ABSTRACTS OF POSTERS

APPLICATIONS OF THE OCEAN BIOGEOGRAPHIC INFORMATION SYSTEM (OBIS) TO HAB DATA

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The Ocean Biogeographic Information System (OBIS) is the information component of the Census of Marine Life (CoML), an international discovery program representing a network of more than 1700 researchers in 73 nations engaged in a 10-year initiative to assess and explain the diversity, distribution, and abundance of life in the oceans - past, present, and future. OBIS is the most authoritative web-based provider of global geo-referenced information on marine species and currently includes 13.2 million records of 82,000 species from 216 databases. In addition to gathering and maintaining marine species-level and habitat-level databases, it provides a variety of spatial query tools for visualizing geographical relationships among species, and between species and their environment. OBIS is growing rapidly to become the national, regional, and international infrastructure for information on marine species and their distribution and abundance, and playing a central research role in ocean biodiversity informatics (Costello and Vanden Berghe 2006).

The current number of HAB-related databases around the world demonstrates both the interest in and the fragmentation of HAB data. There is currently a concerted effort at the international level to consolidate HAB databases. Because it is a worldwide distributed database, OBIS should be an excellent vehicle for this consolidation, though it has not been specifically used for HABs in the past.

OBIS is looking for opportunities to work with HAB scientists and other interested groups to incorporate HAB data sets into OBIS and develop targeted products for end users. We hope to increase the incentives for investigators to supply their research and monitoring data to databases like OBIS, by facilitating data entry and then making this data available in more useful formats that can be compared with others. This should help to improve linkages of HAB data to related environmental data as well as improve taxonomic identification and standardization. A new HAB portal will be created on the OBIS Home Page including useful links to other HAB resources.

On the technical level, the schema specifying the information shared within OBIS could be extended with elements specific for HABs. These extensions could then be incorporated in the database structure and portal site of OBIS. On the community level, the first step is an analysis of needs and expectations of the HAB scientists; results will feed into the technical part and guide development. The new technological platform should provide an incentive for HAB scientists to share their data in the OBIS community; this would bring them several advantages: first of all, their data would become available in a standardized format, together with data from other providers - both within the U.S. and outside. Finally, analytical tools developed by OBIS will automatically be available, such as tools to correlate physical oceanographic measurements with species distributions.

Costello, M. J., and E. Vanden Berghe. 2006. 'Ocean biodiversity informatics': a new era in marine biology research and management. Mar. Ecol.-Prog. Ser. **316:** 203-214.

A FUZZY LOGIC APPROACH TO PREDICTING *Prorocentrum minimum* BLOOMS IN THE CHESAPEAKE BAY

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The dinoflagellate, *Prorocentrum minimum*, forms seasonal blooms in the mesohaline portions of the Chesapeake Bay and its tributaries. Often, these blooms cause hypoxic conditions, which can result in fish kills. Furthemore, certain strains can produce toxins that directly affect the survivorship or behavior of finfish and bivalves. Understanding the environmental conditions preceding a bloom can help scientists understand the mechanisms behind bloom formation. Fuzzy logic provides a novel, flexible solution for predicting blooms, since it incorporates the uncertainty of categorizing and defining a bloom concentration. The Chesapeake Bay and its tributaries are an ideal location for developing predictive models because of the multi-decade, spatially extensive water quality monitoring and phytoplankton datasets available. The results of predictive models at three representative water quality monitoring stations in the Chesapeake Bay will be presented, along with an examination of the sensitivity of the models to the definition of a "bloom" concentration. The usefulness of the model in illustrating potential mechanisms of bloom formation, as well as in guiding efficient best management practices (BMPs), will also be explored.

ASSESSMENT OF MICROCYSTIN PRODUCTION IN CYANOBACTERIA USING A NOVEL WHOLE CELL FLUORESCENT IMMUNOLOCALIZATION METHOD, FLOW CYTOMETRY AND THE ENZYME-LINKED IMMUNOSORBANT ASSAY (ELISA)

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Microcystins are hepatotoxic, non-ribosomal peptides produced by several genera of freshwater cyanobacteria. The detection of microcystin-producing strains of cyanobacteria poses a unique challenge to water management officials because of deficiencies in standardized methods aimed at detecting both the toxin and toxin-producing strains. Routine analyses of phytoplankton from surface waters readily discriminate among cyanobacterial genera; however, morphometric analysis is not sufficient to detect toxic strains. The goal of our research is to develop a rapid and cost-effective microscopic assay that isolates toxic strains using whole cell, fluorescence-based, immunolocalization. Fixation conditions, antigen retrieval and antibody sensitivity were tested by comparing the mean fluorescent intensity (MFI) of labeled cells using flow cytometry to relative toxin concentrations estimated using a commercially available ELISA kit for microcystin. The effects of growth conditions and cell cycle on the efficacy of the immunolocalization method were also examined using *Microcystis aeruginosa*. Results indicated that MFI values measured using flow cytometric analysis regularly correlate with toxin concentrations quantified using ELISA. These results point to a direct relationship between the fluorescent intensity of labeling and toxin content within the cell. An optimized immunolabeling protocol will allow for assessment of relative toxin content within individual cyanobacterial cells and in cell populations, thereby offering new insight into the dynamics of toxin production on a per cell basis. Preliminary investigations with environmental samples seeded with toxic cultures suggest the prospective use of whole cell immunolabeling in field samples collected from at-risk recreational waters and potable water supplies.

GROWTH, TOXICITY AND NITROGEN UPTAKE CAPABILITIES OF THE TOXIGENIC DIATOM *Pseudo-nitzschia cuspidata* FROM THE PACIFIC NORTHWEST

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The toxigenic diatom, *Pseudo-nitzschia cuspidata* (Hasle) has recently been found in bloom concentrations in the Juan de Fuca Eddy region off the coasts of Washington State, U.S.A. and British Columbia, Canada. Isolates collected during the September surveys of the ECOHAB-PNW project during 2004 and 2005 were examined for their growth, toxicity and nitrogen uptake response to inorganic (nitrate, ammonium) and organic (urea) nitrogen. The kinetics of N uptake as a function of substrate concentration were estimated from short (20-min) incubations using the ^{15}N-tracer technique, and are compared to the long-term exponential growth rates of *P. cuspidata* determined in semi-continuous, batch cultures grown on the various nitrogen substrates. Based on the estimated maximum specific uptakes rates (V_{max}), nitrogen preference follows the order: ammonium > nitrate > urea, whereas the nutrient affinity indices - half saturation constants (K_s) and alpha parameter (alpha = V_{max}/K_s), indicate that N affinity follows the order: urea > nitrate > ammonium. Long-term growth experiments conducted at saturating (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) and sub-saturating (40 $\mu E \cdot m^{-2} \cdot s^{-1}$) photosynthetic photon flux densities (PPFDs), demonstrate that *P. cuspidata* grows significantly faster at the higher PPFD for all N substrates, but there are substantial differences in the growth rates achieved on the various N substrates. The exponential growth rate (determined using raw fluorescence units) at high PPFD is slower for cells grown on urea (0.84 ± 0.03 d^{-1}) compared to the cells maintained on nitrate (0.88 ± 0.01 d^{-1}) or ammonium (0.91 ± 0.02 d^{-1}), whereas at low PPFD, urea supported faster growth (0.65 ± 0.003 d^{-1}) than either nitrate (0.55 ± 0.01 d^{-1}) or ammonium (0.51 ± 0.02 d^{-1}).

Recently, Armstrong-Howard *et al.* (2007) showed that both field assemblages and laboratory cultures of *P. australis* produce more domoic acid (both cellular and dissolved DA) when grown on urea versus nitrate or ammonium. In our study, the cellular DA content (determined using cELISA) for *P. cuspidata* did not significantly differ as a function of the nitrogen substrate used for growth at saturating PPFD, but at sub-saturating PPFD, nitrate-grown cells produced 74% and 78% more pDA per cell than ammonium- and urea-grown cells. In contrast to other *Pseudo-nitzschia* species where cellular domoic acid is generally enhanced during stationary phase, the cellular DA concentrations for *P. cuspidata* were always greater during exponential growth compared to stationary growth, regardless of the N substrate or PPFD used for growth. These laboratory results demonstrate the capability of this small, toxigenic diatom to grow and produce DA on both oxidized and reduced N substrates supporting field observations that *Pseudo-nitzschia* species bloom during both upwelling and non-upwelling conditions off the west coast of North America where substantial differences in the nitrogenous nutrition of *P. cuspidata* can be expected.

ARMSTRONG-HOWARD, M. D, COCHLAN, W. P., LADIZINSKY, N. L. AND KUDELA, R. M. 2007. Nitrogenous preference of toxigenic *Pseudo-nitzschia australis* (Bacillariophyceae) from field and laboratory experiments. Harmful Algae **6**: 206-217.

LOW SINKING RATES OF *Pseudo-nitzschia*: A COMPETITIVE FEATURE CONTRIBUTING TO THE DEVELOPMENT AND MAINTENANCE OF TOXIC BLOOMS

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The toxigenic diatom genus *Pseudo-nitzschia*, responsible for amnesic shellfish poisoning, is a significant harmful algal bloom taxon. Efforts to predict the blooms and subsequent fate of *Pseudo-nitzschia* cells require understanding the factors that contribute to the growth and mortality of the diatom. Balancing cell growth and nutrient uptake rates against grazing-determined loss rates is the foundation for many models of bloom maintenance. However, sedimentation is a major loss mechanism for diatoms often not considered in describing bloom development. In addition to their unique cell morphology, *Pseudo-nitzschia* spp. often display behaviours (e.g. optional chain formation) that have the potential to minimize cell loss through sedimentation, and thereby extending the duration and magnitude of toxigenic diatom bloom events. The relative sinking rates of natural communities containing *Pseudo-nitzschia* were studied along the coasts of Washington State, U.S.A., and British Columbia, Canada, as part of the ECOHAB-PNW project. Sinking rates of *Pseudo-nitzschia* were substantially lower than those of the entire phytoplankton community during the early development and midpoint of toxic bloom formation. Measurable sinking rates of *Pseudo-nitzschia* were observed only late in the bloom. Even so, the maximum observed sinking rate of *Pseudo-nitzschia* was less than measured for the phytoplankton community as a whole. This change in *Pseudo-nitzschia* sinking rates corresponded with a sharp reversal of intracellular vs. extracellular domoic acid concentrations, where high concentrations of dissolved DA were released by the cells. The increase in *Pseudo-nitzschia* sinking rates also was accompanied by a higher potential formation of aggregates, measured in a Couette device, that was likely mediated by increased concentrations of exocellular polysaccharides. These results suggest that the low observed sinking rate of *Pseudo-nitzschia* during bloom development could contribute to the competitive success of this taxon, and sedimentation may only be significant after periods of substantial aggregation and subsequent sinking of the diatom flocs.

PHYLOSORT: A PROGRAM FOR DETECTING ENDOSYMBIOTIC AND HORIZONTAL GENE TRANSFER AND ITS APPLICATION TO UNDERSTANDING STX GENE ORIGIN IN *Alexandrium tamarense*

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Genome data offer the potential to clarify long-standing issues in organismal biology. The size and complexity of genome data provides however a significant hurdle in realizing this goal. To aid the identification of genes that have originated through endosymbiotic (i.e., intracellular; e.g., Reyes-Prieto et al. 2006) or horizontal (i.e., foreign source) gene transfer (EGT and HGT, respectively), we have developed a computer program, PhyloSort for automated analysis of trees generated by phylogenomics. Phylogenomic methods allow the user to simultaneously compare dozens of genomes to each other and to generate alignments and phylogenies of each gene homolog. This approach has revolutionized genome annotation and gene family analysis and provided large data sets for multi-gene phylogenetics. Existing phylogenomic pipelines such as PhyloGenie (Frickey and Lupas 2004) and PhyloGena (Hanekamp et al. 2007) generate a large collection, often hundreds or thousands, of phylogenetic trees that the user has to manually inspect to observe general patterns of genome evolution or to address specific hypotheses about gene phylogeny. PhyloSort automates tree topology analysis in a high-throughput fashion. This open-source Java tool allows the user to query the topology of phylogenetic trees to address the most frequently asked question in the field, does a specific gene support the monophyly of specified OTUs? PhyloSort reads Phylip formatted trees as input and can be used via a graphical user interface (GUI) and a text mode command line interface. Initially, the user specifies a source folder containing the input phylogenetic trees and a target folder to where trees that satisfy the search criteria can be copied or moved or alternatively, simply counted. Thereafter sets of taxa (query) that are postulated to be monophyletic are specified and searched in the input trees. The search criteria can be adjusted by setting a minimum bootstrap support value associated with the monophyletic clades and a minimum and/or maximum number of taxa in the trees with matching clades. The trees can also be “clustered” to generate a “uni-tree” set that encompasses gene families. Here we describe PhyloSort using examples of analysis of EGT in different algae. Thereafter, we apply the program to identify homologous genes shared exclusively between the saxitoxin (STX) producing taxa, the dinoflagellate *Alexandrium tamarense* and the cyanobacterium *Anabaena circinalis*. Other prokaryotes and eukaryotes will also be included in this analysis. The goal here is to identify potential HGT events that may have resulted in the origin of STX genes in *Alexandrium* from the cyanobacterium (or some other source). This hypothesis of STX gene origin in *Alexandrium* through HGT is supported by extensive preliminary data that demonstrates that dinoflagellates are masters of harvesting genes from foreign sources.

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PUGET SOUND, WASHINGTON: AN EMERGING HOTSPOT FOR *Pseudo-nitzschia* BLOOMS AND DOMOIC ACID TOXIC EVENTS

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Pacific Northwest inland embayments with limited direct exchange with oceanic waters contain a number of HAB species, including the Paralytic Shellfish Poisoning (PSP) dinoflagellate genus *Alexandrium*, the fish-killing raphidophyte *Heterosigma akashiwo,* and the Amnesic Shellfish Poisoning (ASP) diatom genus *Pseudo-nitzschia*. While blooms of the toxigenic diatom, *Pseudo-nitzschia* dominate public and scientific attention in the well-studied coastal systems of western North America (e.g., Monterey Bay, Santa Barbara and San Pedro Channels, Juan de Fuca Eddy), the recent *Pseudo-nitzschia* blooms observed within the inland waters of Puget Sound, Washington suggest that toxic diatom events are not isolated to these coastal systems. *Pseudo-nitzschia* have been observed in Puget Sound waters since the 1930's (then identified as belonging to the *Nitzschia* genus), definitively identified as *Pseudo-nitzschia* species since 1990 (Horner and Postel, 1993; Trainer *et al*., 1998), and although the neurotoxin domoic acid (DA) has been detected since 1991, only recently have levels exceeded regulatory limits and led to shellfish harvest closures in 2003 and 2005 (Trainer *et al*., 2007). Our monitoring efforts within Puget Sound have revealed the ubiquitous presence of *Pseudo-nitzchia* species, routine detection of DA, as well as adequate macronutrient concentrations and composition to sustain algal blooms, both benign and harmful. Research cruises conducted in June and September 2006 found *Pseudo-nitzschia* cell concentrations as high as 3.4 x10^5 cells L^{-1}, particulate DA concentrations as high as 105.2 pg ml^{-1}, and surface water concentrations of nitrate, ammonium, and silicic acid were routinely 18-20, 1.4-2.0, and 50 μM, respectively, well above macronutrient concentrations limiting for growth. Following the first closures of shellfish harvesting due to DA in 2003 and 2005, it appears that Puget Sound is becoming increasingly conductive to toxic DA events. Due at least partially to increasing human population densities and their use of the marine resources within Puget Sound (including aquaculture activities), the increased frequency of toxic DA events raises questions regarding their impacts on ecosystem and human health. Future sustained monitoring efforts, and research designed to characterize local nutrient dynamics (both natural and anthropogenic) and phytoplankton ecology, are necessary to ensure that commercial, recreational, and Tribal subsistence fisheries continue to provide safe products for human consumption.

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TRAINER V.L., COCHLAN W.P., ERICKSON A., BILL B.D., COX F.H., BORCHERT J.A., AND LEFEBVRE K.A. 2007. Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. *Harmful Algae* 6: 449-459.

LONG-TERM (1992-2006) PATTERNS IN *Phaeocystis* BLOOM MAGNITUDE AND BLOOM DURATION IN MASSACHUSETTS BAY, USA

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Blooms of the colonial prymnesiophyte *Phaeocystis pouchetii* are a regular component of the winter-spring phytoplankton of north-temperate coastal seas, including the Gulf of Maine. These high biomass *Phaeocystis* blooms may sequester large portions of ecosystem resources and can alter trophic pathways; making *Phaeocystis* a 'keystone' phytoplankton taxa that may significantly alter ecosystem function. *Phaeocystis pouchetii* abundance was quantified at two-week to monthly intervals (12 to 17 times per year) in Massachusetts Bay (southern Gulf of Maine, NW Atlantic) for fifteen years (1992-2006) as part of a long-term phytoplankton monitoring program. Related hydrographic, nutrient, and zooplankton data were also collected. Time series and statistical analyses were used to identify patterns and infer mechanisms of *Phaeocystis* bloom initiation, maintenance and termination during 1992-2006.

Time series analyses indicated an increasing *Phaeocystis* abundance trend during 1992-2006, driven by the large blooms in 1997, 2000, and during 2004. Coincident with this *Phaeocystis* pattern, diatom abundance had a long-term decline during 1992-2006, with 2005 - 2006 mean levels (ca. 150,000 cells per liter) that were ca. 27% of the peak level (ca. 550,000 per liter) observed during 1994. Within this long-term decline there were relative peaks in diatom abundance in 1994, 1998 and 2002. The relative diatom abundance peaks approximately corresponded with relative nadirs in *Phaeocystis* abundance and correlation analysis of these two trends ($r = -0.54$, $p<0.0001$) indicated a long-term negative interaction between *Phaeocystis* and winter-spring diatom abundance.

Phaeocystis blooms of greater than one million cells l^{-1} were observed in seven of the 15 years, while greater than 10 million cells l^{-1} were observed during 1997, 2000, and 2004. *Phaeocystis* was present from February through early June, with the annual bloom maximum occurring in April. The maximum *Phaeocystis* abundance of 15.5 million cells l-1 was observed during April 2004. The time of *Phaeocystis* bloom initiation was relatively consistent, however, within the limits of detection imposed by the monitoring schedule, bloom duration appears to have increased significantly (linear regression, $r^2 = 0.41$, $p = 0.0237$) at a linear rate of ca. six days per year during 1992-2006. This increased bloom duration was due to an extension of bloom termination which extended *Phaeocystis* presence into May or early June during 2002-2006. Consistent with its physiological threshold, *P. pouchetii* was not present at water temperature greater than $14^{o}C$, and the date of attainment of the $14^{o}C$ water temperature threshold varied by 39 days, occurring as early as early May (2001) to as late as mid June (1993). Annual variation in winter-spring water temperature explained much (ca. 50%) of the annual variation in bloom duration, with blooms continuing later and subsequently having longer duration in years having cool spring water temperature. Impacts of *Phaeocystis* blooms on zooplankton abundance were also evaluated. A pattern of elevated *Calanus* abundance early in the season (Feb-Mar) and reduced *Oithona* and total zooplankton abundance later in the season (April-May), followed by increased *Oithona* abundance during summer was associated with *Phaeocystis* bloom years.

EVALUATION OF SHORT AND LONG LASTING NEUROLOGICAL RESPONSE TO SINGLE AND REPEATED CIGUATOXIN EXPOSURE IN MICE

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Ciguatera fish poisoning is a common illness in tropical and subtropical regions that manifests complex and long-lived neurological symptoms, often more severe in recurrent cases. To better evaluate the hallmark chronic and repeat ciguatera symptoms we measured based upon batteries of tests the neurological signs and blood toxin levels in mice following one or two administrations of ciguatoxin (P-CTX-1, 0.264 ng/g). Telemetric recording of body temperature showed an early hypothermic response (nadir value at 1hr of 32.2ºC) followed by a long lasting (at least 5 days) thermoregulatory dysfunction displayed as a stabilized body temperature (at 36ºC) with no observable circadian rhythm. The thermoregulatory response was greater following the second exposure, with a significantly sustained (at least 3 days) and stronger (nadir value of 30.13 at 4.5hr) response. Peripheral effect measurement through response latency to radial heat stimulus in the tail flick assay revealed a higher pain threshold in exposed vs. control mice. This long lasting dysfunction of pain perception continued after the recovery from hypothermia. These findings support an acute phase of ciguatoxin toxicity, of short duration, associated as central thermoregulation to lower body temperature and reduced motor activity, and a sensory neuropathy of long duration, featured by persistent reduction in pain threshold and alteration of thermoregulatory process. Biomonitoring of blood toxin levels confirmed ciguatoxin (1.4 pg/ml, limit of detection) for 3 days after exposure, and the concentration measured was significantly higher at 1 hour but not at 4, 24 and 72hr following second exposure. This indicates that repeat exposures lead to larger acute blood levels of ciguatoxin, and these acute internal levels are sufficient for the chronic progression of ciguatera symptoms.

USING REAL-TIME PCR TO DEMONSTRATE CO-OCCURRENCE OF *Karlodinium veneficum* AND THE PARASITIC DINOFLAGELLATE *Amoebophyra* SP. EX. *Karlodinium veneficum*

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Karlodinium veneficum is an ichthyotoxic bloom forming dinoflagellate common to the Chesapeake Bay and other estuarine systems throughout the world. Blooms of *K. veneficum* are thought to be controlled, in part, by the parasitic dinoflagellate *Amoebophrya* sp. ex. *K. veneficum.* This parasite is also common in estuarine environments where it uses its host for carrying out a simple life cycle, including a free-swimming dinospore that infects the host, a vegetative trophont that grows within the host, and a vermiform stage which breaks down into dinospores that get released upon host death. This parasitic infection disrupts reproduction, resulting in a decline of the host population.

The infection of *Karlodinium veneficum* by *Amoebophrya* sp. ex. *K. veneficum* can easily be observed in controlled laboratory experiments using light microscopy. Making these observations in environmental samples becomes difficult when other organisms of similar size are present. Therefore, we designed two real-time PCR assays, based on Taqman methodology, to target the internal transcribed region (*K. veneficum*) and the 18S locus (*Amoebophrya* ex. *K. veneficum*). Annealing temperature, sensitivity and reaction efficiencies were similar for each assay, allowing them to be used effectively for quantifying the co-occurrence of these two organisms throughout controlled infection experiments and over the course of several bloom events. In controlled infection experiments, there is a clear decrease in *K. veneficum* ITS signal six hours post-infection, while in natural samples real-time PCR data demonstrate a definite co-occurrence of these species.

EVIDENCE OF A SELF-RECOGNITION SYSTEM IN THE SEXUAL LIFE-CYCLE OF *Alexandrium tamarense*

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Sexual crosses of Group 1 clones (formerly termed North American ribotype) and Group III clones (formerly termed Western European ribotype) are used to investigate patterns in gamete selection in the dinoflagellate *Alexandrium tamarense*. Cysts resulting from the crosses are analyzed using a nested PCR assay for detection of Group I and Group III ribosomal sequences and also by imaging flow cytometry to assess DNA content. Results are compared to those from natural cysts collected from the Gulf of Maine. These data promise to provide new insights into the cellular mechanism of sexual induction and also patterns of genomic control during the sexual phases of the cells' life cycle. We seek to test several hypotheses derived from a rudimentary model of the dinoflagellate sexual cycle and models derived from other protists. Results thus far suggest a self-recognition system operates as a gate to complete gametic differentiation of these cells in culture. If verified, this finding has significant implications for the interpretation of mating compatibilities and to laboratory study of dinoflagellate sexual processes.

THE NUTRITIONAL ECOLOGY OF THE HARMFUL DINOFLAGELLATE BLOOMS CAUSED BY *Cochlodinium polykrikoides* IN LONG ISLAND BAYS (NY, USA)

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Harmful algal blooms caused by *Cochlodinium* species have been reported worldwide, including both the east and west coast of North America. *Cochlodinium polykrikoides* formed dense blooms in the Peconic Estuary and Shinnecock Bay in Long Island, NY, USA during the late summer and fall of 2004-2006. Bloom waters with cell densities higher than 5×10^4 cells ml^{-1} have been documented as being acutely toxic to multiple fin fish and shellfish species. The mechanisms for the initiation and development of these blooms are not clear. We explored the nutrient preferences and requirements and effect of salinity on the growth rate and yield for a strain of *C. polykrikoides*, CP1, which was isolated from the 2006 fall bloom in Shinnecock Bay. *C. polykrikoides* displayed a higher growth rate and cell yield in GSe medium (Doblin et al. 1999) prepared with artificial seawater compared to those obtained in GSe prepared with filtered seawater at the same salinity. *C. polykrikoides* showed a preference for different nitrogen sources (urea = ammonium > nitrate) with regard to growth rate, while the ultimate cell yield was determined by the initial concentration of nitrogen. It was also shown that *C. polykrikoides* favored the lower salinities in the range of 23-37.5 and that selenium was not required for its growth. Further study on the nutritional effects of other nutrient sources on *C. polykrikoides*, CP1, will also be presented.

The importance of nitrogen supply to the occurrence of *C. polykrikoides* blooms was further substantiated during field incubation experiments conducted during the 2006 bloom event on Long Island. Nutrient amendment, bottle incubation experiments were conducted with varying levels of nitrogen, phosphorus, and organic micronutrients (B-vitamins) using bloom water and the net growth rates of *C. polykrikoides* within each treatment were quantified. During all experiments conducted (n=5), only nitrogen enrichment was capable of significantly increasing *C. polykrikoides* growth rates relative to control treatments ($p < 0.05$; Tukey). In contrast to laboratory findings, the response of field populations to varying nitrogen sources did not differ significantly. These results suggest that nitrogen loads will determine the absolute magnitude of *C. polykrikoides* blooms, which in turn will likely influence the toxicity of a bloom event.

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USING A NOVEL LOW-COST MONITORING ARRAY FOR VALIDATION OF BREVETOXIN-INDUCED RESPIRATORY IMPACT IN SOUTHWEST FLORIDA

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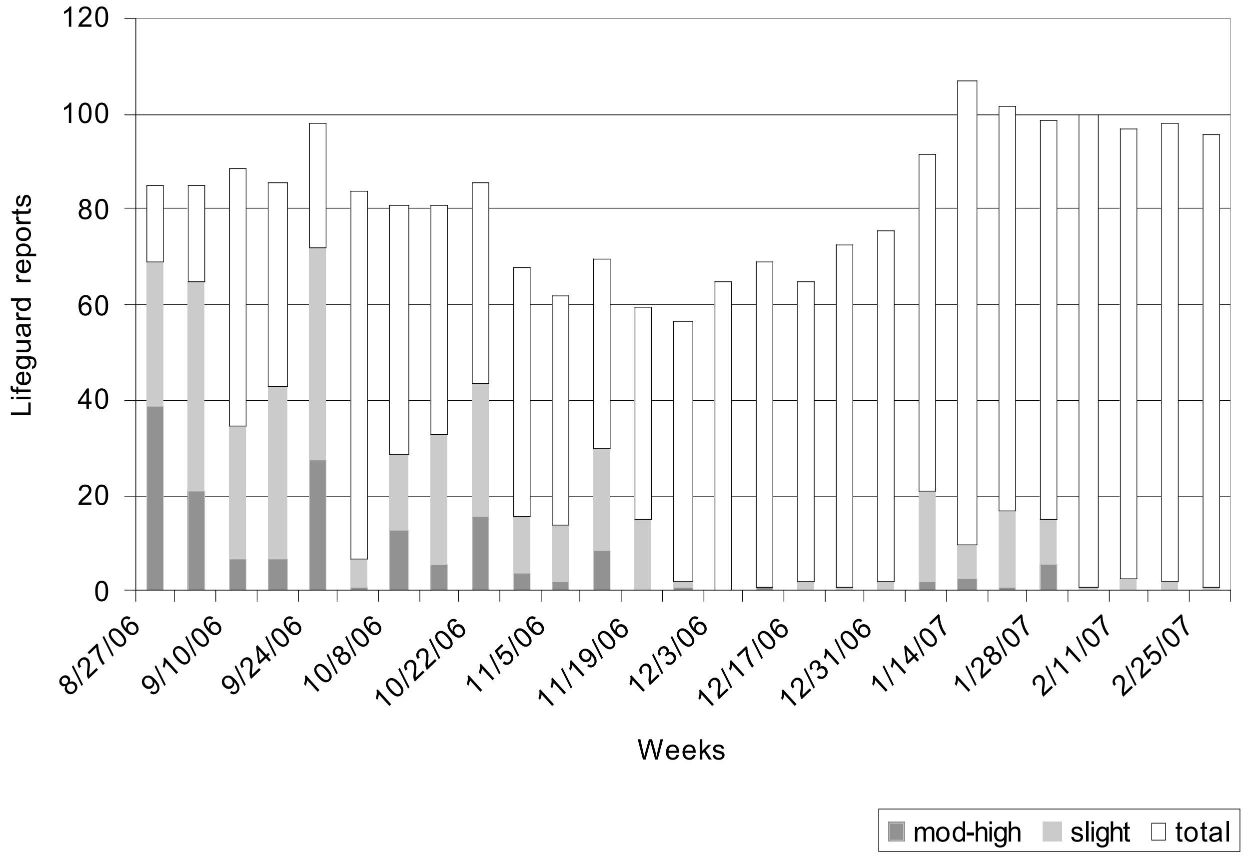
Brevetoxins are a known respiratory irritant, posing a health hazard for beachgoers and local residents, particularly asthmatics. Increased incidence of respiratory irritation related to a HAB event is documented when toxic aerosol concentrations exceed 10 ng/m^{3}of air. This is a major concern for the state of Florida, as it has annual blooms of *K. brevis* off its gulfside beaches. Starting in August 2006, the professional lifeguard corps in Sarasota County began twice daily reports of the presence of respiratory irritation at six permanent sites. This dataset provides systematic results of impact with high temporal and spatial resolution. The benefits of this cheap, low-tech system are real-time monitoring of the health impacts during the presence of a harmful algal bloom and, in addition, validation of the HAB impact forecasts although with the risk of random error between impact classes due to subjectivity. NOAA's HAB Bulletin forecasts the degree of local health impact during a HAB event, considering the location and extent of the HAB as well as the wind direction forecast (e.g. onshore/offshore). Previously only anecdotal or informal reports of impact were available. A comparison of the datasets has found that 67% of high-moderate respiratory impact occurrences were correctly predicted by the HAB forecast bulletin. The dataset allows exploration of incorrect predictions for causation and with the goal of increasing forecast reliability.



120
100
80
60
40
20
0
Lifeguard reports
8/27/06
9/10/06
9/24/06
10/8/06
10/22/06
11/5/06
11/19/06
12/3/06
12/17/06
12/31/06
1/14/07
1/28/07
2/11/07
2/25/07
Weeks
mod-high
slight
total

GENETIC VARIATION AMONG ISOLATES OF *Karenia* brevis AS MEASURED WITH MICROSATELLITE MARKERS

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Karenia brevis is the major harmful algae of concern in the Gulf of Mexico, yet the source of bloom populations, especially in the western Gulf of Mexico, is not well known. Ten microsatellite markers, nine previously described (Renshaw et al. 2006) and one newly characterized, were used to assess genetic diversity among 32 clonal cultures of *K. brevis* isolated over the past 50 years from blooms along the coasts of Florida and Texas. The number of alleles at each microsatellite ranged from three to ten and estimated gene diversity per microsatellite ranged from 0.287 to 0.851 (average 6.67 $\pm$ 0.178). Variation among the clonal cultures was extremely diverse; 21 unique haplotypes were found among 22 Florida cultures, whereas 10 unique haplotypes were found among the 10 Texas cultures. In the latter, eight of the 10 isolates were from the same bloom. Allele distributions, however, were not correlated with bloom (year) or location (Florida vs. Texas). At eight of the ten microsatellites, the most frequently occurring allele was the same for clonal cultures from both Florida and Texas. Microsatellite allele distributions among individual cells sampled during a 2005 bloom along the coast offshore of Corpus Christi, Texas, revealed that the number of alleles found among the available culture collections is less than the number of alleles found in the field sample. These results indicate estimates of genetic diversity in *K. brevis* based on culture collections may be underestimates of genetic diversity in bloom populations. Our observations of genetic diversity in *K. brevis* are similar to results found in blooms of other phytoplankton species. In *K. brevis*, blooms appear not to be clonal but rather to be extremely diverse. Results from this study emphasize the importance of examining multiple individuals of a phytoplankton species to assess genetic variation.

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COASTAL BLOOM DYNAMICS IN SOUTHERN CALIFORNIA - HAVE THERE BEEN ANY CHANGES SINCE 1917?

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Algal blooms may be increasing in frequency and intensity along the coastal waters of the United States. The objective of this research is to determine if these findings are consistent with monitoring programs at Scripps Pier, La Jolla, California, and investigate questions related to bloom dynamics. We have a unique opportunity to compare over 40 years of time series data collected by E. W. Allen (1917-1939), J. McGowan et al. (1983-2000), and L. Busse et al. (2003-2005) to our current monitoring program (2005-2007).

In order to determine if the frequency of algal blooms has increased over time, we have defined a bloom as 1.5 standard deviations above the long-term mean chlorophyll concentration (14.03 mg/m^3, 1983-2000). At least 50 blooms have occurred from 1983-2007, and our results indicate there is a long-term rise in the overall trend of the chlorophyll concentration. The timing of these blooms at Scripps Pier has been found to occur during the late spring and early summer periods.

We will also evaluate the species responsible for the blooms identified and determine if the species assemblage has changed over time or season. Also using the historical Allen data, we will determine if the phytoplankton community structure has changed over time.

LONG-TERM EVALUATION OF A SATELLITE OCEAN COLOR DATASET TO DETECT BLOOMS OF THE TOXIC DINOFLAGELLATE *Karenia brevis* OFF THE WEST FLORIDA SHELF

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Despite the superb splendor of phytoplankton when observed microscopically, and their key importance on the marine biosphere, a minority of them possess a capability to produce toxins that are noxious to other forms of life. In the case of the toxin producing micro-algae, whenever growth rates exceed cell dispersions it causes what is know as harmful algal blooms (or red tides as they are often called). A nearly annual incidence of blooms of the toxic dinoflagellate *Karenia brevis* affects the West Florida Shelf, posing a threat to public well-being and causing massive fish and marine mammal deaths (Van Dolah, 2000). The present work aims to test the long-term stability of three approaches to remotely detect *K. brevis* blooms from satellite ocean color measurements (SeaWiFS and MODIS). Originally, these approaches were each successfully applied to single images, and are based upon the fact that there is less grazing over *K. brevis* due to the toxin it produces (i.e. brevetoxin; PbTx) such that the inherent optical properties of the water impart from other local bloom forming phytoplankton species. The first technique (Canizzaro et al., 2002) makes use of remote sensing reflectance ratios (at 443, 490 and 555nm) to detect *K. brevis*, while the second one (Canizzaro, 2004) suggests a classification scheme based on two criteria: high chlorophyll plus particulate backscatter at 555nm being lower than that calculated by Morel (1988). The performance of a newer methodology (Carvalho et al., 2007), which takes into account only a single water leaving radiance band at 555nm to flag *K. brevis* blooms, will also be tested on a longer period. The in situ dataset, containing abundance of *K. brevis* (cells per liter), was obtained from the Florida Fish and Wildlife Research Institute and will be used to corroborate the retrievals of each of the three algorithms.

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VAN DOLAH, F.M. 2000. Marine algal toxins: origins, health effects and their increased occurrence. Environmental Health Perspectives Supplements, 108(1): 133-141.

MEASUREMENT OF APOPTOSIS IN PHYTOPLANKTON USING FLOW CYTOMETRY AND ANNEXIN V BINDING

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The study of bloom development and the effects of programmed cell death (PCD) on the growth and death of various phytoplankton species is the subject of current investigation. We have examined the role of apoptotic PCD through the use of the annexin V affinity assay and flow cytometry. The mechanism for annexin V binding to phosphatidylserine present on the surface of cells undergoing apoptosis has been widely reported in mammalian cells but application in phytoplankton to detect PCD has been limited. Initial measurements of apoptosis using flow cytometry and annexin V binding were evaluated in the unicellular chlorophyte, *Dunaliella tertiolecta*. Previous studies have shown that *D. tertiolecta* undergoes apoptotic PCD following 12 days of light deprivation indicated by caspase induction DNA fragmentation and morphological indicators (Segovia et. al. 2003). Consistent with these findings we found significant levels of annexin V binding and light scatter changes following light deprivation. Compared to normal light conditions, there was a marked increase (30%) in annexin fluorescence and a corresponding decrease in light scatter properties of cells from light deprived cultures. With the addition of the DNA dye propidium iodide, we could further document membrane damage in cells undergoing apoptosis. Using the described methodology we further evaluated the levels of annexin V binding in light deprived cultures of two dinoflagellates including *Amphidinium carterae* and *Karlodinium venificum*. Our initial findings indicate detectable levels of annexin fluorescence associated with light deprivation. Understanding the role of PCD in control of phytoplankton populations will provide insight into both bloom development and management.

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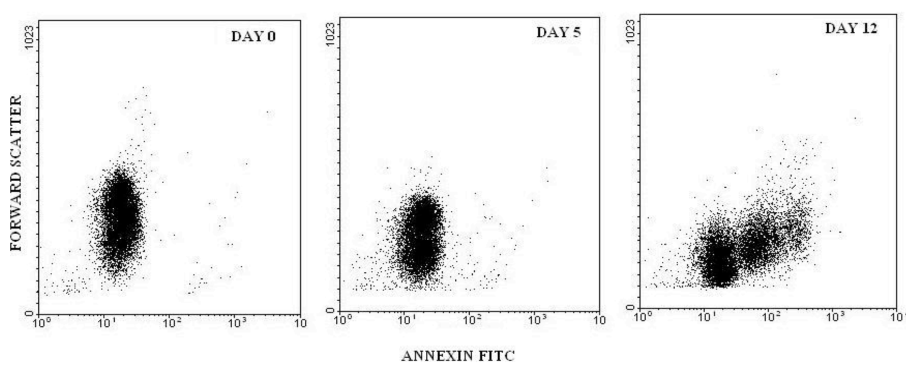

Fig. 1. Increases in Annexin-FITC binding and forward scatter in *D. tertiolecta* at 0, 5 and 12 days darkness.

SINGLE LABORATORY VALIDATION OF THE BREVETOXINS ELISA FOR SHELLFISH CONTAMINATION ASSESSMENT

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The brevetoxins (PbTxs) are a family of lipid-soluble polyether marine neurotoxins produced by the bloom-forming dinoflagellate *Karenia brevis*. These toxins are responsible for annual massive mortalities of fish and are also accountable for reoccurring mortalities of birds, sea turtles, and marine mammals. Lacking a protective theca, *K. brevis* cells lyse easily and the toxins, under the combined actions of wind and waves, can be aerosolized causing a profound concentration-dependent bronchoconstriction upon inhalation. Aside from respiratory impairments, these toxins also present a human health threat as they accumulate to dangerous levels in filter-feeding bivalve shellfish such as clams, oysters and mussels leading to Neurotoxic Shellfish Poisoning (NSP) if consumed.

In recent years, we have developed a high-sensitivity competitive ELISA for the detection of brevetoxins in numerous environmental and biological matrices. This assay is now routinely used as a diagnostic (and sometime forensic) tool to determine brevetoxin exposure, as a research tool to identify pathways of exposure in humans and animals, and to examine the fate of these toxins in the food web and the environment.

Because of the risk for NSP, shellfish contamination is intensively monitored during and after red tide events. As of today, the only approved method for monitoring is the regulatory mouse bioassay, which is slow to provide results and unethical to perform. Here we present results of a single laboratory validation study of a competitive ELISA to monitor brevetoxin contamination in three shellfish species of commercial interest. This study is the first step toward the official validation of the brevetoxin ELISA as an ethical, precise and rapid alternative method for the regulatory monitoring of these toxins in shellfish**.**

POPULATION STRUCTURE OF PARALYTIC SHELLFISH POISIONING (PSP) RESISTANT SOFTSHELL CLAMS, *Mya arenaria*, IN EASTERN MAINE, USA

Laurie Connell, Scott Hamilton, and Amber Bratcher
School of Marine Sciences, University of Maine, Orono, ME

Atlantic Canadian populations of the softshell clam, *Mya arenaria*, with repeated exposure have been shown to be resistant to paralytic shellfish poisoning blooms (PSP) caused by the toxigenic algae *Alexandrium* spp. (MacQuarrie and Bricelj 2000; MacQuarrie 2002; Bricelj et al. 2005; Connell et al. 2006). This resistance is correlated with a mutation in the sodium channel gene pore region, the binding site for saxitoxin, a principle component of paralytic shellfish toxins (PSTs) (Bricelj et al. 2005). This mutation occurs at one base in Domain II resulting in a single amino acid change (Bricelj et al. 2005). A survey of geographically distant *M. arenaria* populations along the eastern seaboard and northwest coast of North America revealed that the resistant genotype occurs in many populations and that the mutation may have occurred multiple times in separate populations. Selective pressures exerted on clam populations by PSTs have not yet been specifically measured, but a survey of *Alexandrium* spp. bloom history coupled with the extent of PST exposure to various *Mya arenaria* populations may provide clues to the rate of expansion of a STX resistant phenotype.

This study surveyed *Mya arenaria* populations from eight locations in Eastern Maine, half of which have a history of repeated PSP blooms. The Domain II sodium channel pore region was sequenced and the populations were compared for percent of homozygous (resistant or sensitive) and heterozygous individuals. These data were then compared with PSP history and dominant ocean currents that can strongly effect larval recruitment. Results from this survey effect management decisions for local shellfish committees on the source of spat to be set in each bay as well as The Maine Department of Marine Resources biotoxin monitoring program.

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Connell, L. B., S. P. MacQuarrie, B. M. Twarog, M. Iszard and V. M. Bricelj (2006). Population differences in nerve resistance to paralytic shellfish toxins in softshell clam, *Mya arenaria*, associated with sodium channel mutations. *Marine Biology* **150**: 1227-1236.

MacQuarrie, S. P. and V. M. Bricelj (2000). Does the history of toxin exposure influence bivalve population responses in *Mya arenaria*?: II) feeding, survival and toxin accumulation. *Journal of Shellfish Research* **19**(1): 636 (abstract).

MacQuarrie, S. P. (2002). Inter- and intra-population variability in behavioral and physiological responses of the softshell clam, *Mya arenaria*, to the PSP toxin-producing dinoflagellate, *Alexandrium tamarense*. Halifax, NS, Dalhousie University**:** 141pp.

DATA MANAGEMENT SUPPORTING REGIONAL VOLUNTEER PHYTOPLANKTON MONITORING EFFORTS

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The Southeastern Phytoplankton Monitoring Network (SEPMN) is an education and outreach program developed by NOAA's National Ocean Service (NOS) to engage school and community volunteer groups in phytoplankton sampling and identification and to raise awareness of harmful algal blooms. Over the past year the program included approximately 2000 volunteers, mostly secondary students and teachers, sampling 80 sites from North Carolina to Texas. Recently, NOAA's Coastal Data Development Center (NCDDC) has partnered with SEPMN to create an end-to-end data-management system that enhances the volunteer experience, facilitates quality control, and ensures the integrity of the data set over the long term.

Volunteers are able to take advantage of an online data-entry tool to submit data, and are then able to visualize and analyze their own validated data as well as data from their Network peers in an online Geographic Information System (GIS) environment. The data-entry tool is a password-protected website offering digital datasheets that perform simple error checking (units, expected data ranges) to ensure accuracy. The resulting data sets enter a workflow that subjects each record to authorization from SEPM administrators before it is automatically entered into a relational database. Data are then exposed to a web GIS application (http://www.ncddc.noaa.gov/website/SEPMN/viewer.htm) that allows students to map phytoplankton abundance measurements across the entire spatial and temporal extents of the SEPMN data set, along with their own submissions. These records include ancillary environmental data (water temperature, salinity), so that by integrating the available data, students can begin to form hypotheses on phytoplankton responses to environmental forcing. In addition, real-time ocean-temperature data from NOAA data buoys can be mapped, allowing students to begin to make testable predictions about phytoplankton occurrences prior to sampling. Finally, the database and associated metadata is periodically uploaded to NOAA's National Oceanographic Data Center for archival, ensuring the long-term integrity of this unique data set.

QUANTITATIVE MAPPING OF CYANOBACTERIAL BLOOMS FROM OCEANSAT-1 OCM SATELLITE DATA: AN EMPIRICAL APPROACH

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[2]University of South Carolina, Department of Biological Sciences, Columbia, SC 29208, USA

We present the preliminary results of the first stage of a NASA-funded project which comprises the development of algorithms for mapping cyanobacterial blooms from Oceansat-1 OCM (Ocean Color Monitor) satellite data. This sensor was chosen over SeaWiFS (Sea-Viewing Wide Field-of-view Sensor) and MODIS (Moderate-resolution Imaging Spectroradiometer) due to its superior spatial resolution (250m compared with 1000m pixels). The OCM and MODIS data were obtained in real-time at the LSU Earth Scan Lab direct-broadcast station for Earth Orbiting satellites. In the years 2006-2007, extensive bi-weekly and weekly field campaigns were undertaken from November 2006 through the present to a freshwater lake (salinity < 1), Lac des Allemands, located in the north-western portion of the Barataria estuary system, Louisiana. Weekly sampling trips will be maintained through summer and then bi-weekly sampling through November 2007, to complete an entire year of field data collection. We have obtained a database of photosynthetic pigment concentrations and phytoplankton composition which are being used in tandem with measurements from the OCM sensor to quantify blooms from space. The field data collection includes water samples for HPLC (High Performance Liquid Chromatography), CDOM (Colored Dissolved Organic Matter), TSS (Total Suspended Solids), microscopic analyses, the cyanobacteria unique pigment phycocyanin, and toxin analyses (Table 1). The results have exceeded our expectations, in particular the number of clear-sky OCM and MODIS images which have been fortuitously obtained on seven days, thus far. Preliminary analyses of the data obtained demonstrate clear seasonal variation in chlorophyll *a* concentrations in Lac des Allemands and in the pigments associated with cyanobacteria. Overall, concentrations of pigments were highest in fall and spring and lowest in winter, corresponding most closely with variation in temperature and light conditions. The microscopic analysis revealed the presence of numerous toxic species of cyanobacteria including *Anabaena circinalis*, *Cylindrospermospsis raciborskii* and *Microcystis* sp. The OCM true color and chlorophyll *a* images and MODIS true color images corroborate with the field measurements and our preliminary results towards the development of regional algorithms for chlorophyll *a* and cyanobacteria concentrations will be presented.

Table 1 Field data

Date	Analyses						YSI	Radiometer	Water Depth
	HPLC	CDOM	TSS	Microscopic	Phycocyanin	Toxin			
Sep 11, 2006	x								
Nov 17, 2006	x		x				x		
Dec 19, 2006	x	x	x	x			x		
Jan 10, 2007	x	x	x	x			x	x	
Feb 19, 2007	x	x	x	x			x		
Mar 23, 2007	x	x	x	x			x	x	
Mar 29, 2007	x	x	x	x	x		x		x
Apr 12, 2007	x	x	x	x	x	x	x		x
Apr 20, 2007	x	x	x	x	x	x	x	x	x
Apr 26, 2007	x	x	x	x	x	x	x		x
May 14, 2007	x	x	x	x	x	x	x		x
May 18, 2007	x	x	x	x	x	x	x	x	x
May 24, 2007	x	x	x	x	x	x	x	x	x
June 01, 2007	x	x	x	x	x	x	x	x	x

June 15, 2007	x	x	x	x	x	x	x	x	x

THE PACIFIC NORTHWEST HAB BULLETIN PILOT PROJECT: TECHNICAL DEVELOPMENT OF AN OCEAN OBSERVING INFORMATION SYSTEM FOR THE PROTECTION OF HUMAN HEALTH

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The Pacific Northwest HAB Bulletin Pilot Project, with support from the Centers for Disease Control, is a web-based information dashboard developed to provide a comprehensive early warning information system for Washington coast HAB events. Traditionally, HAB-related data are maintained by several organizations in separate locations and in a variety of formats. In addition, no single location or system exists to capture the tacit knowledge of local experts who could identify trends and make calculated predictions based on collected data. The separate locations of these resources result in a lag time between acquiring, correlating, interpreting, and distributing HAB event information to coastal managers. The PNW HAB Bulletin Pilot Project builds upon the successful ORHAB (Olympic Region Harmful Algal Blooms) monitoring program by automating the aggregation of HAB information – biological and physical data as well as summarized conclusions by local experts – into a single location. This information dashboard is a dynamic, database-driven web page template comprised of the following components: Real-time drifter tracks, domoic acid in shellfish, *Pseudo-nitzschia* cell counts, sizes, and critical assessment level indicators, winds (Cape Elizabeth buoy data from NDBC), model currents from an existing Navy operational model, Columbia River model, and corresponding analyses by local experts describing the data and the likelihood of occurrence of HAB events. Several technologies and applications were utilized to generate the dashboard from various data sources; these included custom scripts written in UNIX, PERL, Visual Basic, and JavaScript, customized GIS and plot applications, a MySQL database, PHP, XML, and HTML. This combination of technologies allowed data to be automatically retrieved and manipulated from existing resources, including ARGOS satellite servers, websites, spreadsheets, and databases, with minimal or no extra effort required from the data providers. Automating the retrieval of HAB-related data into a single location allowed the PNW HAB Bulletin to significantly reduce the timeline for delivering HAB information to coastal managers, and multiple technologies allowed this data to be aggregated without requiring HAB information providers to modify their existing information storage methods.

NEW PERFORMACE DATA FOR *IN SITU* AND SIMULATED EXPERIMENTS USING THE PHYTOFLASH SUMBERSIBLE ACTIVE FLUOROMETER

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An *in situ* variable fluorescence system has been developed that will allow real-time measurement of the primary variable fluorescence variables: Fv, Fo and Fm. Advances in solid-state light detectors and the development of advanced signal processing circuitry have led to the development of a new generation of fluorescence instrumentation that can be used to measure photosynthetic parameters in a wider range of platforms and locations. Practical applications include the detection of the onset of algal blooms, indication of nutrient status of planktonic algae, ballast water monitoring, as well as the measurement of algal community change. Performance data presented represents red tide distribution, simulated system impacts in relation to the yield and quantum efficiency of *Crocosphaera* (a nitrogen-fixing cyanobacterium) along a transect.

FAST AND ACCURATE DETECTION OF *Alexandrium* SPECIES USING PEPTIDE NUCLEIC ACID PROBES AND SURFACE PLASMON RESONANCE

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Rapid detection of some members of the toxigenic alga *Alexandrium* is of paramount importance in cool temperate waters since these organisms produce a suite of toxins responsible for paralytic shellfish poisoning (PSP). Since *Alexandrium* blooms may not produce cell populations large enough to be visible and these may be below the water surface, early detection can lead to beach closures and fishing restrictions which can prevent human ingestion of contaminated shellfish.

Current identification for filed groups still is based on microscopy, which is time consuming and requires skilled personnel. Our proposed detection mechanism involves functionalizing gold surfaces with peptide nucleic acid (PNA) probes and then sensing binding (hybridization) of target sequences through surface plasmon resonance (SPR).

PNAs, which are DNA analogs with a backbone composed of repeating N-(2-aminoethyl)-glycine units linked by peptide bonds, were selected for the probe layer because of their suitability for field use: PNAs are resistant to cleaving by nucleases and hybridize under a broad variety of conditions. SPR is a good choice for the detection of target hybridization because this technique can accurately measure thickness changes on adsorbed species on surfaces such as gold.

Preliminary experiments using atomic force microscopy (AFM) imaging and ellipsometry show that cysteine-terminated PNA sequences attach to flat gold surfaces through gold-thiol bonds. The PNAs used are biotin-terminated and surface attachment can be verified through biotin quantitation, while hybridization efficacy can be confirmed through binding of complementary labeled DNA.

PIGMENT COMPOSITION OF THE TEXAS STRAIN OF *Prymnesium parvum* DURING LOG, STATIONARY, AND SENESCENT GROWTH PHASES

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[2]Marine Science Program and Department of Biological Sciences, University of South Carolina, Columbia, SC [3]Department of Wildlife and Fisheries Sciences, Department of Oceanography, Texas A&M University, College Station, TX

The harmful algal species, *Prymnesium parvum*, has been identified in fresh, brackish and coastal environments worldwide. In Texas, *P. parvum* blooms have increased in frequency and intensity over the last 7 years. Based on fish kills often associated with *P. parvum* blooms, rapid identification of *P. parvum*'s presence in the water column is necessary for mediation. We employed Chemical Taxonomy (ChemTax) as a rapid, alternative diagnostic tool. Using measured photopigment concentrations, ChemTax can estimate biomasses of bulk or specific phytoplankton taxa; however, ChemTax is sensitive to the initially specified pigment ratios. This sensitivity may prove problematic when different strains of phytoplankton species have different pigment ratios. We performed incubation experiments to determine if the Texas strain of *P. parvum* correlated with pigments ratios identified in a strain of *P. parvum* from Norway (Rodriguez et al. 2006). The Norway strain of *P. parvum* was chosen because of its similar pigment signature to the Texas strain. We determined that Texas *P. parvum* pigment ratios were dynamic as a function of cell density and physiological state. We then used our ratios and the Norwegian ratios for *P. parvum* to estimate *P. parvum* concentrations for in-lake experiments performed in Lake Whitney, Texas. We discovered that the ChemTax model initialized with the Norwegian ratios underestimated *P. parvum*'s contribution to the total chlorophyll *a* signature. In addition, we noted the presences of zeaxanthin within the Texas strain of *P. parvum*. Our results indicate that Texas *P. parvum* may be showing signs of adaptation to the higher light intensity conditions in this semi-tropical habitat.

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FACTORS AFFECTING MICROCYSTIN CONCENTRATIONS AND CELL QUOTAS IN THE GREAT LAKES

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As part of GLERL's new Ocean and Human Health Center, an interdisciplinary research program was initiated to determine the factors controlling microcystin production (cell quota) in the Great Lakes. In Saginaw Bay microcystin concentrations were strongly correlated with correlated with *Microcystis aeruginosa* abundance and total phosphorus concentrations. Microcystin cell quotas averaged 140 fg cell^{-1}, and were not correlated with any environmental factor or growth rates. A series of experiments were conducted with Saginaw Bay and western Lake Erie water to determine the influence of nutrients and light in controlling microcystin cell quota. Significant decreases in cell quota were noted with phosphorus additions and irradiance decreases; however, the decreases were limited (most 2-4X). Environmental factors such as nutrients and light appear to be more important in controlling abundance and growth rate of *Microcystis aeruginosa* than cellular pathways of microcystin synthesis.

CLIMATE INFLUENCES ON HARMFUL ALGAL BLOOMS (HABS) IN SEQUIM BAY, WASHINGTON STATE

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Harmful Algal Blooms (HABs) of *Alexandrium catenella* have been documented in Puget Sound, Washington State, for over 60 years. *A. Catenella* produce a suite of potent neurotoxins, known collectively as Paralytic Shellfish Toxins (PSTs), that accumulate in filter feeding shellfish. Consumption of contaminated shellfish poses a significant threat to human health. Data from the Washington State Department of Health's (WDoH) Biotoxin Program are used to determine variability in PST behavior and determine if local and/or large-scale climate factors contribute to the development of events at Sequim Bay in Puget Sound. This embayment has been identified as a "hot spot" in Puget Sound for toxicity. Correlation analyses of seasonally averaged PST levels in Blue Mussels, *Mytilus edulis*, with local environmental conditions revealed significant relationships with wind speed, stream flow and sea surface temperature (SST). This indicates that low surface wind speeds in the summer and low stream flow and SST in the fall may increase PST risk. A high frequency event scale analysis of PST variability on a daily timescale was conducted for exceptional toxic events in 1992, 1993, 1994, and 2000. This analysis focused on the 14 days prior to the events to determine a combination of favorable conditions that may initiate and sustain high toxicity. Of the preceding favorable environmental conditions, stream flow, wind speed, and air temperature contributed most to the development of toxic events. Thus, a stable water column with warm temperatures and low stream flow conditions may stimulate bloom growth and determine conditions for high toxicity during fall months. We hope to apply these findings to better predict PST risk in Puget Sound.

UNUSUALLY HIGH LEVELS OF DOMOIC ACID IDENTIFIED IN MINKE WHALE (*Balaenoptera acutorostrata*) STRANDING DURING CALIFORNIA *Pseudo-nitzschia* BLOOM

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Blooms of the diatom genus *Pseudo-nitzschia* have been implicated in several large-scale marine animal mortality events along the California coast in recent years, but little toxicological data are available for cetaceans affected by these blooms. The algal toxin domoic acid was detected in several species of cetaceans from an April 2007 marine mammal die-off associated with a Southern California bloom of the diatom *Pseudo-nitzschia*. Domoic acid in minke whale (*Balaenoptera acutorostrata*) feces was detected by liquid chromatography-mass spectrometry (LC-MS) at concentrations of 258 μg/g, exceeding the highest reported values for any marine mammals in this region. Scanning electron microscope analysis of the fecal sample detected a high abundance of frustules of the diatom *Pseudo-nitzschia australis*. The minke whale diet consists mainly of schooling planktivorous fish and krill, organisms that previous research has demonstrated as important vectors of domoic acid to higher trophic levels. These data provide direct evidence for the accumulation of domoic acid in this whale species, at potentially lethal concentrations.

ASSESSMENT OF AN OPERATIONAL HARMFUL ALGAL BLOOM FORECAST SYSTEM FOR THE EASTERN GULF OF MEXICO: A COMPARATIVE ANALYSIS OF SUCCESS AND UTILIZATION THROUGH TWO BLOOM SEASONS

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Blooms of a toxic dinoflagellate, *Karenia brevis*, occur nearly every year on the Gulf coast of Florida with damaging consequences linked to fish kills, marine bird and mammal deaths, human respiratory illness, shellfish bed closures and loss of local tourism. To assist in mitigating local damages resulting from harmful algal blooms (HABs), the Gulf of Mexico HAB Operational Forecast System (GOM HAB-OFS) for HAB detection was transitioned from research to operational status in October 2004 through a multi-office effort of NOAA. Presently in its fourth year of operation, the ecological forecast system issues bulletins twice weekly to coastal resource managers, federal and state agencies, and academic institutions with detailed information concerning the possible presence or confirmation of new harmful blooms, forecasts of spatial bloom extents, movement, intensification conditions, and the daily potential for coastal impacts in the eastern Gulf of Mexico. Since the GOM HAB-OFS' implementation, 7 substantial harmful blooms have been identified and tracked by ecological forecasters in over 250 bulletins utilizing SeaWiFS satellite imagery, past and forecasted winds, a wind transport model and sampling data. The operational system is refining methods for evaluating utilization and skill of the bulletin forecasts based on an assessment of the first two operational years from October 2004 through September 2006. Utilization is determined by the frequency of user response to bulletin information; while skill is evaluated on the accuracy of bloom identification and forecast components. A comparative analysis of bulletin utilization and forecast accuracy during the Forecast System's first two bloom seasons, occurring November 2005 to April 2007, is presented.

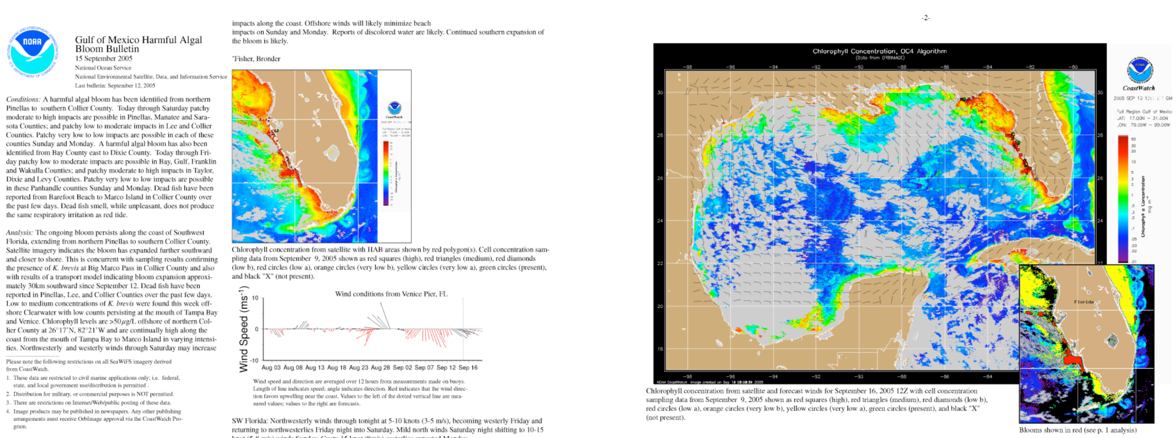

Figure 1. Example Operational Bulletin Distributed by the NOAA Gulf of Mexico HAB Operational Forecast System

DELETERIOUS EFFECTS OF A NON-PSP BIOACTIVE PRODUCED BY *Alexandrium tamarense* ON BIVALVE HEMOCYTES

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The known negative effects of paralytic shellfish toxin (PST)-producing dinoflagellates on feeding, burrowing and survival of some bivalve mollusks has prompted questions concerning whether they might also impair the immune system and thus make the affected bivalves more susceptible to disease agents. The primary components of the cellular defense system are hemocytes, circulating cells similar to mammalian white blood cells. Recent *in vivo* and *in vitro* studies have failed to detect substantial or consistent impacts on hemocyte properties or functions as a consequence of exposure to intact PST-producing dinoflagellates. However, hemocytes would most likely be exposed to intracellular toxins only after the algae are consumed and digested, and the water-soluble toxins released. Therefore, we conducted a series of experiments in which hemocytes from two suspension-feeding bivalves – the Manila clam, *Ruditapes philippinarum*, and the softshell clam, *Mya arenaria* – were exposed *in vitro* to filtered extracts of one highly toxic PST-producing and one non-PST-producing strain of *Alexandrium tamarense* (isolates PR18b, 76 ± 6 STXeq $cell^{-1}$ and CCMP115, containing undetectable PST, respectively). We measured adherence and phagocytosis, two hemocyte attributes previously shown to be compromised by bacterial pathogens and other stressors. The response of hemocytes from individual clams was determined. We found no measurable effect of a cell-free extract from a highly concentrated suspension of the PST-producing strain on hemocytes of either bivalve species. Instead, extract from the non-PST producing strain had a consistent negative effect on hemocytes of the two species, resulting in significantly lower adherence and phagocytosis compared to strain PR18b and filtered seawater controls. The bioactive produced by strain CCMP115, which has yet to be characterized, may be similar to the PST-independent allelopathic compounds described for *Alexandrium* spp., which act on other plankters (Tillmann and John, 2002). These compounds and those produced by other harmful algae are known to cause immobilization, cellular deformation and lysis of co-occurring target organisms, and their cytotoxic effects may be similar to those of ichthyotoxins (Tillmann et al., in press). Thus, non-PST producing *Alexandrium* spp., which do not cause paralysis and burrowing incapacitation of clams (Bricelj et al., 2005), may still produce a bioactive that has negative effects not only on hemocytes, but on other molluscan cell types and their functions, as well.

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TILLMANN, U. and U. JOHN, 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. Marine Ecology Progress Series **230**: 47-58.

TILLMANN, U., JOHN, U., KROCK, B. and A. CEMBELLA. Allelopathic effects of bioactive compounds produced by harmful algae. Proceedings of the 2007 International Harmful Algae Conference, Copenhagen, in press.

COUPLING PHYSIOLOGICAL RESPONSES OF THE TOXIC HAPTOPHYTE *Prymnesium parvum* TO PATTERNS IN GENE EXPRESSION

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A normalized cDNA library[2] from the toxic haptophyte *Prymnesium parvum* was used to design an oligonucleotide-based microarray platform. Allelopathic interactions were investigated between *P. parvum* strain RL10 and the heterotrophic dinoflagellate *Oxyhrris marina*. Cultures of *Prymnesium* were incubated together with both whole cells and cell-free filtrate from *Oxyhrris* cultures. During incubation with *Prymnesium*, the presence of *Oxyhrris* cells, compared to cell-free culture medium, showed no differential effect on the gene expression profile of the haptophyte. A bioassay measuring the toxicity of *Prymnesium* against the sensitive cryptophyte *Rhodomonas baltica* was additionally performed. In this case, *Rhodomonas* viabilities following incubation with *Prymnesium* and *Prymnesium* previously incubated with *O. marina* medium were virtually identical. This suggests no correlation between the presence of *O. marina* medium, and induced toxicity in *P. parvum*. Similar experiments, involving the dinoflagellate *Heterocapsa rotunda* and an unclassified toxic crooccal cyanobacteria strain: NIVA CYA 331, are currently underway. All tentative unique genes (TUGs) identified from the microarray hybridization will be further classified through the use of a *Prymnesium* fosmid library prepared from axenic cultures.

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BIOACCUMULATION OF CYANOBACTERIAL CYLINDROSPERMOPSIS TOXIN IN LOUISIANA BLUE CRAB, *Callinectes sapidus*

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Life history characteristics, specifically a preference for feeding on bottom-dwelling filter-feeding organisms, make the blue crab (*Callinectes sapidus*) vulnerable to the accumulation of toxins. The blue crab commercial fishery of Louisiana is one of the largest crab fisheries in the United States in terms of biomass (Guillory and Roberts 1997). Lac des Allemands, located within the Barataria estuary system of Louisiana, serves as a critical nursery ground for blue crab, but is also an area often abundant in species of *Cylindrospermopsis*. Cylindrospermopsin (CYN), produced by *Cylindrospermopsis raciborskii*, *Umezakia natans*, and *Aphanizomenon ovalisporum*, is an alkaloid hapatotoxin that may also cause damage to the kidneys, thymus, and heart. Previous studies have also shown that CYN can break DNA strands, implicating the compound as a possible carcinogen (Saker et al. 2004). Enzyme-linked immunosorbent assay (ELISA), coupled with epifluorescent and scanning electron microscopy, are used to perform cell counts of *Cylindrospermopsis* species, evaluate their toxicity, and quantify CYN present within tissue samples of blue crab taken from Lac des Allemands. By analyzing the concentrations of CYN specifically within edible portions of blue crab, risk to human consumers is determined.

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Saker, M., J. Metcalf, G. Codd, and V. Vasconcelos. 2004. Accumulation and depuration of the cyanobacterial toxin cylindrospermopsin in the freshwater mussel *Anodonta cygnea*. Toxicon **43:** 185-194.

DOMOIC ACID IN BENTHIC COMMUNITIES OF THE SANTA CRUZ MUNICIPAL WHARF IN MONTEREY BAY CALIFORNIA

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Fish caught from piers are an important and often overlooked part of the recreational fishery. Generally, recreationally caught fish are not subject to the usual state-implemented monitoring programs for phycotoxins. Santa Cruz Municipal Wharf is one such heavily fished pier located in the northern end of Monterey Bay in central California. Some species of fish caught from this pier have been found to possess domoic acid (Fire and Silver 2005), a water soluble phycotoxin locally produced by several species of the diatom *Pseudo-nitzschia*. The diet of fish caught from this nearshore environment varies from species to species and also may differ considerably from that of offshore species. The exact pathway by which pier-caught fish acquire domoic acid (DA) is not well known. This study focuses on the presence of DA in various benthic prey species of fish caught from the wharf. During four separate sampling events, divers collected a variety of benthic invertebrates from the more inshore region of the wharf and another set from the outer, deeper (~6 m) end of the Wharf, including samples taken directly from the pier pilings. The species selected for collection were confirmed prey items, as they had been observed in the stomachs of pier-caught fish (Mazzillo personal communication 2007). Some specimens collected were not confirmed prey items, however, divers collecting the samples had observed fish feeding upon these organsims. Domoic acid levels were measured in the viscera of the invertebrate specimens using both HPLC (High Performance Liquid Chromatography) and ELISA methods, the results of which will be discussed. It is anticipated that the benthic community is an important contributor of DA to the food web, and a few recent studies have shown the presence of DA in several local benthic invertebrates. The extent to which particular benthic organisms acquire domoic acid and also serve as important food sources for commonly caught fish, should be a predictor of contamination of those fish. By understanding the routes by which DA is delivered through the diet of commonly caught pier fish, the regulatory community may better anticipate which fish species will be most contaminated during toxic *Pseudo-nitzschia* events and thereby institute protections for recreational fishermen who consume fish caught from the Wharf.

METABOLISM OF ALGAL STEROLS BY BAY SCALLOPS AND BRINE SHRIMP

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Many HAB species contain sterols with unusual structures. We have hypothesized that these sterols serve as chemical defenses by interfering with the nutrition and growth of marine invertebrates (Giner et al.). There are multiple mechanisms by which such sterols might exert harmful effects. They may be refractory to the normal bioconversion of dietary sterols to cholesterol, leading to a deficiency of the cholesterol needed for cell growth. Alternatively, unusual marine sterols may inhibit enzymes involved in the production of physiologically important steroidal compounds, such as the arthropod molting hormone ecdysterone. To better understand the fate of algal sterols in mollusks and crustaceans, metabolic studies were carried out. Methods for the synthesis of substantial quantities of algal sterols were developed and used to prepare specifically ^{13}C-labeled compounds. These were incorporated into the microalgal diets of juvenile bay scallops (*Argopecten irradiens*) and brine shrimp (*Artemia salina*). Analysis by ^{13}C-NMR spectrometry showed the metabolic fates of the sterols. Addition of a sterol bearing the label in a different position provided a positive internal control in cases where no bioconversion of the test sterol was detected. The brine shrimp were found to be better at metabolizing algal sterols than scallops, and specific sterols were better metabolized than others. The brown tide sterol 24-propylidenecholesterol was metabolized to cholesterol, but dinoflagellate sterols brevesterol and dinosterol were not and accumulated unchanged in the organisms.

Giner, J.-L.; Faraldos, J. A.; Boyer, G. L., "Unique Sterols of the Toxic Dinoflagellate *Karenia brevis* (Dinophyceae): A Defensive Function for Unusual Marine Sterols?" *J. Phycol.* **2003**, *39*, 315-319.

POTENTIAL GOOD IN A "SEA" OF HARM: CHARACTERIZATION OF A MAMMALIAN RECEPTOR ASSOCIATED WITH VOLTAGE-SENSITIVE SODIUM CHANNELS USING BREVENAL PRODUCED BY *Karenia brevis*

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Blooms of the marine dinoflagellate *Karenia brevis* are known to produce brevetoxins, which cause neurotoxic and upper respiratory problems in humans and mammals and are both toxic and lethal to many marine organisms. In contrast, these dinoflagellates also produce an antitoxin, brevenal, shown to be helpful in the treatment of brevetoxin poisoning in manatees and blocks brevetoxin-induced bronchoconstriction in sensitized sheep. Brevenal also protects against the deleterious effects of brevetoxin in fish bioassays. Although the mechanism of action in respiratory systems is poorly understood, it has been established that in mammalian neuronal receptors, brevetoxins act on site 5 of voltage sensitive sodium channels (VSSC) causing four distinct effects: 1) shifting the action potential to more negative values, 2) prolonging channel open time, 3) inhibiting inactivation and 4) inducing subconductance states for Na^+ across the channel. In addition to the brevetoxin receptor, there are five other known receptors located on the alpha subunit of VSSC.

Earlier studies suggested that brevenal is a direct competitor for site 5 using rat brain synaptosomal receptor binding assay (Bouderlais *et al.,* 2004). Our results indicate that brevenal indirectly competes with brevetoxin as the result of an allosteric interaction between a complex of receptors where both brevetoxin and brevenal have unique receptors of their own. Investigation into the brevenal receptor was performed by using a novel radiolabeled ligand, tritiated brevenol (^{3}H-Brevenol)—a reduced product of brevenal. Our receptor binding studies indicate that while brevenal reduces labeled brevetoxin-3 (^{3}H-PbTx-3) associated with receptor site 5, brevetoxin does not reduce specific binding of ^{3}H-Brevenol from its specific binding site. The brevetoxin receptor has an affinity in the range of 1-7 nM while the affinity of brevenal for its receptor is in the 95-100 nM range. Nevertheless, no other VSSC-specific ligand reduces ^{3}H-Brevenol binding. These results suggest the existence of a novel receptor/pharmacore for brevenal/ol. Furthermore, we believe that ^{3}H-brevenol will be an important probe in elucidating the effects of brevenal in several physiological systems. Thus, brevenal is the good in the "sea" of harm caused by the actions of brevetoxins produced *K. brevis*.

Bourdelais, A.J., Campbell, S., Jacocks, H., Naar, J., Wright, J.L.C., Carsi, J., and Baden D.G. (2004). Brevenal is a Natural Inhibitor of Brevetoxin Action in Sodium Channel Receptor Binding Assays. *Cell. Mol. Biol*. 24(4):553-563.

HARMFUL CYANOBACTERIAL BLOOM PROLIFERATION IN FLORIDA BAY, FL, USA: ZOOPLANKTON GRAZING AND THE ROLE OF SALINITY

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Florida Bay has been plagued with a series of ecological disturbances since the late 1980s including the proliferation of harmful cyanobacterial blooms caused by *Synechococcus*. Eutrophication is often suspected to promote algal blooms and nutrients have been a focus of Florida Bay water management and restoration efforts. However, an absence of adequate grazing pressure may also be an important factor in the proliferation of these blooms. Hypersaline conditions are known to occur in Florida Bay and some zooplankton are sensitive to such conditions. Zooplankton grazing can also be disrupted by the biochemical characteristics of cyanobacteria, further promoting blooms. For this study, *in situ* grazing rates of meso- and micro-zooplankton were determined within nine major basins of Florida Bay during all four seasons. We observed that growth rates of the phytoplankton community, up to 1.2 ± 0.1 d^{-1}, typically exceeded microzooplankton grazing rates during algal blooms in the North-Central and Southern basins where blooms are prevalent. At these sites, < 20% of the standing stock was consumed by microzooplankton per day, a percentage below average for estuarine ecosystems. During a July 2006 bloom event, the grazing rate by microzooplankton on the total phytoplankton standing stock was 1.0 ± 0.2 d^{-1} while microzooplankton grazed at a rate of 0.33 ± 0.16 d^{-1} on the *Synechococcus* standing stock. Moreover, increased concentrations of mesozooplankton periodically elicited little or no change in phytoplankton growth and occasionally yielded increases in growth. As such, an absence of adequate grazing pressure may allow *Synechococcus* blooms to occur in Florida Bay. Cell-specific grazing rates by micro- and mesozooplankton and the role of trophic cascades in bloom occurrence will be also presented.

GLYCOLIPID FAMILIES IN PERIDININ-CONTAINING DINOFLAGELLATES

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As the engines of photosynthetic productivity in bloom-forming dinoflagellates, chloroplasts play a crucial role in energy harvesting and conversion. The photosynthetic machinery of dinoflagellates, as well as all other algae and plants, is housed within a matrix thought to be composed primarily of two glycolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). It is only recently, with the advent of analytical techniques such as electrospray ionization-mass spectrometry (ESI-MS) and electrospray ionization-mass spectrometry/mass spectrometry (ESI-MS/MS), that two key questions regarding these lipids can be addressed:

1.) What are the intact forms of MGDG and DGDG present in dinoflagellates, and

2.) How are these forms biosynthesized?

This study addresses the lack of information regarding dinoflagellate glycolipid composition by presenting the results of a full-scan (mass range of 600-1,200 daltons) positive ion ESI-MS survey of the glycolipids of 35 peridinin-containing dinoflagellates, including several harmful species. Further analysis using positive ion ESI-MS/MS was performed to determine the positional distribution of fatty acids associated with MGDG and DGDG.

The dinoflagellates examined were divided into two groups based on the forms of MGDG and DGDG present. The first had lipid masses representing 18:5/18:4 MGDG (with the 18:5 fatty acid attached to the *sn*-1 position of the glycerol and the 18:4 fatty acid attached to the *sn*-2 position), 18:5/18:5 MGDG, 18:5/18:4 DGDG, and 18:5/18:5 DGDG. The second group of dinoflagellates had 20:5/18:4 MGDG, 20:5/18:5 MGDG, 20:5/18:4 DGDG, and 20:5/18:5 DGDG. Of the genera represented by more than one species, 4 genera, including *Amphidinium* and *Coolia*, were present only in the C_{20}/C_{18} glycolipid group, while *Symbiodinium* species were confined to the C_{18}/C_{18} group. *Alexandrium*, *Gymnodinium*, and *Prorocentrum* species were present in both groups. The differentiation between C_{18}/C_{18} and C_{20}/C_{18} glycolipids indicates differing biosynthetic pathways, as well as a possible evolutionary divergence between the two dinoflagellate groups.

DEVELOPMENT AND VALIDATION OF A NOVEL *IN VIVO* NITRATE REDUCTASE ACTIVITY ASSAY

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Differences in nitrogen utilization can significantly impact competitive outcome in phytoplankton communities, leading to blooms of harmful algae. A better understanding of the growth dynamics of HAB species in relation to nutrient input and coastal eutrophication will require a more detailed analysis of their physiological responses to nitrogen source. The enzyme, nitrate reductase, catalyzes the rate-limiting step in nitrate assimilation. Currently there are no methods available to evaluate species-specific nitrate reductase enzyme activities in mixed communities. Bulk assays that determine changes in nitrate reductase activity for the entire community are of little value due to the diverse responses among different algal groups. Here we describe a novel method to evaluate species-specific changes in nitrate reductase activity. This *in vivo* method employs 6-chloro-9-nitro-5-oxo-5*H*- benzo[a]phenoxazine (CNOB) as a fluorescent substrate for nitrate reductase and flow cytometry to evaluate cell specific fluorescence. When excited at 488 nm, the intensity of the emission peak at 583 nm is inversely proportional to the amount of substrate reduced by the enzyme. To validate the proposed method, comparisons to a traditional spectrophotometric nitrate reductase assay were made using a unialgal culture of *Heterosigma akashiwo*. Enzyme activity over time and inhibition studies show similar trends for both methods. With the traditional spectrophotometric activity assay, it was also shown that increasing concentrations of CNOB inhibit the ability of purified nitrate reductase to reduce nitrate to nitrite due to competition for the active site of nitrate reductase. We also confirmed that CNOB is a substrate for purified nitrate reductase using a spectrofluorometer to observe the fluorescence decrease as CNOB is reduced by the enzyme. A similar reduction of CNOB by nitrate reductase in unialgal cultures was also demonstrated. Using a flow cytometer, species-specific enzyme activity within a mixed community was evaluated. The sorting feature of the flow cytometer was used to isolate gated populations of interest in a mixed community based on light-scattering and fluorescence characteristics. DNA extracted from as few as 50 cells collected by the sorting feature was used to identify species within the gated populations by denaturing gradient gel electrophoresis of PCR products. After identification, species-specific reduction of CNOB by nitrate reductase can be calculated from shifts in fluorescence observed in the FL2 channel. In summary, our results suggest that the CNOB method has many advantages over the traditional *in vitro* method for measuring nitrate reductase activity. First, the CNOB method is highly sensitive and requires a minimum amount of culture per analysis. This method also allows *in vivo* investigation of species-specific nitrate reductase activity in mixed populations for the first time compared to the traditional method, which is limited to analyzing only bulk activity. Further molecular identification of populations sorted by flow cytometry enables analysis of mixed communities and environmental samples of unknown composition.

VOLUNTEER-DRIVEN SCIENCE: MAPPING HARMFUL ALGAL BLOOMS IN PUGET SOUND, WASHINGTON

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Puget Sound suffers from annual, costly blooms of *Alexandrium catenella*, which cause paralytic shellfish poisoning (PSP). A primary goal for management in this area is to define PSP dynamics and patterns through increased synoptic sampling and early warning capabilities. The Washington State Department of Health and the Puget Sound Ambient Monitoring Program (PSAMP) uses volunteer samplers to assess the environmental quality of the Sound. Since 1990, volunteer stakeholders and local public health organizations have sampled mussels for PSP analyses every two weeks from 40 sites distributed throughout the Puget Sound. Although PSP monitoring is routine, monitoring for *A. catenella* is not.

As part of an overall goal to interface a proven, high-sensitivity detection method for *A. catenella* into existing PSP monitoring efforts, this network of volunteers have been mobilized for monitoring cell abundances in the Puget Sound. These volunteers were recruited from the Washington State Department of Health, the local tribes and the general community, with the majority working with the State Department of Health in the regular PSP monitoring program. The volunteers were given training on the sampling procedure. In addition to the training, each volunteer was given a sampling pack with all the items necessary to process and archive the samples. Every two weeks, volunteers collected samples for qPCR and for whole cell counts.

The result of this volunteer effort has been tremendous. Almost 500 samples for qPCR and almost 100 samples for whole cell counts were collected by volunteers for the 2006 sampling season. Most of these samples are linked to PSP data also collected by the volunteers. Using a team of volunteers enabled large-scale, repeated, and low-cost qPCR sampling that could not have been achieved otherwise. Moreover, volunteers offer local knowledge, enthusiasm and a sense of ownership and contribution to the area that are a powerful combination in developing a successful monitoring program, reporting on the efficacy of management strategies and raising general awareness of HABs in the environment. In summary, volunteer-driven science partnered with existing monitoring programs enables the collection of a more exhaustive data set needed to support successful management decisions, while also immersing the public in a synergistic view of the scientific process.

DISTRIBUTION OF NUTRIENT DATA IN RELATION TO *Karenia brevis* CELL COUNTS ALONG THE WEST CENTRAL FLORIDA COAST

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Red tides (*Karenia brevis*) are frequent in Gulf of Mexico waters near and around the west central coast of Florida. Routine monitoring of red tide occurrences and nutrient patterns have been ongoing for the past few years (1999 - 2007) and endeavors to capture as many nutrient regimes as possible. With over a thousand sampling periods to date and with red tide present to over three million cells per liter, we have identified ranges of ambient nutrients, both particulate and dissolved and including urea, as a function of counts of *K. brevis*. Data have also been segregated both as to distance from shore and latitudinally to reflect differences in estuarine loadings. Observed concentrations are presented as cumulative distribution functions such that observed concentrations and associated *K. brevis* cell counts can be readily identified and assessed for reasonableness. These can provide useful information for either demonstrating preferred nutrient regimes, draw down of nutrients under bloom progression, the likelihood of terrestrial influences, or setting allowable ranges for ecosystem modeling efforts. Funding support was through the State of Florida and the NOAA EcoHAB:Florida project.

NEW TECHNIQUES FOR NON-LETHAL DNA EXTRACTION FROM, AND PASSIVE INTEGRATED TRANSPONDER (PIT) TAGGING OF, THE SOFT-SHELL CLAM *Mya arenaria*

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Attempts to characterize the population dynamics of the soft-shell clam *Mya arenaria* in relation to paralytic shellfish toxins are complicated by a lack of non-lethal genotyping techniques and reliable tagging methods. An easier and non-lethal technique for clam genotyping is presented here. In addition, I propose a new method for clam tagging, which will increase the consistency of identification when retrieving tagged clams. Both of these techniques will be useful in current and future studies on the effects of red tide toxins on the population genetics of soft-shell clams along the coast of Maine.

A non-lethal method of genotyping, using small amounts of hemolymph, was tested for the first time with *Mya arenaria*. A small syringe was used to extract 200μl of hemolymph from the clams' anterior abductor muscle, which was then applied directly in a polymerase chain reaction (PCR) to successfully amplify a 172 bp DNA fragment for sequencing. Afterwards, all tested clams survived. Using this method, clams can easily be genotyped before placement back in natural conditions for observation in relation to red tide toxins.

PIT tags are a useful way to reliably track individual animals in the field. By inserting PIT tags into soft-shell clams between the mantle and shell, the loss of clam identification could be consistently avoided. This project was designed to determine a method in which PIT tags can be non-lethally inserted, and remain in the clam without rejection. Three groups of clams were acclimatized in a natural sea water flow-through tank. One group acted as a control without tags; while the experimental group had tags inserted. A third group received the same treatment as the tagged group without tag insertion. The clams were then monitored for a number of weeks for death and tag rejection.

GROWTH AND TOXICITY OF *Alexandrium sp.* ON THE NORTH SHORE OF LONG ISLAND, NY, USA: DYNAMICS AND INTERACTIONS WITH NUTRIENTS

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In the spring of 2006 the first case of saxitoxin poisoning of shellfish occurred on Long Island, NY, USA. A bloom in Northport Bay contained *Alexandrium*-like cells and shellfish within this system contained levels of saxitoxin exceeding 80µg STX equiv./100g shellfish tissue causing the New York Department of Environmental Conservation to close the bay to shellfish harvest. Given the recurrent nature of *Alexandrium* blooms, an *Alexandrium* monitoring program was implemented to establish the dynamics and toxicity of *Alexandrium* blooms on Long Island's north shore and to ascertain the role nutrients may play in bloom occurrence. Three estuarine systems were monitored weekly-to-biweekly for densities of *Alexandrium fundyense*, saxitoxin, plankton community biomass and composition, nutrient levels, and standard physical parameters (T, S, light, etc). During May of 2007, an *Alexandrium* bloom associated with elevated levels of saxitoxin in the water column occurred, once again, in Northport Bay. During this bloom, total chlorophyll *a* levels were elevated although concentrations in the >20µm size class were only 3μg L^{-1} (23% of the total chl *a*) suggesting this was a fairly low biomass toxicity event. *Alexandrium* cell densities during the bloom exceeded 1,000 cells ml^{-1}. Elevated saxitoxin levels > 1 ng L^{-1}; determined by an ELISA assay) in the water column were present through the bloom, with levels peaking at 31 ng L^{-1} in late May. During the week following the peak water column saxitoxin concentrations, elevated levels of saxitoxin were also found in shellfish (37µg STX equiv./100g shellfish tissue).

To better understand the role of nutrients in bloom occurrence, nutrient amendment experiments were conducted parallel to the field survey. Replicate bottles filled with water from Northport Bay were amended with environmentally realistic levels of phosphate, nitrate, urea and ammonium. Prior to the occurrence of the *Alexandrium* bloom, the addition of phosphate yielded significantly enhanced growth rates of the total phytoplankton community ($p < 0.07$; Tukey) whereas during the late spring, including the period of the *Alexandrium* bloom, the addition of nitrogen, particularly ammonium, resulted in significantly higher growth rates for the total phytoplankton community ($p < 0.05$; Tukey). This suggests that the overall phytoplankton population was phosphorus limited in the early spring and nitrogen limited in the late spring. During the *Alexandrium* bloom, the addition of ammonium resulted in significantly higher particulate saxitoxin concentrations ($p < 0.05$; Tukey). These results suggest that ammonium may promote the formation of toxic *Alexandrium* blooms. By contrast, phosphorus loading caused a significant decrease in saxitoxin concentrations during experiments ($p < 0.1$; Tukey), suggesting phosphorus may influence bloom toxicity in this system. Data on the densities and growth rates of *Alexandrium fundyense* during bloom events as determine by oligonucleotide probes will also be presented.

LONG-TERM, TEMPORAL VARIABILITY IN *Pseudo-nitzschia* POPULATION DYNAMICS AND DOMOIC ACID TOXICITY IN MONTEREY BAY, CA

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A weekly monitoring program began in May 2003 to assess temporal variability in *Pseudo-nitzschia* population dynamics and domoic acid (DA) toxicity at the Monterey Wharf (MWII; 36.50.993N, 121.49.970W) in Monterey, CA. Water collected from a 5 m vertical net tow (mesh size = 20 μm) was analyzed for phytoplankton community composition, particulate DA, *in vivo* fluorescence, and photosynthetic pigments. Multiple *Pseudo-nitzschia* blooms occurred in 2003, with *P. australis* representing the dominant species and DA concentrations ranged from 0.002 – 2 nM. Beginning in 2004, blooms were less frequent, generally exhibited reduced *Pseudo-nitzschia* abundance and lower DA toxicity (< 0.1 nM). For the next 3 years, the phytoplankton community was mainly dominated by dinoflagellates as evidenced by microscope counts and pigment analysis. Surprisingly, persistent low levels of particulate DA (~0.005 nM) were detected during this 3 year period even when *Pseudo-nitzschia* were not detected by microscope analysis of the samples. In 2007, diatoms reappeared as the dominate phytoplankton group and the largest and most toxic *Pseudo-nitzschia* blooms seen at the MWII occurred in March. To determine if toxic blooms are characterized by distinct species associations within the *Pseudo-nitzschia* community, bulk nucleic acids archived from this time-series were analyzed using a novel species-specific quantitative PCR (QPCR) assay targeting the ITS domain of rDNA. Results from this analysis will be discussed.

EVALUATING GENETIC DIVERSITY OF *Karenia brevis* BLOOMS IN THE WESTERN GULF OF MEXICO

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Karenia brevis is a toxic dinoflagellate in the Gulf of Mexico that blooms almost annually in the eastern Gulf but sporadically in the western Gulf. At present, there is little information about the genetic structure of blooms of *K. brevis* and how different blooms are related, if at all. A single cell, DNA-extraction and PCR-amplification protocol has been developed that permits acquisition of genetic data from historical, Lugol's Iodine-preserved field samples. This protocol allows for testing of various spatial and temporal hypotheses and has been applied to field samples taken over a two-month period from a bloom of *K. brevis* near Corpus Christi, Texas during the fall of 2005. Genotypes at six nuclear-encoded microsatellites obtained from >1000 individual cells revealed extensive genetic diversity. One sample, for example, contained 65 unique genotypes (haplotypes) among 68 cells genotyped. This information will provide a better understanding of the genetic structure and dynamics of a bloom over time while also helping to establish connections, if any, between blooms, potentially allowing the source of an individual bloom to be identified.

Alexandrium catenella CYSTS AND ENVIRONMENTAL CONDITIONS IN PUGET SOUND, WA: RESULTS OF A CYST SURVEY

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Paralytic shellfish poisoning (PSP), caused by *Alexandrium* spp., has been a problem in western Washington marine waters for hundreds, perhaps thousands, of years based on local Indian culture, early explorers, and adaptive responses by native marine mammals, birds, and fish. There have been no recorded human fatalities in Washington since 1942, but even with a comprehensive state monitoring program, human illnesses continue to occur and shellfish harvest closures are now common in all areas except central and southern Hood Canal. To determine the distribution of *Alexandrium* in Puget Sound, we surveyed sediments for benthic cysts in spring 2005 with a more focused study in Quartermaster Harbor and Dyes Inlet in spring 2006. The purpose of the surveys was to determine where cysts are present in surface sediments now and, for some areas, how long the cysts have been present based on cysts in sediment cores dated using ^{210}Pb.

Highest cyst numbers in surface sediments were in Quartermaster Harbor in southern Puget Sound and were present down core in sediment dated to 1955. The second highest cyst numbers were in Sequim Bay in northern Puget Sound and were present to the bottom of a relatively short core dated to 1977, but the first closure there was in 1957. In Penn Cove in northern Puget Sound, cysts were found in core sediment dated to 1957 or at least two decades before a major PSP event in 1978. In Case Inlet in southern Puget Sound, cysts were found in core sediment dated to 1986 or two years before the first harvest closure in that area.

Environmental factors studied when cysts were collected include water properties (temperature, salinity, oxygen, chlorophyll, nutrients), phytoplankton, sediment grain size and organic carbon content (TOC), and metal concentrations. In addition, a 24 hour time series of near bottom current velocity, temperature, salinity, oxygen, chlorophyll and transmissivity and four discrete water samples of suspended sediment and cysts were obtained at key sediment transport locations in Dyes Inlet and Quartermaster Harbor in 2006 to determine if cysts were being transported at maximum ebb and flood tides.

Cysts are transported and deposited as part of the silt-sized fraction of sediment. Thus cyst location may be influenced by sediment transportation and deposition and may also be related to sediment properties such as grain size and TOC content. Grain size of surface and core sediments varied from sand to clay-rich, with coarser sediment generally more common in exposed areas. TOC content averaged 6% by weight with variation between 1-11%. No good correlations have yet been found between either grain size or TOC content with cyst abundance. (See poster by Hubert et al. 2007.)

Sediments in many areas of Puget Sound are contaminated by industrial wastes and runoff leaving non-uniformly distributed metal concentrations that are extremely high in some places. Our results from 2005 show no correlation between total metal concentrations (Cu, Cd, As, and Pb) and cyst abundance. This may be expected from the extremely heterogeneous sampling sites visited that year. However, 2006 results also show poor correlation between metal concentrations and cyst abundance. On the inlet-scale our 2006 results show that metals distributions may be predicted using sediment grain size, suggesting that cyst distribution is dependent at least partially on other factors than sediment transport processes.

Hubert, J., A. Abrahamson, K.S. Davies-Vollum, C.L. Greengrove, and A. Cox. 2007. Do sediment conditions affect the incidence of *Alexandrium catenella* and paralytic shellfish poisoning? A study of sites from Puget Sound. Fourth Symposium on Harmful Algae in the U.S. WHOI, MA.

RELATIONSHIP BETWEEN MICROCYSTIN POTENTIAL AND ENVIRONMENTAL VARIABLES IN LAKES ACROSS NEW YORK STATE

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The concentration of microcystin and presence of microcystin synthetase genes were determined for 62 lake, reservoir and river sites across New York State in 2000. Microcystin occurrence was widespread. Cyanobacterial (CYA, 77%) and *Microcystis* sp. (MIC, 40%) DNA was identified statewide using molecular probes. Three genes targeted within the microcystin synthetase (*mcyA*, *mcyB* and *mcyD*) cluster varied in abundance (*mcyA* and *mcyD,* 42% each; *mcyB*, 26%). *Microcystis* sp. and microcystin potential determined by PCR were highly correlated (>80% similarity). The *mcy* genes were generally found at lake sites with a higher trophic state (oligotrophic – 14%; eutrophic and hypereutrophic – 80%). Principal component analysis indicated *mcy* genes were common in smaller lakes (<20 m depth), at a basic pH (8-9.5), at lower latitude and longitude, and in conjunction with increasing microcystin concentration, algal biomass and total phosphorus (TP). Logistic and linear regression revealed that chlorophyll-a was correlated with the appearance of genes encoding cyanobacteria, *Microcystis* and microcystin, whereas TP, lake depth and latitude were associated with chlorophyll-a production. These results confirm that shallow lakes with increased TP are directly linked to lake productivity, and with higher algal biomass there is a greater potential for microcystin gene presence.

DO SEDIMENT CONDITIONS AFFECT THE INCIDENCE OF *Alexandrium catenella* AND PARALYTIC SHELLFISH POISONING? A STUDY OF SITES FROM PUGET SOUND

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Paralytic shellfish poisoning (PSP) is common in the Puget Sound, resulting in restrictions on shellfish harvesting and health concerns for humans. PSP is caused by *Alexandrium catenella*, a toxic dinoflagellate with cyst and motile phases. The cysts reside in bottom sediment, the motile phase is released after excystment and blooms of the motile phase cause outbreaks of PSP. Although such outbreaks are monitored in Puget Sound by the Washington Department of Health, the factors that control their distribution and intensity are poorly understood. Sedimentological, biological and hydrological factors that might affect the incidence of PSP in Puget Sound were studied. The sedimentological component of the work is considered here. Sediment may affect the incidence of PSP in a number of ways. *Alexandrium* cysts may be transported and deposited with sediment and the location of cysts may be related to sediment properties such as grain size and organic content. Considering cyst abundance and distribution in relation to sediment may help predict areas likely to experience future PSP outbreaks. Surface sediment was collected and box cores 10-40 cm in length were extracted from thirty-two sites throughout Puget Sound. Cysts of *Alexandrium catenella* were found at more than half of the sites. Grain size of bottom sediments at sites varied from sand to clay-rich silt, although most sites were predominantly silty. Total organic carbon (TOC) content averaged 6% by weight varying from 1-11%. No good correlations were observed between either grain size or TOC content with cyst abundance. Sequim Bay, in the north of Puget Sound and Quartermaster Harbor (QMH) in the south had the highest cyst abundances and cysts were present to the bottom of the cores from both of these sites. Quartermaster Harbor and Sequim Bay are in areas protected from strong bottom currents and their sediments are predominantly silty sand. Sequim Bay sediment has above average levels of TOC but QMH sediment has average carbon content. Neither site has unusual sediment characteristics that might account for the high levels of *Alexandrium* cysts observed in their sediments. The lack of correlation between sediment properties and *Alexandrium* cyst abundance indicates that sediment probably does not play an important role in determining the intensity and distribution of *Alexandrium* blooms.

THE VALUE OF HAB PREDICTIONS TO THE COMMERCIAL SHELLFISH INDUSTRIES IN THE GULF OF MAINE

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During the last several decades, harmful algal bloom (HAB) events have been observed in more locations than ever before throughout the United States. Virtually all coastal regions of the United States are now regarded as potentially subject to a wide variety and increased frequency of HABs, and economic impacts can range into the tens of millions for just one event. From a management perspective, it is crucial to begin developing an understanding of the scale of the economic costs to society of HAB events and to determine the extent to which predictive models might be useful in reducing these impacts. Prediction can be based on process-based or empirical models linking the occurrence of HAB events to observable environmental factors. The scale of economic losses and the value of HAB prediction can tell us something about the appropriate scale of public investments in preventing or mitigating the losses. We develop a well-established general formalism for the problem of HAB prediction, and we apply it to commercial shellfishing and growing industries in the Gulf of Maine. We focus on blooms of algae (*Alexandrium spp.*) that produce paralytic shellfish poison (PSP). Blooms of *Alexandrium* frequently result in the closure of productive shellfish beds along the coasts of Maine and Massachusetts, resulting in significant economic impacts. By developing a model for assessing the value of HAB prediction that can be adapted and applied generally, we provide a basis for investment decisions in scientific research and environmental monitoring to support HAB prediction. Using data from commercial shell fishing and growing industries and economic impact estimates for the 2005 HAB event in the Gulf of Maine, we calculate a value of HAB forecasts for the industries in Maine and Massachusetts. Results of our study suggest that the capitalized value of a HAB prediction and tracking system for the Gulf of Maine can range from one to 11 million dollars, depending on the frequency of HAB events, the accuracy of prediction, and the effectiveness of public and private responses.

CHARACTERIZATION OF NITROGEN UPTAKE BY *Heterosigma akashiwo* GROWN IN TURBIDOSTAT CULTURE UNDER TWO LIGHT INTENSITIES

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Raphidophytes were recently identified to contribute significantly to algal blooms and have been implicated in fish kills in the coastal bays of Delaware and Maryland. Ecosystem dynamics facilitating raphidophyte blooms in this system are poorly understood. Nutrient loading, particularly nitrogen in the form of NH_4 and urea, has been increasing for the past decade in these poorly-flushed bays, often reaching levels >5 µM-N. To assess the contribution of these forms of nitrogen to raphidophyte growth, specific uptake rates of NH_4, urea, and NO_3 were determined for the raphidophyte *Heterosigma akashiwo* (CCMP 2393) grown in NO_3-based media in turbidostat culture at 200 and 100 µmol photons m^{-2} s^{-1}. Growth rates were 1.13 d^{-1}. Rates of uptake were determined using ^{15}N-labeled substrates in concentrations bracketing the range observed in Maryland coastal bays. For cells grown at 200 µmol photons m^{-2} s^{-1}, specific uptake rates were highest for NH_4, followed by NO_3, then urea at V_{max}. Rates of V_{max} of NH_4 at 100 and 200 µmol photons m^{-2} s^{-1} were not significantly different and exceeded growth rates by a factor of ~5, suggesting enhanced uptake capability. At 200 µmol photons m^{-2} s^{-1}, the half saturation constant, K_s, was < 1.0 µM-N for urea and NH_4 and was 1.21 µM-N for NO_3. *H akashiwo* often forms blooms in coastal bays during the late summer after ambient nitrogen concentrations have been depleted. *H. akashiwo* thus displays a higher affinity for NH_4 and urea than NO_3 at ambient concentrations typical of late summer in these bays.

IN SITU NUTRIENT MONITORING: AN EXAMPLE OF RESEARCH, DEVELOPMENT, DEMONSTRATION AND TECHNOLOGY TRANSFER

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Technologies for *in situ* nutrient monitoring have advanced considerably in the past several years. These advancements have included the optimization of chemistries for inorganic nutrients (PO_4^{3-}, NH_4^+ and NO_3^- +NO_2^-), the development of a method for urea analysis, adaptive sampling for water sampled triggered by pre-set levels of nutrients, and improved data transmission for real time data access. We have been using WS EnviroTech (formerly W.S. Ocean Systems) automated nutrient analyzers in the tributaries and embayments of the Chesapeake and Coastal Bays to obtain time series of PO_4^{3-}, NH_4^+ and NO_3^- +NO_2^- and urea during periods when HAB outbreaks routinely occur. Common features in the data included highly varying concentrations on the scale of days and significant increases in PO_4^{3-}, NH_4^+ and NO_3^- +NO_2^- following rain events. However, PO_4^{3-} increases were contemporaneous with rain events and ephemeral; NH_4^+ increases were variable and site-specific, but generally contemporaneous with rainfall or delayed for up to a day, while the maximum NO_3^- +NO_2^- responses lagged reactive PO_4^{3-} by a period of several days and generally lasted for several days. The increases for all nutrients tended to be many fold, up to an order of magnitude, higher than pre- rain fall concentrations and would generally be missed by traditional sampling. The varying time scale of these fluxes also yielded highly dynamic nitrogen:phosphorus ratios. Algal responses tended to follow the increases in nitrogen underscoring nitrogen limitation in these systems even when ambient concentrations are not depleted. These innovative monitoring technologies are now being adopted for application by the states of Maryland and Florida.

OCEAN OBSERVING SYSTEMS AND PUBLIC HEALTH: THE FLORIDA BEACH CONDITIONS REPORTING SYSTEM TO MINIMIZE EXPOSURE TO *Karenia brevis* AEROSOLS

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With over 50% of the US population living in a coastal county, the impacts the ocean and coastal environment have on people is substantial. These impacts are both positive, such as tourism and recreation opportunities, as well as negative impacts such as exposure to harmful algal blooms (HABs) and pathogens.

Florida's west coast is an example of a coastal community needing public health information from a beach observing system. The west coast experiences annual blooms of the toxic dinoflagellate, *Karenia brevis*. *K. brevis* produces a potent suite of neurotoxins, brevetoxins. Wind and wave action causes the toxins to become part of the marine aerosol. This toxic aerosol causes respiratory irritation as people inhale. Asthmatics who inhale the toxins report increase upper and lower airway symptoms and have measurable changes on pulmonary function. Real time beach reporting for these toxic aerosols may improve asthmatics and local coastal residents' quality of life

An Integrated Ocean Observing System (IOOS) Public Health Pilot Study in HABs has been designed and implemented in Sarasota and Manatee Counties, Florida. This system is based on condition reports from lifeguards at the 8 public beaches. The lifeguards staff these beaches year round, 7 days a week making them ideal sentinels. The lifeguards provide subjective reports of the amount of dead fish on the beach, the level of respiratory irritation, water color, wind direction, surf condition, and the beach warning flag they are currently flying.

A key component in the design of the observing system was to create an easy reporting pathway for the lifeguards to minimize the amount of time away from their primary duties. The system provides a PDA for each of the 8 beaches. The portable unit allows the lifeguards to report from their guard tower. If conditions at the beach change, the lifeguard can input the data easily on site.

The data is transferred via wireless Internet to a website hosted on the Mote Marine Laboratory Sarasota Operations of the Coastal Ocean Observation Laboratories (SO COOL) server. The user can select the beach of interest and a pop up window provides the most recent report for that beach with a date and time stamp.

The system has proven to be robust and well received by the public. The system has reported variability from beach to beach and has provided vital information to users to minimize their exposure to toxic marine aerosols.

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

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A mesocosm study along the shoreline of Salt Pond (Eastham, MA) was conducted in the spring of 2005 to examine the influence of nutrient composition on toxin composition and content in *Alexandrium tamarense*. In this study, as well as in one conducted in 2003, natural pond water assemblages containing *A. tamarense* along with a clonal culture of the same organism were independently incubated under nutrient replete and nutrient limited conditions in 230-liter tanks for a period of about 30 days. Approximately, on a bi-daily basis, samples for chlorophyll content, cell density, cell volume and life cycle status, cellular nutrient status, saxitoxin content and composition, dissolved and particulate nutrients were collected. In the 2003 study, there was a distinct difference in the cellular toxin composition patterns witnessed in the natural versus the cultured populations in the tanks: the cultured cells had significant shifts in their toxin composition during the course of the experiment while the natural populations did not. Both populations exhibited an increase in toxin content under phosphate stress, and a decrease in toxin content under nitrogen stress as compared to the nutrient-replete controls. These increases and decreases in toxin content due to phosphorus and nitrogen limitation respectively appear to be a typical stress-related response within *Alexandrium* saxitoxin-producing species. However, it is not clear why cellular toxin composition changes did, and did not, occur within the different tank populations of *Alexandrium*. Possible hypotheses for this contrast in toxin compositional change include the effect of life cycle transformations such as those found when sexually compatible cells types co-occur (natural assemblages) or perhaps there is a strain or clone-specific response within *Alexandrium*. Data collected during the 2005 study will be compared and contrasted to those collected in 2003 as well as to those from laboratory studies.

SEDIMENTS AND CYSTS ON THE FLOOR OF HARPSWELL SOUND: IS THERE A LOCAL CYST BED FOR THE HIGH TOXICITIES IN LUMBOS HOLE?

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The Maine Department of Marine Resources PSP monitoring program relies on the Lombos Hole location in Harpswell Sound as an indicator site that signals the imminent onset of HABs in the western Gulf of Maine. One model for this behavior is that Harpswell Sound maintains a local cyst bed of *Alexandrium fundyense* which responds to local conditions with intense blooms. A multibeam backscatter map of the deeper portions of Harpswell Sound indicates that the sound is divided into a northern province of high backscatter and a southern province of low backscatter. Grab samples clearly show that the high backscatter province is floored by sandy gravels and gravelly sands and that the southern province is underlain by muds. Counts of viable cysts in surface sediments show that the gravelly sediments have counts 35% of those in the muddy sediments. While sediment cyst densities are generally higher nearer the mouth of Harpswell Sound in the muddy sediments, chlorophyll levels as measured by an oceanographic monitoring buoy are routinely higher nearer the head of the Sound. Reverse estuarine circulation in Harpswell Sound may promote transport of excysted cells towards the head of the Sound (see Teegarden *et al*. this conference). The presence of cysts in sediments does not necessarily indicate deposition from the immediately overlying waters, as cysts formed near the head of the Sound are in all likelihood transported by near bottom currents to the muddy regions with lower near-bottom current velocities. Our data indicate that comprehensive sediment sampling on fine scales can reveal a complex pattern of cyst distribution, which in conjunction with sonar backscatter and grain analysis data can suggest a process of cyst formation, deposition and transport. Comprehensive sampling can also reveal previously unrecognized "hot spots" of potential *Alexandrium* cyst germination, which in combination with characterization of environmental potential for growth and physical circulation data may provide insight into the spatially and temporally variable nature of *Alexandrium* bloom initiation in the western Gulf of Maine.

THE EMERGING RISKS OF CYANOBACTERIA FOR FISH AND WILDLIFE IN FLORIDA

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Florida's Harmful Algal Bloom Task Force (FHABTF) was established in 1997 and mandated by state legislation in 1999. The FHABTF identified cyanobacteria blooms in fresh, brackish and marine waters as emerging harmful algal bloom (HAB) problems in Florida. For cyanobacteria, six priorities were identified: 1) determine the distribution of toxic and nontoxic strains, 2) develop epidemiological studies to determine public health risks, 3) develop economic impact studies to evaluate losses by location or industry, 4) determine the roles of nutrient enrichment and managed freshwater flow in blooms, 5) determine fate and effects of toxins in the food web, and 6) investigate control and mitigation methods. As part of the FWC's mandate to protect fish and wildlife, we have begun to address some of these priority areas. Cyanobacteria (or blue-green algae) are ubiquitous in Florida's freshwater, brackish, and marine habitats. Major bloom forming species include *Lyngbya majuscula*, *L. wollei*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *Anabaena circinalis*, *Aphanizomenon flos-aquae*, *Trichodesmium erythraeum*, and *Synechococcus* sp. (Steidinger et al. 1999; Williams et al. 2001). In 1999, a major alligator (*Alligator mississipiensis*) die-off in Lake Griffin was suspected, but unproven, to be associated with *Cylindrospermopsis* blooms (Richey et al. 2001). Saxitoxins have been detected at low concentrations in blue crabs (*Callinectes sapidus*) surveyed from freshwater and low salinity areas with chronic cyanobacterial blooms. A recent *Cylindrospermopsis* bloom and co-occurring mallard duck (*Anas platyrhynchos* and mallard hybrids) die-off has lead to an intense investigation for a potential multi-factorial association of etiological factors including cyanotoxins and botulism. An ongoing study funded by CDC/DOH to assess levels of microcystins in four species of freshwater fish from four lakes has confirmed microcystins in the livers of gizzard shad (*Dorosoma cepedianum*) and bluegill (*Lepomis macrochirus*). Surveys for the epiphytic cyanobacterium (family Stigonematales) (Williams et al. 2007) primarily responsible for Avian Vacuolar Myelinopathy have confirmed by PCR several positive substrates for the first time in Florida (J. Williams et al. in prep.). Response efforts and management plans need continuous reappraisal to address the changing scope and impacts associated with cyanoHABs in Florida's waters.

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DEGRADATION OF DOMOIC ACID UNDER COMMON STORAGE CONDITIONS

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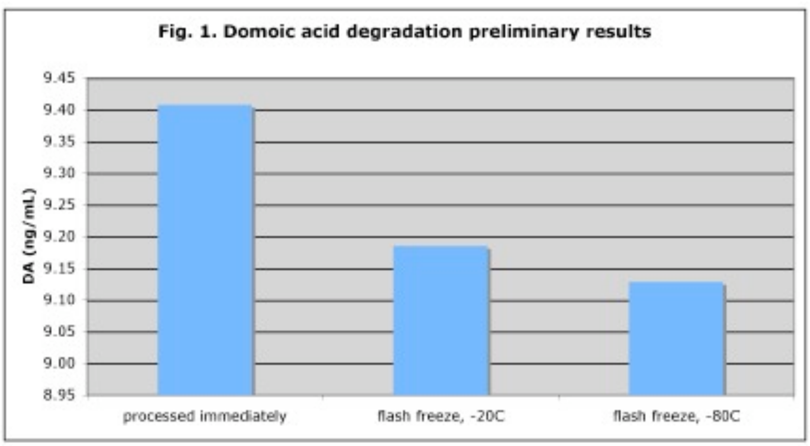
Domoic acid (DA), a potent neurotoxin, is produced by various species of the diatom *Pseudo-nitzschia*. The study of *Pseudo-nitzschia* physiology and toxin production in both the field and in the laboratory commonly involves the collection and (preferably) short-term storage of filtered phytoplankton samples. Because it is generally not practical to process individual phytoplankton samples for DA immediately, the effects of storage on the DA content of the samples becomes an important question. The majority of literature addressing domoic acid degradation has focused on shellfish samples[1,2]. Degradation of DA in filtered phytoplankton samples has been addressed to a lesser extent, predominantly in the context of photodegradation[3,4]. One exception is a study on DA stability in field samples collected during a *Pseudo-nitzschia* bloom[5]. In this study, particulate DA (DAP) samples were sub-sampled, stored under various conditions (room temperature, -20°C, and 4°C), and measured over the course of 6 months. While this study offered recommendations to improve current DAP sample storage methods, it incited additional questions and concern regarding DA stability in DAP samples. Preliminary work for the study presented here strongly suggests that: 1) there is immediate DA degradation taking place in DAP samples, and 2) the rate of DA degradation varies among storage treatments (Figure 1). We will address some of the still outstanding questions surrounding DA degradation through a study of DA degradation in DAP samples recovered from a single toxic uniclonal culture of *P. multiseries*. Recovered DAP samples were stored under a variety of conditions commonly implemented for DAP sample storage (liquid nitrogen [-198°C], -80°C, -20°C, 4°C). To assess relative degradation rates between treatments, filters from each treatment were measured for DA in triplicate roughly every 2 weeks over a 3-month period. More frequent measurements were taken in the first week of storage, since there is some evidence that the most rapid degradation takes place immediately following initial storage.



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COASTAL HAB VOLUNTEER PROGRAMS: STRENGTHS AND LIMITATIONS

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Coastal harmful algal blooms (HABs) and biotoxin monitoring programs have traditionally relied on either the early detection of toxins in seafood as a means of providing public health protection, or, as in the case of Florida, on the presence of the HAB species above 5000 cells per liter inshore at passes. With the discovery of additional toxins and additional species in many regions, new and better tools for early detection of these events became a priority. Monitoring of phytoplankton assemblages was an obvious candidate for improving early warning capabilities, however most state monitoring programs have lacked the financial and institutional resources to build a new government-sponsored monitoring program. Repetitive phytoplankton monitoring, using volunteers, was pioneered by the U.S. Food and Drug Administration as it assisted west coast states in their response to the advent of domoic acid. Over the past 15 years there have been volunteer-based phytoplankton monitoring programs established in several states (e.g., California, Maine, and Florida) in an attempt to detect the onset of HABs. Volunteer HAB programs that collect and ship water samples to a central laboratory for HAB species identification and data management can be advantageous depending on the methods and expertise employed. Some volunteers can be trained to provide accurate field identifications of many toxic species, at least to genus level, particularly for *Pyrodinium, Pseudo-nitzschia, Alexandrium,* and other genera. Some problematic genera are *Karenia, Takayama,* and *Karlodinium*. Within many genera, species identification can be difficult unless the genus is monotypic. The most obvious advantage is wide geographic coverage and high sampling frequency at low cost, from nearshore to offshore, depending on the availability of volunteers and the protocol used. In California and Florida (two states with long coastlines), offshore monitoring can give a forewarning of nearshore events, if sampling and analyses are timely. Volunteer sampling can be done by recreational boaters, recreational and commercial fishermen, ships on regular cruise tracks, bridge tenders, park rangers, existing water or other monitoring programs (government, educational, nonprofit), and citizen volunteers. Volunteer programs function best with a designated point person who acts as the trainer, coordinator and backup sample processor and require an equipment budget (e.g., for water sampling devices or plankton nets, shipping bottles and fixative, even field microscopes), and some infrastructure (prepaid shipping, sample receiving, and someone at the laboratory end who can identify HAB species from the region and enter the data into a spreadsheet or database). Each program should define the data quality objectives (e.g., quantitative or qualitative sample volumes and observations) that best suit their needs and should adopt standard operating procedures. The usefulness of data generated by volunteer samplers and field observers is dependent on the quality of that information, so oversight and quality control is important. Depending on the HAB species of concern, different fixatives may be employed and different sampling depths may be needed, e.g., surface and bottom, or perhaps an integrated water sample inshore. The limit of species detection will depend on the volume sampled and counted. All volunteer monitoring programs have their limitations, e.g., the need for retraining of infrequent samplers and recruitment and training of new volunteers to replace those that drop out. Turnover of volunteers and equipment problems (lost or damaged equipment, malfunctioning boats) can result in periodic inconsistency in sampling effort and field identifications. A secondary disadvantage can be the time between collection and the time of sample observation in the laboratory and subsequent data

entry. Volunteer monitoring programs have thrived despite the limitations of such ventures, and this is no doubt due to the commitment of the participants, the program coordinators, and those in the agencies and scientific community that contribute their expertise and encouragement.

ADVANCED TREATMENT PROCESSES FOR THE REMOVAL OF CYANOTOXINS FROM LAKE ERIE DRINKING WATER

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The presence of cyanobacteria (blue-green algae) and associated cyanotoxins in surface water is of increasing concern in the United States as well as other parts of the world. In the Great Lakes region, blooms of toxic *Microcystis* have occurred in recent years on Lake Erie, which serves as a source of drinking water for over 11 million people (Ouellette, et al., 2006). Cyanobacteria naturally produce deleterious compounds due to cell lysis, which may cause health problems for animals and humans. Microcystins are the most frequently occurring class of cyanotoxins, of which microcystin-LR is the most toxic and frequently detected congener (Antoniou, et al., 2005). Due to adverse health effects, the World Health Organization (WHO) established a provisional concentration limit of 1 μg/L for microcystin-LR in drinking water and the United States Environmental Protection Agency (USEPA) has placed microcystins on the Drinking Water Contaminant Candidate List. Many approaches such as coagulation, chlorination, activated carbon adsorption, and ozonation have been investigated for the removal of microcystins from drinking water, but typically are not effective to meet the WHO guideline (Lawton and Robertson, 1999). Low-pressure membrane processes are of increasing interest to remove organic contaminants from drinking water. In particular, coupling powdered activated carbon to ultrafiltration (PAC-UF) is an emerging technology for the treatment of organic micropollutants in drinking water. In this study, we investigate the removal of microcystin-LR from drinking water using a membrane filtration only and in combination with powdered activated carbon. Process variables examined included PAC type, PAC dosage, membrane characteristics, microcystin concentrations, operating conditions, and the presence of natural organic matter (NOM). Of five different UF membranes, polysulfone (PS) membranes with highest hydrophobicity most significantly adsorbed microcystin-LR (~91%) presumably through hydrophobic interactions whereas hydrophilic cellulose acetate (CA) membranes did not adsorb the toxin. Membranes with a molecular weight cutoff (MWCO) of less than 5,000 Da rejected microcystin-LR through a size exclusion mechanism. Thin-film (TF) membranes with a MWCO of 1,000 Da, which is close to the molecular size of microcystin-LR, adsorbed 70% and also rejected 70% of the toxin. Initial concentrations of microcystin-LR had a significant effect on the degree of the toxin adsorption. Adsorbed amount of microcystins on the membrane surface linearly increased with increasing initial concentration. Operating conditions such as pressure, permeate flux, and water recovery did not affect the adsorption and rejection performance. Of two different PAC materials, wood-based activated carbon was up to 4-times more effective at removing microcystin-LR than coconut-based carbon due to greater mesopore volume. When PAC was coupled to UF using polyethersulfone (PES) membranes, greater removal of microcystin-LR occurred compared to when CA membranes were used due to sorption of the toxin to the PES membrane surface. The presence of Suwannee River Fulvic Acid (SRFA) reduced microcystin-LR removal by PAC-UF, primarily due to competition between SRFA and microcystin-LR for sites on the PAC surface.

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THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE SEASONAL VARIATION OF *Microcystis aeruginosa* CELL DENSITY AND MICROCYSTINS CONCENTRATION IN SAN FRANCISCO ESTUARY

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A bloom of the cyanobacteria *Microcystis aeruginosa* was sampled over the summer and fall in order to determine if the spatial and temporal patterns in cell density, chlorophyll *a* concentration, total microcystins concentration and percent microcystins composition varied with environmental conditions in San Francisco Estuary. It was hypothesized that the seasonal variation in *Microcystis* cell density and microcystin concentration was ecologically important because it could influence the transfer of toxic microcystins into the aquatic food web. Sampling of *Microcystis* cell density, chlorophyll *a* concentration, total microcystins concentration and a suite of environmental conditions was conducted biweekly at nine stations throughout the freshwater tidal and brackish water regions of the estuary between July and November 2004. Total microcystins in zooplankton and clam tissue was also sampled in August and October. *Microcystis* cell density, chlorophyll *a* concentration and total microcystins concentration varied by an order of magnitude and peaked during August and/or September among rivers when $P^B{}_m$ and α^B were high. Canonical correlation analysis identified low streamflow and high water temperature as environmental factors strongly correlated with the seasonal change in *Microcystis* cell density, total microcystins concentration $(\text{cell})^{-1}$ and total microcystins concentration $(\text{chl } a)^{-1}$. Nutrient concentration and nutrient ratios were of secondary importance in the analysis and may be of lesser importance to seasonal variation of the bloom in this nutrient rich estuary. The seasonal variation of *Microcystis* density and biomass and its variation with environmental conditions were potentially important for the structure and function of the estuarine aquatic food web because total microcystins concentration was high at the base of the food web in zooplankton, amphipod, clam and worm tissue during the peak of the bloom.

INFLUENCE OF TEMPERATURE, SALINITY AND NUTRIENT RATIOS ON TOXIN PROFILES OF *Karenia brevis*

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The toxic dinoflagellate *Karenia brevis* forms extensive, annual blooms in the Gulf of Mexico releasing potent neurotoxins having significant impacts on human health, mortalities of marine mammals, birds and fish. This study focuses on the factors affecting cellular virulence. Effects of environmental factors such as temperature (20-30ºC), salinity (20-39) and differing nutrient environments expressed as N:P ratios of 16:1 (balanced), 4:1 (nitrogen limited) and 80:1 (phosphorus limited) were explored with three clones of *Karenia*. The Wilson clone, historically the source of brevetoxin (PbTx) standards for more than 30 years, was used along with the SP3 Super-tox and SP3 Non-tox. Growth as measured by *in vivo* fluorescence was used to assess the effects of temperature and salinity. The SP3 Super-tox and SP3 Non-tox have greater growth rates at a temperature of 20ºC and balanced nutrient conditions than at temperatures of 25 or 30ºC. In contrast, the Wilson clone has the greatest growth at 25ºC and balanced nutrient conditions than at temperatures of 20 or 30ºC. Growth rates for the SP3 Super-tox clone at 20ºC ranged from 0.14 to 0.33 div/day. While those for the Non-tox clone at 20ºC varied from 0.11 to 0.16 div/day. Growth for the Wilson clone at 25ºC ranged from 0.26 to 0.36 div/day. Trends for all clones at 30ºC were similar to growth at 25ºC. At a salinity of 20, none of the clones in any of the temperature/nutrient treatments grew. At a salinity of 25, growth was variable for each clone and temperatures. Good/strong growth occurred at salinities of 30, 35 and 40, with the best growth at salinity of 35. From these results the optimum temperature/salinity environment for growth was determined. Using optimal salinity and temperature conditions, cultures of each clone of *K. brevis* were grown and examined for brevetoxin profiles including PbTx-2, 3, 6, 9 and brevenal, using LC-Mass Spectrometry. The balanced nutrient profile (16:1) contained PbTx-2, 3, 6, 9 and brevenal. The average cellular quota for the *K. brevis* clones was ~15 pg-toxin/cell. A comparison of brevetoxin profiles for SP3 Super-tox and SP3 Non-tox showed both to have significant toxin content, with the same PbTx's present as those for the Wilson clone; however, Non-tox had a notable increase in brevenal. Nutrient stressed experiments (N:P = 4:1 and 80:1) for toxin profiles are presently underway and the effects of the nutrient ratio experiments are yet to be determined. Once completed, these studies will allow a matrix analysis of temperature/salinity and nutrients in determining the cellular production of the various brevetoxins and brevenal. This information will be relevant in evaluating the virulence of strains of *Karenia* and potentially predicting effects of blooms.

EVALUATION OF CYSTEINE EFFICIENCY AS A MITIGATING AGENT OF SHELLFISH CONTAMINATION DURING RED TIDES

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Karenia brevis is a toxic dinoflagellate that frequently blooms along the west coast of Florida. These blooms affect fish, marine mammals and birds, causing large epizootic events. Blooms affect humans by direct exposure to toxic aerosols and by ingestion of contaminated shellfish, which causes neurotoxic shellfish poisoning. During blooms, shellfish beds are closed according to cell counts of the water. They are reopened when shellfish taken from a closed area have depurated and test safe for human consumption. Because shellfish accumulate brevetoxins in their tissue, bed closures are often lengthy and very detrimental to the seafood industry. We recently determined that both pre-treatment and treatment of shellfish with Cysteine and Cysteine congeners during blooms and in laboratory settings reduces the contamination significantly. The objective of this study was to establish what cysteine concentration dissolved in water is necessary to mitigate shellfish contamination with brevetoxins. Shellfish, both clams and oysters, were acquired from a local NC fishery (J&B Aquafoods, Holly Ridge, NC) and exposed to various levels of *K. brevis* in culture while being treated with gradually decreasing concentrations of Cysteine. The results were compared to controls in absence of cysteine treatment and demonstrate that fairly low concentrations of cysteine can reduce significantly shellfish contamination (50 to 95 % according to the treatment used). These results offer new prospects for mitigating the effects of red tides on fisheries.

DEVELOPMENT AND INITIATION OF HABISS; CDC's MULTI-STATE HAB SURVEILLANCE SYSTEM

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The extent of human illness caused by environmental exposure to algal toxins in drinking and recreational waters is unknown. There are guidelines from the World Health Organization, Canada, Brazil, and Australia that public health agencies can follow to make decisions about allowing access to drinking water sources and recreational areas with ongoing HABs. However, there are no U.S. federal regulations, and no official guidance from the U.S. Environmental Protection Agency specifying allowable concentrations of HAB-related toxins in the water. In response to the need to support public health decision-making, NCEH has developed a Harmful Algal Bloom-related Illness Surveillance System (HABISS). HABISS is a unique surveillance system that includes the collection of not only human health data, but data from animals made ill by exposure to HABs and environmental data about the HABs themselves. Data collection is organized in modular format that can easily be adapted to state and local needs. State health agencies are particularly interested in using this database to predict future blooms, thus allowing state public health prevention activities to be in place not only in response to reports of human or animal illnesses, but also in advance of anticipated public health problems.

NCEH is currently collecting environmental data from blooms in each state in order to help facilitate future HAB-related illness prevention and control strategies. In 2005, NCEH began beta-testing HABISS in five states supported by the Pfiesteria cooperative agreement, including Florida, Maryland, North Carolina, South Carolina, and Virginia. With the help and recommendations by these state partners, NCEH is integrating additional data elements into HABISS, such as mapping, modeling, and real-time notification capabilities. In addition to working with State Health Agencies, we are expanding interaction with other public health partners. For example, we are working with the American Association of Poison Control Centers to develop new case definitions for HAB toxin-related diseases that will be included in both HABISS and the national Poisindex database. These new case definitions should allow us to capture cases in real-time, thereby improving emergency response to human illness.

Future HABISS Activities

HABISS continues to evolve, and several new initiatives are already underway.

- Linking reported weather conditions and meteorological factors to HAB outbreaks
- Collaborating with investigators at the National Oceanic and Atmospheric Administration (NOSS) and National Centers for Environmental Prediction (NCEP) to add a simplified prediction component to HABISS
- Collaborating with the Olympic Harmful Algal Bloom Program (ORHAB) to help build a web-based bulletin for early warning of Washington coast HAB events
- Collecting data on ocean-related diseases in animals and people to ensure that data is collected in a concurrent way that it can be linked to and overlaid with data from other systems
- Providing scientific data from HABISS to aid the discussion of HABs and global climate change (e.g., on the effects of increasing ambient air temperature, changing wind patterns, and variable tidal patterns on HABs and human health)

- Expanding the HABISS network to include international partners and participants

INTEGRATING NOVEL DATA SOURCES TO IMPROVE THE GULF OF MEXICO HARMFUL ALGAL BLOOM FORECAST SYSTEM

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Blooms of the dinoflagellate, *Karenia brevis,* are responsible for serious impacts to the ecological, economic, and public health of the Gulf of Mexico. Since 2004, the Harmful Algal Bloom Forecast System has been providing advance warning of *K. brevis* blooms on an operational basis. Traditional techniques such as *in situ* sampling and satellite imagery are used to monitor the spatial extent and movement of the blooms. New methods and technologies are being used to improve the detection and project the impacts of harmful algal blooms (HAB), such as optical data taken from autonomous underwater vehicle (AUV) gliders and moorings and observations of respiratory irritation. AUV gliders are becoming increasingly important observation platforms for investigating HAB initiation sites because of their unique capability for adaptive sampling. The AUVs and several moored sites are equipped with the BreveBuster optical instrument. Developed by Mote Marine Laboratory, the BreveBuster is capable of identifying *K. brevis* blooms by the absorbance signal and provides a similarity index (SI) value that represents the fraction of *K. brevis* biomass in the phytoplankton community. Data from the AUV gliders are sent via satellite to a computer in near real time and are posted to the Internet. Respiratory irritation information collected by volunteers and lifeguards is used to assess health impacts at local beaches. Analysts with the Harmful Algal Bloom Forecast System, a collaborative effort among state and local managers, research scientists, and various offices within the National Oceanic and Atmospheric Administration, use the AUV data to locate subsurface blooms and the respiratory data to communicate potential health impacts to the general public. Open source mapping options such as Google Maps are being considered for displaying these diverse data sets within a system that integrates satellite imagery, field observations, and model output to produce an operational bulletin twice weekly for coastal managers.

PATHOGENICITY, PREY AVAILABILITY AND FUNCTIONAL TYPES IN *Pfiesteria* AND *Pfiesteria*-LIKE SPECIES: THE ROLE OF MICROPREDATORY FEEDING

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Heterotrophic dinoflagellates of the genus *Pfiesteria* are purported to produce a potent ichthyotoxin that stuns fish and causes sloughing of epidermal tissues, which the dinoflagellates subsequently consume. A central notion surrounding *Pfiesteria* toxicity is the existence of distinct "functional types" (e.g., Tox-A, Tox-B, Non-Ind) demonstrating varying degrees of toxicity based primarily on culture age and the type of available prey. It is purported that toxic cultures must be maintained on live fish to stimulate and maintain toxicity, which is lost with extended time in the absence of live fish. Our previous work has demonstrated that, in laboratory assays, these dinoflagellates feed directly on fish epidermis by myzocytosis and, given sufficient time and cell density, this results in fish mortality at rates equivalent to those reported for purportedly "toxic" *Pfiesteria* strains. This direct feeding on fish by myzocytosis suggested an alternative mechanism of pathogenicity in *Pseudopfiesteria shumwayae* that accounted for fish mortalities in laboratory assays without involvement of a toxin. The objectives of this study were to determine if maintenance on fish prey increases pathogenicity in a micropredatory *Pfiesteria* culture (relative to maintenance on algal prey) and to determine the mechanisms that account for any perceived increase in pathogenicity.

We conducted a comparative larval fish bioassay using sub-cultures of a pathogenic *P. shumwayae* isolate (CCMP 2089), previously maintained on either juvenile tilapia (*Oreochromis niloticus*) or a cryptophyte algae (*Rhodomonas salinas* CCMP 1319). Fish mortality was significantly more rapid in the fish-exposed culture (100% mortality in fish-exposed vs 50% mortality in algal-exposed at 72 hrs). However, it was also observed that flagellated-cell density increased more rapidly in the fish-exposed culture [1235 cells/ml (S.E. = 83 cells/ml) in fish-exposed vs 665 cells/ml (S.E. = 36 cells/ml) in algal-exposed at 72 hr], possibly accounting for the increased mortality rate. Subsequent studies examined the response of several pathogenic and non-pathogenic strains of *Pfiesteria* and *Pfiesteria*-like dinoflagellates to fish to determine if the observed increase in cell density in the first study was due to prior exposure to fish prey. Algal-fed strains of *P. shumwayae* and *Cryptoperidiniopsis brodyi* (demonstrated to be pathogenic to fish in previous larval fish bioassays) exhibited a significant increase in mean cell volume when exposed to live fish vs the same strains exposed to cryptophytes only. Strains of *P. piscicida* and *Luciella masanensis* (demonstrated non-pathogenic to fish), did not show an increase in mean cell volume when exposed to fish relative to algal-fed cultures of the same strains. Additionally, morphometric studies were conducted on a pathogenic *P. shumwayae* (CCMP 2089) and a non-pathogenic *P. piscicida* (CCMP 2091) given access to fish tissue for a limited period (8 hr). Strain CCMP 2089 showed a large (3x) increase in flagellated cell volume at 12 hr followed by a decrease in cell density and an increase in cyst density (including doublets and tetrads) at 24 hrs, with a decrease in cyst density and a rapid increase in flagellated cell density from 24- 48 hr. In contrast, in strain CCMP 2091, flagellated cell density and volume remained relatively unchanged over the duration of the experiment (96 hrs) and cyst formation (all single cysts) was negligible. Together, these data suggest that pathogenic (micropredatory) strains of *Pfiesteria*-like dinoflagellates feed rapidly and vigorously on fish tissue, becoming greatly enlarged. These cells subsequently encyst and divide (<24 hr), each producing up to 8 daughter cells. Thus, when a pathogenic fish-fed strain is introduced into a comparative bioassay with algal-fed strains, their densities are able to increase more rapidly and fish mortality rates (by micropredation) are greater as a cell density-dependent effect. In contrast, non-pathogenic strains (e.g., CCMP 2091) do not appear to feed vigorously on fish tissue and do not demonstrate the increase in cell volume and reproductive output. These studies were supported by funding from the CDC, ECOHAB and the Virginia Institute of Marine Science.

THE EFFECT OF CARBON DIOXIDE (CO_2) ON GRAZING ACTIVITIES FOR *Karlodinium veneficum*

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In phytoplankton, growth responses are determined by a number of factors (i.e. dissolved gasses, temperature, light intensity, and nutrients), and the effect of individual variables on growth rate can be strongly influenced by interactions with the other factors. As a response to probable environmental changes, this study focuses on the effect of carbon dioxide (CO_2) on growth rate and grazing activity of *Karlodinuim veneficum*. *K. veneficum* is a photosynthetic, mixotrophic dinoflagellate that is capable of ingesting prey to fulfill limitations in energy and nutrients. Previous studies showed that for *K. veneficum*, heterotrophic activity is the main mode for obtaining energy and carbon; whereas, phototrophy may be a strategy for survival when environmental conditions for heterotrophic activity are poor. Recent studies of other mixotrophic algal taxa, however, have demonstrated that effects of heterotrophy on growth are diverse. Even though the importance of energy (sunlight) and inorganic nutrients as factors that regulate feeding in mixotrophic flagellates has been recognized, detailed examinations of how other factors influence the physiological state of cells and, therefore, feeding capability are scarce. In particular, the effects of global climate change; including increased atmospheric $CO_{2,}$ on heterotrophic activities in mixotrophic dinoflagellates has never been examined. Preliminary data indicate that when the prey (*Rhodomonas)* is present, there is a positive correlation with growth rates in high carbon dioxide systems. For this study, *K. veneficum* and *Rhodomonas* were cultured either alone or together in two different environments: one containing ambient CO_2 (375 ppm) and the other an elevated CO_2 level (750 ppm). Cultures were incubated at 26°C, and at 12:12 light:dark cycle to determine the impact of experimentally increased CO_2 conditions on growth and heterotrophic activity. We also investigated photosynthetic activities of *K. veneficum* cells in laboratory culture using the fluorescent probe, Carboxy SNARF-1 and flow-cytometry to monitor internal pH and physiology of this species as dissolved CO_2 levels was varied. This single-cell method for measuring *in situ* physiology may lead to a better understanding of physiological status of an individual species within a community and how anticipated global atmospheric conditions will contribute to overall species abundance and toxin production in dinoflagellates. This study is a work in progress and the results will be discussed.

IMMUNOLOGICAL RESPONSE OF DISTAL LUNG CELL LINES TO BREVETOXINS

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Brevetoxins, produced by *Karenia brevis*, are marine algal toxins associated with Florida red tides. Brevetoxin exposure can occur through consumption of contaminated shellfish or toxin inhalation. Various ailments have been documented following brevetoxin inhalation, including lung irritation, cough, wheezing, and congestion. While little is known about how brevetoxin exerts its effects on the lung, previous studies have suggested that brevetoxins may have an impact on the lung immune system. In this study, mouse alveolar epithelial or mouse alveolar macrophage lung cell lines were exposed to 0.5-2 μg/ml brevetoxin-2 and various immunological responses were measured. Western blotting for surfactant protein-A, a protein involved in lung innate immunity, identified that brevetoxin-2 decreases the amount of secreted SP-A. Cytokine antibody arrays primarily indicate a T_H1 response following brevetoxin-2 exposure. Microscopic imaging of macrophages incubated with fluorescently labeled particles indicated that macrophage phagocytosis increases after brevetoxin-2 exposure. These results suggest that brevetoxin-2 alters the immune response in the lung and enhances inflammation. Future work will aim to identify the pathways leading to these altered responses. *This research was supported by a training grant from the National Ocean Service.*

SPATIAL DISTRIBUTION OF PHYTOPLANKTON GROUPS AND TOXIC SPECIES IN A NEARSHORE FRONTAL ZONE SYSTEM IN MONTEREY BAY, CALIFORNIA

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Frontal systems are well known as concentration sites for phytoplankton biomass. However, detailed understanding of the interactions between nearshore physical processes and phytoplankton community structure is still missing in Monterey Bay, especially with regards to populations of toxic species. We surveyed the spatial distribution of dominant phytoplankton groups and toxic species across a semi-persistent nearshore frontal zone system in northern Monterey Bay, California, where "red tides" are commonly observed using satellite imagery. In this presentation, we report the changes in the dominant phytoplankton from one side of the front to the other, which includes the observation of a toxic *Pseudo-nitzschia australis* bloom not more than a few kilometers away from a *Ceratium divaricatum* “red tide”. We also describe the physical properties of the frontal system, the biomass differences, and the conspicuous taxonomic changes that occur across the frontal system. In addition, we also compared biomass and taxonomic changes in the presence and absence of the frontal system. We conclude that the observed variability of the dominant phytoplankton groups is a response to the presence of such feature. This particular frontal system appears to act as a physical barrier, separating two different physical environments with distinct competitive conditions which favor the growth of different phytoplankton species. Such fine-scale hydrographic feature may reflect population variability in toxic species that has not yet received much attention and we discuss significant implications of such feature in this presentation.

A STUDY OF *Pseudo-nitzschia* IN THE GULF OF MAINE: DIVERSITY AND TOXICITY

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In July 2003, more than twenty whales died on Georges Bank while additional whales and forty-two seals were found dead in the near-shore waters of Maine. Domoic acid (DA), produced by pennate marine diatoms belonging to the genus *Pseudo-nitzschia,* was detected in several of the whales, in some cases at high concentrations. Humans, marine mammals, and birds are all susceptible to DA, which causes Amnesic Shellfish Poisoning (ASP). The first cases of ASP were reported in 1987 on Prince Edward Island (PEI). One year after the PEI outbreak, DA was recorded in the southwest Bay of Fundy (BOF). Low levels of DA have subsequently been detected in the BOF nearly every year since 1988. Low levels of DA have also been found in scallops on the southeast shore of Nova Scotia, and in clams, mussels and scallops collected on the Scotian Shelf and Georges Bank (Addison and Stewart, 1989). It is now clear from the whale deaths and toxicity records in eastern Canada that DA is an emerging or cryptic problem that has not been adequately characterized in the Gulf of Maine (GOM), in terms of both human health and ecosystem impacts. While some species of *Pseudo-nitzschia* are capable of producing DA, not all are; therefore, it is important to know what species are found in the GOM and which of them are toxic.

Beginning in 2006, water samples from throughout the GOM region were collected and *Pseudo-nitzschia* cells were isolated into culture. Molecular techniques including sequencing of the D1-D3 domains of the large subunit ribosomal DNA (LSU rDNA) and the ITS1, 5.8S, and ITS2 regions (ITS) were then used to identify these *Pseudo-nitzschia* cultures. The resulting sequences were compared to sequences in NCBI's GenBank. Out of the thirty-nine sequences that resembled *Pseudo-nitzschia,* each most closely matched one of the following five unique species or strains: *P. delicatissima, P. pseudodelicatissima, P.* cf. *subpacifica, P. pungens,* and *P. fraudulenta*. Further work will be done using scanning electron microscopy to help corroborate the molecular identifications. These sequences were also used to determine that previously designed probes used to identify west coast *Pseudo-nitzschia* strains (Miller and Scholin, 1998) do not match with 100% identity to these east coast strains.

DA toxicity was measured in the GOM *Pseudo-nitzschia* strains using an Enzyme-Linked Immunosorbent Assay (Biosense, Bergen, Norway). Our results show that 15 of these strains are weakly toxic, with a detectable amount of DA ranging from 92-275 pg/ml in an extracted culture containing cells and media, as compared to our positive control, *P. multiseries*, from Prince Edward Island, Canada, which had a DA level of about 7000 pg/ml. Further toxin analysis using HPLC is in progress to confirm these readings and work is also ongoing to isolate additional strains of *Pseudo-nitzschia* from the GOM.

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CALIFORNIA PROGRAM FOR REGIONAL ENHANCED MONITORING OF PHYCOTOXINS (Cal-PReEMPT)

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California's expansive coastline is threatened by blooms of a variety of harmful algal genera, including *Pseudo-nitzschia* and *Alexandrium*, and these pose a threat to public health, fisheries and aquaculture. New methods for species and toxin detection are available, such as the simple-to-use Jellett tests for domoic acid and saxitoxins in phytoplankton and shellfish samples, the receptor binding assay for saxitoxins, and molecular probes for quantitative species detection. Although these technologies are available, a constraint to adoption of them by the California Department of Public Health (CDPH) and other state monitoring programs is the lack of funds for ground-truthing them, a necessary step to evaluate the efficacy of these tools for improving existing monitoring efforts. To bridge the gulf between availability of new tools and integration of those tools into monitoring efforts, NOAA, through its Monitoring and Event Response Program for Harmful Algal Blooms (MERHAB), is providing funding to perform necessary validation and evaluation of whether to incorporate these methods into the CDPH monitoring program and, if so, how best to utilize them. We have established pilot project sites where new technologies are incorporated into an intensive monitoring program. Our approach is to shift much of the monitoring effort to the field, where field technicians pre-screen samples for toxins and toxin-producing species, thus ensuring early warning of impending blooms while reducing un-necessary and expensive lab-based sample testing. With this presentation we provide a summary of our Cal-PReEMPT project, and include results from field testing Jellett kits for DA and saxitoxins in both plankton and shellfish samples, results from analyses using the receptor binding assay for saxitoxins, and results from a comparison of field observations of potentially toxic species using simple field microscopes versus species-specific molecular probes for quantifying *Pseudo-nitzschia australis*, *P. multiseries*, and *Alexandrium*. We have also been incorporating remote sensing data into the monitoring and decision-making process, and will provide results highlighting the utility and limitations of these methods, focusing on the 2007 domoic acid event in California.

Based on this rigorous intercomparison, we have found that simple field microscopes and phytoplankton net samples provide an effective first line of defense for detecting the onset of HAB events. While using Jellett test kits for detecting phytoplankton and shellfish PSP in the field is easy, there is a learning curve to interpreting the results. Using the kits does not always provide an obvious "yes / no" result, and, at least for PSP toxins, the detection limit of the kits is slightly lower that the regulatory method, giving positive field results for shellfish samples that are non-detectable for these toxins by the standard mouse bioassay. We have begun using a receptor binding assay for quantifying toxin concentrations and we will determine where the Jellett detection limit is for PSP in our samples. Intralaboratory validation of this method will help determine it's suitability as a replacement for the MBA in terms of accuracy and precision, sample throughput, and cost. We have also been conducting an intercalibration of ELISA and FMOC-HPLC methods for particulate domoic acid detection; while overall agreement is excellent, there appear to be differential responses to the presence of DA isomers. Both methods work well, with the ELISA kits providing increased sensitivity and speed, but with higher per-sample costs.

BLOOM-FORMING PHYTOPLANKTON IN SAGINAW BAY (LAKE HURON) AND WESTERN LAKE ERIE: ABUNDANCE, DISTRIBUTION, AND CYANOBACTERIAL TOXICITY DURING LATE SUMMER

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Phytoplankton blooms occur throughout the nutrient-enriched waters of Saginaw Bay (Lake Huron) and western Lake Erie during periods of warm temperature and water-column stratification. Although Great Lakes assemblages exhibit spatial/temporal variances reflecting equilibriums between growth and loss and mediated by system-specific forcing phenomena, the synergistic interactions and/or feedbacks among environmental conditions, assemblage composition/biomass and cyanobacterial toxicity events only partially are understood. As a component of the NOAA's *Center of Excellence for Great Lakes & Human Health* research initiative (http://www.glerl.noaa.gov/res/Centers/HumanHealth/) and to establish a hierarchy of relative importance among environmental conditions influencing Great Lakes bloom-forming phytoplankton, we characterized the synergistic interactions and/or feedbacks among assemblage composition and biomass, toxic phenomena, and system-specific, water-column properties.

Cyanobacteria and diatoms dominated summer phytoplankton assemblages in Saginaw Bay, whereas assemblages were more diverse in western Lake Erie, with diatoms, chlorophytes, cyanobacteria, and cryptophytes dominating assemblages. Although phytoplankton accumulations were spatially and annually episodic throughout both systems, distinct biological 'hot spots' were evident; the greatest accumulations occurred in near-shore water along the southwestern shoreline of Saginaw Bay (near the confluence of the Saginaw River and the Bay) and along western and southern shorelines of Lake Erie (near the confluence of the Maumee River and the Lake and within the lower reaches of Sandusky Bay). Intracellular microcystin concentrations were within the range of concentrations previously reported for these and other Great Lakes' systems, with concentrations (episodically) exceeding the recommended limit for drinking water and (low risk) recreational use.

A suite of variables, indicative of annually-distinct meteorological and hydrological conditions and nutrient-laden inflows, were identified to (collectively) best 'group' sampling stations in a manner consistent with that of chlorophyll *a* concentrations and cyanobacterial biovolumes. However, a great deal of variability between abiotic and biotic patterns remained unexplained and several abiotic variables singularly corresponded with cyanobacterial abundance. Taken together, it appears that multiple environmental conditions (including annual/episodic meteorological patterns, seasonal/intermittent riverine inflows, annual phosphorus loading, etc.) interact with taxon-specific physiological traits to holistically influence late-summer phytoplankton abundance throughout Saginaw Bay and western Lake Erie.

Alexandrium fundyense cDNA MICROARRAY: THE HUNT FOR GROWTH-RELATED GENES

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Understanding of *in situ* cell division rate (CDR) for the PSP-producing dinoflagellate *Alexandrium fundyense* is an important but challenging task. One potential approach is to use cell cycle molecular markers. A correlation between expression of a marker gene and growth rate can be established and *in situ* CDR can be derived from measured gene expression data. This research will screen the *A. fundyense* genome for genes associated with the cell division cycle using cDNA microarray. A full-length cDNA library was created using the 22-bp spliced leader RNA specific to dinoflagellates. Fifteen hundred random inserts from the *A. fundyense* cDNA library were used to construct the microarray. Abundance of transcripts through the diel cycle will be compared and candidate genes showing differential expression throughout the cell cycle will be validated using real-time quantitative polymerase chain reaction (RT-qPCR). Results obtained to date will be presented.

DISTRIBUTION OF KARLOTOXINS AMONG AUSTRALIAN AND NORTH AMERICAN ICHTHYOTOXIC GYMNODINIOID DINOFLAGELLATES (KARENIACEAE)

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Gymnodinioid dinoflagellates from the newly formed family Kareniaceae have long been associated with fish kills, however the precise mechanism by many species remains unsolved. Candidate theories include the production of brevetoxin (in *K. brevis* only), reactive oxygen species, lipid phycotoxins (Arzul *et al*. 2000; Mooney *et al*. 2007) and recently characterized karlotoxins . Karlotoxin has been proposed as the primary cause of ichthyotoxicity in the cosmopolitan *Karlodinium veneficum* and strains known to produce karlotoxins have been isolated from the US Atlantic coast, particularly within Chesapeake Bay, Maryland, USA, and the Swan River, Western Australia (Adolf *et al*. 2005; Deeds *et al*. 2006). Toxin analysis of 13 species of Kareniaceae revealed the presence of karlotoxin, KmTx 2, in only a single species (*Karlodinium veneficum*) but with variable activity in strains isolated from the Swan, Huon and Derwent Rivers in Australia. A newly isolated Southern Ocean species, *Karlodinium conicum*, contained a novel non-hemolytic karlotoxin analogue, confirmed by LC-MS and a characteristic UV-spectrum. Isolated from open water in temperatures as low as 5 °C, the presence of widespread Southern Ocean Kareniaceae (*Karlodinium antarcticum*, *K. conicum*, *K. corrugatum* and *Takayama tuberculata*) (de Salas *et al*. 2008) call for a rethinking of the contribution dinoflagellates may make to the chemical and food chain ecology in the Antarctic environment. Species from the closely related genera *Takayama* (*T. helix, T. tasmanica, T. tuberculata*), *Karenia* (*K. brevis, K. mikimotoi, K. papilionacea*) and *Karlodinium* (*K. antarcticum, K. ballantinum, K. corrugatum, K. decipiens*) were all consistently negative for karlotoxins. The potentially toxic *Karenia papilionacea* and *Karenia umbella* have been associated with fish kills in Australia and New Zealand however their fish killing mechanism remains to be determined. Proposed toxic mechanisms of *Karenia mikimotoi* includes toxic PUFA and gymnocins but there is still no conclusive evidence on their qualitative contribution to ichthyotoxicity. No universal mechanism for ichthyotoxicity in gymnodinioid dinoflagellates has yet emerged and synergistic interactions between "toxins" (e.g. brevetoxin, karlotoxin, gymnocin), free fatty acids and reactive oxygen species are likely to vary between species, strains and even environmental conditions.

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IDENTIFICATION OF *Pseudo-nitzschia* AND DOMOIC ACID FROM A NORTH CAROLINA COASTAL BLOOM: LINKAGE BETWEEN VOLUNTEER OBSERVATIONS AND BIOTOXINS RESEARCH

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The Southeastern Phytoplankton Monitoring Network (SEPMN) is a community outreach program developed to increase awareness of harmful algae to constituent groups and directly involve volunteers in coastal stewardship by participation in phytoplankton sampling and identification. Currently the program has 80 sites from North Carolina to Texas. The majority of the volunteer groups include teachers and students, however, universities, aquariums, parks and recreational facilities, and environmental and citizen groups also participate. During 2006-2007, approximately 2000 participants were actively involved in SEPMN programs and monitoring activities. Volunteers are instructed on algae identification and sample on a weekly or biweekly basis, reporting their data via a secure web portal to researchers at the Marine Biotoxins Program. Results from volunteer groups enable researchers to identify problem areas to isolate for further study. Since 2001 over 50 blooms have been observed by volunteer groups.

During 2006, a multi-species bloom of *Pseudo-nitzschia* was observed by volunteer monitors students of Kill Devil Hills High School, North Carolina and College of the Albemarle. Preserved and live samples sent to the Marine Biotoxins Program were positively identified using scanning electron microscopy as *Pseudo-nitzschia pungens, P. multiseries*, and *P. pseudodelicatissima*. This *Pseudo-nitszchia* bloom peaked at 7,300 cells/L. Analysis of seawater samples detected the toxin, domoic acid using LC-MS/MS techniques at a concentration of 0.6 ng domoic acid/ml of seawater, making this the first report of domoic acid from the Carolina coast of the United States. Oysters collected at the time of this bloom were also positive for domoic acid at a level of 9.6 ng/g. The identification of this multi-species, toxic bloom in North Carolina's waters is an example where a volunteer monitoring program is useful in developing a species list and record of distribution patterns, as well as alerting scientists to the presence of harmful species.

GULF OF MEXICO PHYTOPLANKTON AFFECT FATE OF RED TIDE TOXIN

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The red tide dinoflagellate *Karenia brevis* kills and incapacitates coastal wildlife via the brevetoxins it produces. However, the relationship between *K. brevis* and other Gulf of Mexico phytoplankton species, such as the abundant and widespread diatom *Skeletonema costatum*, is not well studied. We are exploring how chemical cues mediate interactions between *K. brevis* and its phytoplankton competitors, including *S. costatum.* In lab experiments, the addition of live *S. costatum* led to decreased concentrations of brevetoxin B (PbTx-2) associated with *K. brevis* cells. A similar decrease in PbTx-2 concentration occurred when a mixture of brevetoxins (without live *K. brevis* cells) was exposed to *S. costatum*, indicating that *S. costatum* degrades waterborne PbTx-2. LC-MS and ELISA analyses indicated that PbTx-2 was not transformed into other brevetoxins or known brevetoxin metabolites, and instead is decomposed by a previously-unrecognized mechanism. This degradation of PbTx-2 was accomplished by four different *S. costatum* strains from around the world, suggesting that evolutionary experience with *K. brevis* is not a pre-requisite for the ability to degrade PbTx-2. Additionally, phytoplankton-associated bacteria were found to play no role in PbTx-2 degradation, as both axenic and non-axenic *S. costatum* strains degraded PbTx-2. Our results indicate that the metabolic fate of brevetoxins, and therefore the environmental consequences of harmful algal blooms, may depend on which competitors are present during red tides.

THE MITIGATING PROPERTIES OF CYSTEINE ON THE HARMFUL EFFECTS OF RED TIDE

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Karenia brevis blooms, also called Florida red tides, occur almost every year in the Gulf of Mexico resulting in severe economic losses[1], deleterious effects on human heath[2] and devastating consequences to wildlife[3]. The causative agents for these detrimental impacts are the polyether brevetoxins, a family of potent neurotoxins produced by this organism. Brevetoxin exposure results in massive fish kills[4], marine mammal deaths[5,6], and upper and lower airway impairments in humans exposed to contaminated sea-spray[2,7]. Because shellfish accumulate brevetoxins, to prevent outbreaks of Neurotoxic Shellfish Poisoning, frequent bans on recreational and commercial bivalve harvesting are required, thus also hurting fisheries. Here we show that the adverse effects of *K. brevis* exposure can be drastically reduced by cysteine. This molecule was found to complex almost instantly with some of the toxins (including the most abundant) produced by *K. brevis*, to form very polar non-toxic derivatives. Cysteine treatment of water toxic enough to kill fish within minutes prevented fish mortality. Likewise, in a sheep asthma model, the same cysteine treatment prevented the bronchoconstrictor response that was otherwise observed following exposure to toxic aerosols. Furthermore, pretreatment of the sheep with cysteine partially blocked the effects of the exposure to toxic aerosols and reversed the bronchoconstriction when administered after exposure. Thus cysteine suppressed, prevented and ameliorated airway constriction, a major pulmonary consequence of brevetoxin inhalation[8,9]. When applied as a preventive treatment, shellfish treated with cysteine accumulated 50% lesstoxin than untreated shellfish exposed to *K. brevis* under the same conditions. Most remarkably, cysteine treatment during exposure of shellfish to *K. brevis* reduced the toxin accumulation in tissue by 95%. We conclude that cysteine offers new prospects for mitigating the impact of blooms of brevetoxin-producing algae.

REPORTED RESPIRATORY SYMPTOM INTENSITY IN ASTHMATICS DURING EXPOSURE TO AEROSOLIZED FLORIDA RED TIDE TOXINS

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Florida red tides are naturally occurring blooms of the marine dinoflagellate, *Karenia brevis*. *K. brevis* produces natural toxins called brevetoxins. Brevetoxins become part of the marine aerosol as the fragile, unarmored cells are broken up by wave action. Inhalation of the aerosolized toxin results in upper and lower airway irritation. Symptoms of brevetoxin inhalation include: eye, nose, and throat irritation, coughing, wheezing, chest tightness, and shortness of breath. Asthmatics appear to be more sensitive to the effects of inhaled brevetoxin. This study examined data from 97 asthmatics exposed at the beach for one-hour during *K. brevis* blooms, and on separate occasions when no bloom was present. In conjunction with extensive environmental monitoring, participants were evaluated utilizing questionnaires and pulmonary function testing before and after a one-hour beach walk. A modified Likert scale was incorporated into the questionnaire to create respiratory symptom intensity scores for each individual pre- and post-beach walk. Exposure to Florida red tide significantly increased the reported intensity of respiratory symptoms; no significant changes were seen during an unexposed period. This is the first study to examine the intensity of reported respiratory symptoms in asthmatics after a one hour exposure to Florida red tide.

SEASONAL AND INTER-ANNUAL CHANGES IN DINOFLAGELLATES COMMUNITY COMPOSITION IN NEARSHORE ALABAMA WATERS

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Alabama coastal waters have experienced significant blooms of potentially-toxic and toxic diatoms, raphidophytes and dinoflagellates. Our goal is to describe the temporal and spatial variability in community composition and to relate it to environmental changes. Here we evaluate the dinoflagellate community composition over a period of 8 years. Dinoflagellate species abundances from 11 sites in the near-shore Gulf of Mexico have been monitored weekly to bi-weekly since 1999. A further 8 sites in Mobile Bay have been monitored quarterly since 1996. Community compositions in both were grouped and averaged by season and location (Gulf of Mexico or Mobile Bay) and analyzed using PRIMER to determine species interactions, trends and patterns. There was a clear shift in dinoflagellate community composition between seasons and between years (e.g. Fig. 1). For instance, *Alexandrium monilatum* was very abundant in the summer but never recorded in the winter. In contrast, *Dinophysis acuminata* was never present in the summer but did appear in moderate numbers in the winter and spring. Populations of several species of *Ceratium* and *Prorocentrum* were very stable, showing consistent abundances in all season and over the 8-year sample period. Of the common species, *Akashiwo sanguinea* was not recorded prior to 2003 and *Gonyaulax digitale* was observed prior to 2002 and again after 2005. There were major blooms (10^5 – 10^7 cells/l) of *Karenia brevis* in the coastal Gulf of Mexico in October 2005 and of *Heterocapsa triquetra* and *Prorocentrum minimum*, predominantly in Mobile Bay in February 2006 and 2007. Some of these were associated with fish-kills. Where blooms were found in both areas, cell densities were higher inside Mobile Bay than in the near-shore Gulf. For instance, a bloom of *Prorocentrum minimum* had a maximum density of 690,000 cells /l in February and March of 2007, but reached a peak of 1,400,000,000 cells/l in Mobile Bay during the same time-period.

BIOPHYSICAL MODELING OF THE JUAN DE FUCA EDDY IN THE PACIFIC NORTHWEST

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Recent studies indicate that the Juan de Fuca Eddy, a summer nutrient-rich retentive feature off the entrance Juan de Fuca Strait, is an initiation site for the toxigenic phytoplankton *Pseudo-nitzschia* that impact shellfish along the outer Washington coast. In this presentation, we will describe a bio-physical model developed to study eddy generation, bloom dynamics and transport to the coast. Results from the model simulations show that the eddy is generated by enhanced wind and tidally-driven upwelling off Cape Flattery. As the continued upwelling brings more nutrient-rich water to the surface, the eddy forms, phytoplankton grows and is retained in the eddy, and then moves offshore. Variations in the winds and tides will be shown to not only change the location and intensity of this eddy, but also in some cases, to reduce the upwelling and transport the offshore water to the coast. This study is part of ECOHAB PNW, a project funded by the Ecology and Oceanography of Harmful Algal Blooms program to investigate the formation, toxicity, and transport of *Pseudo-nitzschia* spp. blooms in the Juan de Fuca Eddy.

ACCUMULATION AND DEPURATION OF BREVETOXINS AND MAJOR METABOLITES IN SHELLFISH EXPOSED TO RECURRING *Karenia brevis* BLOOMS

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Brevetoxins are accumulated in filter-feeding molluscan shellfish during exposure to blooms of *Karenia brevis*. Although parent algal toxins are rapidly metabolized, shellfish remain toxic causing neurotoxic shellfish poisoning (NSP). Trophic transfer of brevetoxins also occurs, as evidenced by human intoxication from consumption of carnivorous lightning whelk (*Busycon contrarium*) taken from Sarasota Bay, Florida. This study monitored the composition and concentration of brevetoxins and major metabolites in clams (*Mercenaria mercenaria*), oysters (*Crassostrea virginica*), and lightning whelk (*Busycon contrarium*) collected from a common site in Sarasota Bay exposed to several *K. brevis* red tide events, from 11/1/2003 through 7/28/2006. Water samples were collected from three locations around the shellfish study site to estimate exposure concentrations. Samples of water and shellfish tissue were analyzed by LC-MS for quantitative and qualitative determination of parent brevetoxins and major metabolites. Toxicity of shellfish was assessed by the standard mouse bioassay. Water samples contained primarily PbTx-2, followed by PbTx-3, PbTx-2-carboxylic acid, and PbTx-1. As blooms diminished, PbTx-3 and PbTx-2-carboxylic acid became the most abundant brevetoxins. In clams and oysters, PbTx-2 was not found, and PbTx-3 was observed at relatively low concentrations. Most abundant were PbTx-2-cysteine and cysteine-sulfoxide conjugates (MH^+: *m/z* 1018 and 1034, respectively), followed by PbTx-1-cysteine and cysteine-sulfoxide conjugates (*m/z* 990 and 1006, respectively). Clams and oysters retained brevetoxin metabolites long after exposure to *K. brevis* blooms. Oysters were toxic by mouse bioassay longer than clams. In whelks, brevetoxins and metabolites were found at very low levels in muscle tissue, however highly concentrated in viscera.

DETERMING THE ROLE OF *Karenia brevis* BLOOMS IN EMERGENCY ROOM VISITS DUE TO RESPIRATORY AILMENTS IN SARASOTA, FLORIDA

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Harmful algal blooms (HABs) resulting from the marine dinoflagellate *Karenia brevis* are an increasing environmental, economic, and health concern. *K. brevis* blooms, which occur annually in the Gulf of Mexico, produce potent brevetoxins responsible for neurotoxic shellfish poisoning (NSP) in humans as well as mass mortalities of marine organisms. Additionally, anecdotal reports, animal experiments, and pilot studies suggest that aerosolized brevetoxins transported by wind are linked to both upper and lower respiratory symptoms, possibly with long-term health implications. Our research aims to determine whether the number of emergency room visits to Sarasota Memorial Hospital due to respiratory ailments changes as a function of the frequency and intensity of *K. brevis* blooms off the west coast of Florida. Our analysis extends the work of Kirkpatrick et al. (2006) regarding the increased rate of healthcare utilization due to aerosolized brevetoxins. In contrast to previous studies, our research will assess the correlation between red tide blooms and respiratory ailments on a finer time scale, as well as over a longer period and will control for environmental factors that function as significant risk factors/triggers of respiratory ailments. These include: pollen and mold counts, influenza outbreaks, ozone and particulate matter concentrations, and forest fire frequency. We will also account for air temperature, relative humidity, wind speed, and wind direction in the *K. brevis* sampling location, as these measures may influence the production and transport of red tide aerosols (Fleming et al. 2005). This work is the first step in a comprehensive project to determine the economic impacts resulting from various intensities of *K. brevis* blooms, which, in turn, will guide management actions to reduce HAB-induced costs-of-illness.

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AMMONIUM SURGE UPTAKE AND INHIBITION OF NITRATE UPTAKE BY SMALL AND LARGE CELL-SIZED *PSEUDO-NITZSCHIA* SPECIES FROM THE PACIFIC NORTHWEST

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Despite the importance of nitrogen in maintaining blooms of toxigenic diatoms and their production of the neurotoxic amino acid, domoic acid, the nitrogenous nutrition of *Pseudo-nitzschia* species is still poorly known, and is primarily limited to the larger cell-sized species. Quantification of the physiological capacity for nitrogen uptake by *Pseudo-nitzschia* species under both N-replete and N-deplete conditions is a necessary prerequisite for an understanding of the success of *Pseudo-nitzschia* relative to other phytoplankton in coastal systems, and may help to identify the proximate causes of toxin production by these diatoms. The nitrogen uptake capabilities of three *Pseudo-nitzschia* species, isolated from the Pacific Northwest and maintained in unialgal cultures, are presented here: the transient elevated 'surge' uptake rates of ammonium by *P.* cf. *delicatissima* and *P. multiseries*, and the ammonium inhibition of nitrate uptake by *P.* cf. *cuspidata* and *P. multiseries*. Elevated ammonium uptake rates were determined in duplicate, N-starved cultures of the two species (cultures depleted of external nitrate for ~ 2 generations) using both the accumulation of N-15 labeled ammonium into the cells and the disappearance of ammonium from the medium at varying intervals (5-30 min) over a six-hour period. Both the large and small cell-sized species demonstrated a capacity for transient 'surge' uptake in the first minutes following ammonium enrichment, but *P.* cf. *cuspidata* exhibited much faster rates than *P. multiseries*. However within 0.5-1.0 h, ammonium specific uptake rates decreased and stabilized to values sufficient to support the pre-conditioned growth rates observed for both species prior to N starvation. During multi-day experiments, duplicate nitrogen-replete cultures of *P.* cf. *cuspidata* demonstrated complete inhibition (suppression) of nitrate uptake by elevated ammonium concentrations. Only after the ammonium concentrations were decreased by the cells to below the 'threshold' concentration ($< \sim 5\ \mu$M) did these diatoms begin their utilization of nitrate. These laboratory results will be discussed with respect to the relative surface area per unit cell volume of the large and small cell-sized species, and the possible ecological consequences of simultaneous uptake of nitrate and ammonium at the ambient N concentrations normally found in the coastal waters of the Pacific Northwest.

DIEL SYNCHRONIZATION OF EMBRYONIC DIAPAUSE AIDS PREDICTION OF FETAL TOXICITY OF CALIFORNIA SEA LIONS TO DOMOIC ACID-PRODUCING HARMFUL ALGAL BLOOMS

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California sea lions (CSL) have been a repeated subject of investigation for early life toxicity, which has been documented to occur in mass clusters associated with DDT toxicity in the 1970's and domoic acid toxicity in the last decade. The mass early life mortality events result from the concentrated breeding grounds and a long gestation period of 11 months. Days after conception, development of a CSL embryo is suspended and not resumed until a decreasing photoperiod of 11.48 h/day is reached [1], which occurs approximately 90 days after conception at the major California breeding grounds. The photoperiod trigger reactivates the development of embryos, synchronizing an entire population to proceed with development for the next 242 days until birth. Embryonic diapause is a selectable trait thought to optimize timing for food utilization and male migratory patterns; yet, based on the toxicological perspective presented here, also serves to synchronize developmental toxicity of pulsed environmental events, such as exposure to domoic acid. Research studies in laboratory rodents have identified age-dependent neurotoxic effects during development advancing from neuronal migration disorder to hippocampal-localized seizures to full limbic seizures. Parallel studies have characterized unusual susceptibility of the fetus to domoic acid with retention of toxin and potential dermal re-exposure throughout pregnancy via the amniotic fluid. This presentation will describe comparative allometric projections of rodent neurodevelopment to the CSL to analyze prenatal toxicity and exposure susceptibility of the CSL to domoic acid. This analysis will be applied to forecast fetal toxicity based upon photoperiod-synchronized fetal development of animals originating from the Channel Island rookeries and should prove useful for prediction of fetal outcome after domoic acid producing blooms occurring at different times of the year.

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ECOLOGY AND BIODIVERSITY OF TOXIC BENTHIC DINOFLAGELLATES AT JOHNSTON ATOLL, PACIFIC OCEAN

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A major impediment to understanding the seemingly random occurrence of ciguatera toxicity is uncertainty regarding the field ecology of benthic dinoflagellates that introduce toxins into the coral reef food web. Although broad generalizations have been made, the results of past studies have often yielded contradictory results, particularly between ecological patterns documented in the Pacific versus the Caribbean. This study used standardized methodology to investigate the distribution and abundance of toxin-producing benthic dinoflagellates from the genera *Gambierdiscus, Prorocentrum, Ostreopsis, and Amphidinium* at Johnston Atoll, Pacific Ocean to determine how water flow, depth and habitat type influence patterns of biodiversity. Dinoflagellate abundance was highest at stations located in lagoon/channel habitats and was lowest in reef crest/back reef areas subject to wave activity. In lagoon/channel habitats, *P. mexicanum, P. concavum,* and *G. toxicus/P.lima* were consistently the dominant species. However, in back reef/reef crest habitats *O. siamensis*, *O. ovata*, *G. toxicus* and *P. emarginatum* were present in highest proportions, suggesting that these species are better able to persist in turbulent habitats. Grazing activity by herbivorous fishes in reef crest areas is highest relative to the lagoon, suggesting that preferential foraging in reef crest/back reef habitats would result in the uptake and accumulation of toxins predominantly produced by these dinoflagellates. This study shows that remarkably similar patterns of abundance, community composition and species associations exist in the Pacific relative to what has been documented in the Caribbean, which demonstrates that the ecology of this community is consistent among geographic regions and greatly contributes to an accurate and coherent characterization of the population dynamics of ciguatera dinoflagellates.

GRAZING, GROWTH, AND BEHAVIORAL REACTIONS OF A CILIATE FED *Alexandrium* SPP: APPARENT LACK OF RESPONSE TO SAXITOXIN

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Harmful algal blooms can cause economic and ecological damage. Ciliates are important grazers of planktonic algae, and may be impacted differently by harmful algal blooms than are other grazers. The bloom-forming dinoflagellate *Alexandrium fundyense* produces saxitoxin (STX) and a number of similar compounds. STX is considered to be a voltage-gated sodium ion channel blocker, but has been shown also to have an effect on calcium channels, which are important in regulation of motility in ciliates. Experiments were carried out with the ciliate Strombidinopsis sp. fed two members of the *Alexandrium fundyense* complex, *A. fundyense* and *A. tamarense*. *A. tamarense* is considered to be non-toxic or less toxic than *A. fundyense* because it does not produce STX. While both dinoflagellates make other STX-related compounds, *A. tamarense* produces less of these than A. fundyense. The ciliate fed on both species of *Alexandrium*. It survived, but did not grow on A. fundyense; however, there was significant mortality in ciliates fed *A. tamarense*. Behavioral assays showed unexpected differences in reactions to A. fundyense and *A. tamarense* extracts, with avoidance (backwards swimming) being induced by *A. tamarense* extract but not A. fundyense. We are presently developing further the behavior assay and evaluating the effects of calcium channel blockers on motility and grazing in planktonic ciliates. Our goal is to identify the mechanism of toxicity and to assess the role of this interaction in promoting harmful algal blooms.

DOMOIC ACID UPTAKE AND EXCRETION IN FISH, DUNGENESS CRABS, RAZOR CLAMS AND MUSSELS

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Domoic acid (DA) is a neurotoxic amino acid produced by several marine algal species and is the causative agent of amnesic shellfish poisoning in humans, seabirds and marine mammals which have consumed fish that have fed on PN. Profound differences in the toxicokinetics of DA have been identified in a wide variety of shellfish including several species of mussels (*Mytilus* sp.) and razor clams (*Siliqua patula*). We studied the toxicokinetics of DA in razor clams, mussels (*M. galloprovincialis*), Dungeness crabs (*Cancer magister*) and rainbow trout (*Oncorhynchus mykiss*) after intravascular injection and water or oral exposures. Crabs were orally dosed by gavage using homogenized razor clam meat (spiked with DA) and then serially euthanized at selected times and various tissues removed for DA quantification. The uptake of soluble DA in bivalves was studied before and after co-exposure to inhibitors of the multi-xenobiotic resistance (MXR) protein. Oral dosing and initial IV studies used a DA dose of 1 mg/Kg (total weight). Subsequent IV dosing in crabs used a 0.1 mg/Kg DA dose. DA tissue and water concentrations were measured by HPLC-UV / fluorescence or ELISA. After oral dosing in crabs, DA was rapidly and completely absorbed with 2-3 hrs. The majority of the dose was retained in the hepatopancreas, which had DA concentrations 100-200 times that of other tissues such as the kidney and gills. Muscle levels of DA were ~ 10,000 times lower than in the hepatopancreas (Figure 1). After IV dosing in crabs, the initial concentrations of DA in the hemolymph compartment were relatively high and remained largely unchanged for 24 hrs before slowly declining in a log-linear manner. This kinetic behavior was observed for both the 1 and 0.1 mg/Kg doses in crabs. Toxicokinetic analysis using a compartmental model indicated the distributive space of IV injected DA approximated the hemolymph volume of crabs. This is a surprising result given the established ability of the hepatopancreas to extract and retain DA after oral dosing, suggesting a diffusional barrier exists, which prevents DA in the hemolymph from distributing into the hepatopancreas. The kinetic behavior of DA in fish and shellfish will be compared and contrasted including the potential for the involvement of MXR type proteins in the uptake and tissue distribution of DA.

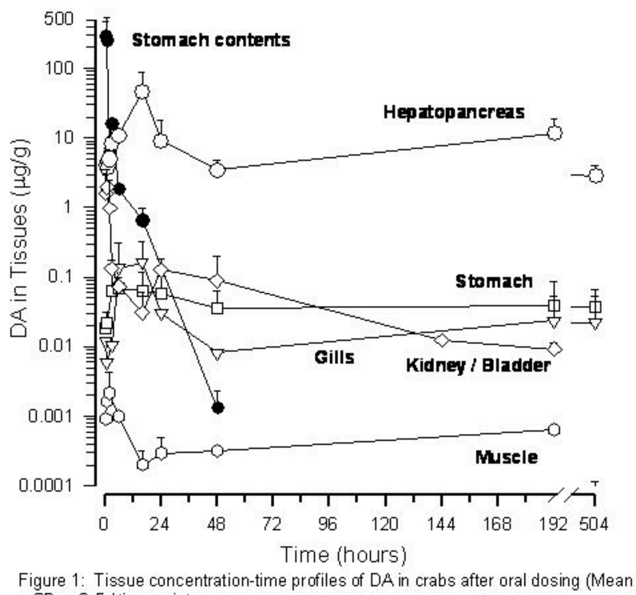

Figure 1: Tissue concentration-time profiles of DA in crabs after oral dosing (Mean ± SD *n*=3-5 / time point

PHENOTYPIC VARIATIONS IN INGESTION OF TOXIC ALGAE WITHIN POPULATIONS OF A MARINE COPEPOD

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Recently, distinct reproductive phenotypes related to PSP resistance have been documented in the copepod *Acartia hudsonica* (Avery and Dam, in press). Furthermore, because a differential population response in ingestion rate of *A. hudsonica* exposed to the toxic dinoflagellate *Alexandrium* spp. has also been documented (Colin and Dam 2002; Colin and Dam in press), we hypothesize that distinct phenotypes for ingestion of toxic algae must exist. Through multiple laboratory studies, this hypothesis was tested. Copepods cultured from regions where toxic *Alexandrium* blooms occur frequently, and others from areas where blooms are infrequent or rare were used in feeding experiments. Ingestion rates of individual copepods were measured when fed either high toxin (*A. fundyense*), or nontoxic (*A. tamarense*) diets. Frequency distributions of ingestion rates revealed distinctive clustering in animals fed the toxic algae. Normal probability plots and corresponding Shapiro-Wilk tests of normality indicate that the data is non-normal, bolstering the argument that the phenotypic groupings are valid. We propose that these phenotypes represent different levels of resistance to toxic algae. The assays described here could provide a quick test for assessing toxin resistance. In addition, future molecular analysis of preserved experimental copepods should elucidate genotypic variations underlying the phenotypic variations. Coupled with HPLC toxin analysis, these studies will be valuable in assessing toxin dynamics in grazers. The presence of toxin resistant phenotypes in grazer populations has important implications for PSP toxin transfer to higher trophic levels and for bloom control.

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NORTH CAROLINA HARMFUL ALGAL BLOOM EVENTS 2005 – 2007

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The frequency and distribution of harmful (toxin-producing) algal bloom (HAB) events are examined to reveal public health gaps in reducing or preventing human exposure to these toxins. A harmful algal bloom event is defined as a human health complaint or concern originating from a water treatment plant, a state agency, or a citizen. From May 2005 to July 2007, twenty-six events were reported to the North Carolina (NC) Harmful Algal Blooms Program. NC blooms occur in fresh and estuarine waters during late spring to early fall seasons across the western, central, and eastern regions of the state. The majority of these events are related to HABs in drinking water (62%). An algal toxin was present in one-third of the events tested (n = 21). Microcystin toxin concentrations ranged from 0.21 to 1.02 µg/L in drinking water and from 0.065 to 0.665 µg/L in recreational (ambient) water. Nearly all of the 2007 events are still pending toxin analyses for anatoxin, cylindropsermopsin, and saxitoxin. The most commonly encountered algae are the cyanobacteria, namely *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, and *Microcystis*. A small number of events are due to other algal groups bloom including *Euglena*, *Chattonella*, and *Karlodimium*. Therefore, a NC HAB event profile is characterized as a cyanobacteria species blooming in the warm months, containing a low amount of microcystin, and occurring in any area of the state. The data generated from this investigation are used to establish historical context and drive public health illness surveillance and outreach activities.

MITIGATING THE RISK OF INTRODUCTION OF HARMFUL ALGAE VIA TRANSFER OF BIVALVE MOLLUSCS

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Bivalve molluscs feed upon microalgae, but may not kill or digest all cells filtered. Shellfish management often involves transplantation, which carries with it the potential for introducing living microalgae in the receiving waters. The risk of introducing some non-native, harmful algal species into new coastal areas within the shells of transplanted molluscan shellfish has been established in the literature, but a wide survey conducted with a consistent protocol has not been done. In the present study, commercially-exploited bivalves and the harmful algal species they can encounter were listed and selected, and laboratory experiments were conducted to assess the risk of introducing theharmful-algal species into new areas when bivalves previously exposed to these algae are transplanted. The selected bivalve species were exposed for two days to an artificial, harmful-algal bloom and then transferred into filtered seawater for several time periods. Fecal samples were collected, observed under the microscope for presence or absence or intact cells, and cultured to assess the potential recovery of viable propagules. Results show that, in most cases, intact, harmful-algal cells were present in the feces. These cells recovered and grew, indicating widespread risk of introducing harmful algae into areas receiving transplanted bivalve molluscs. This risk, however, can be mitigated by 24h depuration in seawater or in air for the species that can survive such emersion (Tables 1A, 1B and 2) . When bivalves are shipped, they very often undergo a long time period out of the water; thus, these results indicate that regular practices may mitigate the risk of inadvertent introductions. Simple precautions taken during transplanting and transfer can be effective safeguards.

	Alexandrium fundyense	*Alexandrium monilatum*	*Aureococcus anophagefferens*	*Heterosigma akashiwo*	*Karenia mikimotoi*	*Prorocentrum minimum*
Argopecten irradians	+			+		+
Crassostrea virginica	+	+		no feces		+
Mercenaria mercenaria	+	-		+		+
Mya arenaria	-			-		-
Mytilus edulis	+			-	+	+
Perna viridis		+				
Venerupis philippinarum					+	

	Alexandrium fundyense	*Alexandrium monilatum*	*Aureococcus anophagefferens*	*Heterosigma akashiwo*	*Karenia mikimotoi*	*Prorocentrum minimum*
Argopecten irradians	-			+		-
Crassostrea virginica	-	+		+		-
Mercenaria mercenaria	-	-		-		-
Mya arenaria	-			-		-
Mytilus edulis	-			-	-	-
Perna viridis		-				
Venerupis philippinarum					-	

Table 1 A and B. Recovery of HAB cells from the biodeposits cultured in the tubes containing FSW after 24 h (1A) and 48 h (1B) depuration in FSW (+ motile cells documented, - no motile or intact cells detected, shaded box, HAB/mollusc pair not tested)

	Heterosigma akashiwo	*Prorocentrum minimum*
Crassostrea virginica	-	-
Mercenaria mercenaria	-	-
Mytilus edulis	-	-

Table 2. Recovery of HAB cells from the biodeposits cultured in the tubes containing FSW after 24 h out of the water and 24h depuration in FSW (+ motile cells documented, - no motile or intact cells detected, shaded box, HAB/mollusc pair not tested)

DOMOIC ACID IN OCEANIC *Pseudo-nitzschia*: IS IT AN ISSUE?

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Pseudo-nitzschia is a nearly ubiquitous genus of pennate diatom that has received greatly increased attention in the last two decades due to the production of the neurotoxin domoic acid (DA) by some of its species. There is now considerable evidence of DA production in many temperate zone coastal environments, sufficient to have led to monitoring programs being instated to protect the public from DA poisoning in commercial and sometimes recreational harvests of shellfish. Interestingly, a considerable amount of the literature on DA coastal "events" is focused on contamination of vertebrate predators such as marine birds and mammals that are often the indicators of toxic blooms in these ecosystems. Thus DA is recognized as a potential toxin capable of being vectored through food chains to result in mortality of animals several trophic levels away from the alga that produced the toxin. The phenomenon of toxicity in the genus has largely been discussed as a nearshore phenomenon.

The goal of this presentation is to present background information to assess the possible production of DA in offshore, oceanic environments and to discuss some concerns about proposed environmental manipulations that may increase *Pseudo-nitzschia* there. We briefly review the state of knowledge about oceanic species of *Pseudo-nitzschia* that may be DA producers *in situ*. We then summarize published results of iron fertilization studies that have found "*Nitzschia*" or "*Pseudo-nitzschia*" to respond to such enrichments. Oceanic iron enrichments are now being widely discussed as a comparatively low cost remedy for carbon dioxide build up in the atmosphere, especially with the possibility of using them to earn "carbon credits" in the global carbon marketplace, as global warming concerns rise. We summarize results from published lab and oceanic experiments that suggest *Pseudo-nitzschia* responses to such iron enrichments and also data that suggest the genus may be particularly suited to respond to such additions. We suggest some possible food web responses that might accompany a response to iron fertilization by blooms of DA-producing *Pseudo-nitzschia*. The second, related talk in this 2-part series will describe some very recent results from a cruise to study *Pseudo-nitzschia* and domoic acid from an oceanic area that contains both naturally iron-enriched regions and ones in iron-limited "high nutrient, low chlorophyll" (HNLC) regions, the type of environment where such iron enrichments are being proposed.

SURVEY OF ALGAL TOXINS IN SOURCE AND FINISHED DRINKING WATERS

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The United States Environmental Protection placed freshwater cyanobacteria, algae and their toxins on its second drinking water Contaminant Candidate List in 2005. Contaminants on this list are considered for possible regulation in drinking water. To make a decision whether or not to regulate them, sufficient information is needed in several areas. These areas include health effects, if they can be controlled by drinking water treatment, and their occurrence in water. More information is needed on the occurrence of algal toxins in water before a regulatory decision can be made. Because there are more than 80 individual toxins or toxin congeners, and not all are equally important in waters of the United States, the USEPA created a short priority list of algal toxins based on the best estimates of their frequency in surface waters of the U.S. and their toxicity. The priority list contains five hepatotoxins, four congeners of microcystin (LR, RR, LA, YR) and cylindrospermopsin, and a neurotoxin, anatoxin-a. This project was a preliminary study of the occurrence of these priority toxins in source and finished drinking waters. Five drinking water utilities, which had a prior history of occurrence of the priority toxins, were sampled. These utilities were in California, Oklahoma, Texas, Vermont, and Florida. Samples were taken from these utilities weekly for 12 weeks between May and August, 2005. Microcystins, cylindrospermopsin, anatoxin-a and cyanobacterial counts for toxin-producers were determined in samples. Microcystin was detected in source water from 4 of the 5 utilities sampled. One of the source water samples had more total microcystins than the 1.0 ppb WHO drinking water guideline value for microcystin LR. Cylindrospermopsin was detected in one source water sample, but anatoxin-a was not detected in any source water sample. None of the toxins were detected in finished water samples. Therefore, at the levels of toxins detected in this study, drinking water treatment appeared to be effective. The detection of toxins in the source waters was not necessarily related to cell density, and highlights the need to analyze water for toxins, rather than rely solely on cell counts as an indication of potential toxin presence.

AXENIC CULTIVATION OF THE HETEROTROPHIC DINOFLAGELLATE *Pfiesteria shumwayae* ON A SEMI-DEFINED MEDIUM

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The potentially toxic dinoflagellate, *Pfiesteria shumwayae*, is an obligate heterotroph, and its abundance is influenced by the availability of suitable prey. Although *Pfiesteria* spp. feed upon various protists and fish, the nutritional requirements of these dinoflagellates are poorly known. Axenic cultivation, the growth of a species in the absence of other metabolizing cells, allows examination of biochemical requirements without the potentially confounding interactions associated with other living organisms. We developed a semi-defined, biphasic culture medium that has supported the axenic growth of three strains of *P. shumwayae* (up to 1.5 x 10^5 cells/mL) for 1.5 years, ongoing. The medium contains high concentrations of certain dissolved and particulate organic compounds, including amino acids and lipids. This culture medium will enable further investigations on the nutritional physiology of *P. shumwayae* under controlled conditions. Development of a semi-defined medium represents significant progress toward defining the nutritional requirements of this species, needed to advance understanding about its ecology in eutrophic estuaries.

DEVELOPMENT OF AN INTERNAL STANDARD FOR THE MEASUREMENT OF FREE MICROCYSTINS IN FISH TISSUE AND SEDIMENTS

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Microcystins (MCs), a class of potent liver/hepatopancreatic toxins produced by numerous species of freshwater cyanobacteria, are well known for their toxic effects on aquatic organisms. As a result, much effort has been put forth over the last decade towards developing efficient extraction methods, clean-up steps, and quantification methods as a means to evaluate exposure routes and accumulation in aquatic organisms. The recovery efficiency of MCs, however, varies greatly between studies due to the different extraction techniques that are utilized (e.g., methanol vs. EDTA) and the matrix being extracted (e.g., fish tissue vs. sediment). We developed a unique internal standard, with a mass different than any known MCs, which can be spiked into field samples and quantified via LC-MS along with natural MCs already present in the sample. The new compound, a derivative of microcystin-LR, has been modified at the Mdha residue (the site of covalent binding with target molecules), therefore providing an accurate measure of only free MCs, the form thought to be most bioavailable. The internal standard and microcystin-LR chromatograph together and interact with the matrices in a similar manner based on dual spike experiments. Examples of the internal standard being used with both fish tissue and sediment field samples will be presented.

SMALL SCALE BLOOM DYNAMICS OF RAPHIDOPHYTE AND DINOFLAGELLATE POPULATIONS IN KING HARBOR, CALIFORNIA

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King Harbor in the City of Redondo Beach, California was the site of massive fish kills during 2005 following intense and prolonged red tide events. Weekly monitoring since early 2006 revealed the presence of an abundant and diverse community of dinoflagellate and raphidophyte species in the harbor with highly heterogeneous spatial and temporal distributions. Vertical migration and photoacclimation of dinoflagellates and raphidophytes were investigated as mechanisms for dealing with changing light levels in the King Harbor marina over a 24-hour cycle on 19-20 June 2007. PAR, CTD, chlorophyll fluorescence, dissolved oxygen concentrations, active chlorophyll fluorescence, backscattering, and light absorption and attenuation data were measured every four hours using sensor arrays. Discrete water samples were analyzed for pigment concentrations, particulate and dissolved inorganic nutrients, and phytoplankton community composition using both microscopical and molecular techniques. The overall phytoplankton community composition changed significantly during the 24-hour cycle, from a raphidophyte-dominated community (mainly *Heterosigma akashiwo*) to a mixed community of dinoflagellates (*Prorocentrum*, *Ceratium*, and gymnodinoids), raphidophytes and diatoms. A shallow subsurface chlorophyll maximum at 1-2 m was observed during the day, but was located near the bottom (3.5 m) in the evening, with the remainder of the phytoplankton biomass more evenly distributed vertically in the middle of the night. The raphidophyte and dinoflagellate populations tended to constitute the daytime subsurface maxima, whereas the less abundant diatoms were more evenly distributed throughout the water column at all time points. These data suggest a high degree of small-scale heterogeneity in vertical distribution of harmful algal populations and provide important insights into mechanisms that impact community composition within red tide assemblages in King Harbor.

RAPID ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF DOMOIC ACID

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Domoic acid (DA) is a potent toxin produced by bloom-forming phytoplankton in the genus *Pseudo-nitzchia* and is responsible for causing amnesic shellfish poisoning (ASP) in humans. ASP symptoms include vomiting, diarrhea, and in more severe cases confusion, memory loss, disorientation and even death. This paper describes the development and validation of a rapid sensitive enzyme linked immunosorbent assay (ELISA) for detecting DA. The assay gives equivalent results to those obtained using standard HPLC and FMOC-HPLC methods. It has a detection range from 0.1 to 3 ppb and can successfully measure DA in razor clams, mussels and phytoplankton. Correlation of ELISA results versus FMOC-HPLC over a broad range of concentrations in razor clam extracts is shown below in Figure 1. Extracts containing high concentrations of DA are quantitated by appropriate dilution. Up to thirty six sample extracts can be analyzed simultaneously in approximately 1.5 hours. The use of a monoclonal antibody and an internal control eliminates the need to run standard curves with each assay. Concentrations of DA are calculated using a predetermined calibration curve specific for this ELISA. The assay uses eight well strips to enable as few as three samples to be analyzed at a time. This eliminates the habit of delaying analysis until a large number of samples are accumulated, thereby saving time and reducing some anomalies that can occur during storage. The ELISA is specific for DA and does not cross-react significantly with glutamate, kainic acid, epi-DA or iso-DA. The relatively low cost, sensitivity, and rapid analysis time provided by this assay make it useful to environmental managers and public health officials for monitoring DA concentrations in environment samples.

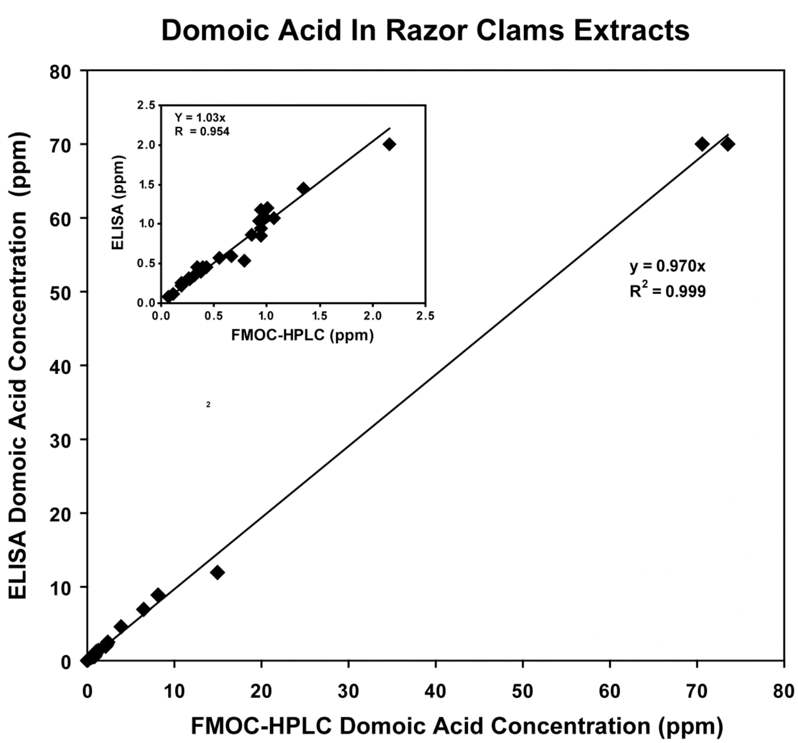

Figure 1. Domoic acid concentrations in razor clam tissues determined from replicate tissue extracts using HPLC and ELISA. The insert shows an expanded version of the regression analysis for sample containing less than 2.5 ppm domoic acid.

NEW SATELLITE DATA PRODUCTS FOR THE DETECTION OF OREGON HABS

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With funding from NOAA's Oceans and Human Health Initiative, we have established a collaborative team to understand and better predict the occurrence of HABs in Oregon coastal waters. Our approach is to use satellite and *in situ* data to further our knowledge of the oceanographic conditions that lead to HABs and their interaction with the coast. The principal phytoplankton genera of interest are *Pseudonitzschia* and *Alexandrium* which can cause domoic acid or saxitoxin accumulation (respectively) in coastal shellfish, primarily razor clams. The Oregon Department of Agriculture (ODA) monitors coastal shellfish for phycotoxins, with a view to protecting public health. This database is most comprehensive for both toxins from about 1998, which corresponds to the era of the SeaWiFS ocean color satellite. NOAA CoastWatch has begun routine dissemination of a chlorophyll anomaly product that can be used to document recent increases or decreases in chlorophyll. This can be used to identify the genesis or demise of blooms, but can not yet definitively identify toxic blooms due to the lack of a known optical signal for *Pseudonitzschia* or *Alexandrium*. However, with the inclusion of ancillary data and careful *in situ* monitoring, we believe these new satellite products will enhance coastal management and human health protection. This work evaluates the efficacy of this new product for detecting blooms, and provides examples of combining the anomaly product with physical oceanographic data and coastal wind data to predict the interaction of blooms with coastal shellfish. We present examples of successful and unsuccessful HAB detection and suggest modifications for more effective monitoring and prediction.

Dinophysis SPECIES AND DIARRHETIC SHELLFISH TOXINS IN MONTEREY BAY, CA

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In 2000, water samples from Monterey Bay, California that were enriched with species of *Dinophysis* tested positive for toxins responsible for Diarrhetic Shellfish Poisoning (DSP) (unpublished data, R. Weber and T.Yasumoto). No shellfish samples were taken concurrently to show the extent to which the toxins were being accumulated, and shellfish have not been tested for DSP in Monterey Bay or California to date, to our knowledge, other than the testing reported here. *Dinophysis,* including species known to produce DSP elsewhere, however, have been recognized for many decades in this region and, indeed, throughout much of California. Our goal was to determine the annual cycle for the local dominant *Dinophysis* species, *D. acuminata* and *D. fortii*, and to discover whether there is a correlation between their abundance and DSP toxins in CA mussels, *Mytilus californianus,* in the bay. We therefore collected weekly water and mussel samples for 16 months at the Santa Cruz Municipal Wharf in Monterey Bay, and measured *Dinophysis* cell densities in our lab and sent the mussel samples (hepatopancreas) to the Canadian Food Inspection Agency (CFIS) in Dartmouth, Nova Scotia for DSP toxin analysis. For the former we counted cells in 100 ml samples using standard Utermöhl techniques and for the latter, the Canadian Agency used LC-MS to detect the suite of shellfish toxins associated with DSP.

We found a significant association between *Dinophysis* abundance and DSP toxins in mussels. Peak densities of *D. acuminata* and *D. fortii* occurred during the summer months when the majority of DSP toxins were detected in the CA mussel samples. A significant correlation ($p < 0.01$) between *D. fortii* cell numbers and okadaic acid (OA) concentrations in mussels during the 2004-2005 sampling period indicates this species may be the OA source. The correlation coefficient weakened with the addition of *D. acuminata*, suggesting that *D. acuminata* may have little to no role in OA production in Monterey Bay. Since DSP toxins are lipophilic, we correlated toxin levels in mussels not just with the cell densities on the week the mussels were harvested but also with densities averaged over the prior several weeks. Results of these correlations indicate toxins in mussels were mostly strongly related to cell averages obtained over the prior several weeks, rather than on the week of collection. Although none of the CA mussel samples contained toxin levels that exceed the regulatory limit set by Canada, (1.0 $\mu g\ g^{-1}$ of digestive gland for any combination of OA/DTX-1), DSP may potentially be a health threat during peak *Dinophysis* events in Monterey Bay, and possibly more broadly in California. In California, recreational harvesting of mussels is prohibited from spring through fall because of the danger of saxitoxin (STX) contamination at this time, which is the anticipated time that DSP toxin levels could be highest in mussels, though commercial harvesting is not similarly prohibited. Instead, for the commercial harvest, mussels are always tested for STX to be sure that the product does not exceed mandated safety limits for STX concentration, a practice that may need to be considered for DSP toxins, given the results presented here.

CHARACTERIZATION, DYNAMICS, AND ECOLOGICAL IMPACTS OF HARMFUL *Cochlodinium polykrikoides* BLOOMS ON EASTERN LONG ISLAND, NY, USA

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We report on the emergence of *Cochlodinium polykrikoides* blooms in the Peconic Estuary and Shinnecock Estuary, NY, USA, during 2004 - 2006. Blooms occurred during late summer when temperatures and salinities ranged from 20-25°C and 22-30 ppt, respectively. Bloom patches achieved cell densities exceeding 10^5 ml^{-1} and chlorophyll *a* levels exceeding 100 μg L^{-1}, while background bloom densities were typically 10^3 - 10^4 cells ml^{-1}. Light, scanning electron and ultrathin-section transmission electron microscopy suggested that cells isolated from blooms displayed characteristics of *C. polykrikoides* and provide the first clear documentation of the fine structure for this species. Sequencing of a hypervariable region of the large subunit rDNA confirmed this finding, displaying 100% similarity to other North American *C. polykrikoides* strains, but a lower similarity to strains from Southeast Asia (88-90%). Bioassay experiments demonstrated that 24 h exposure to bloom waters (> 5 x 10^4 cells ml^{-1}) killed 100% of multiple fish species (1-week old *Cyprinodon variegates*, adult *Fundulus majalis,* adult *Menidia menidia*) and 80% of adult *Fundulus heteroclitus*. Microscopic evaluation of the gills of moribund fish revealed epithelial proliferation with focal areas of fusion of gill lamellae, suggesting impairment of gill function (e.g. respiration, nitrogen excretion, ion balance). Lower fish mortality was observed at intermediate *C. polykrikoides* densities (10^3 - 10^4 cells ml^{-1}), while all fish survived for 48 hr at cell densities below 1 x 10^3 cells ml^{-1}. The inability of frozen and thawed-, or filtered (0.2 μm)-bloom water to cause fish mortality suggested that the thick polysaccharide layer associated with cell membranes and/or a toxin principle within this layer may be responsible for fish mortality. Juvenile bay scallops (*Argopecten irradians*) and American oysters (*Crassostrea virginica*) experienced elevated mortality compared to control treatments during a nine-day exposure to bloom water (~5 x 10^4 cells ml^{-1}). Surviving scallops exposed to bloom water also experienced significantly reduced growth rates. Moribund shellfish displayed hyperplasia, hemorrhaging, squamation, and apoptosis in gill and digestive tissues with gill inflammation specifically associated with areas containing *C. polykrikoides* cells. In summary, our results indicate *C. polykrikoides* blooms have become annual events on eastern Long Island and that bloom waters are capable of causing rapid mortality in multiple species of finfish and shellfish. Results regarding sequences of large subunit rDNA from other North American *C. polykrikoides* strains and the general characterization of North American *C. polykrikoides* clones compared to Asian clones will also be presented.

THREE YEAR ASSESSMENT OF CYANOHAB FORECASTING ON THE TIDEWATERS OF THE POTOMAC RIVER, CHESAPEAKE BAY, USA

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Cyanobacteria (blue-green algae) blooms have been one of the most frequently occurring toxic plankton blooms that present living resource and human health risks in the tidal Chesapeake Bay. Sufficient resolution of water quality monitoring data was available from 1985-2004 on the Potomac River to explore environmental event patterns and assess predictive power for blooms of toxigenic *Microcystis aeruginosa*. In 2005, the first Harmful Algal Bloom forecast for the Potomac River was generated as one of several ecological forecasting communication tools regarding important Bay phenomena. Using a categorical model, the occurrence of bloom activity was predicted months ahead of the actual events. Levels of success are more variable when predicting important characteristics of the blooms (e.g., time of bloom onset, seasonal bloom duration and amount of area coverage or distance in stream miles for a bloom event) leaving ample opportunity for enhancement of the model and regions of application. Model and monitoring results from 2005-2007 are compared and a lag-time regression model of pre-season flows versus bloom detection rates is evaluated in this presentation.

HEMOLYTIC ACTIVITY OF SELECTED HAB FLAGELLATES: A TOXIN COMPLEX STRATEGY

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Selected dinoflagellates consisting of *Amphidinium carterae*, *Prorocentrum minimum*, *P. mexicanum, Karenia brevis* and *Gyrodinium instriatum* as well *as Prymnesium parvum* and the raphidophytes, *Heterosigma akashiwo, Chattonella subsalsa, C. marina, C. antiqua* and *Fibrocapsa japonica* cultures were tested with the Erythrocyte Lysis Assay (ELA) for hemolytic activity. Whole cultures, cell pellets and supernatants were tested with osmotically adjusted samples to determine the effect on lysis of human erythrocytes. The highest ELA values consistently were found in the cell pellet followed by the supernatant fractions. *Prymnesium parvum* had the highest activity with log phase cells nearly having 100% lysis of the saponin control and surpassing equivalent samples of other flagellates having similar chlorophyll biomass. Elevated activity of 76-77% of controls was found with *Heterosigma akashiwo,* 36-57% for *Gyrodinium instriatum* and *Prorocentrum mexicanum*, 24-36% for two clones of *Karenia brevis,* 27-32% for *Amphidinium carterae* and 21-27% for *Fibrocapsa japonica*. Modest activity ranging from 9-21% was found for three *Chattonella* species. No activity was detected for *Prorocentrum minimum*. These results suggest that hemolytic activity is more common among HAB species than previously thought and that this aspect of toxicity may play an important role in the concept of "toxin complex or cocktail" acting in synergy with other toxins. Studies involving the evaluation of toxic virulence of HAB species should therefore take this synergy factor into consideration. In addition, single toxin estimations measured on a cellular basis may underestimate the toxic effects of the complex normally found in these cells. The influence of nutrient stress on hemolytic activity is presently being assessed for the different species.

THE IMPORTANCE OF SURFACE CURRENTS IN HIGH PSP TOXICITY IN LUMBOS HOLE, HARPSWELL SOUND, MAINE

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Over many years, weekly toxicity sampling by the Maine Department of Marine Resources has shown that the Lumbos Hole location in Harpswell Sound is an indicator site that signals through timing and PSP toxicity levels the imminent onset of *Alexandrium fundyense* HABs elsewhere in the western Gulf of Maine. One model for this behavior is that surface currents transport active blooms or viable cysts of *Alexandrium* into Harpswell Sound, or alternatively, that downwelling currents retain developing *Alexandrium* blooms in a favorable environment. In 2007 toxicities in Harpswell Sound began to rise in late April and peaked in late May. Surface currents measured at an oceanographic buoy moored in Harpswell Sound show that the mean residual currents have been almost entirely into Harpswell Sound since mid-February. Preliminary analyses of weekly plankton samples taken in Harpswell Sound indicate that the numbers of *Alexandrium fundyense* began to rise in late April, with significant increases in May. Chlorophyll signals from the oceanographic buoy show seasonal development of the phytoplankton spring bloom, and also higher chlorophyll at the head of the Sound, passing the buoy at ebb tides, and lower levels of chlorophyll from open coastal water moving past in flood tide. During certain tidal phases, surface currents flow towards the head of the Sound continuously, varying in speed but not direction, regardless of flood or ebb cycle. The reverse estuarine flow apparent in Harpswell Sound retains the near-surface populations of *Alexandrium*, which may be derived from local cysts that are spatially variable but of sufficient density to initiate blooms (see Laine *et al*. this conference). Advection of *Alexandrium* cells from outside Harpswell Sound into the Sound, with subsequent retention, is also possible, but not necessary for bloom development in this indicator region. Closures of shellfish beds for PSP contamination indicate toxicity in the Harpswell Sound region, but not in adjacent bays, in late May/early June, suggesting that local conditions govern bloom development and subsequent toxicity. Shellfish toxicity was also apparent to the southwest, near the New Hampshire border, likely derived from offshore *Alexandrium* populations advected southward by the western Maine coastal current (Anderson *et al*. 2005). The distinct spatial character of the two types of closures suggests different mechanisms are probable for bloom development in the Casco Bay region of the western Gulf of Maine. A better understanding of these mechanisms would benefit managers who rely on indicator sites such as Lumbos Hole to inform their monitoring.

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THE EFFECT OF SALINITY ON DOMOIC ACID PRODUCTION BY THE DIATOM *Pseudo-nitzschia multiseries*

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Domoic acid (DA) is a potent algal neurotoxin produced primarily by members of the diatom genus *Pseudo-nitzschia*, most of which are considered cosmopolites and can produce harmful blooms in estuarine and coastal waters. Many of these habitats are subject to extreme fluctuations in salinity and are utilized extensively as shellfish growing/harvesting areas. Knowledge of how salinity influences DA production is essential to evaluating and ultimately predicting the potential impact of *Pseudo-nitzschia* blooms on shellfish resources as well as various wildlife populations (e.g., marine mammals). Herein, we examine the effect of different salinities (10, 20, 30, 40 psu) on the growth and DA production rates of *P. multiseries*. Specific growth rates remained maximal (~0.9 d^{-1}) and essentially unchanged at the three highest salinities tested, but decreased by about half at 10 psu (~0.4 d^{-1}). By comparison, total (particulate and dissolved) DA quotas (~30 fmol DA $cell^{-1}$) and toxin production rates (~12 fmol DA $cell^{-1}day^{-1}$) were similar and maximal at 30 and 40 psu, yet both declined significantly (three- to seven-fold) once adapted to 10 and 20 psu. These results suggest that *P. multiseries* is able to maintain a high growth rate at 20 psu, but at the expense of continuing to produce DA at elevated levels. Since DA production requires a supply of bioenergetic metabolites generated by photosynthesis, we propose that the additional energy needed to grow rapidly while maintaining an osmotic balance at a likely sub-optimal salinity of 20 psu reduces that available for toxin synthesis, leading to the observed decline in DA levels. Our findings suggest that DA levels should be greatest in higher salinity coastal waters versus estuaries, which is consistent with recent field observations along the Louisiana coast and may help to explain the lack of DA outbreaks in this and other low salinity estuarine-based shellfish harvesting areas.

GERMINATION OF *Alexandrium catenella* CYSTS FROM SURFACE SEDIMENTS IN QUARTERMASTER HARBOR, WA

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Paralytic shellfish poisoning (PSP) has a long-term history in the Pacific Northwest of causing regulatory closures of shellfish harvesting. Occurrences of these toxic outbreaks within the Puget Sound have been documented as increasing over the last four decades, however little is known about the biology of the causative organism, *Alexandrium catenella*.

A. catenella has a dual-stage life cycle consisting of motile vegetative cells in the water column and cysts that rest in the sediments. Cyst formation may be an advantageous strategy that promotes survival when conditions in the water no longer support growth. The formation of cysts is thought to be influential in bloom dynamics, but the transitions into and out of the cyst phase are poorly understood. Previous studies have reported a wide range in dormancy period durations from one week to over three months for *A. catenella* from geographically distinct regions (Joyce and Pitcher 2006, Figueroa et al. 2005). This emphasizes the need to determine specific cyst dynamics for geographically distinct *A. catenella* populations

A survey of *A. catenella* cysts in the Puget Sound, funded by NOAA ECOHAB, has found that of 32 locations sampled, Quartermaster Harbor, in south Puget Sound, has the highest concentrations.
Preliminary studies from this project found that *A. catenella* cysts collected from different sites in the Puget Sound (including Quartermaster Harbor) in March and incubated in April and May germinated in approximately 3-6 days. Light was required for germination and the highest excystment occurred around 14˚C (Hoffer et al. 2005). A later study from sediment samples collected in January 2006 found no germination to occur in January and February.

In our study, *A. catenella* cysts collected from surface sediments in Quartermaster Harbor in October of 2006 and maintained in the dark at 4˚C are being used in a time-series germination experiment to evaluate the duration of the dormancy period of Puget Sound *A. catenella* populations. Well plates containing sediments and f/2 medium have been set up new each month for one year from this sediment sample. The well plates are incubated at 13˚C under a 12:12 L:D period and monitored daily for the first week of incubation, and once a week there after for the appearance of motile cells. Preliminary observations have revealed that germination occurs in all months from November through June. Motile cells are not observed on set-up, but germination can occur within 24 hours of being restored to conditions that support growth. These findings differ from the earlier observations of Hoffer et al. (2005). This suggests that cyst germination in the Puget Sound population may occur more rapidly than previously thought. In the next phase of this study, total cyst abundance in sediment sub-samples will be determined using epifluorescence microscopy after sonicating and staining with Primuline (Yamaguchi et al. 1995). Cyst counts will be compared to the number of motile cells observed in fixed well samples to evaluate germination potential.

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THE DEVELOPMENT OF A BEACH IMPACT MODEL FOR FLORIDA *Karenia brevis* BLOOMS

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Since October 2004, the National Oceanic and Atmospheric Administration has provided operational forecasts of beach impacts to the state of Florida in response to recurrent *Karenia brevis* blooms. *Karenia brevis* produces brevetoxin, an aerosolized toxin which causes respiratory irritation in humans. Forecasts provide information as to the likelihood of beach impacts, on a half-county basis. The rule-based model currently being used contains the following: (1) proximity of the bloom to the coast, (2) transport of the bloom, (3) concentration of cells in the water, and (4) wind speed and direction. The resolution of impact forecasts is coarse, providing information daily on a half county basis. Therefore, our current system is capable of predicting whether an impact, and at what level, will occur anywhere within approximately 30 km of coastline on a particular day. However, the forecast lacks the resolution to provide detailed information for a particular beach. In August 2006, professional lifeguards in Sarasota and Manatee Counties began providing real-time respiratory impact information twice a day. These data combined with meteorological measurements are being used to develop a finer resolution respiratory impact model, with the goal of providing more accurate estimates of beach impact, on a twice daily basis.

ECOHAB PACIFIC NORTHWEST (ECOHAB PNW) OUTREACH: OPENING THE SCIENTIFIC JOURNEY TO THE WORLD

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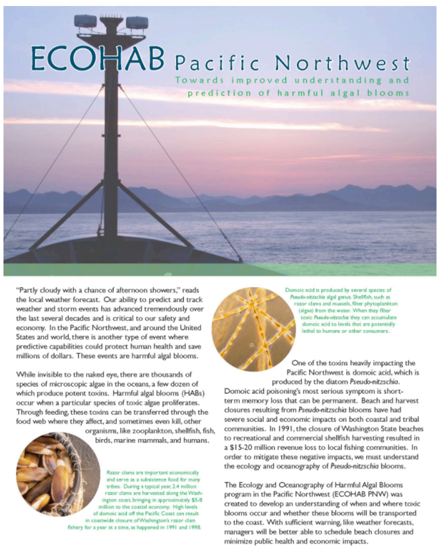

Public and education outreach has been an integral part of the ECOHAB Pacific Northwest project from its inception in 2002. Public outreach activities include a project-dedicated web site, public contact, and multiple interactions with journalists over the course of the study. Educational activities have included participation at in-service teacher workshops, and hosting four teachers-at-sea on research cruises through: 1. the NSF Research Experience for Teachers (RET), 2. the West Coast Center for Oceans and Human Health, 3. the NSF ARMADA program, 4. individual NSF research grants, and 5. the NOAA Hollings undergraduate summer scholarship program. Real-time cruise journals targeting high school level students and the general public were created and maintained while at sea. These journals detailed the scientific journey of oceanographic research, and in particular the challenges investigating harmful algal blooms, collaborative research activities, and human-interest aspects of life at sea. During our final cruise in September 2006, as a collaboration of undergraduate, and graduate students, principal investigators, and Evil Bunny Films, two documentary films (10- and 20-minutes) were produced. These films detail the scientific problem, the collaborative approach to oceanographic research, and the vision for the future of forecasting of harmful algal blooms in the Pacific Northwest. The 20-minute film is targeted for both secondary and undergraduate audiences and details the complexity of science at sea. It is accompanied by the "Harmful Algal Bloom Hunter's Handbook", a series of lesson plans consisting of classroom activities and experiments, cruise journals, a plankton identification chart and a glossary. These outreach materials will be presented, and the films displayed at the poster session during the 4th US HAB Symposium. See also: www.ecohabpnw.org/outreach

Pseudo-nitzschia GROWTH AND TOXIN PRODUCTION IN THE JUAN DE FUCA EDDY IN THE PACIFIC NORTHWEST – ENVIRONMENTAL STIMULATORS OF A TOXIC BLOOM

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As part of the ECOHAB-PNW project to study the community formation, domoic acid (DA) toxicity and transport of *Pseudo-nitzschia* cells from the shelf waters of Washington State and British Columbia to the shoreline, we examined spatial distribution of key HAB parameters: total phytoplankton biomass, contribution of *Pseudo-nitzschia* species, and absolute and cell specific DA concentrations. In September 2003 and September 2004, we used deck-board incubation "grow-out" experiments to evaluate if the natural phytoplankton community could be stimulated with modifications of the water chemistry to either increase the biomass further, increase the relative frequency of *Pseudo-nitzschia,* increase the physiological capacity of the community (P vs. E), or increase the cell-specific or community integrated toxicity due to DA. While there were variations in the community responses to macronutrient additions, cells from several specific water masses responded primarily to nanomolar additions of iron, or to the addition of a nanomolar mixture of iron and copper. We assessed the stimulation of the HAB component of the community physiology by the extent of enhanced growth of *Pseudo-nitzschia*, the cellular and dissolved levels of DA, the recovery of the photosynthetic capacity of the phytoplankton, and to a less frequent extent, alterations in the buoyancy/sinking trend of the phytoplankton. Yearly variation in the late summer phytoplankton communities indicates that there is a complex regional regulation of phytoplankton community rich in *Pseudo-nitzschia*, but the commonly implicated macronutrient concentrations are poor predictors of either *Pseudo-nitzschia* dominance or toxicity. Rather, micronutrient additions of iron and copper stimulated the dominance, toxicity and physiological health in large areas of the Juan de Fuca eddy region and implicate spatial variation in trace metals as the prime proponent of toxicity in these waters.

CHARACTERIZATION OF NOVEL COMPOUNDS FOUND IN *Karenia brevis* CULTURES

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Florida red tides occur in the Gulf of Mexico almost annually as the result of blooms of the unarmored marine dinoflagellate *Karenia brevis*. *K. brevis* is best known for the production of a family of polyether neurotoxins known as brevetoxins. Brevetoxins are compounds found in the organic phase (chloroform layer) of the liquid/liquid extraction of *K. brevis* cultures. Additional compounds produced by *K. brevis* include brevenal, brevisin, brevisamide, hemibrevetoxin B (Prasad and Shimizu, 1989), as well as fatty acids and phytopigments. Although the chloroform layer has been extensively mined for bioactive components, very little emphasis has been put on exploration of the more polar and non-polar components of the *K. brevis* cultures. This project focuses on the petroleum ether (non-polar) layer and the water (polar) layer of *K. brevis* culture. Methods for this project include liquid/liquid extraction, solid phase extraction, LC/MS, bioassay guided fractionation, chromatography techniques, and NMR. The various bioassays used to determine active compounds include antibacterial assays, antifungal assays, cytotoxicity assays, receptor binding assays, and fish bioassays. Previous work with *K. brevis* cultures, or samples from natural blooms of *Karenia* in Gulf Coast waters, have identified polar brevetoxin metabolites related structurally to known A and B type brevetoxins, in which the lactone ring of the brevetoxin backbone structure is open (Abraham et al., 2006). If these brevetoxin metabolites are found, along with any other compounds, they will provide useful standards for analytical work and tools for biological research. Results from this project will help to better characterize *K. brevis* blooms and their associated physiological effects, as any compound produced by *Karenia* has the potential to effect humans and animals. Any new bioactive components found may add to the growing list of marine natural products used as new drug candidates and will be evaluated for physiological effects and contributions to red tides. Preliminary work has determined a method to separate the non-polar petroleum ether layer of *Karenia* culture and has identified both cytotoxic (masses: (m+1)=413, (m+1)=801, (m+1)=871) and antibacterial (masses: (m+1)=373, (m+1)=618) compounds. A method for separating the polar water layer of *Karenia* culture has been determined and has yielded novel cytotoxic compounds with masses of (m+1)=237, (m+1)=251, and (m+1)=546.

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THE RELATIONSHIP BETWEEN COASTAL OCEAN DYNAMICS AND SHELLFISH CLOSURES: A SATELLITE BASED STUDY OF OREGON HABS

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Toxic blooms of Pseudonitzschia or Alexandrium in Oregon coastal waters can lead to accumulation of domoic acid or saxitoxin (respectively) in coastal shellfish, primarily razor clams. Since 1979, the Oregon Department of Agriculture (ODA) has monitored coastal shellfish for phycotoxins, with a view to protecting public health. This database is most comprehensive for both toxins from about 1998, which conveniently corresponds to the era of the SeaWiFS ocean color satellite. This work analyzes seasonal and interannual patterns in the toxin database in conjunction with satellite data. Using satellite chlorophyll data from SeaWiFS, sea surface temperature data from NOAA AVHRR satellites and several wind products (satellite, in situ and an upwelling index) we have quantified the seasonal and interannual variability in coastal ocean physics and biology. There are at least three features which significantly impact the coastal ocean and the frequency of HAB impacts: The Columbia River outflow, Heceta Bank and Cape Blanco. The Columbia and Heceta Bank regions are characterized by a broader near-coastal band of high productivity and more frequent shellfish closures, particularly for domoic acid. Cape Blanco is associated with a 'break' in the patterns of coastal winds and currents. The physics and biology to the north and south of the cape differ in phase and magnitude, with concomitant impacts on HABs and shellfish contamination. At all locations there is significant interannual variability in the timing of HAB events, driven mainly by the major transitions to upwelling and downwelling conditions. The results of these analyses enable us to make generalizations about the likelihood of HABs and shellfish contamination within subregions of the Oregon coast, which in turn increases the efficacy of monitoring programs.

IDENTIFICATION AND ENUMERATION OF *Pseudo-nitzschia* IN PACIFIC NORTHWEST COASTAL WATERS USING THE FLOWCAM® CONTINUOUS IMAGING PARTICLE ANALYZER

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Toxigenic blooms of *Pseudo-nitzschia* are becoming increasingly apparent recently in the Puget Sound waters of Washington State. Until recently, the primary impact has been on the razor clam and Dungeness crab fisheries on the outer coast of the state causing recreational, commercial and tribal subsistence closures on a routine basis. Early detection and rapid screening methodologies are an important component of understanding and mitigating for these bloom events. In this preliminary investigation we focused on the development of a methodology using the FlowCAM for quickly identifying *Pseudo-nitzschia* in the waters of Sequim Bay Washington, a coastal embayment that has recently been plagued with *Pseudo-nitzschia* blooms. The FlowCAM® couples the capabilities of a flow cytometer with a digital-imaging microscope to automate phytoplankton detection and enumeration. For this study, we collected weekly samples during the summer months at the mouth of Sequim Bay on an incoming and outgoing tide. During this time a bloom of *Pseudo-nitzschia* occured which provided the opportunity to explore possible methods of identifying *Pseudo-nitzschia* in natural phytoplankton assemblages using the FlowCAM, with a particular emphasis on identifying bloom conditions. A variety of parameters were examined including length, width, aspect ratio, area based diameter, equivalent spherical diameter, transparency, perimeter, etc…. Based on statistical analysis of these particles, only the aspect ratio was consistent and unique for *Pseudo-nitzschia* during this particular bloom event. Further research is needed to determine the consistency of these results under other bloom conditions and locations with varied community composition. However, these results show promising for rapid detection and screening of phytoplankton samples for *Pseudo-nitzschia* in Pacific Northwest coastal waters.

EFFECT OF CADMIUM, COPPER, NICKEL, AND ZINC ON A MINUTE GOLDEN BROWN ALGA *Aureoccoccus anophagefferens*

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The project focuses on the harmful algal blooms. *Aureococcus anophagefferens* is a toxic pelagophyte observed in the northeast of US. The present study examines the effect of Cd, Cu, Ni and Zn on *A. anophagefferens*. Two ranges of free metal concentrations are studied for accumulation and toxicity of metals. For the low free metal concentration (10^{-12} M - 10^{-9} M for Cd, Cu and Ni, 10^{-12} M - 10^{-8}M for Zn), *A. anophagefferens* is grown in Aquil and the growth kinetics studies are carried out. The free metal concentrations are controlled by adding 100 or 10 μM EDTA and are calculated by MINEQL. Thus, the optimal and toxic free metal concentration for *A. anophagefferens*'s growth and its growth rate are obtained. For the high free metal concentration (10^{-10}M - 10^{-5} for Cd, 10^{-10} M - 10^{-8} for Cu, 10^{-10} M - 10^{-5} for Zn, and 10^{-10} M - 10^{-6} for Ni), *A. anophagefferens* cells are subject to short term exposure (30 min to 24 hr) of single metal in synthetic ocean water (SOW, pH = 8.1, ion strength = 0.7, no EDTA addition). Metal accumulation by cells is determined by inductively coupled plasma mass spectrometry (ICP-MS) upon rinse with SOW-EDTA solution. Therefore, the cellular and intracellular metal fractions, metal uptake, and the affinity of each metal for *A. anophagefferens* are being investigated. Based on these results, the effects of co-contaminants as well as mechanistic uptake of metals on *A. anophagefferens* will be studied.

GROWTH RESPONSE AND GLUTATHIONE PRODUCTION OF BROWN TIDE BLOOM ALGA (*Aureococcus anophagefferens*, CCMP 1984) UPON DIFFERENT SALINITY, METALS, NITROGEN SOURCE, SEWAGE AND HERBICIDE METOLACHLOR EXPOSURE: LAB CULTURE STUDIES AND IN SITU INCUBATION STUDIES

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It is well known that N, P and trace metals strongly affect phytoplankton growth. Brown tide bloom is caused by the overgrowth of pelagophyte *Aureococcus anophagefferens*. Organic nitrogen source, metals, salinity seems to affect bloom formation. We study how environmental factors - salinity, sewage overflow, herbicide metolachlor and trace metals affect the growth and cellular redox status of *A. anophagefferens*. We report the growth and cellular glutathione levels of *A. anophagefferens* (CCMP 1984) upon long term exposure to different salinities, herbicide metolachlor and sewage overflow in artificial sweater media Aquil or L1. We also conduct *in situ* incubations of *A. anophagefferens* in natural seawater, where lab cultured *A. anophagefferens* are inoculated to natural seawater collected from Barnegat Bay-Little Egg Harbor, and Newark Bay, NJ, with amended nitrate, phosphate, Fe, Zn, Cu, glycine, urea, sewage, and herbicide metolachlor, and are incubated in Newark Bay NJ during July 2007. *A. anophagefferens* cells in the *in situ* incubations are monitored overtime by monoclonal antibody according to Caron et al., 2003. Together we hope to find out the controlling factors toward brown tide bloom initiation.

STUDIES ON THE ENZYMATIC HYDROLYSIS OF DSP ESTERS TO PRODUCE THE TOXIN, OKADAIC ACID, IN THREE STRAINS OF *Prorocentrum lima*

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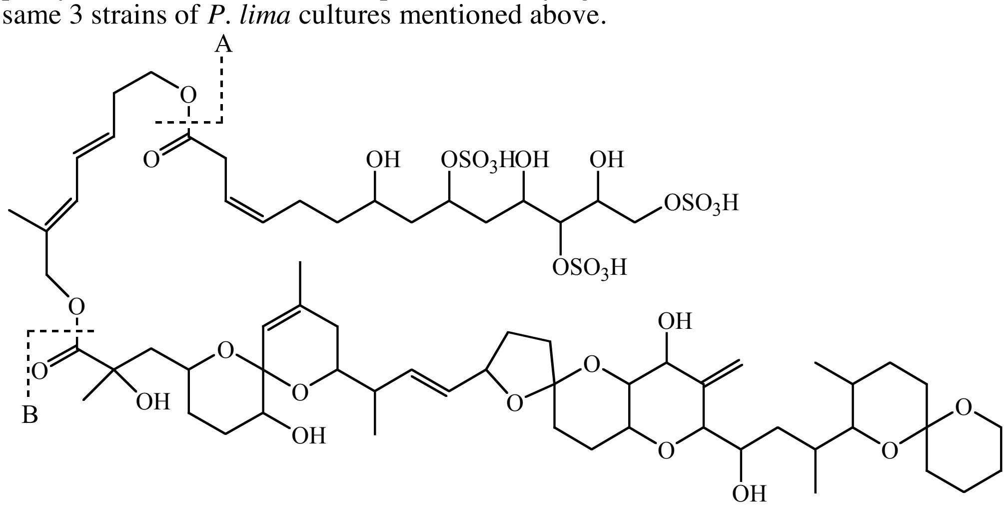
Diarrhetic shellfish poisoning (DSP) is a human gastrointestinal disease that is caused by the consumption of shellfish contaminated by okadaic acid (OA) or one of the OA isomers, dinophysistoxin-1 (DTX-1) and -2 (DTX-2) (Windust et al., 1997). DSP toxins are polyether fatty acids that are all structurally similar and vary only by the number of pendant methyl groups attached to the main chain (Murata, M. et al., 1985). DSP toxins are produced by dinoflagellates belonging to the genera *Dinophysis and Prorocentrum* (Souto et al., 2001), and enter the food chain when filter-feeding bivalves ingest them. Symptoms of DSP include diarrhea, nausea, vomiting, abdominal pain and chills (Morton et al., 1996). DSP poses serious impacts on public health and the aquaculture industry worldwide (Souto et al., 2001). The focus of this research was to examine the hypothesis that in the dinoflagellate, *Prorocentrum lima*, DSP toxins are stored in the biologically inactive form within the cell as water-soluble sulfated diesters (e.g. DTX-4 and DTX-5), and that these storage products can be hydrolyzed via an esterase or lipase to OA diol-ester and OA upon rupture or attack of the *Prorocentrum* cell. As exhibited in the figure below, hydrolysis of DTX-4 at "A" yields the pro-toxin OA diol-ester; hydrolysis of DTX-4 at "B" yields OA itself. Cells and cell medium from 3 different strains of *P. lima* cultures (CMS TAC PL 010, CCMP 1743 NS, and CCMP 1746 Bz) were examined for the presence of an esterase or lipase that specifically converts the DSP derivatives (e.g. DTX-4 and OA diol-ester) to OA. Through the employment of a surrogate substrate, hydrolytic activity was followed throughout the duration of growth in both the cells and cell medium of *P. lima* cultures. This hydrolytic activity was then correlated with the concentration of OA measured throughout the growth period in both the cells and cell medium of *P. lima* cultures. Additionally, the purity of the hydrolytic enzyme was increased through several chromatography steps utilizing a surrogate substrate to guide the purification process. The hydrolytic enzyme of increased purity was then monitored for specific activity against the natural DSP ester substrates isolated from the same 3 strains of *P. lima* cultures mentioned above.



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VARIABLE EXPRESSION OF TOXICITY IN *Prorocentrum minimum*, AND POSSIBLE RELATIONSHIPS WITH TROPHIC STATUS

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Variable toxicity in harmful microalgal species has been long recognized but not thoroughly evaluated in evolutionary and ecosystem contexts. Recent findings suggest that expression of toxicity in several Harmful Algal Bloom (HAB) species represents an active survival strategy. For example, toxicity in *Alexandrium minutum* is induced by the presence of copepod grazers (Selander et al. 2006). *Dinophysis* populations regulate toxin content per cell based upon population density (Lindahl et al. 2007). Karlotoxins are produced by *Karlodinium veneficum* as "predatory venom," slowing the swimming speed of cryptophyte prey (Adolf et al. 2006). Thus, toxins produced by photosynthetic or mixotrophic protists may have defense or nutritional functions, and expression of toxicity, i.e., transcription and translation of the genetic capacity for toxin synthesis, represents a response to a specific environmental stimulus.
The estuarine dinoflagellate, *Prorocentrum minimum*, has been recognized as a "HAB," based upon observations of mortalities of marine organisms coincident with blooms, as well as laboratory experiments showing inimical effects upon grazers (Heil et al. 2005). Nevertheless, blooms of this dinoflagellate can occur without apparent harmful effect, and laboratory experiments yield variable results (Wikfors 2005). Although the chemical structures of putative *P. minimum* toxins have not been described, biological effects caused by pure cultures include bacteriostatic activity (Trick et al. 1984), allelopathy (Denardou-Queneherve et al. 1999), digestive and immunological disruption in molluscs (Wikfors 2005, Hégaret & Wikfors 2005), and acute toxicity to mice when extracts are injected intraperitoneally (Denardou-Queneherve et al. 1999). A repeated theme in these studies is that cultures "in decline" tend to be more toxic than those growing vigorously. We have enhanced expression of toxicity in *P. minimum* cultures by starving them for phosphorus (P), inducing bacteriostatic activity, or restricting air flow in aerated cultures, enhancing molluscicidal activity. An ecological interpretation of these findings suggests that P stress may induce a switch from photosynthetic to bacteriotrophic nutrition, but a nutrient-replete, photosynthetic population limited by inorganic carbon may inhibit grazing to maintain population growth. We have found changes in proteins released into the medium by *P. minimum* when stressed for P or inorganic carbon, as well as changes in fatty-acid composition and pigment content. We hypothesize that expression of toxicity in this dinoflagellate may be associated with a shift from autotrophic to heterotrophic nutrition and are testing this hypothesis experimentally.

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SPECIFICITY OF BACTERIAL ASSEMBLAGES ASSOCIATED WITH THE TOXIN-PRODUCING DIATOM, *Pseudo-nitzschia*

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The marine diatom *Pseudo-nitzschia*, responsible for Amnesic Shellfish Poisoning (ASP) via production of the toxin domoic acid, is widely distributed in Puget Sound, Washington. Over the past four years, three shellfish bed closures have occurred due to domoic acid events in this estuary. Previous laboratory studies have linked enhanced domoic acid production to higher bacterial abundance and morphological diversity, among other potential triggers. The nature of interactions between bacteria and *Pseudo-nitzschia* has not been elucidated, and few data have described bacterial assemblages native to *Pseudo-nitzschia.* We used Automated Ribosomal Intergenic Spacer Analysis (ARISA), a DNA fingerprinting technique based on length differences in the ribosomal RNA intergenic spacer, to characterize bacteria associated with laboratory cultures of 19 *Pseudo-nitzschia* strains (representing 7 species) and 11 other diatom strains. Individual strains supported significantly different bacterial assemblages. Likewise, *Pseudo-nitzschia* species were associated with different bacterial assemblages. However, differences in bacterial ARISA profiles did not exist between diatom genera. These data suggest that specific associations between diatoms and bacteria have evolved at the diatom species and strain levels. For some strains, attached bacteria (> 3 μm fraction) were significantly different from free-living bacteria (0.2 – 3 μm fraction). No significant differences in bacterial assemblages existed during exponential and stationary phases of *Pseudo-nitzschia* growth. One *Pseudo-nitzschia* strain, sampled three times over its initial nine months in culture, did not exhibit any shifts in bacterial assemblages. Furthermore, a substantial number of ARISA operational taxonomic units (OTUs) were shared between such recently-isolated Puget Sound strains and field samples corresponding to the diatoms' origins. Several of these OTUs are responsible for statistically significant differences in bacterial assemblages between *Pseudo-nitzschia* species. We conclude that bacteria coexisting with our Puget Sound *Pseudo-nitzschia* cultures are representative of species-specific associations found in the field. Further work will identify, isolate, and culture *Pseudo-nitzschia*-associated bacteria for physiological experiments testing specificity of associations as well as bacterial influence on toxin production. Analyses of field data will investigate the extent to which species-specific associations between diatoms and bacteria influence the distributions of both groups in Puget Sound.

NUTRIENT-REGULATED TRANSCRIPTOME PROFILING IN THE BROWN-TIDE FORMING ALGA *Aureococcus anophagefferens*

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HABs are a global problem with widespread effects on public health, the coastal environment, and the economy. Understanding the links between HAB dynamics and nutrient supply is a critical area of ongoing study. Assessing the nutritional physiology of a single HAB species in a mixed community remains a challenge, although our increasing molecular-level understanding of nutritional physiology in some species is providing both the knowledge for hypothesis building and testing in the field. *Aureococcus anophagefferens,* the species responsible for "brown-tide" events, provides an excellent model for studying HABs with the upcoming availability of its genome sequence. In this study, we used Long-SAGE (Serial Analysis of Gene Expression) to identify gene expression patterns in *A. anophagefferens* grown under nitrogen (N) and phosphorus (P) deficient and replete conditions. This approach generates tag libraries without *a priori* knowledge of gene sequences via the detection of 21 bp nucleotide sequence tags. These tags were mapped back to individual genes and used to examine transcriptional responses to nutritional state. The sampling frequency of these tags in different libraries indicates their differential gene expression pattern. To date, we have sampled over 86,000 tags representing 8,601 unique sequences and annotation of these tags is currently in progress. A broad-scale comparison shows a greater number of tags showing elevated transcriptional signal in the low P library than in the low N library. We have identified a number of significantly up-regulated tags (R-value >2) in both the low N and low P libraries. Relative to the nutrient replete library, 14 tags from the low P library and 5 tags from the low N library show a higher than 10-fold change in expression levels. At present, none of these highly regulated tags map to publicly available sequence data. However, the pending genome coupled with tag validation may identify genes that can be good diagnostic indicators of cellular nutritional physiology. Through a species-specific quantitative RT-PCR assay that we are developing, it will be possible to examine the expression patterns of these key genes in field populations. Looking at the N and P physiology of the cells in the field over the course of a bloom event will provide insight into the factors influencing bloom dynamics.

DETECTING CYANOBACTERIA BLOOMS USING MERIS

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MERIS, a European ocean color satellite, has increased spectral resolution relative to its domestic counterparts, MODIS and SeaWiFS. Using the increased spectral resolution it is possible to improve differentiation of cyanobacteria blooms from blooms of phytoplankton due to differences in their optical properties. Cyanobacteria often produce surface scum when cells are present in large concentrations. Additionally many species of cyanobacteria can regulate their buoyancy with gas vacuoles, making them extremely efficient at scattering light. Cyanobacteria also have phycocyanin, an auxiliary pigment, which most species of phytoplankton lack. Previously published methods used to detect blooms were run on a time-series from western Lake Erie, where *Microcystis* spp. is a potential bloom forming species. The remote sensing products were then entered as attributes into a rule based model, and the model was trained to produce an output of yes or no, meaning that a cyanobacteria bloom is present or a cyanobacteria bloom is not present. Initial results are promising. The results from this model were run on a quasi-realtime basis during the summer of 2007 in an effort to try and detect and monitor cyanobacteria blooms in the Laurentian Great Lakes.

COMPARATIVE REACTIVITY OF DIFFERENT CYSTEINE CONGENERS AS DETOXIFYING AGENTS OF BREVETOXINS

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Brevetoxins are potent marine neurotoxins produced by the marine dinoflagellate *Karenia brevis*. During *blooms of this organism (red tides) in the gulf of Mexico,* brevetoxin exposure results in massive fish kill, marine mammal deaths, and upper and lower airway impairments in humans exposed to contaminated sea-spray. These toxins also accumulate to dangerous levels in shellfish causing Neurotoxic Shellfish Poisoning if consumed. We have recently demonstrated that cysteine possesses striking properties as it can spontaneously react with some of these toxins to form non-toxic derivatives and thus could be used as a mitigating agent of red tides impacts[1]. The present research has been conducted to evaluate the potential reactivity of different cysteine congeners with brevetoxins to identify potential other mitigating agents. Several congeners have been examined for their efficiency in reacting with brevetoxins in aqueous environment. Both type 1 and 2 brevetoxins (PbTx1 and PbTx2) have been selected to react with these congeners at different molar ratios. Since red tides of *K. brevis* have also been observed in springs and winters, the reactivity of the congeners with brevetoxins was examined at different temperatures (22^{o}C and 8^{o}C). The disappearance of these toxins and the appearance of the non-toxic derivatives of brevetoxins over time have been monitored by LCMS and compared to identify the best conditions for mitigating the harmful effects of brevetoxins.

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FLORIDA'S RED TIDE CONTROL & MITIGATION GRANT PROGRAM: THE BEGINNING

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A newly formed competitive grant program in Florida is dedicated to funding research for control of *Karenia brevis* blooms and strategies to mitigate their effects. The Red Tide Control & Mitigation Grant Program is designed to explore environmentally acceptable techniques or technologies which minimize the size, intensity, or duration of *K. brevis* blooms or reduce environmental, economic, social, or public health impacts of red tide blooms in Florida. The program is implemented by the Florida Fish and Wildlife's Conservation Commission's (FWC) Fish and Wildlife Research Institute (FWRI). A grant to a local non-profit organization, Solutions To Avoid Red Tide (START), provides promotion of the Red Tide Control & Mitigation Grant Program and coordination of the Red Tide Control & Mitigation Panel. A nine-member external panel of representatives from state and local governments, agencies, and advocacy groups has been appointed to three-year, renewable terms to provide peer reviews of grant submissions. The grant panel assisted in the development of the first request for proposals, established the review process and created evaluation criteria. Panelists then reviewed, recommended, and prioritized projects for funding. Based on the panel's recommendations, funded research covers a broad range, including determining socioeconomic costs associated with various elements of Florida red tide, investigating reduction of toxicity though a variety of controls, and the production of a documentary on *K. brevis*. This program addresses an identified need in funding for applied research and responds to a public outcry in the State of Florida. As called for in Harmful Algal Bloom Research and Response: A National Environmental Science Strategy (HARRNESS, 2005), the Red Tide Control & Mitigation Grant Program is directed at identifying practical strategies to mitigate bloom impacts on the people and resources of Florida. The backbone of this program will possibly serve as a model for other regions in the United States hoping to proactively address mitigation and control of harmful algal blooms.

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