NO3 < 0.1) or NO3-limiting media (Si/NO3>2) using a wide range of supply rates (0.2/d to 1.2/d). In Si-limiting media, DA content increased by over 100-fold in cells subjected to low Si (μ = 0.2/d) compared to high Si (μ = 1.2/d) supply rates or in NO3-limiting media. Although absolute FAA content decreased under NO3 limitation, relative FAA content (FAA/PON, molar) increased with growth rate in both media types and ranged from 1% to 5% of the PON content. In contrast, no significant growth rate dependent trends in GLU or PRO pools were observed in Si-limiting media. Overall, DA accumulation was enhanced in cultures with POC/PON <8 and GLU contents >5 fmole/cell. The GMA model, as initially parameterized, points to growth rate-dependent changes in enzyme abundance as underlying the observed changes in the FAA pools. Intriguingly, model results indicate that maintenance of GLU pools in conditions promoting DA production, may reflect enhancement of GLU biosynthetic (GOGAT, glutamate synthase) and PRO catabolic (ProDH, proline dehydrogenase) activities in Si-limited relative to NO3-limited cells. Under these model conditions, Si-limited *P. multiseries* cells may be subject to stronger transients in the accumulation of the potentially toxic (ROS-active) intermediate of PRO catabolism, *del*^{1}- pyrroline-5-carboxylate (or hydroxylated derivatives).

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THE DISTRIBUTION OF BREVETOXIN (PbTx-3) TO SPECIFIC LIPOPROTEIN FRACTIONS IN MOUSE BLOOD

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Brevetoxin, the neurotoxin produced by *Karenia brevis*, has had adverse effects on the environment, humans, and marine organisms for hundreds of years. Following consumption of shellfish infected with brevetoxins, or simply inhaling aerosolized brevetoxins, humans can show symptoms ranging from respiratory to digestive distress. Blood is an essential tissue to analyze when a human or animal is suspected of having brevetoxicosis because it serves to distribute toxins to the tissues. Many small molecules are bound to carrier proteins, such as albumin or specific binding proteins, or to larger lipoprotein particles in the blood. Lipophilic contaminants such as o',p'-DDT are known to accumulate in lipoprotein particles and because brevetoxin is also lipophilic, we have investigated the distribution of the brevetoxin congener PbTx-3 to blood lipoproteins. The first stage of the experiment was conducted using mouse plasma spiked with PbTx-3. This plasma was fractionated into different size lipoproteins by iodixanol gradient ultracentrifugation. Each fraction was then characterized and quantified by Lp(a) lipoprotein affinity agarose gel electrophoresis and radioimmunoassay (RIA). The PbTx-3 was restricted to only those gradient fractions confirmed to contain high-density lipoproteins (HDLs). None of the fractions containing low-density lipoproteins (LDLs) contained PbTx-3. We are currently analyzing the distribution of brevetoxin in lipoprotein fractions of blood from mice exposed to sublethal doses of PbTx-3. New information on the distribution of brevetoxins in blood and the process by which the toxin is delivered to tissues may permit more effective therapeutic measures to treat intoxication from brevetoxins and the related ciguatoxins.

EFFECTS OF *Alexandrium fundyense* CELL CONCENTRATION AND CELLULAR TOXICITY ON COPEPOD FEEDING BEHAVIOR

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Mesozooplankton such as copepods are major grazers of phytoplankton, and their feeding activity can potentially control harmful algal blooms (Campbell et al., 2000; Teegarden et al., 2001), as well as provide vectors for food web transfers of toxin (White, 1981; Teegarden and Cembella, 1996; Turner et al., 2001; Teegarden et al., 2003). Laboratory experiments suggest that copepods can and will avoid toxic *Alexandrium* sp. cells in mixtures of prey types, and that cellular toxin content is a principal cue for selective feeding (Teegarden, 1999). Field studies of zooplankton grazing on *Alexandrium* sp. at lower concentrations indicate that non-selective feeding is common when toxic cells are only a minor portion of available food (Teegarden et al., 2001). From these studies we hypothesized that selective feeding on *Alexandrium* spp. is dependent on cell concentration, or cellular toxicity, or both. To address and prioritize these hypotheses, we performed a series of experiments challenging three species of copepod grazers (*Acartia hudsonica*, *Centropages hamatus*, *Eurytemora herdmani*) with mixtures of natural water samples containing non-toxic algae (diatoms and flagellates) and three clones of *Alexandrium* spp. – GTCN16 (toxin content below detection), GTCA28 (moderate toxicity, ~25 pgSTXeq cell^{-1}), and BC1 (high toxicity, ~75 pgSTXeq cell^{-1}), each at high (10^5 cells L^{-1}) and low (10^4 cells L^{-1}) concentrations.

Ingestion of *Alexandrium* sp. by all copepod species depended more on concentration than cellular toxicity. Within any one copepod species and *Alexandrium* sp. clone treatment, clear differences existed in clearance rate, selection indicated by electivity index, *Alexandrium* cells ingested, and total food ingested. In low *Alexandrium* sp. concentration treatments, copepod clearance rates on *Alexandrium* were usually higher and electivity indices less negative, indicating less avoidance compared to high concentration treatments. Effects of cellular toxicity were not however consistent and predictable. For example, there was little difference between *E. herdmani* rates of consumption or selectivity of *Alexandrium* between the BC1 high toxin clone treatment and the GTCN16 low toxin clone treatment, while the moderate toxicity GTCA28 clone was shunned. In high toxicity (BC1) treatments, one result consistent among copepod species was that total food consumption was lower at high *Alexandrium* sp. concentrations, suggesting that high cellular toxicity and high cell concentration suppresses overall feeding, but this is not solely a result of selective *Alexandrium* sp. avoidance, as all food species were consumed at lower rates. The low toxicity GTCN16 clone was also consistently consumed by all copepod species at the highest rates and greatest positive electivity compared to more toxic clones fed to the same grazer species, but differences were not dramatic. To summarize, toxicity of individual *Alexandrium* sp. cells appeared to have only minor influence on feeding selectivity, unless combined with the factor of high concentration. Concentration however had an effect on selectivity and total food consumption at any level of cellular toxicity.

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THE PRESENCE OF DOMOIC ACID IN *Pseudo-nitzschia* FROM THE CHOPTANK RIVER, A CHESAPEAKE BAY TRIBUTARY

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Pseudo-nitzschia is a toxic diatom that produces domoic acid (DA), the neurotoxin responsible for Amnesic Shellfish Poisoning (ASP) and Domoic Acid Poisoning (DAP) which affects humans through contaminated shellfish and marine mammals and birds through contaminated fish. Three clones of *Pseudo-nitzschia* were isolated from the Choptank River, one in November 2002 and two in April 2003. All three clones were tested for domoic acid activity using the receptor binding assay and ASP direct cELISA test kits. Only the November clone was found to be toxic, displaying 0.08 pg DA-equl/cell in exponential phase and .438 pg DA per cell in stationary phase. While the presence of *Pseudo-nitzschia* has been documented in the lower Bay since the early 1980's, this is the first record of toxic *Pseudo-nitzschia* in the Chesapeake Bay area. Historical data suggests that *Pseudo-nitzschia* abundances have been increasing and spreading throughout the Bay over the past five years. Based on algal community monitoring by Maryland Department of Natural Resources between 2000-2002, 50% of the water samples containing *Pseudo-nitzschia* had concentrations above levels requiring mandatory testing of shellfish meats in Denmark and New Zealand. No known toxic events have occurred in Maryland, however, increasing *Pseudo-nitzschia* abundances and the presence of domoic acid in the food chain could have an impact on the future of the Cheapeake Bay shellfish industry, wild vertebrate populations, and public health.

OBSERVATIONS ON MIXOTROPHIC FEEDING BY THE TWO AUTOTROPHIC HAB SPECIES *Amphidinium carterae* AND *Prymnesium parvum* IN ESTUARINE WATERS

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Clonal cultures of the two HAB species *Amphidinium carterae* and *Prymnesium parvum* were established from natural samples and studied in batch culture. *Amphidinium carterae,* originally isolated from a sample taken in the coastal Yucatan Peninsula, Mexico, and a *P. parvum* clone isolated from an aquaculture facility having large fish kills in North Carolina, were both grown in F/2 medium in 6 well tissue culture plates. Cultures were further conditioned to phosphorus limiting media by transferring them to F/2 having $10\mu M \cdot L^{-1}$ PO_4-P. Cell growth was monitored over a 21 day period and observations were made relative to their behavior when ciliates or dinoflagellates were introduced. For the *A. carterae* cultures, cells of the ciliate *Euplotes* sp., abundant in an original raw sample, were introduced into wells when *A. carterae* densities of 5×10^4 cells$\cdot L^{-1}$ occurred. Within a few hours, *A. carterae* cells were found aggregating near the *Euplotes* and while the ciliate kept moving away from the mass of algal cells, a trailing stream of *A. carterae* continued to follow the ciliate eventually surrounding it and rendering it immobile. The mass of *A. carterae* cells eventually totally enveloped the *Euplotes* appearing to attach to the surface of the ciliate. Within four hours, the ciliate was completely devoid of cellular material with a remaining empty cast. After consumption of the *Euplotes, A. carterae* cells dispersed and were found regularly distributed throughout the volume of the wells.

For *Prymnesium parvum*, phosphorus limited cells were also conditioned in the manner described above and attained densities of 6.6×10^5 cells$\cdot L^{-1}$. When these densities were achieved, cells of a clonal culture of *Gyrodinium instriatum* were introduced into the *P. parvum* wells and followed for a period of 6 days. The *G. instriatum* cells were highly motile, exhibited normal morphology and for the first day showed no effects of *P. parvum*. Within 48 hours, however, enlarged *G. instriatum* cells showing a rounded morphology began to appear in the cultures with at first few (2-5) *P. parvum* cells attached. The enlarged spherical infected cells continued to be motile but had slower motion than those showing no symptoms of attachment by *P. parvum.* By the third day of incubation, numerous *G. instriatum* showed the unusual morphology, no motility as completely spherical cells with attached swellings of *P. parvum* plainly visible. These cells persisted for a few days before they were lysed releasing the *P. parvum* to the media as motile cells. By the end of the 6 day study, over 80% of the *G. instriatum* cells had been infected and the *P. parvum* cells had increased to 3.3×10^6 cells$\cdot L^{-1}$.

Both organisms studied naturally co-occur with the prey items tested. In the case of *A. carterae*, a sand dwelling dinoflagellate, *Euplotes* is present in its immediate environment in the highly saline waters of the Gulf of Mexico. In contrast, *P. parvum*, found at salinities as low as 4, co-occurred with *G. instriatum* competing for the same resources. Infection of a co-dominant under the conditions of the aquaculture ponds offered an effective nutrient gathering method as well as reduction in competition for any available resources. Both organisms when nutrient stressed exhibited the same strategy of mixotrophy to allow them to maintain their densities and bloom populations.

VALIDATING REMOTE SENSING TECHNIQUES FOR DETECTING *Karenia brevis* IN THE GULF OF MEXICO

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Since 1999, the Center for Coastal Monitoring and Assessment (CCMA) has been using ocean color imagery from SeaWiFS to detect and monitor blooms of the toxic dinoflagellate, *Karenia brevis*, in the eastern Gulf of Mexico. Anomalously high chlorophyll is used for detection, where the anomaly is defined as the difference between real-time observations and a two-month running mean of SeaWiFS-derived chlorophyll. This method has proven accurate in detecting blooms >83% of the time along the Florida Panhandle, from Tampa Bay to Cape Romano and the Florida Keys.

Although this technique is successful in detecting blooms in which concentrations exceed 10^5 cells L^{-1}, the level at which marine mammal deaths and respiratory irritation occurs in humans, other techniques may aid in detecting blooms which exceed $0.5x10^5$ cells L^{-1}. Empirical optical techniques show promise in distinguishing between various phytoplankton groups. One optical algorithm distinguishes between *Karenia brevis* and other phytoplankton (such as *Trichodesmium* spp.) by comparing particulate backscattering to chlorophyll *a*; this has potential application in water with low scattering and low colored dissolved organic matter. In addition, the CCMA is developing a technique which separates resuspended benthic algae from the water column phytoplankton. This involves identifying chlorophyll associated with resuspended sediment (as determined by the 670 reflectance), as resuspension events often produce elevated chlorophyll along the Texas coast. The methods are being evaluated to determine their accuracy and reproducibility. These methods offer the potential to expand the detection of *Karenia brevis* to various regions in the Gulf.

THE FATE OF DOMOIC ACID IN BENTHIC COMMUNITIES OF MONTEREY BAY, CALIFORNIA

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Domoic acid (DA) is produced by the diatom, *Pseudo-nitzschia sp.*, along both the east and west coasts of North America. It is responsible for Amnesic Shellfish Poisoning, and has been shown during toxic blooms to neurologically affect marine birds and mammals, in cases resulting in massive mortality events. Along the west coast, preliminary studies have been done on the trophic transfer of DA through the pelagic food web, leading to a better understanding of the fate of this toxin in the environment. However, the presence of DA in benthic communities is poorly understood. Recent research indicates that DA may be highly concentrated in certain species of benthic invertebrates and that DA may be present in the sediment underlying toxic blooms. To date, there have been no studies examining whether DA is reaching higher trophic levels in the benthos. The primary objective of this research is to identify whether DA contaminates benthic fish communities, and if so, to what degree. Specific aims include: 1. Determine the toxin concentration in whole viscera of bottom-feeding fish over a two year period, 2. Explore possible toxin vectors, including both lower trophic level benthic organisms and sediment/detrital sources, using both newly available data on DA concentration in benthic invertebrates and sediment, as well as stomach content analysis from contaminated fish in this study, 3. Investigate whether there is a correlation between toxin concentration in benthic fish and toxin concentration in the overlying water during toxic bloom events. Species of rockfish and flatfish, as well as water samples, were collected from nearshore and offshore sites within Monterey Bay, CA. HPLC-UV and HPLC-FMOC methods were used to analyze toxin concentration in pooled individuals of fish species and water samples, respectively. While dissecting the individual fish, stomach content (including both organisms and the presence of sediment) was observed and recorded. Here we report domoic acid concentrations for benthic fish and water samples over the first 9 months of this study, along with preliminary suggestions of possible benthic contamination sources. Examining the presence of DA in higher trophic levels of the benthos will lead to a better understanding of both the extent of DA contamination in marine food webs during toxic blooms and the fate of DA at the end of these blooms.

LC/MS ANALYSIS OF BREVETOXINS AND THEIR METABOLITES IN THE EASTERN OYSTER (*Crassostrea virginica*)

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Brevetoxins from *Karenia brevis* are rapidly metabolized in the Eastern oyster (*Crassostrea virginica*), as evidenced by LC fractionation and *in vitro* assay of toxic oyster extracts (Dickey et al., 1999). In laboratory studies with pure brevetoxins (B-type, PbTx-2 and -3), we previously identified by LC/MS two of these metabolites, cysteine-PbTx and its sulfoxide (MH^+: *m/z* 1018 and 1034), as derivatives of PbTx-2 (Plakas et al., 2002). PbTx-2 is also metabolically reduced to PbTx-3 in the oyster. In the present study, we further explore brevetoxin metabolism in oysters naturally exposed to *K. brevis* red tide, by using LC/MS and *in vitro* assay. In addition to the previously identified metabolites of PbTx-2, we found a cysteine conjugate and its sulfoxide (MH^+: *m/z* 990 and 1006) with A-type backbone structure, as probable derivatives of PbTx-1. We also found glycine-cysteine-PbTx (*m/z* 1047 and 1075), glutamyl-cysteine-PbTx (*m/z* 1147), and glutathione-PbTx (*m/z* 1176 and 1204) conjugates with A- and B-type backbone structures. Amino acid-toxin conjugates can further react with fatty acids in oysters through amide linkage to form a series of fatty acid-amino acid-toxin conjugates. These fatty acid conjugates are apparent major contributors to the composite cytotoxic responses obtained in extracts of brevetoxin-contaminated oysters. Other brevetoxin compounds found in oysters were consistent with hydrolysis and oxidation/reduction reactions. Brevetoxins and metabolites observed in field-exposed oysters were confirmed in oysters exposed to *K. brevis* cultures in the laboratory. Of those analyzed, the previously identified cysteine-PbTx metabolite of PbTx-2 was by far of the highest relative abundance by LC/MS. Cysteine-PbTx and its sulfoxide are being evaluated as potential biomarkers for monitoring brevetoxin contamination in oysters.

References:

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THE RELATIVE IMPORTANCE OF DOM UPTAKE, GRAZING AND PHOTOSYNTHESIS OVER DIEL CYCLES IN THE MIXOTROPHIC DINOFLAGELLATE *Akashiwo sanguinea*, IN THE LAFAYETTE RIVER, A TRIBUTARY OF THE CHESAPEAKE BAY

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The Lafayette River, a tributary of the Chesapeake Bay, experienced dense concentrations (6,000 cells/ml) of the mixotrophic dinoflagellate, *Akashiwo sanguinea,* during the summer in both 2002 and 2003. Mixotrophic species such as *A. sanguinea* may have a competitive advantage over strictly autotrophic species that can only fix carbon during the day through photosynthesis. In previous studies, we determined that *A. sanguine*a has the capacity to take up organic N and C and that both contribute substantially to their growth. Urea and amino acid C were major sources of C for *A sanguinea* and their uptake exceeded photosynthetic C-uptake during blooms in 2002. At the beginning of this bloom, total C uptake measured during the day was insufficient for meeting cellular C demands based on the observed N uptake. Later in the bloom, uptake of organic C could almost completely satisfy the C demand based on the observed N uptake and the molar C:N ratio. Grazing and nighttime uptake of organic C were not considered in this first study, nor were changes in the physiological state of cells as blooms progress. So, in order to determine the relative N and C contributions from dissolved organic matter (DOM) uptake, grazing and photosynthesis over the progression of blooms, we measured uptake of dually-labeled ^{15}N- and ^{13}C organic compounds (urea, amino acids and dipeptides), ^{15}N-labeled nitrate and ammonium, and ^{13}C-labeled bicarbonate and glucose at 6-12 hour intervals over several 24-hour periods during which natural populations were increasingly dominated by *A. sanguinea*. In addition, we estimated grazing rates by feeding them cryptophyte prey and examining changes in prey concentrations using microscopy. Results suggest that *Akashiwo sanguinea* takes up organic C and grazes to supplement photosynthetic C acquisition and that rates of these processes vary on diel time scales and as blooms initiate and develop.

CHEMICAL CHARACTERIZATION OF THE ALGISTATIC FRACTION OF BARLEY STRAW INHIBITING *Microcystis aeruginosa*

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Using barley straw for algal growth management has become routine in parts of the U.S. after gaining wide acceptance in the U.K. Nevertheless, there are conflicting reports of its effectiveness *in situ,* and recent laboratory studies have shown that, in addition to inhibition, stimulation is observed in species of marine and freshwater algae. Limited research conducted to identify algistatic agents in barley straw has suggested oxidized phenolics or free radicals from their photodecomposition are the inhibitory components.

To isolate and identify the inhibitory components of barley straw extract, a microplate assay system was developed using *Microcystis aeruginosa. M. aeruginosa* has been consistently inhibited by barley straw extracts in studies conducted in our laboratory and by others. The 24-well plate assay utilizes *in vivo* fluorescence monitoring for determination of chlorophyll levels in each 2-ml culture. Barley straw extracts used in this study were aerobically decomposed for 2-3 days up to 6-7 months, with inhibition present in all of the aqueous samples tested.

One difficulty in a bioactivity-guided fractionation study is preparing enough material for assays and for analysis. This study involved extracting kilogram-sized samples of straw, yielding multiple grams of extract for testing. Initially, samples of freeze-dried extract were reconstituted in water/ethanol (1:1), refrigerated overnight, and centrifuged to separate the supernatant from precipitated constituents. This step reduced the sample mass by half, but concentrated the active components into the supernatant. The precipitate showed no activity. Next, a portion of the supernatant was percolated through a bed of Polyamide CC6 resin (for irreversible adsorption of larger polyphenolic compounds such as tannins), which resulted in the loss of activity. Additional supernatant was then passed through a filter with a nominal molecular weight cutoff of 1000, which resulted in much less growth inhibition when compared with the unfiltered material. An aliquot of supernatant was further passed through filters with nominal MW cutoffs of 3000 and 10,000 to further classify the approximate size of the molecule responsible for the growth inhibition.

Results to date suggest the inhibitory component(s) are polyphenolics of a larger size than previously reported. Structural characterization of the algistatic fractions by HPLC/MS, coupled with UV/Fluorescence/ELSD detectors is underway and these results will also be reported.

THE PRESENCE OF DOMOIC ACID AND *Pseudo-nitzschia australis* IN SEDIMENTS OF MONTEREY BAY, CALIFORNIA

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The entrance of HAB toxins into food webs has major impacts on fisheries, marine mammals, and human health. In Monterey Bay, domoic acid (DA) has caused bird and marine mammal deaths, as well as restrictions on the collection of shellfish. These mortality events have all been attributed to contamination of the pelagic food web. The HAB diatom, *Pseudo-nitzschia australis*, an important local source of DA is known to aggregate and sink and has been found in large numbers in the water directly above the sediments, possibly contaminating the sediments. The contamination of marine food webs from true sedimentary sources of DA has not been documented, to our knowledge.

This study reports the presence of DA and *P. australis* cell equivalents in the sediments at nearshore sites in Monterey and several other locations in the Monterey Bay region. Sediment samples were taken systematically over a 15 month period near the city of Monterey and sporadically at the other sites. During the sampling period there were blooms with flocculation events as well as periods when no toxic *Pseudo-nitzschia* were detected in the water column.

During flocculation events such as that observed at the Monterey site, toxic *P. australis* cells, cell fragments and associated DA would be expected to be delivered to the sediments. In the sediments, cell breakage could result in some of the particulate DA being converted to dissolved DA. Both the particulate and dissolved DA in the sediments would be available for transfer into benthic food webs via various processes. In our study we measured the concentration of the total DA pool in the sediments using standard HPLC methods.

Since it is extremely difficult to cleanly separate cells and cell fragments from the organic-rich sediment matrix at our sites, we found it necessary to use a method that provides a proxy for cell concentrations. We chose the sandwich homogenization method developed by Scholin (Scholin et. al. 1997. Limnol.Oceaogr. 42:1265-1272.), a technique that uses optical density to count rRNA strands in a lysed homogenate, providing a measure of cell equivalents. As all cells are disrupted during processing, the resulting cell equivalents include rRNA strands from cells that were living at the time of sampling as well as from other sources, including dead cells or cell fragments.

The samples obtained in this study were collected during and between blooms, and thus provide insights as to how often domoic acid enters the sediments, how long it remains, and the likelihood that the benthos is a regular site of contamination of the food chain. Comparisons of the amount of *P. australis* cell equivalents and domoic acid in the sediments will begin to illustrate the relationship between *P. australis* cells and toxin in the sediments. Such information is critical in understanding whether DA can enter benthic food webs from the sediments, a route for contamination of marine food webs that is presently extremely poorly known.

WILL OFFSHORE WASTEWATER DUMPING LEAD TO HARMFUL ALGAL BLOOMS IN THE GULF OF MEXICO?

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The Florida Department of Environmental Protection is looking to avoid what could be an environmental disaster for the Tampa Bay, Florida area, by dispersing millions of gallons of treated wastewater into the Gulf of Mexico. There are over 1.2 billion gallons of wastewater held in gypsum stacks and ponds at the defunct Mulberry Corporation's phosphate fertilizer plant located near Port Manatee, Florida. Heavy rainfall or hurricane conditions could cause water levels in the stacks and ponds to rise to a point that would lead to a breech in the gypsum stack and pond dikes unleashing this wastewater directly into the Bishop Harbor section of Tampa Bay.

A short-term measure was put in place to prevent the gypsum stacks from overflowing. Starting in January 2003, about 2 million gallons of treated wastewater began to be discharged in Bishop Harbor per day in an effort to lower water levels at the site. By February 2003 two HAB events had been documented, a *Heterosigma akashiwo* bloom followed by a *Prorocentrum minimum* bloom. Will the trend be the same for the Gulf of Mexico after treated, but still highly nutrient rich, wastewater is released there?

Every 4-6 days from late July through the end of November 2003, about 7.5 million gallons of treated wastewater will be shipped 100 miles offshore, where it will be sprayed into the Gulf of Mexico. Following the wastewater dispersal, water quality samples, including samples for harmful algal bloom analysis, are being collected. Analysis of monitoring efforts both in Bishop Harbor and the offshore dumping site will be presented alongside baseline data collected before the wastewater dumping began. Included will be results from culture studies on inoculum size and treated wastewater suitability conducted using *Karenia brevis*.

BIOMONITORING BLOOD BREVETOXIN IN STRIPED MULLET (*Mugil cephalis*) AFTER SUBLETHAL LABORATORY EXPOSURE TO *Karenia brevis*

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There is a critical need to simply and reliably monitor brevetoxins routinely in the blood of humans and aquatic animals. The blood collection card method in conjunction with the brevetoxin radioimmunoassay (RIA) has proven successful in determining the toxicokinetics of blood brevetoxin levels in laboratory mice. Most recently this method has successfully identified blood brevetoxin levels in bottlenose dolphins and West Indian manatees exposed to red tides. Our newest studies are using striped mullet as laboratory test animals to better define the kinetics of aqueous exposure to *Karenia brevis*. To do this we have exposed striped mullet to sublethal densities of *K. brevis* (~250 000 cells/liter) for 4, 8, 12, and 24 hours. At each time point a water sample was collected and the fish bled for further analysis by RIA. The RIA results indicate that blood brevetoxin levels increased to values significantly different from that of the controls at 8 to 12 hours of exposure ($p < 0.05$), this was followed by levels not significantly different from controls at 24 hours. Striped mullet were also exposed to a *K. brevis* culture with a known brevetoxin concentration of 0.5 nM. Even at low brevetoxin concentrations the RIA was able to detect significant amounts ($P < 0.05$) of brevetoxin in the blood of the mullet at 8 hours of exposure. This method of analysis, using RIA in conjunction with blood collection cards, has proven to be an effective method to detect blood brevetoxin in finfish exposed to brevetoxin via *K. brevis* even at concentrations as low as 0.5 nM.

COMPLEX GENE STRUCTURE OF THE FORM II RUBISCO IN THE DINOFLAGELLATE *Prorocentrum minimum* (DINOPHYCEAE)

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We analyzed the unusually complex organization of the nuclear-encoded (form II) ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) gene in the potentially harmful dinoflagellate *Prorocentrum minimum* (Parvillard) Schiller by intensive genomic DNA and cDNA sequencing and Western blotting. Over ten transcribed units (TUs) were detected, which varied dramatically in their 3' untranslated region. Each TU appeared to contain four tandem copies of the Rubisco coding region (1.46 kb each; coding unit, or CU) interspersed by a 63-bp spacer; the four CUs in each TU were co-transcribed and apparently co-translated to a tetrameric polyprotein that may undergo successive cleavage steps to yield mature Rubisco. By means of Real-Time PCR analysis, it was estimated that each of the *P. minimum* genome harbored 148 ± 16 CUs. Although nucleotide sequences varied by 1-9% among the detected CUs, their inferred amino acid sequences were essentially identical. Our results suggest that the complex structure of *Pmrbc* has been derived from extensive and repeated gene duplications, an evolutionary process that has also been observed for other dinoflagellate genes.

NUTRIENT EFFECTS ON GROWTH AND PHOTOSYNTHESIS OF *Chattonella subsalsa* (RAPHIDOPHYCEAE) ISOLATED FROM INLAND BAYS, DELAWARE (U.S.A)

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The Inland Bays of Delaware are shallow, highly eutrophic embayments that have been plagued by frequent and severe harmful algal blooms. During the past several years, novel blooms of the toxic Raphidophytes *Chattonella*, *Heterosigma* and *Fibrocapsa* have caused fish kills and raised concerns for human and ecosystem health. We examined the effects of nitrate, phosphate, ammonium and urea availability on the biomass yield, growth rate, and photosynthesis of unialgal *Chattonella subsalsa* cultures isolated from the bays, using classic Monod-type experiments. This isolate was unable to grow on urea, suggesting that it does not possess a urease enzyme and may require inorganic sources of nitrogen. Values for μ_{max} (maximum nutrient-saturated growth rates) in the various experiments ranged from 0.64 to 0.7 d^{-1}. Half-saturation concentrations for growth ($K_{1/2}$) were 8 μM for nitrate, 3 μM for ammonium, and 0.7 μM for phosphate, suggesting that this species has a high requirement for inorganic nitrogen and should thrive in the highly N-enriched environment of the Inland Bays. Experiments also examined photosynthetic efficiency as a function of nutrient availability, supporting the conclusions from the growth rate determinations. We are currently comparing the nutrient requirements of *Chattonella subsalsa* with several sympatric Raphidophyte species and other local algal isolates to assess the effects of eutrophication on competition and succession both within the Raphidophyte group, and in comparison to other groups such as diatoms and dinoflagellates.

IDENTIFICATION OF EUGLENOID ALGAE THAT PRODUCE ICHTHYOTOXIN(S)

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We report toxin production by eucaryotic freshwater microalgae by members of the genus *Euglena*. Fish mortalities (sheepshead minnows, catfish, striped bass, and tilapia) occurred in pond fish and when fish were exposed to unialgal isolates of two species of *Euglena [E. sanguinea* Ehrenberg and *E. granulata* (Klebs) Lemm.-see Figure 1]. Erractic swimming behavior of fish, as well as death of rat cell lines suggest the toxin may be a neuro-transmitter analogue. At least one toxic fraction has been isolated from unialgal isolates of both species, and is water-soluble. The toxin(s) is/are stable at –80° C for at least 60 days and are heat stable to 30° C.

Table 1. Fish killing potential of *Euglena* spp. in laboratory and field samples.

Date	Location	Fish killed	Sample Type	Algal Density (cells/mL)
July-August	field	striped bass	wild (*E. sanguinea)*	unknown
Early August	laboratory	talapia	wild (*E. sanguinea)*	unknown
August 23	laboratory	catfish	wild (*E. sanguinea)*	3,500*
November 7	laboratory	catfish	isolate (*E. sanguinea)*	982
December 13	laboratory	catfish	culture (UTEX LB2345)	1,220
January 15	laboratory	sheepshead	culture (UTEX LB2345)	1,345**

*sample also contained over 1,500 resting cysts
**sample also contained over 1,100 resting cysts

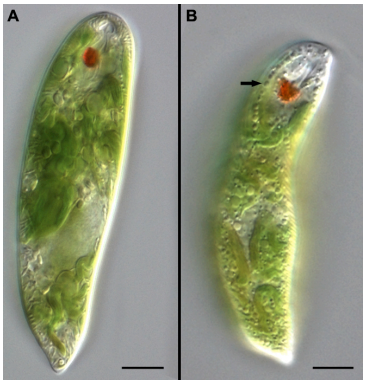

Fig.1. Toxin producing photosynthetic euglenoids. (A) *Euglena sanguinea* isolated from a freshwater pond in North Carolina. (B) University of Texas Culture Collection strain LB2345, now identified as *Euglena granulata*. One of the many rows of subsurface mucocysts is seen at the arrow. Bars, 10 µm.

THE GROWTH AND SURVIVAL OF THE COASTAL DINOFLAGELLATE, *Gyrodinium instriatum*, IN DIFFERENT SALINITIES

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Harmful algal blooms (HABs) are a specific problem and concern for the delicate coastal ecosystem. These blooms, or the toxins they produce, result in fish kills and some have been shown to cause adverse effects on human populations. The dinoflagellate *Gyrodinium instriatum* Freudenthal and Lee was selected for this study because it commonly forms extensive blooms in temperate estuaries. This species, identified in waters with salinities ranging from 2 to 36, formed blooms in estuaries along the eastern seaboard of the United States, as well as worldwide. These estuaries varied in salinity and in location, including some in Florida, North Carolina, New York, Ecuador, and Japan. The apparent extraordinary tolerance to variations in salinity stimulated this study. Growth studies were conducted, to examine the capacity for growth of the species, by performing visual counts on cultures grown in a series of salinities, from 5 to 30. Growth was seen in all six of the salinities, 5, 10, 15, 20, 25, and 30, indicating that this species is very tolerant to salinity, but the most dense growth was seen at the lower salinities of 5 and 10. In order to mimic what might be expected in natural environmental systems, cultures were preconditioned in each of the six salinities and then transferred to the salinity series (5-30). Growth was monitored daily for a period of 8 days by measuring in vivo fluorescence. Cultures grown at the lower salinities, 5 and 10, increased biomass when introduced to the highest salinities, 25 and 30. When cultured in the intermediate salinities, 15 and 20, growth occurred in all salinities tested, from 5-30. *Gyrodinium instriatum* grown at the highest salinities of 25 and 30 showed low growth when introduced to the salinity range. Adjustment of this organism to changes in salinity seems to most readily take place at salinities below 20, with the most rapid growth occurring at extremely low salinity levels. The species is definitely estuarine in nature, with a remarkable capacity to adjust to salinity changes of estuarine environments; this specific trait is essential for it's capacity to form blooms. Along with tolerance to salinity changes, the morphology of the species shows a great deal of plasticity with the shapes ranging from broadly oval, to rotund, to completely spherical. When monitored by microscopic observation, stressed *G. instriatum*'s morphology changed drastically. The cells changed shape from a fairly uniform oval to a very spherical shape. This suggests that *G. instriatum* has difficulty osmoregulating, yet adapts its shape to the salinity in which it resides. This observation agrees with the field studies involving this species, in samples from Delaware, Maryland, and North Carolina where highly spherical cells were collected. Once these cells are isolated and placed into an appropriate media, they resume the shape described for the species. These morphological observations may help to explain the confusion encountered when identifying *G. instriatum* and why it may be confused with a similarly described species, *G. uncatenum*. Further investigation is needed to determine if these species are indeed different and, if so, what characteristics should be used to distinguish between the two species.