ABSTRACTS OF ORAL PRESENTATIONS

THE USE OF MICROSATELLITE MARKERS TO COMPARE THE POPULATION STRUCTURE OF *Pseudo-nitzschia pungens* FROM THE PACIFIC NORTHWEST AND THE NORTH SEA

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Pseudo-nitzschia pungens is a pennate diatom commonly found in the waters of the Pacific Northwest and the North Sea. Due to the frequency of domoic acid events in the Pacific Northwest an understanding of the population and bloom dynamics of *Pseudo-nitzschia* species in this region is needed. Our research compared various population genetic parameters of *P. pungens* isolated from the Juan de Fuca eddy region in the Pacific Northwest and from the North Sea. These data along with population differentiation statistics indicated that *P. pungens* from the Pacific Northwest had a different population structure than *P. pungens* from the North Sea. Genetic data from a North Sea *P. pungens* sample provided evidence for the presence of a single unstructured population while a more complex population structure was found in Pacific Northwest *P. pungens* samples. Microsatellite data indicated that two genetically distinct populations were present in all Pacific Northwest *P. pungens* samples. These results implied that either the two populations of *P. pungens* could have recently mixed in the Juan de Fuca eddy region but had not exchanged genetic material by sexual reproduction, or that there may be cryptic species (morphologically identical but reproductively isolated species). The detection of multiple populations or cryptic species of a potentially toxic diatom suggests a more complex cause of HABs in the Pacific Northwest than was hitherto assumed, and calls for additional studies investigating physiological and genetic differentiation between the two strains.

CRYPTOPHYTES IN CHESAPEAKE BAY AND THEIR POTENTIAL RELATIONSHIP TO MIXOTROPHIC HARMFUL ALGAL BLOOMS

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Cryptophytes represent a substantial portion of phytoplankton biomass in eutrophic coastal estuaries such as Chesapeake Bay, but their ecology is poorly understood due in part to difficulty in identifying species by light microscopy. We hypothesize that cryptophyte abundance drives the formation of mixotrophic harmful algal blooms (HABs) in eutrophic environments. *Karlodinium veneficum* grows 2-3 fold faster when feeding on cryptophytes and produces an allelochemical, karlotoxin (KmTX), that allows it to effectively compete for cryptophytes, even in the presence of another heterotrophic predator, *Oxyrrhis marina*. *Karlodinium veneficum* feeds on a broad array of cryptophyte species, but other mixotrophic bloom formers such as *Myrionecta rubra* (Gustafson et al. 2000) and *Dinophysis acuminata* (Nishitani et al. 2005), show a high degree of specificity in prey selection. We have begun to investigate the *in situ* diversity of cryptophytes in Chesapeake Bay by generating clone libraries from sub-populations of phytoplankton sorted by flow cytometry to enrich for phycoerythrin-containing cryptophytes. Preliminary results from one sample collected during spring 2007 from the Patapsco River, MD showed a high abundance of *Amoebophrya* (Dinophyceae) sequences and one sequence identified as *Teleaulax acuta* based on a BLAST search. This survey will result in the development of molecular tools for identifying and tracking cryptophyte species *in situ* and will play a role in a broader project aimed at improving our ability to predict mixotrophic HABs, including attempting to forecast *K. veneficum* blooms by using phycoerythrin fluorometers on Maryland Department of Natural Resources real-time continuous monitoring platforms to detect potential prey populations in locations where *K. veneficum* blooms have occurred annually for the last two years (Corsica R., MD).

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NISHITANI, G., YAMAGUCHI, M., ISHIKAWA, A., YANAGIYA, S., MITSUYA, T., IMAI, I. 2005. Relationships between occurrences of toxic *Dinophysis* species (Dinophyceae) and small phytoplankton in Japanese coastal waters. Harmful Algae **4**: 755-762.

Alexandrium fundyense CYST DYNAMICS IN THE GULF OF MAINE

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Resting cysts play important roles in bloom initiation, termination, and species dispersal for several important HAB species. Several studies have examined linkages between cyst abundance and bloom timing and location, but these efforts have typically been over relatively small areas due to the difficulties and expense of mapping cysts over larger regions. Here we present the results of four cyst mapping surveys for *Alexandrium fundyense* in the Gulf of Maine, each covering hundreds of km in the alongshore direction, and 50 – 100 km in the offshore. The first survey (in 1997 (augmented with Bay of Fundy data from 1982, 1983 & 1984) revealed a widespread cyst distribution, with two centers of abundance, one in the Bay of Fundy, and a second offshore of Casco and Penobscot Bays in western Maine. The second survey (2004) shows a much larger cyst population, with approximately 10x as many cysts in the western Maine seedbed area. This was the cyst distribution that existed prior to, and undoubtedly contributed to, a massive 2005 *A. fundyense* bloom in southern New England. The third survey was conducted after the 2005 bloom, and reveals levels of cysts in western Maine that are ~5X the 1997 levels, but ½ of the 2004 levels. No significant geographic expansion of cysts into southern waters was seen, as had been feared based on the high motile cell concentrations that occurred in those waters during the 2005 bloom. The 2006 cyst map showed similar patterns to other years, with approximately 30% fewer cysts than observed in 2005. The overall pattern is thus one of large interannual variability, with a gradual decrease in cyst abundance in recent years. This presentation will discuss these interannual changes in the context of the blooms that occurred before and after the mapping efforts and in the temporal trends in PSP toxicity in the region over the last several decades as well. Newly acquired data on cyst deposition fluxes from sediment traps, and the abundance of resuspended cysts in the benthic nepheloid layer will also be discussed.

THE EVOLUTION OF TOXIN RESISTANCE IN COPEPODS: HOW DO COPEPODS RESPOND TO BLOOMS OF TOXIC *Alexandrium fundyense*?

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We have been using the calanoid copepod *Acartia hudsonica* and the dinoflagellate *Alexandrium fundyense* as a model system to study the interactions of planktonic grazers and toxic algae. The foundation for these studies was laid when it was discovered that some copepods appeared to be resistant to toxins while others were susceptible (Colin and Dam 2002, 2004). Since then, we have made several advances understanding the mechanism and the consequences of resistance. First, life history experiments revealed discrete reproductive phenotypes related to resistance (Avery and Dam 2007). When we used fitness as an index of resistance, it was apparent that the most resistant copepods were not extreme members of the population with respect to various characters, but middling members. As a result, the discrete phenotypes have been hypothesized to represent a simple genetic system of one or two genes showing heterozygote advantage. An immediate consequence is that heterozygote advantage would prevent the fixation of resistance alleles in a population so it appears that no population would become fixed for resistance. This hypothesis is supported by results of experiments lasting three generations, in which resistant and nonresistant alleles would appear to alternate through generations due to segregation and selection. Hence, there is no guarantee that evolution of resistance would necessarily lead to bloom control. If our model applies to other copepods co-occurring with *Alexandrium* sp., then we can expect measures of population growth rates, ingestion rates, and egg production rates to reflect this polymorphism for resistance.

References:

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Colin, S.P. and H.G. Dam. 2004. Testing for resistance of pelagic marine copepods to a toxic dinoflagellate. Evol. Ecol. 18: 355-377.

Avery, D.E. and H.G. Dam. 2007. Newly discovered reproductive phenotypes of a marine copepod reveal the costs and advantages of resistance to a toxic dinoflagellate. Limnol. Oceanogr. In press,

RECREATIONAL EXPOSURE TO MICROCYSTINS DURING A *Microcystis aeruginosa* BLOOM IN A SMALL LAKE

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Evidence of adverse human health events from exposure to freshwater cyanobacterial blooms is primarily anecdotal. Our objective was to document recreational exposure by measuring microcystins in blood samples from people at risk for swallowing water or inhaling spray (e.g., water skiers, jet skiers) during a *Microcystis aeruginosa* bloom. We found very low levels of microcystins (1 µg/L to 6 µg/L) in the water. Blood levels of microcystins were below the limit of detection (0.147 µg/L) for 97 people who did recreational activities on the lake with the *M. aeruginosa* bloom (exposed participants) and 7 people who did recreational activities on a different lake with no algal bloom (unexposed participants). Using technology developed for the assessment of aerosols containing brevetoxins, we detected microcystins (<0.1 ng/m^3) in aerosol samples (both personal samplers and ambient environmental samplers), indicating that recreational activities are potential sources of exposure to these potent toxins. We plan to conduct a similar study in a lake where microcystin concentrations are at least 20 µg/L to assess whether we can detect the toxin in blood samples from people with higher exposures.

Pseudo-nitzschia AND DOMOIC ACID IN NATURALLY IRON-ENRICHED AND IRON-POOR AREAS OF THE GULF OF ALASKA

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Although a number of cosmopolitan species of *Pseudo-nitzschia* are now known to produce the neurotoxin domoic acid (DA), very little is known about the ability of the truly oceanic species to produce DA *in situ*. There is considerable literature available to show that *Pseudo-nitzschia* are a common component of subarctic and subtropical oceanic systems, including ones in iron-limited HNLC (high nutrient, low iron) regions of the world ocean, as reviewed in the first talk in this 2-part series. With recent concerns about global warming and discussions of iron enrichment of HNLC regions of the oceans to remedy the warming, the possibility of promoting the growth of a known toxin producing genus needs to be further explored. This presentation describes some new data we acquired on a recent expedition to the Gulf of Alaska, which includes an HNLC area known to contain members of this pennate diatom genus.

Here we present data from the northern Gulf of Alaska, an oceanic system that has both iron limited and naturally iron enriched (from major eddies that contain river discharge) regions, obtained on a cruise devoted to the study of the various forms of iron and their concentrations in seawater. Our studies in the Gulf of Alaska are focused on determining whether the naturally occurring Pseudo-*nitzschia* in this area are producing DA under natural, *in situ* conditions both in the iron rich and iron poor (HNLC) areas. We will present some data on the cellular DA quotas in the field relative to various forms of iron in those waters. If successful, we will also present results from iron fertilization experiments conducted aboard ship during grow-out experiments. Field data will come from stations along north-south transects in the Alaska region where *Pseudo-nitzschia* cells are sufficiently abundant to measure DA using highly-sensitive enzyme-linked immunosorbent assays (ELISA), with corresponding cell numbers being obtained by epiflourescence microscopy aboard ship. Species of *Pseudo-nitzschia* present in the field and in grow-out experiments are being identified using suitably fixed or prepared materials from stations and grow-out experiments. Species designations will rely on SEM and TEM analysis for morphological discrimination of the species in the Gulf of Alaska. This type of field analysis of *Pseudo-nitzschia* populations, we suggest, is needed to determine whether local populations of oceanic *Pseudo-nitzschia* are already producing domoic acid in HNLC areas and whether iron fertilization, as proposed as a solution to global warming, may not only produce phytoplankton blooms but also may inadvertently increase the abundance of neurotoxin-producing species of *Pseudo-nitzschia*.

USING SOCIAL SCIENCE TO DEVELOP COMMUNICATION MESSAGES THAT FACILITATE PUBLIC TRUST AND UNDERSTANDING REGARDING HARMFUL ALGAL BLOOM CONTROL

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Harmful algal bloom (HAB) control encompasses mechanical, biological, chemical, genetic, and environmental strategies that kill HAB organisms, limit their growth and proliferation, or remove them from the water column. Despite studies suggesting that clay dispersal is a particularly promising method for controlling HABs (Sengco, 2001; Sengco and Anderson, 2004), public attitudes toward the strategy range from reasonably doubtful to strongly negative. At a 2006 public forum on red tide research in the Gulf of Mexico, for example, stakeholders voiced concern that clay dispersal (a) introduces an "unnatural" substance into coastal waters and (b) may have unforeseen environmental and public health impacts that are worse than the blooms themselves.

In many ways, societal decisions and public reactions will influence whether and how promising research on HAB control will move beyond an experimental scale to field demonstration and implementation. For example, societal decisions weighing the risks and expected benefits of alternative control options will influence the level of funding investment and design of permitting processes. In addition, public outcry stemming from misperceptions and mistrust could directly impede field demonstration and implementation. Public trust, understanding, and informed participation in decisionmaking are critical to ensure the responsible development and effective implementation of HAB control. Public education and outreach are critical toward this end.

A report by the National Science and Technology Council, *Grand Challenges for Disaster Reduction,* emphasizes that social science research is needed to design communication messages appropriate for public education and outreach. Specifically, designing effective communications requires an understanding of diverse stakeholder audiences – e.g., their knowledge of HABs, concerns about HAB control, perceptions of risks, and level of trust in scientists and decisionmakers. It also requires understanding and improvement of the institutional capacity of the HAB science-management community to communicate with stakeholders (e.g., interagency communications and media relations). Systematic study of public audiences and institutional capacity is needed to design effective communication messages and strategies related to HAB control.

This presentation reports on a nine-member interdisciplinary project to develop communication messages and strategies that help resource managers (e.g., Florida Fish and Wildlife Conservation Commission), citizens groups (e.g., Solutions to Avoid Red Tide), scientists, and others foster public trust, understanding, and meaningful participation in decisionmaking regarding HAB control in Florida. Communication design will be informed by social science research (e.g. interviews and surveys) to better understand stakeholders. The project will involve HAB scientists and managers, and investigate the impact of the research process on their knowledge, attitudes, and perceptions related to the human dimensions of coastal management.

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APPLICATIONS OF VIDEO-ENDOSCOPY TO STUDY THE EFFECTS OF HAB SPECIES ON SUSPENSION-FEEDING BIVALVES

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Bivalve suspension-feeding is a complex process involving particle capture and ciliary transport on the gills, and pre-ingestive particle selection at the gills and/or labial palps via rejection of pseudofeces. *In vivo* observations of bivalve feeding, using video-endoscopy, have been used in the past decade to elucidate mechanisms of particle processing in adult bivalves (Ward et al., 1998), but have rarely been applied to study the effects of harmful algae (Bricelj et al., 1998). This work demonstrated a novel mechanism of particle rejection of toxic *Alexandrium tamarense* cells by oysters which did not result in the formation of consolidated pseudofeces, but rather the loss of free cells from the gill dorsal tract, typically considered an acceptance tract. The present study extends the work of Bricelj et al. (1998) by applying video-endoscopy to study the effects of two non-dinoflagellate HAB species: *Aureococcus anophagefferens*, the causative agent of brown tide (BT), and *Pseudo-nitzschia multiseries*, a domoic acid (DA)-producer.

Hard clams, *Mercenaria mercenaria*, were exposed to non-toxic algae, *Isochrysis galbana*, and then supplemented with bloom levels of either a toxic or non-toxic isolate of *A. anophagefferens* for 2h. Our results indicate that clams exposed to the toxic BT (in contrast to the non-toxic BT) showed intermittent cessation and reversal of the inhalant flow above the frontal surfaces of the gills, and intermittent convulsions/twitching of the gills within ~4-60 min of exposure. Such effects are likely due to inhibition and lack of coordination of the gill lateral cilia, which create the inhalant feeding current, as well as effects on the gill musculature. During exposure to toxic BT, while major disturbance effects were occurring at the level of the gills, clams showed fully extended siphons with dilated apertures and extended guard tentacles, generally associated with active feeding. Therefore, the siphon aperture cannot be used as a reliable index of feeding activity. Our results support *in vitro* observations of dissected gills in which toxic BT caused dopamine-mimetic inhibition of lateral ciliary activity (Gainey and Shumway, 1991), yet suggest a more complex mechanism of action whereby BT also caused convulsions of gill musculature that will contribute to reduced feeding efficiency. The *in vivo* response was more rapid than that reported *in vitro*, as ~7h of exposure to comparable BT levels were required for maximal reduction of the rate of ciliary beat in excised gills of this species.

Recent advances in endoscopy-guided sampling of gill tracts can also answer key questions about the site and mechanism of particle selection, thus contributing to our understanding of species-specific differences in toxin uptake capacity under varying particle scenarios. Most prior video-endoscopy studies used relatively small algae (≤ 15 μm diameter). Less is known about bivalve feeding on large, non-spherical particles such as *P. multiseries*, that attain up to ~150 μm cell length and can vary greatly in size due to asexual division. It is also known that oysters, *Crassostrea virginica*, accumulate significantly lower DA levels than mussels, *Mytilus edulis*, when exposed to toxic *Pseudo-nitzschia* spp. under both field and laboratory conditions. We therefore used endoscopy-guided sampling to test whether oysters, which possess a highly complex, evolved gill, can selectively reject larger *P. multiseries* cells from mixed phytoplankton assemblages, thus providing a mechanistic explanation for their low DA accumulation.

BRICELJ, V.M., J.E. WARD, A.D. CEMBELLA, and B.A. MACDONALD, 1998. Application of video-endoscopy to the study of bivalve feeding on toxic dinoflagellates. In: Harmful Algae, B. Reguera, J. Blanco, M.L. Fernández & T. Wyatt, Eds., Xunta de Galicia and IOC of UNESCO Publishers, pp. 453-456.

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IMPLEMENTATION OF A HARMFUL ALGAL BLOOM PREDICTION SYSTEM IN CHESAPEAKE BAY

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A variety of harmful algal blooms (HABs) impact Chesapeake Bay, degrading the bay's health and jeopardizing the viability of this important natural resource. We are developing an operational system that will predict the likelihood of three important HAB species in Chesapeake Bay and its tidal tributaries: the dinoflagellates *Karlodinium veneficum* and *Prorocentrum minimum* and the cyanobacteria *Microcystis aeruginosa*. Warnings of these events will aid in mitigating their deleterious effects on human and ecosystem health. The approach uses real-time and 3-day forecast data derived from a variety of sources and techniques to drive multi-variate, empirical habitat models that predict the probability of blooms of these HAB species. A prototype prediction system generates daily nowcasts and 3-day forecasts of *K. veneficum* using environmental conditions simulated by the hydrodynamic Regional Ocean Modeling System (ROMS) configured for Chesapeake Bay and a habitat model constructed using an artificial neural network. The predictions, in the form of digital images, are available via the World Wide Web to individuals and interested agencies to help guide research, recreational and management activities (Fig. 1). In particular, these predictions will be employed by the Maryland Department of Natural Resources to guide their response sampling efforts for HAB monitoring.

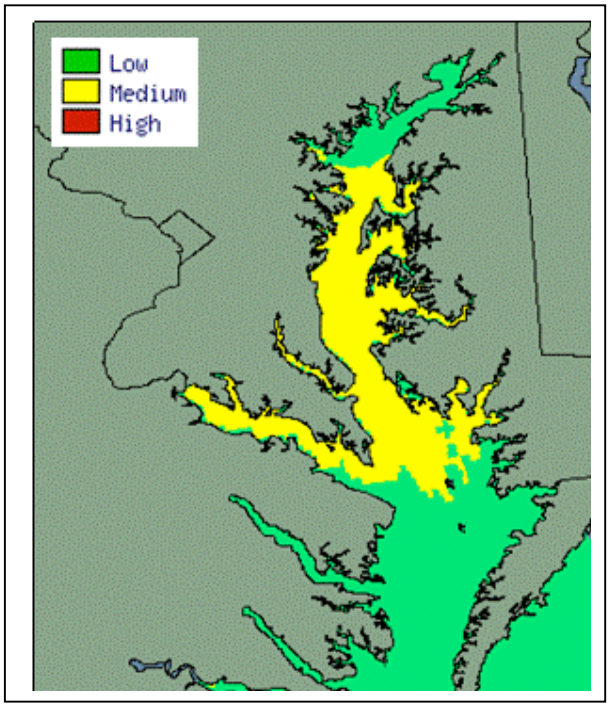

Figure 1. Predicted relative abundance of *Karlodinium veneficum* in Chesapeake Bay for July 11, 2007.

ISOLATION OF THE SODIUM CHANNEL GENE FROM THE COPEPOD *Acartia hudsonica* AND ITS POTENTIAL LINK TO SAXITOXIN RESISTANCE

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Coastal environments worldwide are experiencing increases in harmful algal blooms that pose serious threats to human health and fisheries. Several species of the dinoflagellate genus *Alexandrium* produce paralytic shellfish poisoning (PSP) toxins, also known as saxitoxins, which accumulate in shellfish and water column grazers and are then transferred to higher trophic levels. Saxitoxins kill shellfish and finfish, marine mammals and humans by interfering with nerve function via blockage of the sodium channel, resulting in respiratory failure due to muscle paralysis. Mutations in the sodium channel gene, even if only in the form of a point mutation of one nucleotide (e.g., Bricelj et al. 2005), could confer resistance to this toxin. Copepods are the most abundant metazoans in the ocean, and hence a key link between primary producers and higher trophic levels. In previous studies in our laboratory, we used *Acartia hudsonica* as a model to demonstrate the development of resistance to toxic *Alexandrium* in some populations and a fitness advantage for the putatively adapted population (Colin and Dam 2004). Here, we examine whether the mechanism of such resistance may be related to mutations in the sodium channel.

We began with *A. hudsonica* population New Jersey coastal water where there has been no report of *Alexandrium* blooms. cDNA libraries (5'-end cDNA, Hexamer cDNA, regular oligo-dT cDNA, and modified oligo-dT cDNA) were synthesized using reported method (Lin and Zhang 2003, AEM 69: 343-349). Common primers were designed based on the alignment of the amino acid sequence of the sodium channel alpha subunit from other organisms. Using the combination of these primers, several fragments of copepod sodium channel were PCR amplified and sequenced. Specific primers were designed based on these fragments and combined with common primers to obtain the 7.1 kb full-length cDNA of *A. hudsonica* sodium channel alpha subunit (AhSC).

This is the first report of sodium channel alpha subunit from copepods. AhSC shares 50-60% similarity in amino acid level to counterparts in other organisms and has a typical channel structure comprising four domains with six motif? in each. Phylogenetic analysis reveals that AhSC is closest to the sodium channel alpha subunit of insects, consistent with taxonomic phylogeny. Strikingly, we found two types of sodium channel alpha subunits in *A. hudsonica*, which are identical **except that one (type 1) has a three-amino-acid insertion** between the third and the fourth functional domains of the protein. This insertion is located very close to an "IFM" or "MFM" [Isoleucine (Methionine, Valine), Phenylalanine and Methionine] motif that acts as an inactivation gate, opening and closing the channel. The insertion may shift the positioning of this motif when it is plugged in the channel, thereby affecting the sodium ion inflow. We hypothesize that this insertion may change the effect of saxitoxin on the sodium channel, and account for saxitoxin resistance in copepods.

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SILICIC ACID LIMITATION IS NOT A TRIGGER FOR DOMOIC ACID PRODUCTION BY *Pseudo-nitzschia* BLOOMS IN THE PACIFIC NORTHWEST

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A central paradigm in the study of toxigenic diatoms is that concentrations of cellular DA (particulate DA per cell) become elevated as silicic acid (silicate) concentrations become limiting for growth and/or uptake by these diatoms. We have tested this hypothesis during numerous ECOHAB-PNW cruises from 2003-2006 in the coastal waters off Washington State, U.S.A. and British Columbia, Canada where toxigenic blooms of *Pseudo-nitzschia* typically occur. During 2004, we studied a massive toxic bloom measuring up to 48 km in diameter and reaching cell concentrations of 11-13 million cells/liter of *P. cuspidata* - the overwhelmingly dominant *Pseudo-nitzschia* species present. Results from this bloom event demonstrate that the highest levels of cellular toxin (5-64 pg DA/cell) correlate poorly with ambient silicate concentrations, and typically occur where dissolved silicate concentrations were 5-50 μM. None of the ~400 particulate DA (pDA) analyses conducted in 2004 (determined using cELISA) showed elevated cellular toxin concentrations when ambient concentration of silicate were < 4 μM, rather elevated pDA was generally associated with ambient silicate levels well above those considered limiting for its uptake and growth by most neritic diatoms. Increased cellular toxin levels also did not correlate with decreased ambient concentrations of nitrate or orthophosphate, indicating that toxin production in this natural *Pseudo-nitzschia* bloom was not governed by macronutrient availability. A similar result was found during the intense toxic *Pseudo-nitzschia* bloom in Monterey Bay, CA in 1998. The most established correlate for elevated domoic acid concentrations in 2004 was low dissolved iron concentrations; a finding consistent with laboratory culture experiments and our field incubation studies in the ECOHAB-PNW study region. These findings provide perhaps the most detailed insight to date into the environmental triggers for toxin production in natural assemblages of *Pseudo-nitzschia*, and demonstrate that commonly implicated macronutrient factors such as silicate limitation are poor predictors of either *Pseudo-nitzschia* dominance or toxicity in the Pacific Northwest.

THE EFFECTS OF TEMEPRATURE AND EUTROPHICATION ON TOXIC AND NON-TOXIC STRAINS OF *Microcystis* WITHIN NEW YORK LAKES

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During recent decades, toxic cyanobacteria blooms have been increasing in frequency and intensity across the globe. The cyanotoxin, microcystin, which can be produced by *Microcystis*, has been linked to incidences of hepatic cancer and other grave medical conditions. This study focused on the effects of altered temperatures (± 4°C) and nutrient levels (N, P) on toxic and non-toxic strains of *Microcystis* in Missisquoi Bay (MB) of Lake Champlain, which lies between New York and Vermont. Since it is not possible to distinguish between the different strains of *Microcystis* by means of traditional light microscopy, toxic and non-toxic strains were differentiated via quantification of the microcystin synthetase gene (mcyD) and ribosomal DNA gene, 16S. During the summer of 2006, Missisquoi Bay hosted chlorophyll concentrations of which ranged from 17.2 to 145 μg L^{-1}. The ratio of the cyanobacteria specific pigment phycocyanin-to-chlorophyll *a* within the phytoplankton community peaked the in early August, suggesting dominance by cyanobacteria at this time. *Microcystis* was not detectable in this system until mid-July when the non-toxic strain dominated the *Microcystis* community (> 94% of the population). During an August *Microcystis* bloom, non-toxic and toxic populations peaked at 1.5 ± 0.8 x 10^7 cell equivalents L^{-1} and 9.8 ± 2.3 x 10^5 cell equivalents L^{-1}, respectively. In a manner similar to the *Microcystis* population, microcystin was not detectable until early August but remained elevated through October ranging from 0.8 to 2.0 μg L^{-1}, with peak concentrations coinciding with the peaks in toxic cell densities. Nutrient manipulation experiments suggested that MB was primarily phosphorus-limited throughout the summer months (May-September) when ambient DIP concentrations were low (0.2 ± 0.1 μM) and water column alkaline phosphatase activities were elevated (94 ± 37 pmol h^{-1} ml^{-1}). During 60% (3 of 5) of the experiments conducted at this time, increased levels of phosphorus lead to an increase in growth rates of the total phytoplankton community as well as of cyanobacteria (phycocyanin-specific growth rates). However, this system changed to being nitrogen-limited during the fall (October) when ambient DIN concentrations were low (1.1 ± 0.1 μM) and experimental N additions yielded significantly increased growth rates for the total phytoplankton community. In experiments where increased cyanobacterial growth rates were elicited by increasing either N or P, an increase in temperature coupled with elevated nutrient concentrations often lead even higher growth rates. For example, in an experiment conducted during the peak of the August *Micocystis* bloom, increases in P significantly increased the growth rate of non-toxic *Microcystis* cells ($p \leq 0.001$, 2-Way ANOVA). In contrast, growth rates of toxic *Microcystis* cells were significantly enhanced by both N and P additions at this time ($p \leq 0.001$, 2-Way ANOVA). While similar trends were observed at higher incubation temperatures (4°C above ambient), the growth rates for each population associated with the significant treatments (N, P) were significantly greater than they were at ambient temperatures ($p \leq 0.002$, 3-Way ANOVA for both populations). Importantly, temperature also changed the relative abundance of toxic *Microcystis*. At ambient temperatures, the toxic population comprised between 0.45 and 0.96% of the total community in all treatments, whereas at elevated temperatures, toxic cells comprised 5 - 14%. In summary, the results suggest that increases in surface temperature coupled with nutrient loading could initiate a shift in dominance within the *Microcystis* population, causing toxic cells to comprise a greater percentage of the total population. Results on the response of toxic and non-toxic strains of *Microcystis* to nutrients and temperature from other New York lakes will also be presented.

FORMULATION OF ADVISORY LEVELS FOR CARIBBEAN AND PACIFIC CIGUATOXINS AND TIERED METHODS FOR THEIR DETERMINATION

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Ciguatera fish poisoning is a seafood hazard caused by consumption of fish that have accumulated lipid-soluble ciguatoxins. Ciguatoxins traverse the food web from the primary producer(s), Gambierdiscus spp., to piscine predators in tropical and subtropical regions of the world. Expanding trade in fisheries from these regions gives rise to wider distribution and increasing frequency of ciguatera outbreaks among seafood consumers. Information derived from the study of ciguatera outbreaks has improved clinical recognition, confirmation, and timely treatment of this disease. Such studies are equally important for the differentiation of ciguatoxin profiles from one region to the next, the determination of ciguatoxicity thresholds in humans, and the formulation of policy for public health, regulatory and industry organizations. Review of case studies of the past decade suggests that 1.0 ppb Caribbean and 0.1 ppb Pacific ciguatoxins in finfish are approximate thresholds for adverse effects in seafood consumers. These estimates are derived from analyses of fish tissues implicated in ciguatera outbreaks using a two-tiered protocol to assess ciguatoxicity and confirm molecular presence of ciguatoxins in fish tissues. To these threshold estimates are added safety factors to address 1) individual human risk factors (e.g. pre-existing health status which influence susceptibility to ciguatoxins); 2) variation or uncertainty in the amount of fish consumed; and 3) uncertainty in assay accuracy (e.g. only C-CTX-1 and P-CTX-1 are available as reference standards, yet multiple ciguatoxin congeners are present in toxic fish). Using this approach toxin advisory levels for South Atlantic, Gulf of Mexico, Caribbean, and Pacific regions can be assessed for acceptable levels of consumer protection.

AUTONOMOUS, SUB-SURFACE DETECTION OF THE ALGAL TOXIN DOMOIC ACID ONBOARD THE ENVIRONMENTAL SAMPLE PROCESSOR

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The ability to detect harmful algal bloom (HAB) species and their toxins in real or near-real time has been identified as a critical need in the recently revised U.S. National Plan - Harmful Algal Research and Response: A National Environmental Science Strategy 2005-2015 (HARRNESS). Over the past decade considerable effort has been directed toward development of remote, in-situ diagnostic capabilities for harmful algal bloom (HAB) taxa, their genes, gene products, and metabolites, paralleling the rapid emergence of ocean observing initiatives and infrastructure that will accommodate the resulting technologies in the future. Both researchers studying HAB/toxin dynamics and coastal resource managers responsible for monitoring bloom populations as well as mitigating their socioeconomic impacts eagerly await the transition of such tools to operational status.

The Environmental Sample Processor (ESP; www.mbari.org/microbial/ESP), developed for the autonomous, sub-surface application of molecular diagnostic tests, has successfully detected several HAB species using DNA probe arrays (e.g., Greenfield et al., 2006). Since toxin production and thus the potential for public health and ecosystem effects can vary widely with algal physiological status as influenced by a number of environmental factors, the concurrent detection of algal toxins onboard the ESP is essential. We have developed methods for extracting domoic acid (DA) from toxic *Pseudo-nitzschia* cells (extraction efficiency > 90%) and testing of samples using immuno-based membrane arrays on the ESP platform. Current toxin detection limits in extracts are in the 10's of ng DA per mL and the average repeatability between independent analyses ranges from 15-20%. Through the conduct of species- and toxin-specific arrays, potentially toxic species of *Pseudo-nitzschia* and domoic acid were detected concurrently on a second generation (2G) ESP instrument during 2006 deployments in Monterey Bay, CA, USA, representing the first remote, integrated assessment of algal cell abundance and toxin presence in coastal waters. Efforts are now underway to increase the assay sensitivity and refine its calibration in order to obtain more accurate estimates of a bloom's toxicity and thus its potential impacts. Deployment of the modified DA assay method is planned for August-September 2007 in Monterey Bay. Ultimately, integration of multiple ESPs with ocean observing systems will enhance our monitoring, prediction, and management capabilities for HABs and their adverse effects.

Greenfield, D.I., R. Marin III, S. Jensen, E. Massion, B. Roman, J. Feldman, C.A. Scholin. 2006. Application of environmental sample processor (ESP) methodology for quantifying *Pseudo-nitzschia australis* using ribosomal RNA-targeted probes in sandwich and fluorescent in situ hybridization formats. Limnol. Oceanogr.: Methods 4: 426-435.

ASSESSING THE ROLE OF ENVIRONMENTAL STRESSORS AND GENETIC COMPOSITION ON MICROCYSTIN PRODUCTION IN LAKE ERIE *Microcystis* POPULATIONS

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Blooms of the cyanobacterial HAB Microcystis have recently resurged in some regions of the Great Lakes, coincident with the invasion of zebra mussels to the system. Due to the use of these waters for drinking water and recreation, there is a significant need to understand the factors contributing to bloom toxicity and develop the ability to forecast when the presence of these blooms will be a threat to human health. Microcystis bloom toxicity is regulated both by environmental factors and genetic composition of the strains present in the bloom. Some of the environmental factors that have previously been shown in culture experiments to influence HAB toxicity (nutrients, light and grazers) were manipulated in laboratory experiments using natural communities of the cyanobacteria HAB Microcystis from western Lake Erie. The response of the Microcystis community to these environmental stressors was assessed by measuring changes in growth rates and in the concentration of the toxin microcystin. Additionally, a quantitative PCR assay was used to quantify changes in the number of toxic colonies using the mcyB gene, which is involved in the synthesis of the toxin microcystin. The genetic composition of the Microcystis community in western Lake Erie was also assessed over the course of a bloom season using both the highly variable phycocyanin intergenic spacer region (PC-IGS) and mcyB. Sequence analysis showed that there is shift in community composition over time and that strain composition plays a role in Microcystis bloom toxicity in this region.

MONITORING TOXIC *Alexandrium catenella* IN THE PUGET SOUND USING REAL-TIME QUANTITATIVE PCR (QPCR)

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Dinoflagellates of the genus *Alexandrium* can produce a suite of potent neurotoxins that cause paralytic shellfish poisoning (PSP) in humans, and can have serious deleterious impacts on public health and economic resources. *Alexandrium* and related PSP-toxicity is a problem of global scale. Within this genus, *Alexandrium catenella* is widespread in the northwestern part of North America, including the Puget Sound, and is responsible for seasonal harmful algal blooms (HAB) in these regions. Even at low cell densities, *A. catenella* toxins can accumulate in shellfish and result in PSP. As a result, accurate measurements of *A. catenella* distributions, particularly at low cell density, are critical to continued PSP monitoring and mitigation efforts. For example, detection of low, but increasing cell densities may trigger increased PSP monitoring, or help to target PSP monitoring to specific locations or time-periods. Towards this end a specific, sensitive, and high throughput real-time quantitative PCR (qPCR) method has been developed to assay the abundance of *A. catenella*.

In this study, Puget Sound surface water samples for qPCR analyses, microscope cell counts, and shellfish for PSP analyses (typically *Mytilus edulis*) were collected every two weeks from April 2006 through October 2006 by community volunteers and local public health organizations from 41 Sentinel Sites distributed throughout the Puget Sound. qPCR amplification of DNA extracted from field samples and standards was performed with a SYBR Green detection system. With the qPCR assay, low water column abundances of *A. catenella* of less than 10 cells per liter were measured. The detection of low cell numbers by qPCR resulted in the ability to report cells at all Sentinel Sites before these sites reached the USDA's regulatory PSP limit. Monitoring cell abundance by qPCR predicted, at times, an increase in PSP toxicity. This was seen for roughly half of the sampled Sentinel Sites. Often the increase in cell abundance occurred a week or two in advance of the increase in PSP toxicity. However, given the variability associated with the sites, qPCR cell counts were unable to define an absolute or threshold cell number necessary to predict PSP toxicity. There is a clear seasonality to *A. catenella* bloom dynamics in Puget Sound, as cell numbers increased substantially in nearly all Sentinel Sites from May to October. This first sampling season has begun to establish the utility of qPCR in providing early warning of PSP toxicity and its utility as a tool for deriving the seasonal bloom patterns of *A. catenella* within the Puget Sound. These bloom patterns are some of the first data for *A. catenella* cell numbers in this region and will ultimately help guide management practices. These results will be coupled with an additional field season to establish a more concrete relationship between cell number, bloom dynamics and PSP toxicity.

POPULATION GENETICS OF TOXIC *Alexandrium* BLOOMS IN THE GULF OF MAINE

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Coastal waters of the northeastern U.S. are subject to recurrent outbreaks of paralytic shellfish poisoning (PSP) caused by the toxic dinoflagellate *Alexandrium fundyense*. PSP toxicity shows considerable spatial and temporal variability, due in part to growth and accumulation zones defined by coastal circulation patterns. Another likely contributing factor is phenotypic heterogeneity in the *A. fundyense* population, evidenced by dramatic differences in inherent toxicity amongst clonal *A. fundyense* isolates from the region. Overall, this variability in PSP toxicity represents a significant challenge from public health and fisheries management perspectives. As part of the Woods Hole Center for Oceans and Human Health, one of our aims is to investigate the manner in which genetic diversity affects bloom dynamics. More effective management of shellfish resources threatened by PSP toxins requires an understanding of the population biology of *Alexandrium* species, as well as the manner in which these different genotypes respond to environmental conditions, as this ultimately affects the quantity and types of toxins accumulating in shellfish.

A set of microsatellite markers, developed for *A. tamarense*, has been used to study the genetic diversity of bloom populations of *A. fundyense* throughout the Gulf of Maine. These markers are highly polymorphic in *A. fundyense*, and are able to resolve fine-scale genetic differences between isolates. Toxic *Alexandrium* blooms occurred in the Gulf of Maine in 2005 and 2006, with the 2005 bloom being the largest toxic bloom in at least several decades. Several hundred clonal isolates were established from water samples taken throughout the blooms, in different geographic locations and at different times. The genotypes of these toxic bloom isolates were determined using microsatellite markers and used to assess spatial and temporal changes in the genetic composition of the bloom. In 2005, early-bloom populations from Bay of Fundy, Casco Bay, and MA Bay were not significantly different from one another. However, late-bloom populations collected near Martha's Vineyard (the southern extent of the bloom) were genetically distinct from early-bloom samples. In 2006, populations were collected from across the Gulf of Maine region, and none were significantly different from one another. The bloom that year did not extend to the Martha's Vineyard area. Further, a comparison of 2005 and 2006 samples showed that, in general, populations from the two different years were not genetically distinct. We also sampled a 2006 bloom in an isolated embayment, Salt Pond, MA. Populations in Salt Pond were genetically distinct from those in the wider Gulf of Maine, and they changed over the course of the 3-week bloom. From these two years of data, it appears that overall genetic composition of *Alexandrium* blooms in the Gulf of Maine is not significantly different from year to year. Within a year, however, we did observe changes in bloom populations on the timescale of approximately one month. This could result from the natural progression or 'turnover' of genotypes during a bloom, or from the mixing of genetically distinct cells from other (unknown) sources. Results of the 2006 analysis of the Salt Pond bloom provides support for the former hypothesis, although the mixing of different source populations cannot be discounted.

DETERMINATION OF PARALYTIC SHELLFISH POISONING TOXINS USING THE LAWRENCE METHOD: APPLICATION TO HUMAN URINE AND SERUM

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The AOAC Lawrence HPLC method for determining paralytic shellfish poisoning (PSP) toxins in shellfish was evaluated on the human clinical matrices urine and serum. Initial analysis revealed an interfering, naturally-occurring fluorescent compound in urine. Further analysis by high resolution mass spectrometry identified the compound to be hippuric acid, a major constituent in human urine originating from dietary sources. The hippuric acid was removed from samples by adjusting the pH to 4 prior to sample clean-up and by doubling the SPE cartridge bed volume. Interference by naturally-occurring fluorescent compounds was found to be minimal in the serum matrix. Quantitation of a range of PSP congeners spiked in these matrices will be presented and implications for public health will be discussed.

FLORIDA AQUATIC TOXINS HOTLINE: FORMAL EVALUATION OF HAB OUTREACH AND EDUCATIONAL ACTIVITIES

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With the increasing number of persons interacting with the coastal areas (both freshwater and marine) and with the apparent increase of harmful algal blooms (HABs) around the world, healthcare providers, public health personnel and coastal managers are struggling to provide scientifically-based appropriately targeted outreach and education to people with possible marine and freshwater toxin diseases and to persons with possible exposure to the marine and freshwater toxins, as well as the media and the general public. A variety of outreach and education materials and services have been created, but there has been almost no formal evaluation to determine whether these materials are effectively reaching their target audience and meeting the audience's expectations.

The Florida Poison Information Center-Miami (FPIC-Miami) has provided 24 hour 365 day/yr toll free Aquatic Toxins Hotline (1-888-232-8635) in several languages which has received over 25,000 calls since its inception in 1998. All calls are answered by highly trained Poison Information Specialists. These calls are reported as a form of passive surveillance of HAB-related illness and information requests to the Aquatic Toxins Program of the Florida Department of Health and to the Centers for Disease Control and Prevention. Recently, the Hotline was expanded to include an automated call processing menu system that allows callers to access information in English or Spanish. Callers can get information about the health effects and locations of the Florida red tide (including the NOAA HAB Bulletin), ciguatera fish poisoning, and blue green algae (cyanobacteria), and resources for learning about general marine toxin issues. Callers also have the opportunity to speak directly with a Poison Information Specialist.

This Pilot Study is the first known evaluation of the use of and satisfaction with outreach and education materials for HABs. Overall, the majority (68%) of callers reported that they were satisfied with the information provided by the Aquatic Toxins Hotline. Most callers were also satisfied with specific services offered by the Aquatic Toxins Hotline automated system (including speaking directly with a Poison Information Specialist), and some callers provided specific suggestions for improvements. This study demonstrated that the new automated system quickly provided useful HAB-related information for the large majority of the callers (78%), thus decreasing the workload of routine informational calls for the Poison Information Specialists and allowing them to focus on the those persons who may truly be ill. The results from this study will lead to the expansion and improvement of this valuable HAB outreach, education and surveillance tool, as well as recommendations for the evaluation of other HAB outreach and educational materials.

IMPACTS OF EUTROPHICATION-RELATED BLOOMS OF *Prorocentrum minimum* and *Karlodinium veneficum* ON EARLY LIFE STAGES OF OYSTERS IN CHESAPEAKE BAY

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Eutrophication-related algal blooms, especially *Prorocentrum minimum* and *Karlodinium veneficum* are now common in Chesapeake Bay. Of their documented impacts, high biomass leading to low oxygen and fish kills are the most recognized. Here we report on a suite of impacts that these blooms have on early life stages of oysters. Native oyster (*Crassostrea virginica*) populations are now <1% of their historical values in Chesapeake Bay and a major restoration effort is underway using hatchery-produced spat for restocking natural habitat. Furthermore, there is also consideration of introduction of a new oyster species, *Crassostrea ariakensis,* which is thought to be more disease resistant and faster growing than its native counterpart. All phases of hatchery operations depend on ambient water, which can be filtered or treated prior to use. To test the effects of HABs, exposure experiments of various life stages of oysters to cells and their exudates were conducted. When embryos from freshly spawned *C. virginica* were exposed immediately to *K. veneficum* at 10^4 cells/ml , either alone or in combination with other algae, virtually all of the developed larvae were deformed within 24-48 hours and were dead within 72-96 hours. Similar effects were found for *C. ariakensis* exposed to *K. veneficum* at a single concentration of 10^4 cells/ml. No deformities, and mortalities of <40%, were observed in controls to which a standard diet of the haptophyte *Isochrysis* was added. Effects of exudates of *K.. veneficum* and of whole cells of *P. minimum* have been fewer. Viability increased somewhat when older larvae were exposed to the same HAB. Blooms of *P. minimum* and *K. veneficum* are most prevalent during spring and summer, when spawning of *C. virginica* occurs. Eutrophication and associated harmful algal blooms have the potential to reduce survival of early life history stages of oysters and hence to reduce oyster recruitment. Any reduction in recruitment either spatially or temporally, combined with an overall reduction in sheer numbers of larvae that survive, will make establishment of significant, self-sustaining populations of natural or introduced oyster species more difficult.

PRELIMINARY INSIGHT FROM THE FIRST GENOME-SEQUENCE OF A HARMFUL ALGAL BLOOM SPECIES, THE BROWN TIDE ALGA, Aureococcus anophagefferens

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Aureococcus anophagefferens is the causative species of harmful brown tide blooms which have plagued the eastern US seaboard for more than twenty years. While brown tides can be sporadic, the concurrent outbreak of massive *A. anophagefferens* blooms in NY, NJ, and MD during 2007 attested to their persistence and pervasiveness. The sequence of the *A. anophagefferens* genome has been completed by the Department of Energy's Joint Genome Institute, a process which will facilitate a better understanding of this organism's ecology, as well as provide impetus for the development of novel molecular tools. A combination of expressed sequence tags (ESTs, ~ 50,000) and raw genomic data (~ 7-fold coverage of the organism's 56 megabase genome) have been completed and are undergoing analysis. Preliminary bioinformatic predictions suggest that > 11,500 identifiable genes exist within the genome with an average gene length of 2,138 base pairs, an average transcript length of 1601 base pairs, and an average protein length of 523 amino acids. Gene density was estimated to be 205 genes per megabase pair. Many gene models were supported by EST presence and/or putative gene homologs found in public informatics databases. A total of 49% of the genes were predicted by homology, while Ab-Initio prediction and EST clusters were used to predict the remaining 49% and 2%, respectively. Between 68 and 77% of the predicted proteins were homologous with published gene sequences found in the P-FAM and Swissprot databases. This preliminary introduction to the *A. anophagefferens* genome will emphasize the presence and expression of genes involved in nutrient utilization and cycling, as well as photosynthesis.

DOMOIC ACID NEUROTOXICITY IN NATIVE AMERICANS IN THE PACIFIC NORTHWEST: HUMAN HEALTH PROJECT METHODS AND UPDATE

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A five-year epidemiological cohort study is currently underway in the Pacific Northwest to determine whether or not there are human health effects of low-level exposure to Domoic Acid through Razor Clam consumption. The cohort is comprised of a random sample of 653 Native Americans, stratified by age (6 months to 80 years) from three coastal tribes in Washington State. The project is currently in year three of data collection. The methodology includes a broad range of valid and reliable standard behavioral, dietary, medical history and symptom assessment tools. This includes the Occupational and Environmental Neurology Questionnaire, Shellfish Assessment Survey, Block Food Frequency Questionnaire and Daily Food Records. Specialized, state of the art methods for assessing cognition and memory across the lifespan are also being utilized including select subtests of the Wechsler Child and Adult Intelligence Scales, Ravens Progressive Matrices, California Verbal Learning Test, Mullen Scales of Early Learning and the Fagan Test of Infant Intelligence. Domoic Acid levels are being obtained on a regular basis (every two weeks) from razor clams harvested from the regions under study.

Baseline findings indicate that 1) low levels of Domoic Acid have been consistently reported in the regions under study, 2) a significant number of persons in the cohort eat razor clams, 3) the general intellect of the cohort is distributed normally and similar to the overall U.S. population and 4) behavioral variables such as depression and substance abuse are also normally distributed in the study sample. Available data to date suggests that persons under study are at risk of low-level exposure to Domoic acid. Whether or not there is health risks associated with low-level exposure remains to be determined.

APPLICATIONS OF THE SECOND-GENERATION ENVIRONMENTAL SAMPLE PROCESSOR (2G ESP) FOR REMOTE DETECTION OF HARMFUL ALGAE: 2007

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Molecular approaches for identifying harmful algal bloom (HAB) species and affiliated toxins are central to research and monitoring, but such methods typically require the return of discrete samples for laboratory analysis. We are working to overcome this impediment by using the Environmental Sample Processor (ESP; http://www.mbari.org/microbial/ESP). The second-generation (2G ESP) was deployed for the first time in Monterey Bay, CA during 2006 and has the capacity to develop DNA probe arrays for target HABs, archive samples, and detect the phycotoxin domoic acid (DA). During 2006, the 2G ESP successfully automated detection of a number of harmful species, including diatoms of the genus *Pseudo-nitzschia,* some of which produce DA. The ESP and affiliated assays also detected DA itself.

Deployments of the 2G ESP in Monterey Bay are on-going. At the time of this submission, we completed 2 deployments during April -June 2007. A number of HABs, including members of the genera *Pseudo-nitzschia*, *Alexandrium*, and *Heterosigma* were detected. We are also refining methods that validate instrument performance by periodic water sampling and analyses using laboratory versions of molecular assays that are emulated aboard the ESP. In addition to field studies, we are generating standard curves for a number of HABs as part of an effort to become more quantitative with our DNA arrays. Finally, we are investigating the utility of qPCR for both *Heterosigma akashiw*o and *Alexandrium* sp. for comparisons to instrument results. Here we present findings from the 2007 field sampling season as well as examples of laboratory studies aiming confirm results from the field.

EFFECT OF THE HARMFUL ALGA *Prorocentrum minimum* ON THE HEMOCYTE RESPONSE OF QUAHOGS *Mercenaria mercenaria* WITH VARIOUS LEVELS OF QPX INFECTION

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Northern quahogs Mercenaria mercenaria are regularly exposed to harmful algal blooms (HABs), which can impact the immune system and, thus, susceptibility to infection by parasites. Moreover, quahogs in a given population can be more- or less-severely infected by a parasite, Quahog Parasite Unknown, or QPX. HABs occur in areas where parasite-stressed shellfish are located. As many parasites can modify hemocyte characteristics in affected bivalves, we conducted experiments to assess the combined effects of both stresses on clams.

To evaluate the possible individual or combined effects of the harmful alga Prorocentrum minimum and the parasite QPX, on hemocyte characteristics of quahogs M. mercenaria, an experiment was conducted exposing quahogs with varying levels of QPX infection to a simulated bloom of cultured P. minimum for five days. The simulated bloom was created by adding cultured P. minimum to a natural phytoplankton assemblage, and control clams were exposed to just the natural phytoplankton with no amendment. Hemocyte characteristics measured with flow-cytometric analyses (hemocyte concentration, morphology, percentage of phagocytic cells, viability, adhesion, apoptosis and production of reactive oxygen species), histology and prevalence and intensity of QPX were assessed for individual quahogs after microalgal exposure.

The results indicate that an exposure of quahogs to P. minimum for 5 days triggers an increase in the size of the hemocytes and in the production of reactive oxygen species of hemocytes as well as a decrease in the percentage of phagocytic hemocytes. Histological observations also shown the presence of some significant inflammations in the tissues of quahogs exposed to P. minimum. A lack of infected animals fed the natural plankton did not permit us to assess the effect of QPX alone. But the results indicate that the presence of QPX infection affect the hemocyte parameters of quahogs exposed to P. minimum by decreasing the size of the hemocytes.

This study revealed that the harmful alga P. minimum can alter hemocyte morphology and functions after only five days of exposure. In contrast, the presence of the parasite modified only the size of the hemocytes, no other responses of the hemocytes of the quahogs to this dinoflagellate. Thus, P. minimum effects upon quahogs are consistent, regardless of parasite QPX burden.

REGIONAL OCEANOGRAPHY LEADING TO TOXIC *Pseudo-nitzschia* EVENTS ON BEACHES IN THE NORTHERN CALIFORNIA CURRENT

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Recent interdisciplinary studies of *Pseudo-nitzschia* off the Washington/Oregon coasts have improved our understanding of the physical/chemical/biological oceanographic elements leading to significant toxic conditions in razor clams on coastal beaches. First, phytoplankton must become concentrated in a bloom source region such as the Juan de Fuca eddy. ECOHAB PNW studies suggest this requires a period of downwelling-favorable or lightly fluctuating winds. 2) Environmental conditions leading to elevated domoic acid (DA) levels must be present. In this region, cellular DA levels appear to be influenced by iron and copper availability and not by macronutrient levels. 3) The Juan de Fuca eddy region, which has been shown to be a regional source of domoic acid, is located offshore and well to the north of coastal beaches that have experienced toxic outbreaks. Patches of toxic phytoplankton must first escape this eddy-like offshore source region--escape is favored during upwelling-favorable wind conditions, which allow the geostrophic constraint of the eddy circulation pattern to be broken. 4) The patch must then move alongshore to sites with shellfish populations and 5) the cells must retain their cellular toxin content during the time period of transport. For a toxic source in the Juan de Fuca eddy this requires southward advection along the shelf, as occurs during periods of upwelling-favorable winds in summer and early fall. ECOHAB PNW studies show that toxin can be maintained in the 7-14 days required for transport. For an Oregon source of toxin such as Heceta bank to impact the Washington shelf, this requires northward advection along the shelf, as occurs during periods of downwelling-favorable winds in spring. Last, 6) the patch of toxic phytoplankton must move onshore to coastal beaches and/or estuaries and 7) must remain there for a period sufficient for significant ingestion by shellfish. In the northern California Current this requires a period of significantly strong downwelling-favorable winds, as occurs during a storm. The requirement to fulfill each of these several steps illustrates why toxic events occur sporadically on coastal beaches in spite of the frequent observations of toxicity in offshore locations.

THE NEW ENGLAND 2005 *Alexandrium* BLOOM: ESTIMATES OF THE ECONOMIC EFFECTS ON COMMERCIAL SHELLFISHERIES

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Over the last several decades, harmful algal bloom (HAB) events have been observed in more locations than ever before throughout the United States. The 2005 bloom of *Alexandrium fundyense* was the most widespread and intense in New England waters since a similar event more than three decades ago. Oceanographers have argued that another significant *Alexandrium* bloom could recur in the future due to the deposition of large quantities of algal cysts in New England ocean sediments. If true, then the results of our study should provide useful information for the public and natural resource managers. Using historical data from the National Marine Fisheries Service, the Maine Department of Marine Resources, the Massachusetts Division of Marine Fisheries, and other sources, we develop estimates of the direct economic impacts of the 2005 event on commercial shellfishing and growing industries in Maine and Massachusetts. We identify the effects of the event on market supply channels and prices using empirical data from the shellfishing and growing industries. Results of our analysis suggest that the 2005 event had broad spatial and temporal effects on certain components of the shellfish market. In response to a supply shortage resulting from local closures, there was an increase in shellfish imports to New England during the red tide. Our results indicate that the low-end estimate for total direct impacts in Maine was $2.4 million, including lost revenues in the softshell clam and mussel fisheries. Shellfish closures in Maine were the most likely cause of observable price changes for softshell clams on the Fulton Fish Market in New York. The total direct impacts on the commercial shellfish industry in Massachusetts may have been as high as $18 million. (Because of serious data limitations, however, this estimate should be viewed with caution.) To improve estimates of HAB impacts in Massachusetts, the consistent compilation of monthly shellfish landings is essential. We compare these results with estimates of economic impacts from other locations in the nation, which have been compiled in a continuing effort to characterize the economic effects of HABs in the United States.

NEW TRICKS WITH OLD TOYS: APPLICATION OF MASS SPECTROMETRY TO THE ANALYSIS OF PEPTIDE TOXINS

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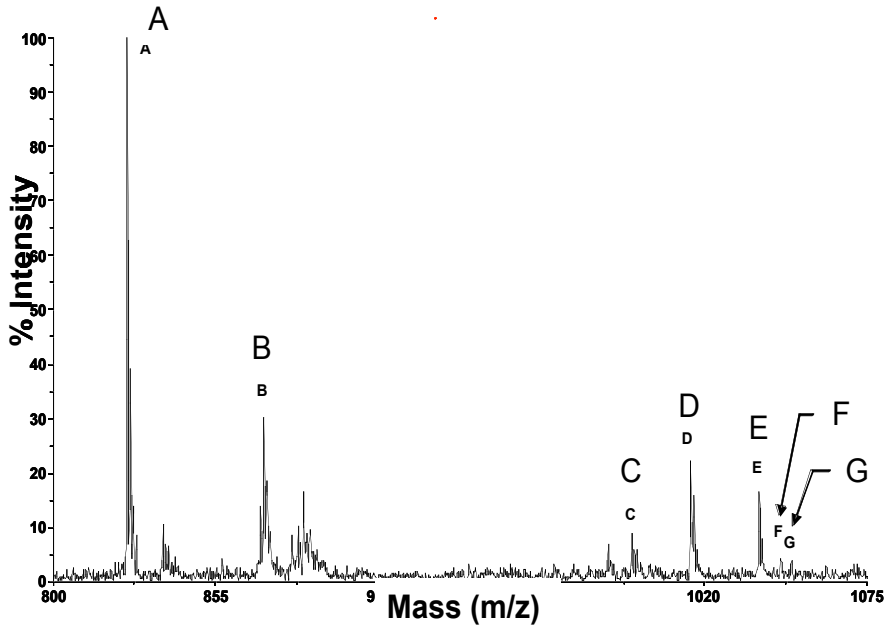
Microcystins (MCs) are potent toxins produced by some species of cyanobacteria. Rapid analysis of natural waters for MC content is essential to protect the public from exposure through drinking water or recreational contact. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) offers several advantages for analysis of MCs over current methods, including speed, reduced sample handling, and congener identification, but has previously been limited to qualitative analysis of MCs. In this study, a recently developed quantitative MALDI-TOF method for analysis of MCs was used to analyze intact cells and pre-extracted samples, with comparison to the results obtained using PPIA and by HPLC with electrospray ionization mass spectrometry (ESI-MS). Addition of MALDI matrix to intact cell samples provided simple, rapid identification and quantitation of MCs. Consistent results were obtained across a wide range of cyanobacterial and diatom densities, with recoveries from 87.2% (MC-LR) to 113.2% (MC-YR). Samples that were pre-extracted using a traditional approach were analyzed in parallel by PPIA, MALDI, and ESI-MS. The initial correlation between the MALDI and PPIA results ($R^2 = 0.69$) improved significantly ($R^2 = 0.81$) when the MALDI results were adjusted based on the mouse bioassay toxicity of each identified congener. The results confirm the utility of MALDI-TOF MS as a quantitative screening method for MCs, and the potential for its use as a stand-alone technique. Advantages and disadvantages of this technique, the selection of the appropriate internal standard, as well as a comparison with the more traditional LCMS-ESI will be presented.



Maldi TOF Mass spectrum of an intact cell protocol (ICP) field sample from Lake Neatahwanta, NY. Peak identification based on *m/z* ratio: (A) nodularin (internal standard); (B) phaeophytin *a*; (C) MC-LR; (D) desmethyl MC-FR; (E) MC-RR; (F) MC-YR; and (G) MC-(H_4)YR.

TEMPORAL AND SPATIAL VARIABILITY IN PACIFIC NORTHWEST Pseudo-nitzschia POPULATIONS

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Diatoms in the genus *Pseudo-nitzschia* are a common component of phytoplankton assemblages, although little is known about the distribution of individual species and/or strains in any given location. Automated Ribosomal Intergenic Spacer Analysis (ARISA), a form of DNA fingerprinting, was developed to recognize specifically at least 14 different species of *Pseudo-nitzschia*. This technique allows the rapid determination of species distributions over time and space. Puget Sound, WA is an estuary connected to the Pacific Ocean by the Strait of Juan de Fuca; within the estuary, there are four interconnected but hydrographically distinct basins. We examined species distributions within the different basins of Puget Sound during a two-day window in June, 2006. Overall, six *Pseudo-nitzschia* species were detected throughout the Sound, but species composition varied among the different basins. For example, some species such as *P. multiseries* and *P. australis/P. seriata* were restricted to a subset of the basins, whereas the distributions of other species such as *P. pungens* and *P. delicatissima* were much more widespread. Preliminary results from a time series collected at single site in Puget Sound indicated that *P. pungens* and *P. delicatissima* persist across multiple seasons, and PCA analyses suggest that these species may have lower environmental constraints than other species. Environmental clone libraries were generated to investigate the role of intraspecific diversity in distribution patterns. Interestingly, *P. pungens* and P. *delicatissima* exhibited the most and least intraspecific diversity, respectively, of the ten Pseudo-nitzschia species detected in clone libraries. At this time, 13 distinct genotypes of *P. pungens* have been detected in Pacific Northwest waters, 12 of which appear to be unique to the Pacific Northwest, and 5 of which appear to be unique to Puget Sound. Nucleotide divergence for Pacific Northwest *P. pungens* (4.7%) is higher than other species detected in clone libraries (0-3%), which may be indicative of multiple populations or cryptic or pseudo-cryptic species. Only a single genotype of *P. delicatissima* has been detected in Pacific Northwest samples, and this same genotype has been detected in isolates from Denmark and Portugal. We are currently generating clone libraries for additional basins in Puget Sound, and for samples from toxic *Pseudo-nitzschia* blooms, to further investigate the relationship between genetic composition, distribution, and the environment.

RECENT ECOSYSTEM SHIFT IN CENTRAL CALIFORNIA ALTERS HARMFUL ALGAL BLOOM PATTERNS

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In California, the toxic species of primary concern are the dinoflagellate *Alexandrium catenella* and members of the pennate diatom genus *Pseudo-nitzschia*, both producers of potent neurotoxins that have sickened and killed marine life and humans. During the summer of 2004 in Monterey Bay, we observed a dramatic change in the taxonomic structure of the phytoplankton community – the typically diatom-dominated community shifted to more of a red-tide, dinoflagellate-dominated community. Here we use a six-year time series (2000-2006) to show how the dominant harmful algal bloom (HAB) species in the Bay up to that point, *Pseudo-nitzschia*, was replaced as a major toxin producer by two genera of toxic dinoflagellates, *Alexandrium* and *Dinophysis*. This change represents a shift from a genus of toxin producers that typically dominate the community during a toxic bloom, to HAB taxa that need only be a minor component of the community to create a toxic event. This has significant implications for monitoring because toxic events are therefore not dictated by the relative dominance of a species. To strengthen that point, this change in the local HAB species was also reflected in the toxins present in higher trophic levels. Despite the small contribution of *A. catenella* to the overall phytoplankton community, the increase in the presence of this species in Monterey Bay was associated with an increase in the detection of paralytic shellfish toxins in shellfish and clupeoid fish. We also provide evidence, based on the statewide biotoxin monitoring program, that this increase in the frequency and abundance of *A. catenella* events occurred not just in Monterey Bay, but also in other coastal regions of California. Our results demonstrate that changes in the taxonomic structure of the phytoplankton community influences the nature of the algal toxins that move through local food webs and also emphasizes the importance of monitoring for the full suite of toxic algae, rather than just one genus or species.

DEVELOPMENT OF QUANTITATIVE PCR-BASED TECHNIQUES FOR ASSESSING ZOOPLANKTON GRAZING ON HARMFUL ALGAE: A TALE OF TWO SPECIES

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Quantitative polymerase chain reaction (qPCR) techniques can be used to sensitively detect and quantify DNA of harmful algae. By using the amount of target DNA as a biomarker for cell number, the approach has been successfully used to quantify the concentration of single algal species in mixed field samples. Similarly, it may be possible to use qPCR to enumerate ingested cells of harmful algae in zooplankton gut contents. Eventually, a qPCR-based measure of gut contents could be used to derive predation rates on field populations of harmful algae. Such an approach offers several advantages over current techniques for measuring grazing on harmful algae and could increase sampling frequency in both space and time. However, detailed experimentation is required to ground truth the method prior to its use in the field.

We conducted laboratory grazing experiments using two model grazer species, adult *Acartia hudsonica* copepods, and *Nassarius* sp. veliger larvae, both fed *Alexandrium fundyense* (GTCA 28) as the harmful algal prey. DNA extraction procedures were optimized to ensure quantitative recovery of *Alexandrium* DNA. QPCR primers amplified a 183 base-pair fragment of large subunit rDNA. It has been tested against numerous algae and is specific for North American populations of *Alexandrium fundyense/tamarense/catenella* (Dyhrman et al. 2005). The detection limit is <1 cell and standard curves with known numbers of *Alexandrium* cells showed a linear signal response between 5 – 10,000 cells. *Alexandrium*-free extracts of either predator did not produce false positives when analyzed alone or PCR inhibition when added to *Alexandrium* standards.

When fed *A. fundyense* in the absence of other prey, a positive qPCR signal for *Alexandrium* was found in samples of 5-15 individual predators, indicating that a detectable amount of *Alexandrium* DNA survived ingestion and digestion. The *Alexandrium* signal in veliger larvae increased with the prey concentration, reaching 1-2 ingested cells per animal. In *A. hudsonica*, by contrast, the *Alexandrium* signal was detectable but exceedingly low, generally << 0.1 cell per copepod, even when other measures indicated that the copepods were eating >100 cells indiv.$^{-1}$ h^{-1}. Microscopic inspection of the copepods confirmed the presence of fecal material in their guts. However, samples of isolated fecal pellets and dissected gut contents also had very low *Alexandrium* signals. The veligers ingested *A. fundyense* at a relatively low rate but had a strong qPCR signal, while the copepods ingested *A. fundyense* at a high rate but had a barely detectable signal. We hypothesize that differences between the predators' ingestion and digestion mechanisms account for the differences in qPCR signal strength from ingested *Alexandrium*. In particular, copepods are known to mechanically damage large algal cells during ingestion, which may promote very rapid digestion of algal DNA. Based these early results, a qPCR-based measure of gut contents offers a specific and rapid approach for evaluating the impact of grazers on harmful algal blooms but it may be best suited for use with predators like veligers and some protists, that ingest algal prey whole.

DYHRMAN, S. T., D. L. ERDNER, J. LA DU, M. GALAC, AND D. M. ANDERSON. 2005. Molecular quantification of toxic *Alexandrium fundyense* in the Gulf of Maine using real-time PCR. Harmful Algae **doi:10.1016/j.hal.2005.07.005**.

THE OPTICAL-BASED HAB DETECTION OBSERVATORY: LESSONS LEARNED DURING 4 YEARS OF IMPLEMENTATION

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The first *in situ* BreveBuster, optical-based HAB detector, was deployed May 20, 2003. The first BreveBuster-equipped autonomous underwater vehicles began missions in November 2003. Fixed installations on buoys and channel markers took place in the summer of 2004. Presently, there are four AUVs and one vertical profiler equipped with BreveBusters, and seven regularly instrumented fixed sites. Current plans call for six more fixed installations in the eastern Gulf of Mexico and three near Vera Cruz, Mexico. By the end of the year there will be approximately 23 BreveBusters in existence. Besides the many engineering and funding complications that had to be addressed, there have been and continue to be many issues that must be dealt with on a day by day basis. For instance: Hurricane Charley removed our first fixed installation just weeks after it was deployed; cables, no matter how well secured, work loose on open water installations; satellite phone data telemetry can be interrupted by higher priorities; VHF radio telemetry rarely meets the manufacturers range specifications; installers and operators make mistakes; and of course there is biofouling. In addition to these 'mechanical' issues there are the issues of how to handle the information output. The internet provides a very functional dissemination infrastructure, but there have been many questions about who the end users should be and in what format should the information be presented. Our experiences with these issues will be itemized and our responses discussed.

GENE EXPRESSION IN ZEBRAFISH AFTER ACUTE AND SUB-ACUTE EXPOSURE TO THE MARINE NEUROTOXIN DOMOIC ACID

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Domoic acid (DA) is a neuroexcitatory amino acid that is naturally produced by some species of the diatom genus Pseudo-nitzschia. The toxin accumulates in filter-feeding marine shellfish and is transferred through the food web resulting in a severe neurotoxic illness known as amnesic shellfish poisoning (ASP). Acute signs of ASP include vomiting, diarrhea, confusion, disorientation, seizures, memory loss, coma, and death. Dose-response relationships for the acute signs of DA-induced neurobehavioral excitotoxicity have been well defined for primate, rodent, and fish model species. However, little is known about the effects of sub-acute DA exposure (levels below those shown to induce overt toxicity). Furthermore, there is growing concern regarding the potential human health impacts of long-term low-level exposure to DA particularly in Washington State coastal Tribal communities that are dependant on shellfish for subsistence. In the present study, the zebrafish (Danio rerio) model was used to identify gene expression effects in the central nervous system (CNS) associated with acute and sub-acute DA exposure. Differential gene expression as evidenced by microarray analysis was observed in the brains of both sub-acute and acute treatments compared to controls. The observed gene expression patterns indicated that sub-acute DA exposure impacted the zebrafish CNS, and also that the mechanisms of DA toxicity may be different under conditions of acute and sub-acute DA exposure. Collectively, the dose-responses of DA-induced behavioral injury coupled with the microarray-generated gene expression data suggest that zebrafish are a useful model for exploring the mechanisms of chronic algal toxin exposure relevant to the vertebrate central nervous system.

SEASONAL AND INTERANNUAL VARIABILITY OF *Pseudo-nitzschia* AND DOMOIC ACID IN THE JUAN DE FUCA EDDY REGION AND ITS ADJACENT SHELVES

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The Ecology and Oceanography of Harmful Algal Blooms of the Pacific Northwest (ECOHAB PNW) program was motivated by a hypothesized physical pathway between the Juan de Fuca Eddy, an apparent initiation site for toxigenic *Pseudo-nitschia* blooms, and coastal clamming beaches on the Washington coast. Data from 6 cruises over 3 summers have shed light on the processes controlling the presence of domoic acid (DA)-producing *Pseudo-nitzschia* in the eddy region, those species responsible for toxic events, and the environmental parameters controlling bloom development. The field program used a combination of synoptic surveys and lagrangian drifter studies. The synoptic surveys provided information on the scales of variability and insights into the factors governing *Pseudo-nitzschia* and toxin occurrence. We identified more than 10 species of *Pseudo-nitzschia* off the WA coast, but those responsible for the most toxic blooms were *P. cuspidata* and *P. australis*. However, the presence of a particular species cannot be used as an absolute indicator of toxicity due to the high level of variability in DA production by field assemblages of *Pseudo-nitzschia*. Although sometimes achieving high abundances (>10^6/L), *Pseudo-nitzschia* spp. were always a small component of the total phytoplankton biomass, therefore satellite imagery is not useful for bloom prediction in this region. Throughout the study area, *Pseudo-nitzschia* usually occurred as a minor member of diatom-dominated assemblages; notably, it was often the major diatom taxa present in euglenoid and dinoflagellate blooms that occurred in the eddy region. Over the entire data set, no simple predictive relationship was found between environmental parameters (nitrate, phosphate, silicate, chlorophyll, temperature or salinity) and either *Pseudo-nitzschia* abundance, species or DA. Both *Pseudo-nitzschia* abundance and DA were highly variable in time and space. On a 21 day time scale, measurable toxin was always observed in the eddy region, and the highest level of toxin (>90 nM) and *Pseudo-nitzschia* cell numbers (>11-13x10^6/L) were observed in an eddy bloom in September 2004. The field as well as modeling studies clearly demonstrated that toxic blooms can escape the eddy and move southward along the WA coast. On two occasions, toxin found in the coastal region was associated with the presence of Columbia River plume water. Our observations confirm the idea that the eddy region and not recently upwelled coastal water is the primary initiation site for most toxic blooms on the WA coast.

CHARACTERIZATION AND REGULATION OF GENE EXPRESSION NETWORKS IN RESPONSE TO ACUTE STRESS IN *Karenia brevis*

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Karenia brevis is a dinoflagellate whose expressed genome is of significant interest because of its role in producing harmful algal blooms. The longevity of a *K. brevis* bloom is dependent on the cells'ability to adapt to changing conditions in the coastal environment. The induction of stress response genes has been shown at the protein level in *K. brevis*. To identify if this induction is controlled at the level of transcription initiation, we used a *K. brevis* microarray to measure changes in transcript abundance in response to acute stresses, including heat and oxidative stress. Consistent with a general stress response that includes a transient shut-off of general mRNA transcription, genes involved in ATP driven processes were downregulated following each of the treatments. However, transcription of stereotypical heat shock proteins and other stress related genes, known to be induced at the protein level in *K. brevis*, were not seen, implicating post-transcriptional regulation of these mRNAs.

There is precedence for widespread post-transcriptional control in dinoflagellates. Recently, we identified an RNA mediated *trans*-splicing mechanism in *K. brevis* that may play a role in this process. Consistent with the hypothesis that the stress response in *K. brevis* under post-transcriptional control, we found the SL sequence on many of the *K. brevis* stress response genes. To investigate control at the level of translation, polysomal fractionation was used to separate polysome bound mRNAs from the translationally inactive pool following peroxide stress. The microarray was then used to interrogate over-representation in the polysome fractions. We found many of the stress response genes, whose transcript levels remained constant, were present in the polysome bound fractions following peroxide treatment. Based on the results at the protein level, this suggests that the rate of protein synthesis may be accelerated via an increase in translational efficiency following environmental insult in *K. brevis*. This study provides the first comprehensive investigation into the stress responses networks present in a dinoflagellate responsible for harmful algal blooms (HABs).

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GENETIC NETWORK REGULATING CELL DIVISION AND TOXIN PRODUCTION IN *Karlodinium* AND *Amphidinium*: A GENOMIC APPROACH

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Karlodinium and Amphidinium are distinct dinoflagellate lineages but produce similar toxins. To understand what regulates population and toxin dynamics in these species, it is necessary to uncover biochemical or genetic network involved in the regulation. Given that their genomes are too large to sequence currently, analysis of expressed genes is the only way to gain information of this kind. Based on our recent discovery of widespread trans-splicing using a common splice-leader gene (Zhang et al. 2007), we undertook a project to sequence the full-length cDNAs for Karlodinium veneficum (CCMP 2778; 60k clones) and Amphidinium carterae (CCMP 1314; 30k clones) with a goal to assemble regulatory pathways for cell division and toxin production. Conditions necessary for production of billion cell cultures, optimization of cDNA library construction, and sequencing results will be presented. Insights into features of the dinoflagellate genome and the genetic network of cell division and toxin production will be discussed.

Zhang, H., Hou, Y., Miranda, L., Campbell, D. A., Sturm, N. R., Gaasterland, T. and Lin, S. 2007. Spliced leader RNA trans-splicing in dinoflagellates. Proc. Natl. Acad. Sci. USA 104: 4618-4623.

DEVELOPMENT OF A TOXIC DINOFLAGELLATE (*Karlodinium veneficum*) BLOOM IN A SHALLOW, EUTROPHIC, LAGOONAL ESTUARY

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A dense bloom of the ichthyotoxic dinoflagellate *Karlodinium veneficum* was discovered in the Neuse River Estuary, NC on 19 October, 2006 and was associated with four subsequent fish kills. This bloom was fostered by runoff following tropical storm Ernesto which input particulate and dissolved nutrients. Initially, runoff lead to increased flushing, light limited productivity and low algal biomass. As riverine discharge declined and the water column stabilized a surface frontal zone with lowered dispersion rates favorable for biomass accumulation became established. At the same time a prolonged period of low wind allowed vertical stratification and development of hypoxic bottom conditions that produced the among the highest hypolimnic concentrations of remineralized NH_4^+ ever measured in the estuary. A brief wind event mixed regenerated nutrients throughout the water column. A subsequent period of stable runoff, calm winds and a highly stratified water column provided salinity, light, nutrient and hydrologic conditions ideal for phytoplankton growth. The resultant community became dominated by dinoflagellates, the most successful of which was the mixotroph *K. veneficum* (>200,000 cells/ml, 740 ng / ml karlotoxin). The success of this species is probably due to its ability to produce the karlotoxin KmTx2, which aids in the capture of algal prey during mixotrophic feeding and in deterring microzooplankton grazers. Once the bloom was established, small-scale estuarine physical processes coupled with vertical migration behaviors acted to further concentrate cells. The bloom demise was linked to disruption of an already senescing population by a turbulent wind mixing event. Toxin released from these cells was postulated to be the cause the concurrent fish kills. Data that supports this assumption includes likely movement of the disrupted bloom into the fish kill area, the presence of *K. veneficum* at the kill sites, and the characteristic premortem symptoms of karlotoxin poisoning which include air gulping and lethargy despite high ambient DO conditions. This bloom underscores the important linkages between meteorological forcing and benthic-pelagic coupling in fostering phytoplankton blooms in shallow estuaries.

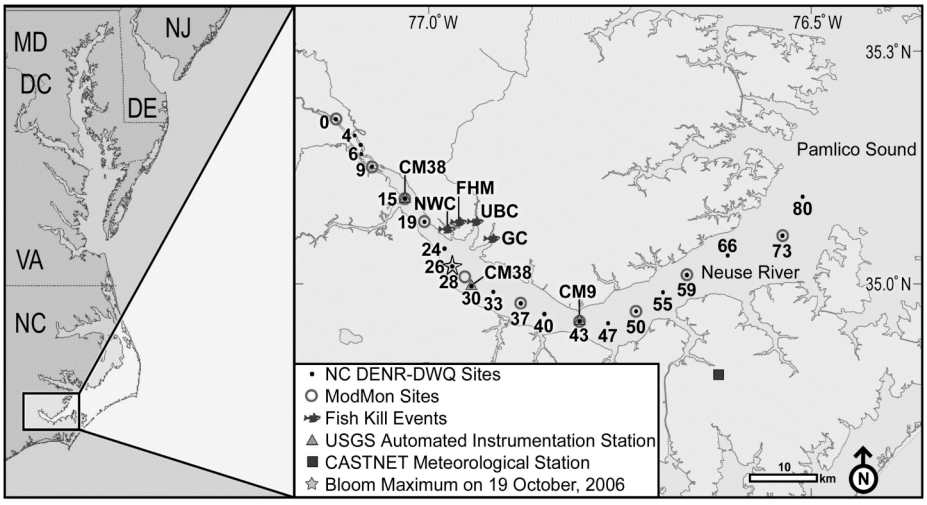

Fig. 1. Map of the Neuse River Estuary showing locations of sampling stations and documented fish kill events. NWC= North-west Creek. FHM= Fairfield Harbor Marina. BC= Broad Creek. GC= Goose Creek. Water quality station identifiers are expressed as km downstream. USGS automated instrument-ation stations are labeled by the channel marker (CM) to which they are fixed.

A GENOMIC APPROACH FOR IDENTIFYING THE SAXITOXIN (STX) SYNTHESIS GENES

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Anabaena circinalis ACBU02 and *Alexandrium tamarense* synthesize STX. A single plate of *A. circinalis* DNA was sequenced by 454 Life Sciences™, providing a total of 317,395 quality-filtered sequence reads. Total sequence output was 34,158,371 bases and average read length was 107 bp. These reads were assembled into 8340 contigs which varied in length from ~170 to ~4000 bp. Approximately 90% of the contigs gave BLASTn e-value scores better than 0.001. Many ORFs were identified that could play a role in the synthesis of STX, for example, 21 contigs encoded genes recognizable as non-ribosomal peptide synthetases (NRPSs), consistent with the hypothesis of Kellmann et al. 2006 that the STX genes in cyanobacteria are a cluster of non-ribosomal peptide synthetases (NRPS) and polyketide synthases. Of particular interest was an NRPS A domain that is likely to have specificity for arginine, based on predictions of the 10 amino acids that line the arginine binding site.

Alignment of a putative NRPS A domain from *Anabaena circinalis* with MycC, an arginine-binding A domain of *Microcystis aeruginosa*. Residues involved in arginine recognition are in **bold**.

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MycC,          GAYVPLDPNYPPERLDYMISDSAISLLLTQQSLVQF-----------LPENQAEILCLDT
A.circinalis   GAYLPLDPKYPQARLADILDDSQVSIILTQEKLLTSPSSPLQTGETSLSPYQGKIILLDT
               *** **** **  **     **  *  ***  *              *   *  *  ***

MycC,          DWSRIANYSQENLTSPVKTENLAYVIYTSGSTGKPKGVMNIHQGICNTLKYNIDNYNLNS
A.circinalis   DLTIISQQNIETPISAIKPENLAYVIYTSGSTGKPKGVMITHQNIVNHATSIIDKYQINS
               *   *     *   *  * ********************  ** * *     ** *  **

MycC,          EDRILQITPFSFDVSVWEVFSSLTSGATLVVTKPDGYKDIDYLIDLIVQEQVTCFTCVPS
A.circinalis   HDRILQFTRFIFDVAAEEIFPAWLSGATLIMRPQEMFTNLIEFSEFLGQESLTVVNLPAP
                ***** * * ***   * *    *****                    **  *

MycC,          ILRVF---LQHPKSKDCHCLKRVIVGGEALSYE---LNQRFFQQL--NCELYNAYGPTEV
A.circinalis   YWQEWVLEIDRKVSQIPDSLRLVITGSDQVLPEKLALWQKLVAEKGQNIQWINAYGLTET
                           *     *  ** *      *   * *        *    **** **
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The predicted *A. circinalis* ORFs were used to scan an *A. tamarense* EST database of unique cDNAs (Hackett et al. 2005) for similarities. Of the 8340 *A. circinalis* contigs, 476 gave e-values better than 0.001 when BLASTed against the *A. tamarense* unigenes. A phylogenomic analysis was conducted with the 476 matches against a database that included 14 species of algae and other diverse eukaryotes. This resulted in 97 trees that contained *A. tamarense* and *A. circinalis*. These trees were reduced manually based on assumptions about gene products expected in STX synthesis (e.g., NRPS A domains specific for ARG, amidinotransferase, sulfation). The findings thus far are speculative, but remarkable given that only 1/3rd of the *A. circinalis* genome has been sequenced, the short contig lengths, and considering how few genes in *A. tamarense* are annotated. Though speculative at this point, a clear hypothesis that emerges is that a set of gene transfers have, over time, enriched the *A. tamarense* genome with sequences from the cyanobacterium. Testing this hypothesis will require complete, or nearly complete, genome data from the cyanobacterium and dinoflagellate with access to comparative data from other STX-producing taxa. We have recently received NSF support to complete three genomes of STX producing cyanobacteria and three transcriptomes of dinoflagellates. Available data will be discussed.

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OBSERVATIONS AND MODELS OF *Alexandrium fundyense* BLOOMS IN THE GULF OF MAINE AND GEORGES BANK: FROM CLIMATOLOGY TO FORECASTING

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Coupled physical-biological models are used to study fluctuations in Alexandrium fundyense populations in the Gulf of Maine in a) climatological and b) data-assimilative modes.
Climatological distributions of A. fundyense are investigated through simulations of population dynamics of A. fundyense within the seasonal mean flow. A model that includes germination, growth, mortality, and nutrient limitation is qualitatively consistent with observations. Cells germinated from cyst beds in the Bay of Fundy and near Penobscot and Casco Bays are advected from east to west in the coastal current. Growth of vegetative cells is limited by temperature from April through June throughout most of the region, whereas nutrient limitation occurs in July and August in the western gulf.

The coupled model is used to hindcast the historic 2005 A. fundyense bloom and to investigate the relative importance of factors governing initiation and development. Sensitivity experiments distinguish the roles of three major factors hypothesized to contribute to the bloom: 1) the high abundance of cysts in western GOM sediments; 2) several strong storms with prevailing downwelling favorable winds; and 3) anomalously large fresh water discharge entering the gulf due to abundant rainfall and heavy snowmelt. Our results suggest that high abundance of cysts in western GOM was the main cause of the 2005 bloom. Wind forcing was an important regulator, as episodic bursts of northeast winds caused onshore advection of offshore populations. These downwelling favorable winds also accelerated the along-coast flow, resulting in transport of high cell concentrations into Massachusetts Bay. Anomalously high river runoff in 2005 resulted in stronger buoyant plumes/currents, but had limited impact on the gulf-wide bloom distribution.

Initial results from 2006 and 2007 will also be discussed, emphasizing comparisons between observations and forecast/hindcast predictions.

NOVEL STRUCTURE OF POLYKETIDE SYNTHASE GENE TRANSCRIPTS IN THE FLORIDA RED TIDE DINOFLAGELLATE, *Karenia brevis*

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Karenia brevis is the Florida red tide dinoflagellate responsible for detrimental effects on human and environmental health through production of brevetoxins. Brevetoxins are polyketide compounds thought to be synthesized by a modified polyketide synthase (PKS) complex, but the gene cluster for this PKS has yet to be identified. Eight PKS transcripts were identified in *K. brevis* by high throughput screening of two *K. brevis* cDNA libraries. However, because axenic cultures are unavailable, the origin of PKS transcripts remains controversial. Through phylogenetic analysis of PKS transcripts encoding ketosynthase domains and the presence of a dinoflagellate – specific spliced leader sequence, these transcripts have been confirmed to be encoded by *K. brevis*. Identification of the spliced leader at the 5′ end of the PKS transcripts indicates that the sequences described are full-length transcripts, which was further confirmed by northern blot analysis. Although most similar to type I modular PKS, sequence analysis determined that seven of the transcripts encode single catalytic domains, six KS domains and one KR domain. This is the first study to describe full-length PKS transcripts in a dinoflagellate and identifies a novel PKS organization, with sequence most similar to type I modular PKS, but structure most similar to type II.

OBSERVATIONS ON THE EPIPHYTIC RELATIONSHIP BETWEEN *Gambierdiscus* SPP. AND SEVERAL MACROALGAL HOST SPECIES

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Specimen of twenty six different macroalgal species were collected, rinsed, and placed in petri dishes containing sterile, modified Keller's media and monoclonal *Gambierdiscus* cells (approximately 110 per dish). The dishes were placed in an incubator at 27C, 12 L/D, 100 μE, and examined periodically over a one-month period to count the number of attached, unattached, and dead *Gambierdiscus* cells. *Gambierdiscus* cells displayed one of three attachment modes, depending on the host species: no attachment at all (e.g., *Portieria hornemannii*), attachment in the early stages of the experiment, followed by unattachment (*Jania* sp.), or unattachment followed by later-stage attachment (*Dasya* sp. 1). *Portieria hornemannii* was the least favorable host, with zero *Gambierdiscus* cells attaching over the course of the study, the highest *Gambierdiscus* mortality (68%), the quickest time to 50% mortality (168 hours), and the fewest live cells (maximum of 34 cells). *Chaetomorpha* sp., *Galaxuara marginata*, *Jania* sp., and an unidentified cyanobacteria all exhibited good host characteristics. *Chaetomorpha* sp. had the lowest *Gambierdiscus* mortality rate (0.8%), whereas *G. marginata* had the maximum number of *Gambierdiscus* cells observed (4,532 at the end of the experiment). *Jania* sp. had the highest average number of cells over the course of the study (1,189), and the second highest average and maximum % attached cells (17 and 57%, respectively), with the unidentified cyanobacteria exhibiting the highest average and maximum % attached cells (33 and 73%, respectively). These results demonstrate that 1) some macroalgae species are better hosts than others (corroborating published field results); 2) attachment is not simply a matter of macroalgae morphology, but that chemical exudates likely play a significant role (corroborating published results); and 3) the composition of the chemical exudates likely changes as a macroalgal host proceeds through its life cycle and subsequent death phase.

DISSOLVED DOMOIC ACID: A COMPETITIVE ADVANTAGE FOR *Pseudo-nitzschia* IN COASTAL AND OFFSHORE HNLC WATERS

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The toxigenic diatom genus *Pseudo-nitzschia* is known to release variable amounts of the potent neurotoxin, domoic acid (DA) during both exponential and scenescent growth stages. The environmental conditions that regulate DA production and release by *Pseudo-nitzschia* are poorly understood but recent evidence shows that it can be associated with micronutrient (Fe, Cu) limitation. Because DA is a charged molecule, its release from exponentially growing cells implies an active transport process, which in turn, suggests dissolved DA provides a benefit to the cell. We investigated the effects of elevated dissolved DA concentrations on the species composition of natural population cultures in coastal waters of the Pacific Northwest and in offshore high nitrate low chlorophyll waters of the subarctic Pacific. These experiments used a novel sea-going continuous culture system that accentuates differences in growth adaptations of the community by selectively "washing" out those organisms having lower growth rates, thereby providing unique insights to bottom-up control of phytoplankton community trajectories. In both coastal and offshore regimes, overall chlorophyll biomass increased significantly with the addition of either DA or DA bound to Cu, a hypothesized purpose for DA release (Wells et al. 2005). Moreover, community analyses showed that growth of *Pseudo-nitzschia* spp. was strongly selected over that of other diatoms present, resulting in an overwhelming dominance of *Pseudo-nitzschia* in these treatments. This finding was consistent in both coastal and offshore waters indicating it may be a universal pattern. To our knowledge these findings provide the first direct evidence that DA is produced and released by *Pseudo-nitzschia* as a dissolved tool to facilitate its competition for growth. By implication then, it may follow that increased cell toxicity may signal environmental conditions where there is less cellular need to deploy the internal reserves of this competitive tool.

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SCRABBLED MODULES, SPLICED LEADERS, CAP DEPENDENT TRANSLATION CONTROL – WHAT NEXT IN DINOFLAGELLATE POLYKETIDE TOXIN SYNTHESIS?

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Polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPs) catalyze chain elongation from simple building blocks to create a diverse array of natural products including algal toxins. Modular PKS and NRPS proteins consist of one or more polyfunctional polypeptides, each of which is made up of as few as 1 or as many as 11 modules, with the amino-terminal to carboxy-terminal order of the individual modules paralleling the sequential order of polyketide or nonribosomal polypeptide chain elongation. In contrast, the corresponding structural genes for discrete PKS or NRPS open reading frames may be transcribed in an order that directly parallels the sequence of the eventual biosynthetic steps, as is the case for the erythromycin PKS. Each PKS or NRPS module is made up of a set of three mandatory or core domains, two of which are catalytic and one which acts as a carrier, that together are responsible for the central chain-building reactions of polyketide or polypeptide biosynthesis, as well as a variable set of auxiliary domains that mediate the modification (e.g. reduction, dehydration and methylation) of the newly extended polyketide or polypeptide chain. Based on the recent cloning of the putative PKS genes involved in amphidinolide synthesis by Koboyashi's group, the dinoflagellates break all known PKS paradigms. Not only do the genes not contain three mandatory core domains but the genes are not tandem arrangements but disperse over nearly 10, 000 bp of genomic sequence. Our own work with *Amphidinium carterae* found a keto-reductase (KR) module dispersed over nearly 10, 000bp of genomic sequence with frequent introns and no apparent nearest neighbor PKS modules. Perhaps an operon arrangement for transcriptional control of these genes is not needed given the recent discovery of trans-splicing in dinoflagellates (Zhange et al. 2007, Lidie and Van Dolah, 2007). All transcripts are treated equally and just need to have the appropriate mRNA CAP structure to be recruited for translation. We will present our current attempt to characterize the 5' CAP structure in dinoflagellates and how this might relate to constitutive expression of PKS genes.

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MONITORING OF BREVETOXINS IN *Karenia brevis* BLOOM-EXPOSED EASTERN OYSTER

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Brevetoxins are produced by the marine alga *Karenia brevis* and accumulated in filter-feeding molluscan shellfish. Consumption of brevetoxin-contaminated shellfish causes neurotoxic shellfish poisoning. Previous studies in Eastern oyster (*Crassostrea virginica*) established the extensive metabolism of algal brevetoxins and the persistence of brevetoxin metabolites after controlled exposures to *K. brevis* culture. The present study examines uptake and persistence of brevetoxins and metabolites in Eastern oyster exposed to recurring blooms of *K. brevis* at a single study site in Sarasota Bay, FL, over a three-year period. Brevetoxins were monitored by several methods, both structure-based (LC-MS, ELISA) and pharmacology-based (cytotoxicity assay, receptor binding assay). Toxicity of shellfish was assessed by traditional mouse bioassay. Measurements by all methods reflected well the progression and magnitude of the blooms. Shellfish toxicity above the guidance level (20 mouse units/100 g) was associated with cell densities >5,000 cells/L, and retained for up to two weeks after bloom dissipation. By in vitro methods, brevetoxins were measurable for several months after a bloom. By using LC-MS, we find that brevetoxins persist in the field-exposed oyster mainly as metabolites of the algal brevetoxins PbTx-1 and PbTx-2, as previously established in laboratory studies. Most abundant of the brevetoxin metabolites in shellfish were cysteine and cysteine-sulfoxide conjugates of PbTx-2; lesser amounts of PbTx-1 metabolites reflect the lower abundance of parent toxin in the bloom water. Levels of PbTx-2 conjugates as determined by LC-MS were highly correlated ($r^2 = 0.9$) with composite toxin measurements by ELISA (antibody directed against B-type brevetoxin backbone). LC-MS and ELISA values also correlated well ($r^2 = 0.7$) with those of mouse bioassay. Cytotoxicity and receptor binding assays did not correlate as well. ELISA and LC-MS methods offer rapid screening and confirmation, respectively, of brevetoxin contamination in Eastern oyster, and an alternative to mouse bioassay in assessing toxicity following *K. brevis* blooms.

CHEMICALLY-MEDIATED COMPETITION: INTERACTIONS BETWEEN THE RED TIDE DINOFLAGELLATE, *Karenia brevis*, AND CO-OCCURRING PHYTOPLANKTON

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The red tide dinoflagellate *Karenia brevis* blooms seasonally and often nearly monospecifically in coastal areas of the Gulf of Mexico, producing neurotoxic brevetoxins which kill fish and marine mammals. However, the mechanism that *K. brevis* uses to dominate the phytoplankton community is not well understood. We considered how *K. brevis* interacts with competing phytoplankton species: whether it releases inhibitory chemical compounds to suppress competitors, what effect these compounds have on competitor growth and physiology, and how competitors respond to compounds produced by *K. brevis*. We found that compounds exuded during *K. brevis* blooms and in *K. brevis* cultures inhibited the growth and lowered the photosynthetic efficiency of competing phytoplankton. Exudates of *K. brevis* cultures also increased membrane permeability of three phytoplankton species. Compounds produced during *K. brevis* blooms were allelopathic to the diatom *Skeletonema costatum*, but *K. brevis* bloom samples lost their allelopathic effect when exposed to *S. costatum*, indicating that *S. costatum* possesses a mechanism for undermining *K. brevis* allelopathy. Efforts to isolate and identify the responsible compounds are ongoing. Our results indicate that although *K. brevis* produces potent allelopathic compounds, competitors vary in their susceptibility and resistance strategies, and competitive interactions in the marine plankton appear to be complex, multi-directional, and part of an ongoing co-evolutionary battle over limiting resources.

A POTENT EFFECT OF IN VITRO GASTRIC DIGESTION ON THE OVERALL TOXICITY OF BREVETOXIN-LADEN ATLANTIC MENHADEN (*Brevoortia tyrannus)*

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Brevetoxins, produced by dinoflagellate *Karenia brevis*, have increasing impact on marine biodiversity, costal health and economy. These toxins were responsible for severe dolphin fatalities. A large dolphin mortality event was recorded in March and April of 2004, in which 107 bottlenose dolphins found dead along the Florida panhandle, although neither an observed algal bloom nor toxin present in water samples. Analysis of stomach contents, suggested that these animals have died of gorging enormous amount of toxin- exposed Atlantic menhaden fish, *Brevoortia* sp [1,2]. Although high levels of toxin were reported in undigested menhaden in the dolphin stomach contents, the total toxin load appears substantially less than what would be projected to be a lethal dose, based upon oral toxicity studies of purfied toxin in mice. In light of the above, we investigated the effect of gastric digestion on the brevetoxin composition and toxicity of Atlantic menhaden exposed to cultures of *K. brevis*. Viscera were submitted to an *in vitro* digestion experiment in presence of a synthetic or a natural gastric juice collected from a stranded bottlenose dolphin (*Tursiops truncatus*). Brevetoxins were extracted from the gastric suspensions and alterations in toxin composition were examined using liquid chromatography / mass spectrometry (LC/MS) and radioimmunoassay (RIA). Alterations in toxin activity were measured using receptor binding assay (RBA) and Neuro-2A cytotoxicity assay (N2A). Unlike the undigested extracts, *in vitro* digestion produced a dramatic increase in biological activity in parallel with depletion of the predominate brevetoxins and cysteine conjugate metabolites. LC / assay guided fractionation of the post-digestion toxic products revealed that the peaks of biological activity are mostly due to unknown hydrophobic metabolites, which still remain to be determined. Taken together, our report suggests that gastric digestion play a significant role on the quantity and the composition of brevetoxins which are bioaccessible for further intestinal absorption. Digestion may trigger a brevetoxin transformation along with a possible discharge of more potent lipophilic brevetoxin conjugates extracted by the digestion process.

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FEATURES OF NEUROTOXIC SHELLFISH POISONING FROM RECREATIONALLY HARVESTED CLAMS IN FLORIDA, 2006: EPIDEMIOLOGIC AND CLINICAL FACTORS

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A series of Neurotoxic Shellfish Poisoning (NSP) cases occurred along the south-west coast of Florida during 2006. NSP is caused by consumption of shellfish contaminated with brevetoxins originating from the marine dinoflagellate, *Karenia brevis*. *K. brevis* blooms cause the phenomenon known as red tide, a recurrent environmental hazard in the Gulf of Mexico. Florida state agencies routinely sample coastal waters for *K. brevis*; testing of regulated shellfish beds has been in place since the 1970's.

Descriptions of NSP cases are rare. In this series, 8 clusters representing 17 individuals were identified. All cases were associated with consumption of recreationally-harvested clams from coastal areas not officially approved or open for harvesting. Most (n=15) cases sought medical attention and treatment at surrounding hospitals. A variety of gastrointestinal and neurological symptoms were reported; neurological symptoms being the predominant clinical presentation. Incubation was from 1-6 hours, symptom duration was a few hours to 2 days, and 7 cases were admitted after emergency room presentation. Available samples of clams from case meals, implicated shellfish beds, and urinalysis from cases were positive for brevetoxins.

Most cases were tourists of Asian ethnicity who were unaware of shellfish bed closures. Increased public health warnings, including public services announcements, radio interviews and door-to-door reverse 911 contacts were initiated, cases abated after July. Despite local knowledge about red tides, routine monitoring of *K. brevis* cell counts in seawater, successful monitoring of state-regulated shellfish beds and local dissemination of shellfish-risk information; education of transient groups such as tourists remains a challenge.

A COMPARATIVE STUDY OF PERCEIVED RISK FROM TWO COASTAL COMMUNITIES: IMPLICATIONS FOR COMMUNICATION AND EDUCATION

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Risk perception is the characteristic way individuals approach, think about and interpret environmental risks and hazards. An individual's perception of risk guides their response to environmental threats. Moreover, it provides a framework for interpreting medical symptoms or illnesses. Since perceived risk is not always correlated with actual risk, obtaining risk perception data for a community is important for effective public health communication, education and intervention programs. Toward this end, we studied the perceived risk of harmful algal blooms (HABs) and HAB-related illnesses in a coastal community in the Mid-Atlantic States (Maryland Eastern Shore) and the Pacific Northwest (Northwest Washington). Since coastal communities often differ in history, culture and environmental hazards, it was hypothesized that they would differ with respect to the nature and extent of environmental worry, environmental health knowledge, and credible sources for information. Approximately 500 persons from the Maryland Waterman community (MD group) and seafood dependent tribal coastal communities in Washington (WA group) were studied with a standard risk perception questionnaire. The questionnaire was comprised of 17 questions in Likert-type or rank-order format regarding environmental worry, environmental health knowledge and sources for reliable HAB-related information. Findings indicated that although both communities worried about HAB's, there were significant differences in many areas. This included differences in how the groups perceived the effectiveness of scientists and where they would turn for reliable information. The WA group was more confident than the MD group that scientists would find a solution to their HAB problems. Moreover, they were more likely to turn to state and Tribal agencies for accurate HAB-related information. In contrast, the Maryland group had significantly more environmental anxiety and was more likely to turn to the media for reliable HAB-related information. The results support the need to take into consideration specific community perceptions and behavioral tendencies in the development of community and culturally-appropriate communication pathways.

ENVIRONMENTAL FACTORS ON MICROCYSTIN CONCENTRATIONS IN LAKES

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Invasion of Midwestern lakes by the zebra mussel (*Dreissena polymorpha*) has altered the prevailing limnological paradigm of a positive influence of nutrient loading on cyanobacterial dominance (Raikow et al. 2004, Sarnelle et al. 2005). A multi-lake survey by our group (Knoll et al. *in press*) has indicated an average 3X elevation in microcystin (toxin) concentrations lakes that have been invaded by the zebra mussel, but this survey was limited to lakes with total phosphorus concentrations less than 20 μg L^{-1}. Microcystin concentrations in the mixed layer were highly correlated with the biomass of *Microcystis aeruginosa*, but not with the biomass of *Anabaena*. As a follow up, we enlisted citizen monitors to sample 75 lakes distributed across broad environmental gradients throughout the state of Michigan. Preliminary analysis has revealed a positive influence of total phosphorus concentrations on microcystin concentrations (as measured by ELISA), but only for lakes lacking zebra mussels. The influences of lake morphometry and mean annual air temperature (based on lake location) will also be examined to increase predictive power and suggest the impacts of global warming.

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TOXIC BLOOMS OF *Pseudo-nitzschia* spp. AND THEIR IMPACT ON COASTAL MARINE LIFE IN THE SOUTHERN CALIFORNIA BIGHT AREA NEAR LOS ANGELES

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Blooms of *Pseudo-nitzschia* spp. and domoic acid concentrations in the plankton have been documented in coastal waters near the Los Angeles metropolitan area on an annual basis since 2003. Field observations have demonstrated that major bloom characteristics such as temporal bloom dynamics or level of toxicity varied considerably among years, but general trends were also apparent for coastal waters along Los Angeles and Orange County shorelines: 1) Particulate domoic acid concentrations commonly exceeded those reported from other geographical areas (ie, 2003, 2006 & 2007) indicating that regional environmental conditions particularly favor *Pseudo-nitzschia* and domoic acid production. 2) Highest concentrations of domoic acid within the study area (≈ 400 km^2) repeatedly occurred inside or immediately adjacent to the breakwater of the Los Angeles harbor (2003, 2006 & 2007). 3) Local *Pseudo-nitzschia* blooms impacted a wide array of species through food web interactions. The latter observation was based on domoic acid detection and quantification in a large number of animal samples collected from marine mammals and seabirds (body fluids, stomach contents and feces). In 2006 and 2007 eleven coastal species that had not been implicated previously tested positive for the algal toxin. The spatiotemporal relationships between bloom dynamics and physiochemical parameters were examined.

THE FATE OF SAXITOXINS IN *Alexandrium tamarense* DURING INFECTION BY *Amoebophrya* sp., AND INITIAL OBSERVATION OF HOST-PARASITE DYNAMICS FROM FIELD STUDIES IN A SMALL CAPE COD EMBAYMENT

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Laboratory studies were conducted to examine the fate of saxitoxins in *Alexandrium tamarense* (strain SPE10-1) during the course of infection by the parasitic dinoflagellate, *Amoebophrya* sp. Host cultures were grown in f/2-Si medium until they reached late exponential phase. Parasite dinospores of known age were harvested through gravity filtration. Small volumes of host culture were placed in duplicate scintillation vials to which dinospores were added at a 10:1 parasite-to-host ratio. A parallel set of duplicate vials were used as controls (i.e. no parasites). Subsamples were taken every 12 hrs from 0 (initial conditions) to 96 hrs. The samples were processed to determine host abundance, dinospore abundance, parasite prevalence and toxin content. For the infected cultures, host abundance decreased steadily over the course of the study. Dinospore abundance initially decreased as the cells entered the host. This was followed by a small increase in dinospore abundance at 36 hrs and large increase at 72 hrs. Parasite prevalence reached 49% at 36 hrs. Host abundance in the controls was unchanged during the experiment. There was no significant difference in total toxin content per cell between the infected cultures and controls. Similarly, there were no significant differences in the toxin profiles per cell (i.e. quantity and quality of each toxin) between infected and control cultures during the course of the study. There was a decrease, however, in the total amount of toxin in the infected *culture* relative to the uninfected controls, which corresponded to the decrease in the number of hosts as the infection proceeded. The fate of these toxins – whether they were destroyed by the developing parasite, remained intact within the emerging dinospores, and/or released into the medium following host lysis – is currently under investigation.

To understand the role of *Amoebophrya* sp. in controlling an *Alexandrium sp*. population in the field, studies were conducted in Salt Pond (Eastham, MA) during the spring bloom in 2006 and 2007. Integrated water samples were collected weekly prior to the bloom to monitor host abundance. As host density increased to > 1,000 cells/L, integrated water samples were collected every two or three days. For greater spatial resolution, water samples were also collected at three locations across the length of the pond from the surface down to depth at 1-m intervals using a submersible pump. The water samples were preserved and analyzed for host abundance. Parasite prevalence was examined using microscopic observations, fluorescent in situ hybridization (FISH) probes, and quantitative protargol staining. Preliminary results from the 2007 study showed the initiation, development and decline of the host population during which three peaks in host abundance were seen. The second and third peaks were followed by two peaks in parasite prevalence based on FISH probing and microscopic observations. An initial peak in parasite prevalence following the first peak in host abundance was found only in microscopic observation, which suggests the presence of another parasite that affects *Alexandrium* sp. cells that was not detected by the probes. Protargol staining and other analyses are currently being applied to characterize this further. The potential implications of these data on the role of *Amoebophrya* sp. in controlling *Alexandrium* sp., naturally or as a possible control agent, will be discussed.

CAN BENTHIC-PELAGIC COUPLING BY *Karenia brevis* SUPPORT PERENNIAL OFFSHORE SEED POPULATIONS FOR COASTAL BLOOMS?

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Multiple hypotheses have been presented to explain how nutrients are delivered to populations of *Karenia brevis* in oligotrophic water columns in the Gulf of Mexico. Vertical migration behavior coupled with the physiology of *K. brevis* may help alleviate bottom-up controls and permit populations to persist as vegetative cells near the sediment-water interface throughout the year. Aggregations of natural *Karenia brevis* populations near the sediment-water interface suggest that cells may derive nutrients from the sediment in oligotrophic water columns. How cells interact with the sediment, however, remains uncertain. Video of cells near the sediment-water interface suggest that cells may either access nutrients that flux out of the sediment or migrate into the sediment pores where higher nutrient concentrations exist. Experiments to test the ability of *K. brevis* to migrate into the sediment were devised using chambers divided by a 100 μm mesh overlain with a thin layer of sediment collected from the Gulf of Mexico. Since the diel vertical migration of *K. brevis* typically displays a nocturnal descent, experiments tested migration response during the day and night and with and without a sub-sediment nutrient source. In order to determine the ability of *K. brevis* to exploit elevated nutrients associated with the sediment, we examined the diel rates of uptake and assimilation of different nitrogen substrates (NH_4^+, NO_3^-, Urea). Uptake and assimilation rates were measured during the day with the light intensity under which the cells were grown (< 30 μmol quanta m^{-2} sec^{-1}) and in the dark at night. Growth under a 12:12 light/dark cycle produced average growth rates of ~ 0.15 divisions day^{-1} for all the experiments. The chamber experiments suggest that while the sediment affects the progress of descending cells, migration occurs through thin layers of sediment and increases in response to elevated nutrient concentrations below the sediment. Since all cells found below the sediment had significantly higher C/N ratios than those remaining above the sediment, the motivation for migration appears related to a cell's internal biochemical state. The flexible exploitation of sediment-derived nutrients combines with the diel uptake and assimilation of a variety of N substrates to support low growth rates. The ability to maintain low growth rates in low light environments while accessing sediment derived nutrients permits *K. brevis* to persist as vegetative cells near the sediment-water interface. This ability to maintain slow growing populations associated with the sediment may substitute for life cycle strategies that involve encystment under unfavorable conditions that are observed in other dinoflagellates. Populations of vegetative cells associated with the sediment may provide seed populations that are advected onshore, under upwelling favorable conditions, to environments that promote cell aggregation and growth on higher light and opportunistic nutrient sources. The combination of cell aggregation and growth would result in the near-surface harmful algal blooms that are observed near-shore.

HARMFUL ALGAL BLOOMS AND THE 15^{O}C BARRIER

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Experimental data on the temperature-cell division relationships of ca. 45 dinoflagellate, raphidophyte and other flagellate species representing various toxicity modes, bloom behavior and Life-form Types recognized by Smayda and Reynolds (2001, 2003) were analyzed. HAB and red tide species exhibit an unexpected, general sensitivity to temperature - an ignored factor in HAB ecology. A 15^{O}C barrier to bloom development of the major, harmful flagellates and red tide species is evident, with two distinct thermal groups separated by this "bloom threshold temperature" recognizable. Temperatures below, or near 15^{O}C suppress cellular growth of the raphidophytes, other "naked" flagellates, and almost all toxic dinoflagellates examined - the optimal growth temperature for these species is usually $\geq$ 20^{O}C. The dinoflagellate species that are inhibited at, or below 15^{O}C cluster into "mixing-drift" Life-form Types IV,V and VI, while those species that can grow below 15^{O}C, and often bloom close to this temperature, belong primarily to Life-form Types I, II and III. The latter species -"cold water tolerant" - tend to be non-toxic or, if toxic, ichthyotoxic. *Alexandrium tamarense* is a conspicuous transitional species among the PST-producing *Alexandrium* spp. in bridging the 15^{O}C barrier. The ecological consequences of the 15^{O}C barrier and the multiple effects of temperature on motility and other behavior are considered, including the potential impacts of global warming on HABs. The relevance of the findings to Margalef's Mandala is discussed, and the hypothesis developed that it is temperature - not biophysics or ecology - which generally constrains flagellate blooms to periods when stratified waters prevail. The ecological corollary that HAB flagellates exploit, rather than require stratified waters for their growth, life cycle completion, and in meeting their nutritional (energetic) needs is considered

Smayda, T.J. and C.S. Reynolds 2001. Community assembly in marine phytoplankton: Application of recent models to harmful dinoflagellate blooms. J. Plankton Res. **23**: 447-461.

Smayda, T.J. and C.S. Reynolds 2003. Strategies of marine dinoflagellate survival and some rules of assembly. J. Sea Res. **49**: 95-106.

BLENDING OF OBSERVATIONS AND MODELS IN FORECASTING TRANSPORT OF HARMFUL BLOOMS

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Forecast systems for harmful algal blooms (HABs) have a key objective of identifying where a HAB will have an impact. This requires coupling of HAB locations with forecasted currents in a transport model. Assimilation of HAB fields is the most problematic part of forecasted locations. Water samples are point measurements describing only a part of a HAB concentration field. Satellite imagery, when usable, is constrained by both clouds and the need for interpretation. Other data types, such as gliders and moored instruments, also are limited in coverage. As a result of these limitations, the location data will include a range of points in space and time, which points to a need for interpreted HAB fields, rather than automated assimilation. A transport model must then merge the interpreted fields and the forecast circulation.

One modeling environment, the General NOAA Operational Modeling Environment (GNOME) provides a tool and potential capability to examine this assimilation problem. GNOME was designed for horizontal two-dimensional transport, and has been configured for integration of modeled currents and fields. The transport can be examined, including consideration of the influence of characteristics and uncertainty of the HAB location field on the forecasts. Examples of transport using GNOME with fields derived from satellite and observations, and model-derived currents are made for *Microcystis aeruginosa* in Lake Erie and *Karenia brevis* in the Gulf of Mexico. The results are examined to capture the influence of uncertainties in the HAB field on the forecasts, and lead toward broadly applicable capabilities.

RED TIDE RELATED LOSSES AND SMALL BUSINESS ADMINISTRATIONS LOANS: A 20 YEAR RETROSPECTIVE

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An unusual sequence of events in the fall of 1987 lead to the transport of a Florida red tide (*Karenia brevis* bloom) to the coast of North Carolina (Tester et al. 1991. Limnol. Oceanogr. 36:1053-1061). Before the end of this red tide event in May 1988 more than 145,280 hectares of shell fishing waters were closed leaving 5,000commercial fishermen were without a resource to harvest. The estimated loss of revenue to the coastal community was estimated to be $24.7 million. In an attempt to mitigate the economic damages to the local community the Small Business Administration (SBA) was requested to lend aid. It was then North Carolina shell fishermen and other small business owners discovered that loss of revenue from red tides was not covered in the SBA's definition of disaster. Testimony from NOAA staff and the combined efforts of the North Carolina congressmen resulted in changes in the SBA's definition of "disaster" to include ocean conditions that resulted in the closure of customary fishing waters as stated in the Small Business Administration Reauthorization and Amendment Act of 1988. The bill amended the Small Business Act and the Small Business Investment Act of 1958. This study reports the SBA assistance for red tide related disasters under this bill. Disasters due to red tides have been declared only 7 times since the legislation was changed in 1988. Businesses in four states have been provided loans by the SBA for red tide related losses. Forty-one SBA loans were made to North Carolina businesses in 1988 for a total of $1,334,526. In 1996, 1999, 2001 and 2002 a total of 32 loans were made to Florida businesses for a combined total of $850,515. During the 2005 *Alexandrium* bloom from Maine through Massachusetts the fishery was declared a "failure" and shell fishermen were eligible for grants. The SBA legislation has never been invoked to assist with loses in west coast states of the US or in states bordering the Gulf of Mexico west of Florida.

Table 1. Small Business Administration loans for losses related to Red Tides or harmful algal blooms summed over all businesses from 1988 to 2005 by declaration date and by state. Loan values are given in two formats. The original loan amount is given (unshaded) and the loan amounts converted to 2006 dollars (shaded).

		Number of Loans	Max	Max 2006 $	Min	Min 2006 $	Total	2006 $
State	**NC**	51	$114,500	$189,394	$1,200	$1,985	$806,800	$1,334,526
Date	**1988**							
	FL	4	$28,700	$35,177	$4,900	$6,006	$61,700	$75,625
	1996							
	FL	16	$73,500	$81,912	$4,900	$5,461	$390,700	$435,414
	1999							
	FL	12	$589,000	$64,682	$4,400	$4,832	$174,500	$191,630
	2001							
	FL	4	$61,300	$66,885	$5,000	$5,456	$135,500	$147,845
	2002							
	MA	3	$46,500	$46,500	$5,000	$5,000	$67,200	$67,200
	2005							
	ME	6	$26,000	$26,000	$2,500	$2,500	$67,600	$67,600
	2005							
Totals		96					$1,704,000	$2,319,840

GENE EXPRESSION PROFILES OF *Karenia brevis* DURING LYSIS BY ALGICIDAL BACTERIA

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Flavobacterium sp. (strain S03) and *Cytophaga* sp. (strain 41-DBG2) are algicidal bacteria active against the brevetoxin (PbTx)-producing dinoflagellate, *Karenia brevis*. Both algicidal bacteria cause lysis of *K. brevis* (Fig. 1), but *Flavobacterium* sp. requires physical contact with the target algal cells (i.e., direct attack), whereas *Cytophaga* sp. releases a dissolved algicidal agent (i.e., indirect attack). However, little else is known about the specific mechanisms by which these algicidal bacteria target and lyse *K. brevis*. Time-course experiments involving exposure of this dinoflagellate to algicidal strains S03 and 41-DBG2 were conducted, utilizing an 11000 feature *K. brevis* microarray (Lidie et al., 2005) to ascertain transcriptional profiles prior to algal cell lysis. Algal RNA was collected at 12 and 36 h following the inoculation of individual algicidal bacteria into exponentially growing cultures of bacteria-free *K. brevis* (C2 isolate). RNA was amplified and fluorescently labeled prior to hybridization with time-matched control RNA collected from *K. brevis* cultures exposed to the non-algicidal bacterium *Stanierella latercula*. Relative to control cultures, algicidal strain S03 induced 625 and 366 differentially expressed *K. brevis* genes at 12 and 36 h, respectively, following inoculation. Similarly, strain 41-DBG2 induced 736 and 675 genes at 12 and 36 h, respectively. In each algicidal treatment, the majority of differentially expressed *K. brevis* genes were up regulated. Genes with known annotations (~35%) were compared based on functional groups between exposures to the two algicidal bacteria. A consistent transcriptional response induced by both algicidal strains was the up regulation of chloroplastic and photosynthetic genes (i.e., light harvesting proteins, flavoproteins) prior to cell lysis. In addition, other genes involved in translation, protein recycling, and ion signaling were up regulated by both algicidal treatments. In contrast, strains 41-DBG2 and S03 also elicited a response from functionally distinct gene sets. The indirect attacking *Cytophaga* sp. (strain 41-DBG2) induced various stress response and signaling genes consistent with an antimicrobial response (i.e., permease, neomycin fusion protein, integrin complex binding). Actin-related genes involved in motility (i.e., various flagellar proteins) and dormancy or encystment were also identified. The direct attacking *Flavobacterium* sp. (strain S03) induced more structurally-related (i.e., ankyrin, myosin heavy chain) and cell adhesion/interaction genes. Our findings suggest that algicidal bacteria induce an early transcriptional response consistent with algal cell lysis and apparently unique to the mechanism of attack. These data will be useful for assessing natural causes of *K. brevis* bloom termination and for evaluating HAB control/mitigation strategies leading to lysis of *K. brevis* cells.

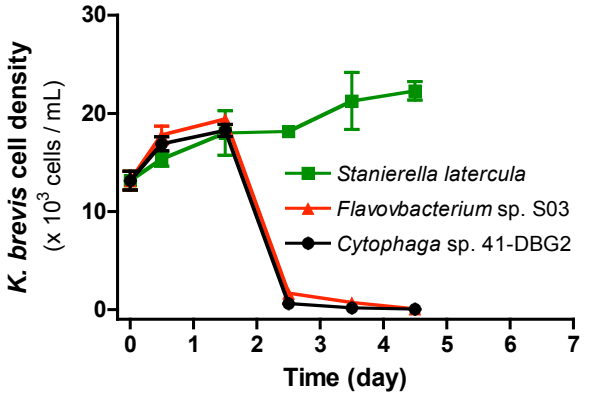

Figure 1. Growth of *K. brevis* in controls and algicidal bacteria treatments. Algal cell counts in bacteria-free cultures of *K. brevis* (C2) inoculated with the non-algicidal bacterium *Stanierella latercula* (100 cells/mL), algicidal strain S03 (10 cells/mL), or algicidal strain 41-DBG2 (100 cells/mL). Values are mean ± SE (n=3).

Lidie, K.L., J.C. Ryan, M. Barbier and F.M. Van Dolah. 2005. Gene expression in the Florida red tide dinoflagellate *Karenia brevis*: analysis of an expressed sequence tag (EST) library and development of a DNA microarray. Mar. Biotechnol. **7:**481-493.

CIGUATOXICITY IN THE NORTHERN GULF OF MEXICO

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The northern Gulf of Mexico has historically been virtually free from ciguatera. Although included within maps for regions that are potentially ciguatoxic, there is little historical data to suggest a problem. Ciguatoxic barracuda were caught from the continental shelf of Texas in 1988 and 1998, but there is no public perception of ciguatera and no public health warnings have been issued. No other fish has been linked to a confirmed case of ciguatera, although there is a 20+ year old report of neurological symptoms from a grey snapper caught on the coral reefs at the Flower Garden Banks National Marine Sanctuary. A follow up study noted the presence of *Gambierdiscus* on oil platforms and *Sargassum* along the Texas coast as well as low levels of toxin in a small percentage of barracuda (Villareal et al. 2007).

In 2006-2007, an unusual number of ciguatera cases were noted. At the time of this writing, there are 9+ cases linked to both grouper and barracuda landed in Florida and Texas that are traceable to fish caught either at the Flower Gardens or along the Florida/Alabama section of the northeastern Gulf. Where available, fish has been sampled and the presence of ciguatoxin confirmed by LC-MS. A follow-up survey of 31 fish collected at the Flower Gardens found toxin levels greater than 0.2 ppb in 2 of 31 fish with trace levels reported in two more. *Gambierdiscus* has been cultured from the Flower Gardens as well, extending the range of this genus to the most northerly coral reef in U.S. waters.

These cases may represent the beginnings of a broad incursion of ciguatera into the northern Gulf of Mexico. The sudden increase in ciguatera in general and presence of toxin in a previously safe guild of fish (grouper) suggests significant changes are occurring in the Gulf of Mexico. In the Gulf of Mexico, barracuda migrate extensively following isotherms; however, grouper are generally more territorial except when assembling for spawning aggregations. The hypothesis that an endemic ciguatera food-web is present, as opposed to a migratory introduction, cannot be rejected and is consistent with predictions that the increase in artificial reefs and oil platforms, as well as warmer sea-surface temperatures could lead to increased ciguatera (Villareal et al. 2007).

Villareal, T.A., S. Hanson, S. Qualia, and R.L. Dickey. 2007. Petroleum production platforms as sites for the expansion of ciguatera in the northwestern Gulf of Mexico. *Harmful Algae* 6:253-259

MOLECULAR CHARACTERIZATION OF TOXIC CYANOBACTERIAL COMMUNITIES IN THE LOWER GREAT LAKES: A SEVEN YEAR SYNOPSIS.

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Lab experiments as well as field surveys and experiments have been ongoing in the lower Great Lakes (Lakes Erie and Ontario) since 1999 to gain some insight into the processes associated with the proliferation of toxic cyanobacteria. Cyanobacteria of the genus *Microcystis* have reoccurred at bloom levels in most years since 1995 with cell densities exceeding 1 million cells per liter and toxin concentrations above suggested World Health Organization standards. As better management of these events can only occur with a better understanding of their ecology, molecular biological tools, designed to phylogenetically characterize and quantify all cyanobacteria, all *Microcystis* or potentially toxigenic *Microcystis* have been developed and employed in combination with analytical chemistry and standard limnology to ascertain why blooms may occur and how environmental parameters may influence toxin production. Phylogenetic analyses of toxin genes indicate that *Microcystis* spp. are the primary producers of the hepatotoxin in microcystin in this region, although other toxin producing cells (*e.g., Planktothrix*) proliferate in some regional embayments, and genetic indicators of an as-of-yet unknown to science population of cells are also present. Quantitative analyses further suggest that only a subset of the total *Microcystis* community (mean ~ 10 %, range ~ 5 - 50% across all years and stations) is potentially toxic, implying that these populations are diverse and that toxin production may carry some costs (and potential benefits) that affect competition and proliferation within the microcystis-producing community. When combined with data on environmental physiochemistry, our observations suggest a disconnect between the causes of blooms and toxin production in natural systems. These observations will be presented in the context of future management and research directions that are needed for this and other HAB-impacted aquatic systems.