ABSTRACTS OF POSTERS

DEVELOPMENT OF A TECHINICAL GUIDE FOR PUBLIC HEALTH RESPONSE TO HARMFUL ALGAL BLOOMS IN FLORIDA

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A global increase in harmful algal bloom (HAB) frequency and expansion of their geographic range, together with the appearance of new toxic species is causing growing concern for public health. Florida has more than 70 microalgal species, some of which pose risks for human health. Because of these increasing threats, the Florida Harmful Algal Bloom Public Health Technical Panel was convened as an ad hoc committee to the Florida Harmful Algal Bloom Task Force created in 1997. The primary goal of the Technical Panel was to aid in the development of a resource planning guide for county health response to HABs in Florida. The Technical Panel, comprising of public health, environmental, natural resource, agriculture, and research professionals, met in 2004, 2005 and 2006 to determine the information needed. Based on their input, a technical guide was developed identifying HABs in Florida, the potential public health and environmental effects of a particular bloom, and the required resources needed to develop plans for the detection, response and management of HABs potentially affecting public health in Florida. The guide includes a review of known harmful algae and the consequences of their presence in Florida waters. In addition, it identifies current resources (e.g. diagnostic and analytical laboratories, scientific experts), regulatory guidelines, management strategies, and authorities for monitoring, research, and public health information. The technical guide encourages rapid coordinated response efforts by state and local governments in an effort to reduce the negative impacts of HABs on aquatic resources and public health.

POPULATION STRUCTURE OF *Pseudo-nitzschia australis* AND ITS ASSOCIATION TO DOMOIC ACID PRODUCTION IN THE WATERS OF WASHINGTON STATE

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Since 1991, contamination of razor clams with the neurotoxin domoic acid has resulted in frequent closures of this important recreational and commercial fishery on the outer coast of Washington State. However, prior to September 2003 domoic acid had not caused a closure of shellfish harvesting in the inland waterways of Washington State. In September 2003 domoic acid concentrations reached 29 ppm in blue mussels (*Mytilus edulis*) from Kilisut Harbor in northern Puget Sound. Although this was the first closure due to domoic acid in the inland waters of Washington State, diatoms of the genus *Pseudo-nitzschia*, which can produce domoic acid, have been observed in Puget Sound waters since at least 1990. One of the species responsible for the 2003 closure, as well as a subsequent closure in Puget Sound at Penn Cove in 2005, was *Pseudo-nitzschia australis*. This species is frequently observed on the outer coast of Washington State as well as in Puget Sound, and was thought to be the causative organism in the first documented domoic acid event on the outer coast of Washington in 1991. Microsatellite markers were used to characterize two distinct populations of a different species, *Pseudo-nitzschia pungens*, on the Pacific coast of Washington State and British Columbia, but this species produces low levels of domoic acid and has not been responsible for shellfish harvest closures in Washington State waters. Microsatellites will be used in a fashion similar to the work with *P. pungens* to determine the population structure of *P. australis* in Washington State waters. Additionally, the population specific production of domoic acid in *P. australis* will be assessed in laboratory culture experiments using highly sensitive antibody-based methods. The ultimate goal of this work is to determine whether certain genetically distinct populations of *P. australis* are predisposed to producing higher concentrations of domoic acid and to characterize those environmental conditions that are conducive to elevated production of domoic acid. If certain populations prove to be more toxic than others, specific molecular markers will be developed to pinpoint highly toxic populations. This will allow the development of risk assessments for toxic *P. australis* blooms and optimal focusing of monitoring efforts so that the effects of domoic acid contamination to human health in Puget Sound waters can be mitigated.

GENE EXPRESSION OF AMMONIUM TRANSPORTER AND UREASE C SUBUNIT IN THE TEXAS BROWN TIDE *Aureoumbra lagunensis.*

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Understanding the influences of nutrient type and availability in harmful algal blooms will help to define the dynamics of these events. One approach for understanding nutrient use is to examine the expression of genes responsible for nutrient assimilation. The pelagophyte *Aureoumbra lagunensis* causes ecosystem disruptive algal blooms, and is responsible for the longest recorded harmful algal bloom (1989-1997). Because of *Aureoumbra*'s small size and its inability to use nitrate, it has been hypothesized that its ability to use ammonium and organic nitrogen, especially at low concentration, led to the unusual persistence of this bloom. Overall, our aim is to determine gene expression changes under growth on ammonium and urea, with an eventual intent of developing expression assays that are indicative of nitrogen source use and/or sufficiency in *Aureoumbra*. We have cloned the genes encoding ammonium transporter and urease C subunit from *Aureoumbra* and developed specific primers. We are using quantitative real-time PCR to determine the expression of the genes in cells grown on ammonium and urea, and these results will be presented. The data generated will provide insight into algal nutritional physiology, and ultimately help us to understand nitrogen use and sufficiency in natural populations of *Aureoumbra lagunensis*.

HAB CAMP: A COOPERATIVE ADVENTURE IN COMMUNITY EDUCATION IN A CoASTAL COHORT COMMUNITY AT RISK OF DOMOIC ACID POISOINING

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Extant data indicates that adult members of our CoASTAL cohort Native American communities have serious concerns about the impact of their ocean environment and marine biotoxins on future generations. Results of domoic acid perceived risk studies also indicate they feel there is little they can do to improve the situation. Instead, they turn to the scientific community and local tribal fisheries and environmental agencies for answers and assistance. In an effort to improve the self-efficacy of the CoASTAL cohort communities, provide educational outreach to children, and increase enthusiasm for the environmental and harmful algal bloom (HAB) related sciences, a unique multidisciplinary HAB Summer Camp Program was designed, developed and implemented in Neah Bay, WA. in July, 2009. The HAB Camp targeted middle-school aged students who worked side by side with professionals in the field for one week. The curriculum provided cultural information about the tribe's relationship with the sea in the past as well as contemporary, state-of-the-art HAB monitoring laboratory and field experiences. The program helped campers develop a broad understanding of marine life and the associated ecological systems and introduced a holistic environmental perspective that could lead to healthy lifestyle choices. This included an introduction to epidemiology and an outbreak simulation. All campers were formally assessed with pre and post measures of environmental knowledge, attitudes and enthusiasm for studies of HABs or environmental sciences. Findings are reported in terms of curriculum development and evaluation as well as camper based outcome measures.

SPATIAL DISTRIBUTION OF *Pseudo-nitzschia* ITS1 TYPES DETECTED WITH ARISA IN GLACIER BAY, ALASKA

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Pseudo-nitzschia is a genus of marine diatoms comprised of at least 30 species. Eight of the 11 species known to produce the neurotoxin domoic acid (DA) have been detected on the west coast of North America. Low levels of DA have been reported in shellfish as far north as Alaska. Toxin production is known to vary between different species and strains of *Pseudo-nitzschia*, as well as between different environments. Cryptic diversity in *Pseudo-nitzschia* cell morphology can confound taxa identification. The molecular fingerprinting technique ARISA has been used to identify different *Pseudo-nitzschia* species in coastal, estuarine, and offshore waters in the Pacific Northwest. To determine if ARISA could be used to identify *Pseudo-nitzschia* species in Alaska, a survey of the Glacier Bay Marine Protected Area was conducted during March 2008. Glacier Bay is an estuarine fjord in Southeast Alaska that receives freshwater input from 16 glaciers and is connected to the Pacific Ocean by Icy Strait. Samples for DNA analysis were collected in Glacier Bay and Icy Strait from surface waters at 18 stations. *Pseudo-nitzschia* specific PCR primers were used to amplify the internal transcribed spacer 1 (ITS1) rDNA for ARISA; six ITS1 lengths were detected. Environmental sequencing confirmed the identity of *P. delicatissima, P. multiseries* and *P. granii*. The sequence of another *Pseudo-nitzschia* ITS1 type was similar to *P. micropora* and *P. delicatissima* sequences, but did not match either one. Sequences corresponding to the remaining two ITS1 lengths were not obtained, although one was putatively identified as *P. australis* or *P. seriata*, based on the length of the ITS1 amplicon. All six of the ITS1 lengths detected in Alaskan waters were also detected with ARISA in the Pacific Northwest. *Pseudo-nitzschia* species were present throughout Glacier Bay. Diversity was highest in Icy Strait and at the entrance of Glacier Bay, and decreased at stations located further within Glacier Bay. *Pseudo-nitzschia* species showed distinct patterns of spatial distribution throughout Glacier Bay and Icy Strait. One species, *P. delicatissima,* was detected at all stations. Other species were present only at some stations. For example, *P. granii* was detected in Icy Strait and near the mouth of the bay, but not at the head. *Pseudo-nitzschia multiseries*, a species known to be toxigenic in other waters, was detected at the mouth and at the head of Glacier Bay, but not in between. This snapshot view of *Pseudo-nitzschia* in an Alaskan estuary suggests that species distributions can be patchy over relatively small spatial scales.

TOXIC *Pseudo-nitzschia*: AN EMERGING THREAT TO NORTHERN GULF OF MEXICO

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Harmful/toxic phytoplankton blooms (HABs) have been the subject of regional interest in the northern Gulf of Mexico for many decades. Among many HAB species, *Pseudo-nitzschia* spp. and its toxin domoic acid (DA) in coastal waters are one of the most immediate concerns. Studies of Louisiana coastal and estuarine waters document the occurrence of the toxic diatom *Pseudo-nitzschia* spp. often in bloom quantities. *Pseudo-nitzschia* has been found to be present throughout the year and occurs in high numbers ($>10^6$ cells/L) in both inshore and offshore water bodies of Louisiana. Associated toxin production has also been documented in the field. High toxin levels are corresponding to high cell abundances. *Pseudo-nitzschia* is most abundant, and consequently water contains the highest toxin levels, usually in early spring, when riverine input increases and brings excess of available nutrients. To date, maximum DA was found to be about 13 µg DA/L from April 2007 and highest cellular DA was found to be 19 pg DA/cell from April 2008, when cell numbers in both cases exceeded 10^6 cells/L. The variability in cell toxin levels among sites likely reflects both differences among species mixes and environmental growth conditions. The major importance of their high cell numbers and toxicity is of course due to the potential threat of contamination within the pelagic and benthic system. These toxin values are not far from what has been previously found in California where animal mortalities were observed. These results will lead to discussion on whether local *Pseudo-nitzschia*, which respond rapidly to nutrient enrichment, could possibly – due to heightened abundance - generate DA levels that threaten ecosystems.

The ecology of these specific groups in this area is poorly understood. What is most lacking is knowledge of which species are toxic and what controls their toxicity in a large river plume, thus complicating efforts to control outbreaks or to determine to what extent their toxin can be transferred through the food web. Despite the presence of abundant and toxic *Pseudo-nitzschia*, there have been no known confirmed incidents of Amnesic Shellfish Poisoning (ASP) reported from the northern Gulf of Mexico. However, filter-feeding menhaden have been shown to be potential vectors of DA to higher trophic levels. Furthermore, several lines of evidence provide strong proof that *Pseudo-nitzschia* spp. are stimulated by high nutrient inputs, thereby increasing the potential for DAP incidents in areas where eutrophication is increasing. Widespread occurrence of toxic *Pseudo-nitzschia* spp. poses a significant potential health problem not only for humans but also for marine animals in the region. Thus, a better understanding of the role of *Pseudo-nitzschia* in the Gulf of Mexico is essential.

SOUNDTOXINS: FIRST YEAR FINDINGS OF A HARMFUL ALGAL BLOOM AND MONITORING PROGRAM FOR PUGET SOUND

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SoundToxins is a monitoring program that was formed in 2007 to provide advanced warning of harmful algal bloom (HAB) events in Puget Sound, WA. Advanced warning of HAB events enables shellfish and finfish growers to take action to reduce the detrimental effects of HABs. Such actions may include early or selective harvesting of shellfish and taking steps to minimize exposure of fish to the HABs. SoundToxins draws its strengths from a diverse partnership of shellfish and finfish growers, environmental learning centers, volunteers and Native American Tribes. The project goals are to determine: 1) which environmental conditions promote the onset and blooming of HAB species and 2) which combination of environmental factors might be used for early warning. Sampling is performed at twelve sites throughout Puget Sound and weekly water samples are analyzed for temperature, salinity, macronutrients, chlorophyll *a*, particulate and dissolved marine toxins (Paralytic Shellfish toxins and domoic acid), and phytoplankton species abundance with a focus on the HAB species *Alexandrium catenella*, *Dinophysis* spp., *Pseudo-nitzschia* spp., and *Heterosigma akashiwo*. Currently, no monitoring is performed by the Washington State Department of Health for *Dinophysis*, which causes Diarrhetic Shellfish Poisoning, even though the organism has been found at several sites and at significant densities. The potential danger for *Dinophysis* toxins needs to be fully assessed for the Puget Sound region. During the first year of sampling, *Pseudo-nitzschia*, *Alexandrium*, and *Dinophysis* were observed throughout the region, exemplifying the need for a phytoplankton monitoring program in Puget Sound that will provide early warning for potential harmful blooms so that steps can be taken to mitigate the effects of marine toxins on human health.

APPARENT BIOACCUMULATION OF CYANOBACTERIAL TOXINS BY SPORTFISH FROM SEVERAL LAKES IN WESTERN WASHINGTON STATE

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Toxins from freshwater cyanobacteria ("blue-green algae") have been widely associated with various, potential threats to human health. In particular, direct exposure to these toxins via drinking water and other related routes has been implicated. However, growing evidence suggests that these toxins may also accumulate in freshwater food-webs, including trophic transfer to species of fish and other animals consumed by humans, posing a largely uncharacterized threat to human health. In order to investigate the potential for exposure to cyanobacterial toxins in the food-web, we quantitatively analyzed the neurotoxic anatoxin-a and hepatotoxic microcystins in various species of sportfish collected from lakes in several counties (Pierce, Jefferson and Snohomish) of western Washington State where "blooms" or general dominance of relevant genera of cyanobacteria, including *Anabaena*, *Microcystis* and *Aphanizomenon*, have been previously reported. Samples of both muscle and liver tissue from fish caught in these lakes were extracted, and subsequently analyzed for anatoxin-a and microcystins by appropriate methods for each, including HPLC-FL and ELISA, respectively. Cyanobacterial toxins were detected in both liver and muscle from all six species of fish, including Rainbow Trout, Yellow Perch, Kokanee, Brown Bullhead, Largemouth Bass and Largescale Sucker, collected from all six of the lakes investigated. Measured concentrations of toxins included levels that both indicated possible bioaccumulation, and are above recommended guidelines for intake, of these toxins. Taken together, these data suggest a potential for exposure to toxins via consumption of sportfish in these freshwater lakes following blooms of cyanobacteria.

DOMOIC ACID PRODUCTION, GROWTH RATE AND NITROGEN UPTAKE KINETICS OF *Pseudo-nitzschia* SPECIES: A COMPARISON OF OCEANIC AND COASTAL SPECIES

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A neritic and an oceanic species of *Pseudo-nitzschia* were investigated using unialgal batch cultures to determine the effects of inorganic and organic nitrogen substrates on exponential growth and domoic acid (DA) production. The coastal species (*P. australis*) also was assessed for differential growth rate and DA production as a function of three different macro-nutrient limiting conditions (N, P and Si), as well as variable PPFD levels. The exponential growth rates achieved under saturating PPFD (ca. 120 μmol photons m^{-2} s^{-1}) ranged from 1.8 to 2.2 d^{-1}, and the cellular toxicity (DA concentration normalized to cell abundance) decreased during stationary growth under phosphate limitation, but not silicate limitation. The N-15 isotopic tracer technique was employed to determine the first nitrogen uptake kinetic parameters for the oceanic species *P. turgidula* isolated from the HNLC region of the northeastern, subarctic Pacific Ocean. Maximum specific uptake rates and affinity values were estimated for all three N substrates for *P. turgidula* and demonstrate nitrogen uptake preference (using V_{max} values) of the order: $NH_4^+ > NO_3^- >$ urea. *P. turgidula* was found to have minimal, although detectable, particulate DA concentrations in the range of 50 - 600 pg DA L^{-1} and maximal specific growth rates averaging 1.35 d^{-1}. These are the first results to demonstrate that an oceanic isolate from a HNLC region can utilize both inorganic and organic nitrogen for assimilation and has DA production capabilities. Consequently the potential for toxic diatom bloom formation cannot be dismissed during mesoscale iron fertilization efforts to alleviate iron deficiency and enhance carbon dioxide sequestration.

CRYPTIC POPULATION DIVERSITY IN *Heterosigma akashiwo*

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A harmful algal bloom occurrence can actually represent a series of cascading blooms by several cryptic strains of the same alga. Such diversity could account for the lack of a clear relationship between cell number and toxicity in some algal populations. Population diversity can also present a confounding challenge in developing models of bloom dynamics because strains can have varied excystment cues, swimming abilities, growth rates, temperature robustness, predation rates, etc.

Unfortunately, population diversity within a species of bloom forming algae is difficult to determine. Bar-coding strains via molecular sequencing addresses this problem. However, in order to be ecologically meaningful, the amount of variability measured must reflect some degree of functional change.

The nuclear encoded ribosomal ITS region has proven to be an excellent species level sequence marker in the raphidophyte, *Heterosigma akashiwo*. Unlike most organisms, *H. akashiwo*, has a short 576 base pair ITS1-5.8S-ITS2 region, where nearly no base pair changes (SNPs) are observed among strains with vastly different physiological characteristics.

Non-coding micro-satellites, in contrast, provide a level of detail that is so fine scale that it is useful to discriminate at the individual/clonal level.

Intermediate to these sequence choices are five genes in the mitochondria that provide group-level information. These mitochondrial genes code for essential proteins in the electron transport chain to make ATP. By comparing mitochondrial genomes from vastly different Pacific and Atlantic strains, we identified the cytochrome oxidase 1 (COX1), NADH dehydrogenase 2 (NAD2), NAD4, NAD5, and NAD7 genes as having high variability of approximately one change per hundred base pairs.

Fifty *H. akashiwo* cultures from around the world were assembled to create a reference library. PCR primers were developed and optimized for each of the five genes. Cells were harvested by centrifugation, lysed, and DNA amplified. The PCR product was then enzymatically cleaned, and directly sequenced with internal sequencing primers. The sequence reads were assembled, and then aligned. Individual gene sequences were aligned to compare relatedness and look for single nucleotide polymorphisms (SNPs). Relatedness was also compared by parsimony analysis of all five genes together. In all nearly 8000 base pairs were sequenced per strain.

Twelve haplotypes have been identified in this ongoing work. These haplotypes show some geographic affinity, although some regions, such as the Puget Sound, clearly have mixed populations. Also noteworthy is that sub-cultures kept separate over thirty years in the laboratory have remained constant in strain signature.

Although, most of the nucleotide changes encode synonymous amino acids, some changes are more drastic and code for a different amino acid in the protein. Some substitutions (polar/non-polar) have the potential to alter the shape and efficiency of the protein. These mitochondrial gene haplotypes also mirror measurable functional changes in the cells.

MERHAB-LGL: THE ROLE OF EMBAYMENTS, NEARSHORE NUTRIENT SHUNTS AND OFFSHORE WATERS IN THE INITIATION AND MAINTENANCE OF TOXIC ALGAL BLOOMS IN THE LOWER GREAT LAKES.

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Lake Erie, one of the Laurentian Great Lakes re-experienced toxic cyanobacteria blooms starting in the mid 1990's. Since that time, toxic blooms have reoccurred (primarily in the western basin). In 2000, extensive monitoring of this lake and connecting Lake Ontario were started under the auspices of NOAA's MERHAB-LGL (Monitoring and Event Response of Harmful Algal Blooms in the Lower Great Lakes) program. This program coupled physical, biological, chemical and molecular techniques to develop a holistic monitoring strategy for the lower Great Lakes. At a similar time, Environment Canada started an extensive sampling program in the Bay of Quinte area of concern. This intensive monitoring program has led us to reexamine several of the basic assumptions that surround the occurrence of toxic cyanobacteria blooms. Classical taxonomy was of limited use in identifying the presence of toxic species. *Anabaena* species were widespread throughout the lower Great Lakes, but the occurrence of anatoxin-a was ephemeral. Similarly the production of microcystin toxins was generally associated with occurrence of *Microcystis* species, however other microcystin-producing species were present in these lakes. Populations of cyanobacteria often contained mixtures of toxic, non-toxic and potentially toxic species. These subpopulations could be separated by genetic techniques examining the *mcy* operon, but understanding the occurrence of toxic blooms requires an understanding of those factors responsible for shifting population through these different genotypes. Toxic populations were not necessarily correlated with the highest nutrients, though oligotrophic waters rarely developed a significant degree of cyanobacterial toxicity. A comparison of environmental parameters with toxin production between nearshore and offshore waters of Lake Erie and Lake Ontario will be presented.

TRANSITION IN *Microcystis* POPULATION GENETICS IN SHORELINE ACCUMULATIONS DURING 2007 BLOOM SEASON IN COPCO RESERVOIR, KLAMATH RIVER, NORTHERN CALIFORNIA

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Toxic *Microcystis* blooms associated with very high levels of the liver toxin microcystin have occurred in Copco Reservoir on the Klamath River in Northern California during the last several summers. We have characterized the genetic diversity of *Microcystis* present throughout the 2007 bloom season (June through October) in the shoreline windblown scum that constitutes the major public health risk. We have determined the DNA sequences of two target loci, *cpcBA* (phycocyanin B/A intergenic region) and ITS (16S/23S ribosomal RNA gene internal transcribed spacer) by constructing clone libraries of PCR products produced with cyanobacterial-specific primer pairs. Based on information from both target loci, the populations of *Microcystis* variants (strains) were distinct at the early and late ends of the bloom season. The early population persisted from June until late August, the peak of the bloom, while the late population was present from late August through October. Although both populations were quite varied, including multiple strains, there was little evident overlap between the two populations. A single sampling (21 August) from the water column of Upper Klamath Lake (UKL, 55 miles upstream of Copco Reservoir), which suffers blooms that are dominated by *Aphanizomenon*, indicated that its minority *Microcystis* population was related to the early season highly toxigenic population in Copco Reservoir.

Quantitative PCR was used to quantify the relative gene copy number of *cpcBA* and *mcyB*, one of the genes needed for toxin production, in the Copco Reservoir and UKL samples. The *cpcBA* locus is a single copy gene that should be present in all *Microcystis* strains, but blooms often contain a mixture of strains with the *mcy* genes (toxigenic) and lacking those genes (non-toxigenic). We observed a high proportion of toxigenic strains early in the bloom (90% in July), declining to very low levels (<2%) in September and October. The August UKL sample indicated the presence of >90% toxigenic *Microcystis*.

These studies illustrate the extensive *Microcystis* population changes that can occur within a bloom season. In the cells concentrated in the windblown scum of Copco Reservoir in 2007, there was a transition from a highly toxigenic population early in the season and at the peak of the bloom to a less toxigenic population as the bloom declined.

DEVELOPMENT OF A DIRECT DETECTION METHOD FOR *Alexandrium* IN THE GULF OF MAINE USING SURFACE PLASMON RESONANCE AND PEPTIDE NUCLEIC ACID PROBES.

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Paralytic shellfish poisoning caused by consumption of shellfish that have fed on toxic algae is a major health issue worldwide. Dinoflagellates of the genus *Alexandrium* can produce dangerous amounts of paralytic shellfish toxins (PSTs) at such low cell densities that water discoloration often associated with Harmful Algal Blooms (HABs) is not always evident, making detection of blooms during early stages extremely important. Species that produce PSTs are difficult to distinguish morphologically from non-PST producing species, and current identification methods are expensive, time-consuming, and require special training. Development of a rapid, low-cost and easy-to-use device to detect and monitor *Alexandrium* would be an important advancement for coastal managers, as HABs vary interannually in location, intensity, and duration.

Our research focuses on RNA detection of multiple species of *Alexandrium* using peptide nucleic acid (PNA) probes and surface plasmon resonance (SPR). PNA are short-sequenced DNA mimics where the negatively charged sugar-phosphate backbone is replaced by a neutral peptide chain. PNA probes have a high discrimination for mismatches, are resistant to protease and nuclease degradation, and will hybridize in low salt concentrations, making them ideal for use in field settings. The PNA probes are covalently bound to a gold surface sensor unit and samples are flowed over the probe surface in a portable, field-compatible SPR instrument developed with collaborators in Seattle, WA (Seattle Sensor Systems). SPR is a label-free, optical detection method that measures the change of refractive index of a gold surface after binding (hybridization) of the target sequence to probe and yields results in seconds.

Our preliminary work has shown reliable detection of oligonucleotide sequences specific to *A. fundyense, A. tamarense, A. catenella,* and *A. ostenfeldii*, as well as very good discrimination between negative control PNA probes and *Alexandrium*-specific PNA probes. We have also been able to regenerate the probe layer on the sensor surface for use in multiple tests by removing the target RNA, making this method economical in the long run. Currently we are focusing on determining minimum detection limits using oligonucleotides, and refining a rapid RNA extraction protocol to test this method first using pure cultures of each *Alexandrium* species and later testing mixed field samples.

HABITAT SUITABILITY MODELS FOR THREE HAB SPECIES IN THE CHESAPEAKE BAY

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We are developing and implementing a system to routinely provide short-term predictions of the likelihood of blooms of the following three harmful algal bloom (HAB) species in Chesapeake Bay and its tidal tributaries: the dinoflagellates *Karlodinium micrum* and *Prorocentrum minimum* and the cyanobacterium *Microcystis aeruginosa*. The method involves using real-time and 3-day forecast data acquired and derived from a variety of sources to drive empirical habitat suitability models of the target species. In this poster, we will discuss the approach, development and verification of the habitat suitability models for each of the three HAB species.

THE NUTRITIONAL ECOLOGY OF THE HARMFUL DINOFLAGELLATE BLOOMS CAUSED BY *Cochlodinium polykrikoides* IN NEW YORK ESTUARIES

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The harmful dinoflagellate *Cochlodinium polykrikoides* is well-known for forming ichthyotoxic blooms in coastal regions of Asia and North America, but the nutritional factors supporting and promoting these blooms have not been well-studied. To better understand the nutritional ecology of harmful dinoflagellate blooms caused by *C. polykrikoides* in Long Island estuaries (NY, USA), laboratory and field studies of this species were conducted. The spatial and temporal dynamics of *C. polykrikoides* cells, nutrients, and co-occurring phytoplankton were quantified within two New York estuaries from 2006 - 2008. The growth response of *C. polykrikoides* and co-occurring phytoplankton during experimental loading of different nitrogen sources was established. Furthermore, growth rates of clonal isolates of *C. polykrikoides* on a variety of nitrogen sources (urea, ammonium, glutamic acid, nitrate) and through a range of concentrations (2-200 μM) were assessed. Finally, uptake rates of various N compounds in both field and laboratory conditions were examined. *C. polykrikoides* cultures grown on glutamic acid displayed significantly faster growth and N-uptake rates compared to cultures grown on urea, ammonium, and nitrate. From 2006 – 2008, blooms of *C. polykrikoides* occurred in regions with a variety of N concentrations, but were only monospecific in locations with lower levels of nitrate and ammonium (< 2 μM). During bloom experiments, the loading of a variety of N compounds (urea, ammonium, glutamic acid, nitrate) were all capable of significantly increasing the growth of *C. polykrikoides*. Finally, the dominant N-compounds assimilated by bloom populations differed among sites, with nitrate and nitrite being taken up fastest at the most eutrophic locations and urea and glutamic acid being assimilated quickest within open water, mesotrophic sites. The sum of these observations suggests that *C. polykrikoides* is a nutritionally flexible species, capable of assimilating and growing on a variety of N-compounds, with the compound yielding maximal growth or uptake changing with prevailing nutrient conditions. Results further suggest monospecific blooms may be promoted by modest loading rates of N.

A SURVEY OF TEN TEXAS INTERTIDAL RIVERS FOR *Prymnesium parvum*

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Since 2001 Texas has experienced a marked increase in the frequency, duration, and intensity of blooms of the toxic haptophyte *Prymnesium parvum*, commonly known as the golden alga. *P. parvum* blooms have affected five Texas river basins: the Canadian, Red, Brazos, Colorado, and Rio Grande. Though most of these blooms have occurred in the upper portions of these river basins, in early 2006 two fish kills were confirmed along the lower Brazos River, marking the first occurrence of *P. parvum* near the coast. In 2008 blooms were confirmed in an additional four waterbodies along the lower Texas coast. There is concern over the possibility of *P. parvum* entering and blooming in Texas' estuaries because the species is primarily an estuarine organism in other parts of the world. This study surveyed *P. parvum* concentrations in the tidal portion of ten Texas rivers: the Sabine, Trinity, Brazos, Colorado, Lavaca, Guadalupe, Aransas, Nueces, Arroyo Colorado, and the Rio Grande. The 2008 season proved to be atypical, as the warm, wet winter may have prohibited large-scale blooms from occurring. No *P. parvum* were found in any of the samples. In 2009 the typical large inland blooms returned, and sample analysis is ongoing to determine if *P. parvum* was present near the river mouths during this time.

VIRAL-PHYTOPLANKTON INTERACTIONS: ISOLATION, CHARACTERIZATION AND ECOLOGY OF VIRUSES INFECTING THE DIATOM *Pseudo-nitzschia*

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Viruses are important regulators of phytoplankton communities, contributing to bloom termination and preventing transfer of fixed carbon to higher trophic levels. Although diatoms are one of the most important marine primary producers, only a few diatom-infecting viruses have been characterized. The cosmopolitan diatom genus *Pseudo-nitzschia* is most noted for the ability to produce domoic acid, a neurotoxin that can reach ecologically dangerous levels during large blooms. Toxic blooms of *Pseudo-nitzschia* are common in coastal Washington waters but in 2005 a toxic bloom of *Pseudo-nitzschia* was detected inside Puget Sound. Preliminary data suggest the viral size fraction of water collected during this bloom event is lethal to some cultures of *Pseudo-nitzschia*. We hypothesize that there may be several viruses that can infect *Pseudo-nitzschia,* ranging from single stranded RNA viruses to double stranded DNA viruses from the *Phycodnaviridae*. Isolation and characterization of the putative *Pseudo-nitzschia* virus(es) is currently underway. Exponentially growing *Pseudo-nitzschia* cultures are challenged with environmental samples and monitored for potential lytic events via both fluorescence changes and epifluorescence microscopy to quantify virus-like particle production. We have also developed a protocol for growing lawns of *Pseudo-nitzschia* on solid media to enable individual plaques to be picked for characterization. Our ultimate goal is to develop a genomically characterized virus-host system to better understand bloom dynamics in the field.

THE U.S. FRESHWATER HARMFUL ALGAL BLOOM RESEARCH & CONTROL ACT OF 2009

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U.S. Congressional legislation is needed to establish a National Freshwater Harmful Algal Bloom Research Plan (FHABRP). The U.S. Environmental Protection Agency (EPA) has purview over all U.S. freshwater bodies, but has not made regulatory determinations or established Federal policy due to freshwater harmful algal blooms (FHAB) data deficiencies. States and localities developing strategies to prevent, control and mitigate FHABs in recreational and drinking waters must rely on regulations and guidelines developed by the World Health Organization and other countries. The Agency has not established the FHABRP because of the lack of a clear Congressional directive. A clear Congressional directive, the proposed Freshwater Harmful Algal Bloom Research & Control Act of 2009 (FHAB Act), is needed to establish the FHABRP so crucial FHAB data can be obtained. U.S. FHAB policy must be developed to protect human health, aquatic ecosystems and economies from the looming crisis posed by FHABs.

The EPA listed Microcystins, Cylindrospermopsin and Anatoxin-a as highest priority cyanotoxins, and Saxitoxin and Anatoxin-a(s) as medium to high priority. Research is needed to assess the frequency and concentrations with which cyanobacteria and cyanotoxins occur in recreational and finished drinking waters. Health research is needed to obtain cyanotoxin dose-response data for establishing Reference Doses (ingested compounds), Reference Concentrations (inhaled compounds) and cancer assessments. Risk management research is needed to assess the efficacy and sustainability of ecological and chemical approaches to FHAB control, develop improved and lower cost control technologies and devise enhanced mitigation strategies. No Federal research funds currently target this research. The FHAB Act and subsequent fund allocations are needed to establish the FHABRP, obtain the needed data and establish U.S. policy.

Congress was informed of the need for the FHAB Act through oral and written testimony (Hudnell, 2008). The proposed FHAB Act is modeled after the Harmful Algal Bloom and Hypoxia Research and Control Act (1998, 2004) that directed the National Oceanographic and Atmospheric Administration (NOAA) to establish a research plan for coastal HABs. FHAB Act funds would be administered through the three competitive, research grant programs established by NOAA – ECOHAB, MERHAB & PCM HAB. An informal coalition is petitioning House and Senate committees to demonstrate the public support needed to advance the FHAB Act. This effort is supported by a website through which individuals can join the coalition, access the Emails sent to members and view, sign and Email letters of support to Congress – http://www.FreshwaterHABlegislation.com/.

Hudnell, HK, Congressional Testimony presented to the U.S. House of Representative's Committee on Science & Technology, July 9, 2008, Washington, D.C.
http://science.house.gov/publications/hearings_markups_details.aspx?NewsID=2255

PATTERNS OF CHLOROPHYLL VARIABILITY AND PHYTOPLANKTON COMMUNITY COMPOSITION OFF SOUTHERN CALIFORNIA AND THE INFLUENCE OF MACROSCALE AND REGIONAL OCEANOGRAPHIC PROCESSES

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Understanding the processes that influence the biological response of primary producers can aide in many aspects of oceanographic and climate research. Over twenty years of surface chlorophyll measurements collected off southern California (SC) for the California Cooperative Fisheries Investigation (CalCOFI) and the Scripps Pier Chlorophyll programs were analyzed in relation to various macroscale processes for the North Pacific and to regional processes off SC, including the strength and position of the California Current. Principal component analysis was used to resolve the dominant spatial and temporal patterns of the surface chlorophyll and dynamic height anomalies. Correlation analysis determined which relationships were significant, while composite maps illustrated the mean oceanographic conditions of temperature, salinity, and nitrate corresponding to specific periods of chlorophyll distribution and physical forcing. The macroscale indices associated with large scale warming and cooling of the ocean represented the greatest influence on chlorophyll and dynamic height over the study period, years 1984-2006. The strength of the currents within the region was the second greatest influence. Phytoplankton community composition of harmful algal bloom species collected at Scripps Pier were compared to basin wide and regional variations to determine if these processes influence the timing or abundance of these species.

EVIDENCE OF AN APOPTOTIC PROGRAMMED CELL DEATH PROCESS IN THE TOXIC DINOFLAGELLATE *Karlodinium veneficum*: EFFECTS OF LIGHT DEPRIVATION AND CHANGES IN SALINITY

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We have previously reported induction of programmed cell death (PCD) in phytoplankton using flow cytometry and the annexin V affinity assay (Measurement of Apoptosis in Phytoplankton Using Flow Cytometry and Annexin V Binding. Fourth Symposium on Harmful Algae in the U.S., October 2007). Our results provided preliminary evidence of an apoptotic PCD process in the toxic, cosmopolitan dinoflagellate *Karlodinium veneficum*. In this study we further examined the effects of light deprivation and changes in salinity in *K.veneficum*, using additional flow cytometric measurements of PCD and cell cycle properties. Consistent with our initial findings where we examined apoptosis with annexin V FITC staining and propidium iodide uptake, we demonstrate using the TUNEL (TdT-mediated dUTP-biotin nick-end labeling) assay an apoptotic PCD process in light deprived cultures of *K. veneficum.* In addition we examined the effects of light deprivation and salinity changes on cell cycle distribution. The results of cell cycle analysis using flow cytometry and SYBR green staining reveal the presence of a G_1 arrest and subsequent cell death in high salinity cultures. Further analysis of the light scatter and cell cycle properties of SYBR green stained *K. veneficum* provide additional evidence of a sub G1-like apoptotic process.

The results of this study provide additional evidence of an apoptotic PCD process in *Karlodinium venificum.* Understanding the nature of PCD induction in HAB species may provide important insights into bloom decline and possible mechanisms for management.

RED TIDE: SEMANTICS AND STATISTICS

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When monitoring for harmful algal blooms part of the problem is recognizing when a bloom is occurring and what the likely trajectory is. As a result, in coastal monitoring programs for harmful algal blooms, extensive coastlines such as Florida can be problematic for sampling strategy. This is particularly true for species such as *Karenia brevis* if three dimensional sampling is not practical because of areal expanse, depth, and rapid assessment needs. Until coastal ocean observing systems are in place for harmful algae and/or their toxins, point sampling is the usual strategy of data collection but its usefulness depends on location, depth, and timing. Where point sampling has been particularly effective has been on ship cruises that sampled fixed stations along transects from inshore to offshore, surface to bottom. Such cruises are in the Florida Fish and Wildlife Conservation Commission's Red Tide Database, e.g., cruises by USFWS, NASA, Florida Department of Natural Resources, Florida Department of Environmental Regulation, Mote Marine Laboratory, and ECOHAB. Compounding the potential difficulties of the choices of sample station location, depth and frequency of sampling are the choices of sample and subsample sizes for determining abundance of cells. For example, in the Florida program, up to a one liter water sample may be collected and fixed with Lugol's solution, then a pooled 3 ml subsample is enumerated to derive an estimate of abundance. The limit of detection for this protocol is 333 cells L^{-1} and the results are scaled up with the attendant increase in the uncertainty of the estimate. This can be an issue when 1000 cells L^{-1} represents background levels in the Gulf of Mexico and 5000 cells L^{-1} is the regulatory limit that activates closure of shellfish beds inshore. On the other hand, red tides are detectable from satellites typically at >50000 cells L^{-1} which is a developing or ongoing bloom. In addition, *K. brevis* blooms have penetrated coastal waters along the bottom without surface expression until nearshore and so would not be detectable by satellite or by data collected only in surface waters. The Florida Red Tide database, principally from event response cruises, is composed of about 80% surface sample results and about 20% bottom results (>90000) samples. If *K. brevis* is not recorded in surface samples or listed as "present" does that mean that a bloom is not somewhere around, perhaps on the bottom? What is the probability of a *K. brevis* bloom being somewhere in nearby coastal waters if a sample has a count of 20000 cells L^{-1}? What is the probability of a bloom in nearby coastal waters if surface water from 30 stations had "zero" records? Is a "negative" or "zero" record a result of small sample size, e.g. 1 liter vs. 10 liters, or counts less than the detection limit? Is it necessary in the case of *K. brevis* to monitor at least surface and bottom rather than just surface water samples to detect potential blooms or bloom development or is surface sampling enough depending on what stage of the bloom is being sought and at what distance offshore? What *K. brevis* surface count estimate is indicative that a bloom is developing? What is the probability, based on a single count or a cluster of counts, that a red tide is present, if a red tide is defined as 10000, 50000 or 100000 cells L^{-1}? To address these questions and others, we apply Bayesian and other i statistical approaches to data from the Florida database and results will be discussed.

CONDENSATION OF GERANYL DIPHOSPHATE AND GLUTAMATE IN THE BIOSYNTHESIS OF DOMOIC ACID

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Domoic acid (DA), a member of the kainoid class of marine toxins, causes amnesic shellfish poisoning in humans and is one of the leading causes of marine mammal mortality on the Pacific coast of North America. Levels of DA production by marine diatoms vary markedly from year to year, but the molecular events that regulate DA biosynthesis are poorly understood. Understanding the regulation of DA biosynthesis first requires a description of its biosynthetic pathway. Precursor feeding experiments indicate DA is formed from the condensation of geranyl diphosphate with glutamate to generate a pyrrolidine ring skeleton that is subsequently converted to DA. However, the specific pathway for formation of the pyrrolidine ring has not been elucidated. Here, we incubated *Pseudo-nitzschia* cultures with [1-$^2H_2$]geraniol to differentiate between pathways that involve hydrolysis of geranyl diphosphate and oxidation to the aldehyde prior to condensation with the amino nitrogen of glutamate, and pathways that involve direct nucleophilic substitution of the pyrophosphate of geranyl diphosphate by the glutamate amino group. Analysis of DA generated in the cultures incubated with [1-$^2H_2$]geraniol suggest both ^2H are retained, which is consistent with a pathway that involves nuclear displacement of the pyrophosphate. Future precursor feeding studies will address the specific mechanism of pyrrolidine ring closure.

OREGON'S HARMFUL ALGAL BLOOM SURVEILLANCE PROGRAM: OVERVIEW OF PUBLIC HEALTH ADVISORIES ISSUED TO PROTECT RECREATIONAL USERS DURING CYANOBACTERIA BLOOMS IN LAKES AND RESERVOIRS

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Freshwater harmful algae blooms (HABs) are predicted to increase in severity due to changes in land use and climate, and are an emerging public health concern. A variety of species of freshwater cyanobacteria are capable of producing toxins that pose potential risks to people, pets and wildlife. The Office of Environmental Public Health (OEPH) in Oregon's Department of Human Services is collaborating with the Centers for Disease Control and Prevention to enhance surveillance of the impacts of cyanobacteria blooms on public health. OEPH has issued public health advisories to protect recreational users during blooms of toxigenic cyanobacteria since 2004. Blooms in Oregon are typically dominated by cyanobacteria of the genera *Microcystis* and *Anabaena*. Health advisories for cyanobacteria blooms have been issued in 12 of Oregon's 36 counties. The number of health advisories issued per year has increased as well as the duration of the bloom events. These data support the hypothesis that cyanobacteria blooms are increasing in severity but may also be indicative of enhanced surveillance efforts among local, state and federal partners. Future efforts to educate and inform the public about the causes and consequences of freshwater HABs would be valuable to minimize both public health and socioeconomic impacts.

HAB INFORMATION: THE ROLE OF INFORMATION TECHNOLOGIES IN INTEGRATING AND COMMUNICATING HARMFUL ALGAL BLOOM RESEARCH

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Increasingly, Harmful Algal Bloom (HAB) research is conducted using collaborative, cross-disciplinary approaches. The trend towards multidisciplinary research calls for a more accessible means of aggregating, combining, displaying, and communicating the multitude of data in a meaningful and useful way to various types of users, including scientists, managers, and the general public. Communication to each of the various user classes requires inherently different considerations in selecting methods for addressing their HAB information needs. HAB research collaborators and partners generally require accessibility and security of information, while the general public requires educational outreach communicated in a clear and concise way. Both user classifications require timely delivery of information in an appropriate format in order to maximize its use. Here, we survey the traditional scientific and outreach efforts in delivering and communicating HAB research. Next, we discuss the trend and advantages in the Information Age towards communicating HAB research using cloud computing technologies such as Web 2.0. Finally, we discuss the Pacific Northwest Harmful Algal Bloom (PNW HAB) Bulletin as a model of this development process.

GULF MENHADEN *Brevoortia patronus*: A POTENTIAL VECTOR OF DOMOIC ACID (DA) IN COASTAL LOUISIANA FOOD WEBS

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Harmful algal blooms are an increasing problem for coastal waters world-wide. The diatom genus, *Pseudo-nitzschia*, is of particular concern in Louisiana, due to the potential for several species to produce the neurotoxin domoic acid (DA). While trophic transfer of DA to consumers has repeatedly occurred along the California coast, little is known about trophic transfer of recently detected DA in the Gulf of Mexico. In this study, the presence of DA in gulf menhaden (*Brevoortia patronus*) and the potential for trophic transfer to higher order consumers was investigated. Species identification was conducted with transmission electron microscopy (TEM). DA quantification in corresponding water and tissue samples was determined by Enzyme-Linked Immunosorbent Assay (ELISA). Examination of the phytoplankton revealed four species of *Pseudo-nitzschia*: *P. calliantha*, *P. pseudodelicatissima*, *P. pungens*, and *P. americana*, with *P. calliantha* as the dominant species. The results of the toxin assay illustrated that low-levels of DA existed in both water and tissue samples, with a significant correlation between the two ($n = 25$, $p = 0.025$, $\alpha = 0.05$). The discovery of *P. calliantha* is the first identification of this toxic species in Louisiana waters. The present study is also the first documentation of a DA vector in the entire Gulf of Mexico and indicates possible DA contamination in food webs of coastal Louisiana.

ON THE DISTRIBUTION AND ECOPHYSIOLOGY OF A RECENLTY DESCRIBED RAPHIDOPHYTE

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In August 2004, a new raphidophyte species, superficially resembling *Heterosigma akashiwo* under light microscopy, formed dense blooms in Delaware's Inland Bays (DIB). During environmental sampling in the DIB, it became evident that although it is a marine species, it has a unique tolerance to low salinity. QPCR analysis of both archived and recently collected molecular samples demonstrated large variability in its abundance in three DIB sites over the years 2003-2006. Additionally, broad scale distribution analysis using PCR, yielded detection in coastal South Carolina and the Delaware Bay estuary in 2001, although samples from New York, Georgia, Florida were negative.

Fine scale distribution data in DIB obtained in conjunction with environmental parameters showed a strong positive correlation with lower salinity (< 20) but not with temperature. These environmental observations were followed up in laboratory culture experiments focusing on bottom-up control effects on growth. PO_4^{3-} concentrations supporting maximum growth rates were 3-20 µM, although active growth was maintained even at 0.5 µM. PO_4^{3-} requirements of this new species place it with *Chattonella subsalsa* rather than *H. akashiwo* in terms of environmental niche, with a µmax:Ks of 0.85. Culture experiments also confirmed that this species has a wide salinity (3-18) tolerance towards the lower end of the spectrum. This is probably the reason why it can bloom up to extremely high densities following a rapid salinity change. Top-down control of microzooplankton grazing was measured in samples collected in June 2005 in Love Creek in DIB. We determined that microzooplankton do at least occasionally graze on this species. This study is a first look into the distribution and ecophysiology of this newly described algae.

TWO CYANOPHAGES CAPABLE OF LYSING MICROCYSTIS FROM KLAMATH RIVER

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For the last several years, Copco Reservoir on the Klamath River in far northern California has suffered from highly toxic blooms of the cyanobacterium *Microcystis aeruginosa*. With a view to examining the role of virus infection in *Microcystis* population dynamics, we have embarked on a search for cyanophages in this reservoir. We have isolated two *Microcystis*-infecting cyanophages from water samples taken from Copco Reservoir in September 2008. One virus is able to amplify in and lyse cells of *Microcystis aeruginosa* UTEX LB2386. On the basis of pulse-field gel electrophoresis and restriction analysis, the genome of this virus is double-stranded DNA about 180 kbp in length. Negative staining electron microscopy shows this virus has a 90-95 nm isometric head and a 170 nm tapered tail (15-25 nm wide) with an apparent base plate. Based on these properties, this virus is a putative myovirus. The second virus is able to amplify in and lyse cells of an isolate of toxigenic *Microcystis* cultured from Upper Klamath Lake. The genome of this virus is dsDNA about 50 kbp in length. Progress on the characterization and genome sequencing of these cyanophages will be presented.

UPTAKE AND DEPURATION OF DOMOIC ACID, A PHYTOTOXIN, BY WASHINGTON CLAMS

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In Washington State, blooms of the toxin-producing phytoplankton *Pseudo-nitzschia* have been responsible for numerous beach closures for shellfish collection. A number of *Pseudo-nitzschia* species have been shown to produce the neurotoxin domoic acid, which bio-accumulates in the soft tissues of suspension-feeding shellfish and can cause death in vertebrate predators that consume tainted shellfish.

Since 1991, beach closures for razor clam collection have occurred regularly along the outer Washington coast. In Puget Sound, however, beach closures for domoic acid have been less frequent and only first occurred in 2003. In order to understand the potential impacts of domoic acid in Puget Sound, we fed domoic acid-producing *Pseudo-nitzschia* to nine species of Puget Sound clams, and measured feeding rates and domoic acid uptake and depuration over time. Experiments were also performed on razor clams to provide a comparison between suspension feeders in Puget Sound and on the outer Washington coast.

Currently, blue mussels are used as the sentinel species in Puget Sound for detecting domoic acid in shellfish. Results from this experiment indicate that other species such as Manila clams or purple varnish clams may provide a more conservative estimate of domoic acid in shellfish tissue, as these species uptake domoic acid at a faster rate than mussels, and depurate the toxin at a slower rate.

DIVERSITY AND DISTRIBUTION PATTERNS OF *Pseudo-nitzschia* SPECIES OFF THE UNITED STATES NE COAST DURING SUMMER 2008

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The diatom genus *Pseudo-nitzschia* contains several HAB species that are capable of producing the neurotoxin domoic acid (DA). Toxin production is known to vary among species and strains, and between different environments. In 2003, over 20 whale deaths in eastern Georges Bank were attributed to DA poisoning; multiple *Pseudo-nitzschia* species were detected in water samples during that event (1). Although *Pseudo-nitzschia* is known to be a constituent of phytoplankton assemblages in the Maine Coastal Current system, DA concentrations are not routinely monitored in shellfish there, and reports of species distributions are limited. To facilitate the rapid and high-throughput identification of *Pseudo-nitzschia* species in the Maine Coastal Current system, we examined the utility of ARISA (2), a molecular fingerprinting technique that has been used to identify *Pseudo-nitzschia* species in the Pacific Northwest.

Pseudo-nitzschia isolates and samples for DNA analysis were collected on weeklong cruises to Georges Bank and the Gulf of Maine during May, June, and July of 2008. Isolates were tested for DA production and identified to species using a combination of electron microscopy and internal transcribed spacer 1 (ITS1) rDNA sequencing (3). The sequences from these isolates, combined with ITS1 environmental sequencing, indicated that *Pseudo-nitzschia* DNA could be amplified and that species could be distinguished using ARISA, based on the sequence and length of the ITS1, with few exceptions. The ARISA approach was then used to characterize *Pseudo-nitzschia* species distributions at 20 stations from Georges Bank and the Gulf of Maine during May and June, and at 14 stations from Georges Bank during July. A total of 17 distinct ITS1 lengths were detected, half of which matched isolate and/or environmental sequences. Additional environmental sequencing is being conducted to determine ITS1 sequences for those fragments not yet identified. Six ITS1 lengths, including those that corresponded to *P. pungens*, *P. seriata*, and *P. delicatissima,* were detected throughout the three cruises*,* whereas several other ITS1 lengths were detected during one cruise only. A similar pattern was detected between Georges Bank and the Gulf of Maine, with some ITS1 lengths distributed throughout, and others limited to either location during a single cruise. The highest *Pseudo-nitzschia* species diversity was detected in July at Georges Bank, when temperatures were warmer than on previous cruises. We are currently investigating associations of individual species with environmental gradients and hydrographic features to better understand links between *Pseudo-nitzschia* species and environmental variability in this system.

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A QUANTITATIVE REAL-TIME PCR ASSAY FOR THE IDENTIFICATION AND ENUMERATION OF PLANKTONIC CELLS AND RESTING CYSTS OF *Alexandrium fundyense*

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Harmful algal blooms (HABs) are a global problem that affect both human and ecosystem health, and HAB incidence appears to be increasing worldwide. As a result, monitoring and study of HAB events have also increased, creating a need for rapid yet sensitive methods that can accurately identify and enumerate HAB organisms. We have developed a quantitative real-time PCR method (qPCR) for enumeration of both vegetative cells and resting cysts of the toxic North American/Group I ribotype of the *A. tamarensis* species complex, a group of organisms responsible for paralytic shellfish poisoning events worldwide. To characterize the performance of this method in the field, we used multiple independent analysis methods, including qPCR, to count *A. fundyense* in replicate subsamples of >500 plankton samples collected on 5 cruises during three years, and >100 sediment cores.

For planktonic vegetative cells, the qPCR method used cultured cells for a standard curve and was able to quantify as few as 5 cells•L^{-1}. The qPCR data were compared primarily to cell counts obtained using fluorescent in situ hybridization (FISH), and the relationship between the qPCR and FISH counts varied between years and also within a single bloom year. In 2005, qPCR values overestimated those determined by FISH by approximately 2-fold. In contrast, qPCR counts were lower than the corresponding FISH values, by a factor of 1.1-6.8, in both 2007 and 2008. In 2008, a cloned large subunit ribosomal RNA (LSU rRNA) gene fragment was used as a standard, in hopes of reducing variability associated with cultured cells. Comparison of the FISH and qPCR data from 2008 shows a 3-fold difference in the ribosomal copy number of *Alexandrium* cells collected during two cruises approximately one month apart. The apparent ribosomal copy number in field cells ranged from ~500,000 to ~1.5 million; in cultured *Alexandrium* cells, extracted ribosomal copy number was ~500,000.

For cysts, the qPCR method used the cloned LSU rRNA gene standard curve and was able to reliably quantify 10cysts/cc sediment. Field-collected cysts were determined to contain an average of 28,400 extracted LSU rRNA gene copies. The qPCR data were compared to counts obtained using the primulin-staining method; overall, the qPCR counts were lower than the primulin values by ~25%. This underestimation was less pronounced in those samples collected from the top 1 cm of sediment, and more pronounced in those derived from the next 1-3 cm of the core.

The qPCR method was developed to provide an alternative to the FISH and primulin-staining methods, and its primary advantages over these methods are a higher throughput and fewer person-hours required per sample. However, because the qPCR method detects ribosomal gene copies and not cells *per se*, variation in ribosomal gene copy number can affect the results. Our analyses indicate that ribosomal gene copy number in *Alexandrium* can change greatly over time and with different life cycle stages. Thus, the assay should be calibrated against field-collected samples in order to provide the most reliable estimate of cell or cyst numbers.

TRANSCRIPTOME ANALYSIS OF BACTERIA-PHYTOPLANKTON INTERACTIONS IN *Alexandrium tamarense*

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The extent and nature of bacteria-phytoplankton association is a subject of considerable interest and debate. Such interactions may significantly affect harmful algal bloom dynamics by influencing multiple physiological processes such as phytoplankton metabolism and growth, in addition to HAB toxin production. We examined potential interactions between bacteria and the red-tide dinoflagellate, *Alexandrium tamarense*, using transcriptome analysis by massively parallel signature sequencing (MPSS). *A. tamarense* CCMP1598 (axenic) and CCMP1493 (xenic clone of CCMP1598) were grown in replete semi-continuous culture. After harvest, mRNA was isolated and then MPSS analysis was performed by Illumina, Inc. MPSS signatures were assigned to genes using BLAST searches vs. an *A. tamarense* EST database, and significant variations in gene expression between culture conditions were determined using a Z-test for proportional equality. A total of 1,486 unique genes were identified as having MPSS signatures matched to ESTs. In the presence of bacteria, 21% of MPSS signature-matched ESTs were expressed at significantly different levels than in axenic culture ($p < 0.001$; 9% up-regulated, 12% down-regulated). Transcription of genes critical to several physiological processes is significantly altered in the presence of bacteria. Potentially regulated processes include bioluminescence, carbon fixation and metabolism, programmed cell death, and toxin production. These results indicate that the presence of bacteria in culture significantly alters the *A. tamarense* transcriptome by affecting several key physiological processes. Bacteria-phytoplankton associations therefore likely play a critical role in HAB dynamics not only by influencing toxin production but also via direct effects on phytoplankton growth and metabolism.

APPLICATION OF IMAGING FLOW CYTOMETRY TO MONITOR LIFE CYCLE TRANSITIONS IN *Alexandrium tamarense* SPECIES

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The *tamarense* species complex of *Alexandrium* is globally distributed and includes strains that produce toxins associated with paralytic shellfish poisoning (PSP). The complex can be divided into 5 clades called Groups I – V. Previous research has shown that species from Group I and Group III can interbreed in culture and in nature, but the resulting progeny of these hybrids are inviable. These findings are particularly interesting because Group I cells produce PSP toxins while Group III cells do not. Therefore, the introduction of nontoxic cells into the marine environment could be an effective means of mitigating toxic *A. tamarense* blooms. Before such a strategy can be pursued, a more complete understanding of the factors governing sexuality is needed. In general, light microscopy is used to monitor life cycle transitions. However, this method is inefficient and possibly unreliable because sexual stages are difficult to differentiate from asexual vegetative cells. To address the problem of low sample throughput, we have configured the Imaging Flow Cytobot (IFCB), a submersible flow cytometry system, for automated detection of *A. tamarense* cells that have been stained with a Group I specific ribosomal RNA probe. The system measures cellular DNA content in order to confirm diploidy in swimming planozygote cells. The IFCB system will be used to assess the appearance and overall abundance of sexual stages in a series of mating experiments between Group I and Group III clones. A modified IFCB will also be used to analyze samples taken over the course of a natural bloom in the Nauset Marsh area (Cape Cod, MA).

SODIUM CHANNEL AMINO ACID SUBSTITUTIONS UNDERLYING RESISTANCE OF CLAMS TO PSTs

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Paralytic shellfish poisoning toxins (PSTs) act by blocking Na^+ channels in clam nerves and in the muscles and nerves of vertebrates that eat them. The amino acids that bind PSTs are found in the extracellular vestibule of the pore through which sodium flows through the channel into the cell. These amino acids are highly conserved between species. We previously identified a naturally occurring amino acid substitution in the Na^+ channel of soft shell clams (*Mya arenaria*) from areas with frequent exposure to harmful algal blooms. This altered amino acid renders the clam sodium channels profoundly insensitive to saxitoxin and tetrodotoxin. Clams from nearby areas with no history of PST exposure lack this substitution and are far more sensitive to PSTs. However, the nerves of these sensitive clams, as well as other molluscs, are still far more resistant to PSTs than mammalian nerve and muscle. We sought to identify the molecular basis for this basal resistance of *M. arenaria* and other molluscan sodium channels to PSTs. To do this we examined differences in the amino acids forming the pores of clam and vertebrate sodium channels and tested their involvement by introducing substitutions into the mammalian brain Na^+ channel. The sodium channel protein is comprised of 4 homologous domains (I-IV) and the pore is lined by amino acids from each of these. We identified one substitution in the pore of domain III that explains a large part of the resistance to tetrodotoxin and is partially responsible for the resistance to saxitoxin. A second substitution in the pore of domain IV causes a large increase in resistance to both tetrodotoxin and saxitoxin. These mutations in combination with or without the previously identified substitution (found in domain II) further explain the molecular basis for the PST resistance that underlies bivalves' ability to remain viable as well as to accumulate toxins in an environment containing PSTs.

EVALUATION OF DIETARY ASSESSMENT TOOLS USED TO ASSESS DIETARY EXPOSURES OF ADULTS PARTICIPATING IN THE COMMUNITIES ADVANCING THE STUDIES OF TRIBAL NATIONS ACROSS THE LIFESPAN (CoASTAL) COHORT

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Accurate assessment of dietary intake is essential for researchers and public health practitioners to make advancements in health. This is especially important in Native Americans who display disease prevalence rates that are dramatically higher than the general U.S. population. Obtaining information on the dietary profiles of a population is also important for understanding and characterizing the risk for specific food borne illnesses or toxic exposures such as amnesic shellfish poisoning or exposure to mercury or organophosphates. The objective of this study was to evaluate three dietary assessment tools: 1) dietary records, 2) a food frequency questionnaire (FFQ), and 3) a shellfish assessment survey (SAS) among Native American adults from the Communities Advancing Studies of Tribal Nations Across the Lifespan (CoASTAL) cohort.
CoASTAL was comprised of randomly selected individuals from three tribal registries of Pacific Northwest Tribal Nations. This cross-sectional study used data from the baseline of CoASTAL and was restricted to the non-pregnant adults (18+ yr) who completed the SAS (n=500), a FFQ (n=518), dietary records (n=444), weight measures (n=493), and height measures (n=496). Paired t-tests, Pearson correlation coefficients, and percent agreement were used to evaluate the convergent validity of the dietary records and the FFQ. Validity was also evaluated while accounting for plausibility of reported energy intake (rEI). Sensitivity and specificity as well as Spearman correlation coefficients were used to evaluate the SAS compared to the FFQ and dietary records.

Statistically significant correlations between the FFQ and dietary records for selected nutrients were not the same by gender. Accounting for plausibility of rEI for the dietary records and the FFQ improved the strength of the correlations for percent energy from protein, percent energy from carbohydrate, cholesterol and calcium for both men and women. Dietary fiber maintained a statistically strong association regardless of plausibility classification. In addition, significant associations between rEI (dietary records and FFQ) and body weight were more apparent when using only rEI considered plausible. The SAS was found to similarly assess shellfish consumption in comparison to the FFQ but did not correlate well with the dietary records.

Dietary assessment methods will likely always have some level of error and the type of error differs between methods. These results support the benefit of multiple measures of diet, including regional and culturally specific surveys that assess exposure to region-specific risk associated foods. For example, local shellfish in the Pacific Northwest can be characterized differently using a variety of dietary measures to accommodate a breadth of research objectives examining exposures in populations.

EXPOSURE AND EFFECT ASSESSMENT OF AEROSOLIZED RED TIDE TOXINS (BREVETOXINS) AND ASTHMA

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In previous studies, we have demonstrated statistically significant changes in reported symptoms for life guards, general beach goers, and asthmatics, as well as statistically significant changes in pulmonary function tests in asthmatics, after exposure to brevetoxins in Florida red tide (*Karenia brevis* bloom) aerosols. In this study, we explored the use of different methods of intensive ambient and personal air monitoring to characterize these exposures to predict self-reported health effects in our asthmatic study population. Health effects were evaluated in 87 subjects with asthma before and after 1 hour of exposure to Florida red tide aerosols assessed for aerosolized brevetoxin exposure using personal and ambient samplers. After only 1 hour of exposure to Florida red tide aerosols containing brevetoxin concentrations above 57 ng/m^3, asthmatics had statistically significant increases in self-reported respiratory symptoms and total symptom scores. However, we did not see the expected corresponding changes in pulmonary function test results. Significant increases in self-reported symptoms were also observed for those not using medication and those living $\geq$ 1 mile from the coast. These results provide additional evidence of health effects in asthmatics from ambient exposure to aerosols containing very low concentrations of brevetoxins, possibly at the lower threshold for inducing a biologic response (i.e., toxicity). Consistent with the literature describing self-reported symptoms as an accurate measure of asthmatic distress, our results suggest that self-reported symptoms are a valuable measure of the extent of health effects from exposure to aerosolized brevetoxins in asthmatic populations.

MONITORING OREGON'S COASTAL HARMFUL ALGAE (MOCHA): A LOGISTICAL APPROACH TO HAB EVENT RESPONSE

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The Oregon Department of Fish and Wildlife has routinely monitored nearshore waters along the Oregon coast for the presence of Harmful Algal Blooms (HABs) since 2004. Closures of shellfish harvest areas due to contamination by phycotoxins are frequent, and have a considerable economic impact on coastal communities. In 2003, the Oregon Department of Fish and Wildlife (ODFW) estimated that the closure of the razor clam fishery at Clatsop Beach alone cost the local communities $4.8 million. In order to mitigate such losses to coastal communities in Oregon, an integrated HAB monitoring and event response program was implemented in 2007.

As part of this effort, ODFW was able to enhance nearshore phytoplankton sampling to include 12 sites sampled weekly for the presence of HABs. A recent event response included the following;

- increased sampling effort of both water samples and tissue sampling
- alert notifications of increased HAB levels via a pre-established e-mail distribution list
- inter-agency collaboration to confirm species identification, biophysical conditions and bloom tracking

As the HAB numbers begin to fluctuate sampling strategies are adjusted to include; increased nearshore water analysis, the use of domoic acid (DA) field testing kits, increased laboratory tissue testing and increased alert notifications. With tens of thousands of harvesters expected to descend upon the beach during any one tide series the necessity to avoid negative human health and socioeconomic impacts is great. With out the integrated monitoring in place, resource managers would be unable to facilitate the necessary shift in priorities to proactively mitigate for the potential impacts.

MATRIX-SPECIFIC PROTOCOLS FOR THE DETECTION OF DOMOIC ACID IN MULTIPLE MARINE MAMMAL SPECIES

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We present the first several chapters from a manual which will be published on-line with the aim of establishing standard protocols for the detection and quantification of algal toxins in multiple sample matrices from a wide range of marine mammal species. The first algal toxin we focus on is domoic acid (DA). Very sensitive methods for detecting DA are now commercially available in the form of enzyme linked immunosorbent assay (ELISA) kits. Increasingly widespread use of these kits has highlighted the need to create a standardized sample preparation protocol in order to generate reliable, repeatable results. These kits were developed for testing shellfish tissue and the dilution and extraction methods were optimized for that specific sample matrix. Performing the same procedures for marine mammal samples such as feces or urine could lead to false positives or unreliable measurements due to matrix effects. Matrix effects testing for matrix types including feces, urine, and serum was carried out in samples from several marine mammal species including California sea lions (*Zalophus californianus*) and northern fur seals (*Callorhinus ursinus*). The minimum dilution required to avoid matrix effects in each sample type from each species was determined from these curves and is reported here. Tandem LCMS analysis was also performed on these samples, which allows for confirmation and quantification of DA thus validating the accuracy and range of the ELISA results.

ABUNDANCE AND DYNAMICS OF *Alexandrium catenella* OFFSHORE REDONDO BEACH (CA) AS DETECTED BY QUANTITATIVE PCR

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Toxic dinoflagellate species belonging to the genus *Alexandrium* recur over vast stretches of the U.S. coastline where they can produce saxitoxins, a neurotoxin that is the cause of paralytic shellfish poisoning (PSP) in birds, mammals and humans through contaminated seafood consumption. PSP outbreaks are known to be especially frequent and acute in the northeastern U.S., but 25 years of monitoring on California coasts has shown that *A. catenella* can also result in measurable levels of saxitoxins in the plankton during late summer/fall. The temporal and spatial dynamics of *A. catenella* blooms are highly variable, and environmental factors promoting outbreaks are poorly understood. This situation is partly because no accurate and rapid quantitative method has thus far been applied to study the ecology of this harmful species on the U.S. west coast.

We employed quantitative PCR (qPCR) for rapid identification and enumeration of *A. catenella* strains occurring in California coastal waters. A qPCR assay based on a molecular beacon targeting the 28S ribosomal RNA gene was adapted from a previous approach used in Japanese waters (Hosoi-Tanabe and Sako, 2006, Mar Biotech 7:506-514). The *A. catenella* strain used to develop the method was isolated from coastal waters off the City of Redondo Beach (CA) in May 2006. The method was applied to analyze environmental samples collected weekly over approximately a 3-year period, in order to provide a temporal survey of *A. catenella* abundance in the Redondo Beach area in relation to environmental parameters. These results are relevant for better prediction and prevention of harmful consequences of PSP outbreaks.

NEAR BOTTOM DINOFLAGELLATE POPULATIONS ON THE NORTHWEST FLORIDA SHELF

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Harmful Algal Bloom dinoflagellate populations are most commonly sought near the sea surface in the Gulf of Mexico. Seed populations of harmful dinoflagellates usually exist 18-74 km from the coastline on the shelf in the Gulf. Succession models and computer models have suggested that dinoflagellates populations could exist near the sediment interface, separate from surface populations. While the water column is extremely oligotrophic in the spring and fall, the sediments on the shelf contain higher nutrient levels, and larger diatoms will grow near bottom in depths where the euphotic zone reaches the sediments. In deeper stratified columns, as the euphotic zone separates from the nutrient rich sediments, dinoflagellates potentially have the ability to dominate over diatoms as they can migrate vertically through the water column between the nutrient rich sediments to through the base of the euphotic zone higher in the water column. Three cruises (May 2008, October 2008, and July 2009) occurred on the Northwest Florida shelf to investigate the spatial and temporal distributions of these near bottom dinoflagellate populations. Cross shelf transects between the 20-60m depth contours were taken on each cruise. Samples focused on the sediment interface were analyzed on the FlowCAM for dinoflagellate populations. Hydrographic, nutrient, and chlorophyll *a* concentration data were also collected in conjunction with near bottom samples. HPLC samples were also taken to compare to FlowCAM results of the diatom vs. dinoflagellate contribution to chlorophyll *a*. Nitrate samples showed low concentration throughout the water column, with higher concentrations at the sediment interface. Bottom oriented water samples generally showed that low concentrations of dinoflagellate populations were found to dominate between 30-50m depth contours, while diatoms dominated between 20-30m depth contours. In water columns deeper than 50m both dinoflagellates and diatoms populations decline as the euphotic zone separates from the sediment interface by more than a 12hr. swimming speed ascent.

THE UTILITY OF THE FAGAN TEST OF INFANT INTELLIGENCE TO EVALUATE INFANT NEUROBEHAVIORAL OUTCOMES IN HAB RESEARCH: PRELIMINARY RESULTS FROM THE CoASTAL COHORT

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The effect of marine biotoxins on infant growth and development is an important public health issue but largely unexplored in the scientific arena. As part of a larger on-going study focused on the cognitive effects of Domoic Acid (DA) in Native Americans living on the coast of Washington State, a group of 27 infants were tested on the Fagan Test of Infant Intelligence to evaluate early visual memory. This test has been successfully used in field and laboratory studies to examine the behavioral consequences of prenatal exposure to chemical compounds such as methylmercury, lead, polychlorinated biphenyls and alcohol. The Fagan test is based on the infant's selective visual attention to targets such as geometric shapes and human faces. Measuring the visual fixations of infants to test stimuli provides a vehicle to study attention and visual recognition memory in infants ranging from five to twelve months of age. Infants in the Coastal Cohort ranged from nine to twelve months and all infants completed the ten test problems. The preliminary results from this study show that infants in this cohort are, as a group, performing well within the normal range on this test (mean novelty preference of 63 ±7%). There is some evidence supporting enhanced performance on this test measure that may be related to maternal diets high in polyunsaturated fatty acids. We are now in the process of evaluating the relationship between infant performance on the Fagan test and maternal exposure to Domoic Acid during pregnancy.

CYANOBACTERIA BLOOM DYNAMICS IN SOUTH CAROLINA BRACKISH DETENTION PONDS: A PILOT STUDY

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Coastal South Carolina (SC) is undergoing some of the most rapid urbanization in the United States. This development entails residential neighborhoods, golf courses, and extensive stormwater drainage systems. Fresh and brackish detention ponds are frequently used as catchments of stormwater flow in SC coastal developments, but due to their limited flushing capabilities, detention ponds accumulate nutrients and other non-point source pollutants making them susceptible to eutrophication and algal blooms (Lewitus et al., 2003, 2008). Research and monitoring efforts revealed that detention ponds are the single greatest promoter of harmful algal blooms (HABs) in SC (Lewitus et al., 2003, 2008; Brock 2006). Cyanobacteria HABs in particular are a common feature of SC detention ponds, and they are unique because they occur in brackish systems as well as freshwater systems, their typical habitat (Lewitus et al., 2008). *Microcystis* is the most abundant cyanobacteria genus (Brock 2006; Lewitus et al. 2008), but the environmental factors associated with the proliferation of cyanobacteria blooms, including *Microcystis*, are not well understood in brackish systems. Moreover, the extent to which toxic and non-toxic *Microcystis* occur, or even co-exist, in SC brackish detention ponds is not known. Elucidating both the genetic variability both within and among *Microcystis* blooms, as well as the environmental factors that are linked with bloom development, will provide valuable information for management of detention ponds in coastal SC systems. Here we present preliminary results from a pilot field study conducted during the summer of 2009 exploring the environmental factors associated with the proliferation of toxic and non-toxic cyanobacteria in SC brackish detention ponds. Seasonal variability in nutrients, microcystin toxin levels, cyanobacteria abundances, and environmental variables are depicted from select brackish detention ponds. Further analyses into the population structure of *Microcystis* populations are anticipated.

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PHYTOPLANKTON COMMUNITIES OF THE WEST COAST OF FLORIDA – RESPONSE TO NUTRIENT ENRICHMENT

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Six bioassay studies were conducted in March, August, and November of 2007-2009 to determine the response of *Karenia brevis* and other local phytoplankton communities from the west coast of Florida to different nutrients and estuarine waters (Tampa Bay, Charlotte Harbor, and the Caloosahatchee River). In only one of the bioassays was *K. brevis* present in above background concentrations (11,000 cells per liter). Coastal bulk seawater was collected and immediately filtered through a 150µm mesh screen to exclude zooplankton. Aliquots of samples were transferred to 20 liter incubation chambers and were spiked with different treatments. Treatment levels for estuarine waters approximated a 1:25 dilution of estuarine water with bulk coastal water, while the addition of inorganic single nutrients was similarly scaled based on existing information of typical concentrations in estuarine outflow. Treatments, in addition to the individual 25 PSU estuarine waters, included NH_4, NO_3, Urea, PO_4, SiO_2, glycine, and arginine, with l-glutamine, and urea plus PO_4 added during later efforts. Incubations took place in ambient seawater, near surface, at the Mote Marine Laboratory facility. Treatments were sampled at initiation and at 24 and 48 hours post-treatment. Preliminary results show that diatoms dominated the ultimate response regardless of *K. brevis* presence, and that uptake rates of NO_3, NH_4, and urea, while differing after 24 hours, resulted in very similar phytoplankton community responses after 48 hours.

CYANOTOXICITY STUDIES IN WESTERN WASHINGTON LAKES

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There is concern over the increased occurrence of toxic cyanobacteria in Pacific Northwest lakes yet extremely little is still known about the frequency and dynamics of toxic blooms in this region. Currently there are three different programs from various funding sources that are providing much needed data on cyanobacterial neurotoxins and hepatotoxins throughout the state of Washington. Information from these studies will advance our understanding of bloom dynamics in these lakes and provide necessary information for lake management and protection of human health.

King County has the only long term monitoring program for the detection of cyanobacterial toxins in this region. Mycrocystins and anatoxin-a are measured regularly at public beaches in three major urban lakes (Washington, Union and Sammamish). To date, anatoxin-a has not been detected in these lakes and microcystin levels have remained below the 1 µg/L World Health Organization threshold for drinking water even though the toxin has been consistently detected in all three lakes.

In response to increased public awareness of health risks posed by algal blooms Washington State established funding for a statewide program to assist local governments in the management of toxic blooms. In its third year, this program is rapidly providing much needed data on species composition and toxin levels in target lakes throughout the region. The data available to date suggest that toxigenic species are common and persistent in the region's small lakes and that microcystin and anatoxin-a levels can often exceed recreational guidelines.

CDC has recently funded a five year study to evaluate cyanotoxin levels in 30 western Washington lakes, consisting of a systematic monitoring program for recreational lakes in the heavily populated Puget Sound lowland region. The study also includes cylindrospermopsin and saxitoxin, two toxins previously undetected in the state.

PATTERNS OF *Pseudo-nitzschia* AND DOMOIC ACID RELATED TO PHYTOPLANKTON COMMUNITY STRUCTURE IN THE BENGUELA CURRENT

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Members of the cosmopolitan diatom genus *Pseudo-nitzschia* produce a toxin called domoic acid (DA), which is responsible for Amnesic Shellfish Poisoning in humans and negative impacts on marine ecosystems. Phytoplankton community structure and DA levels were analyzed from samples collected from the Benguela Current, along the southwestern coast of Africa, in December 2007. *Pseudo-nitzschia* cells were measured for identification through the use of light microscopy and assigned to one of three morphological groups. Other organisms present were defined to the genus level. Phytoplankton community structure was dominated by either diatoms or dinoflagellates at each of the thirteen stations sampled. *Pseudo-nitzschia* was present at seven stations; at most of these stations, all three morphological groups were represented. Particulate DA was detected at five stations and ranged between 2.7 and 184 ng/L (averaging 3.0 pg/cell). The *P. australis/fraudulenta* group was the dominant type of phytoplankton detected at the station with the highest DA level. To help identify *Pseudo-nitzschia* species and more fully characterize *Pseudo-nitzschia* diversity, a genetic fingerprinting technique called Automated Ribosomal Intergenic Spacer Analysis (ARISA) will be performed. These community composition data, in conjunction with environmental parameters, could help elucidate *Pseudo-nitzschia* species and conditions correlated with DA production in this region.

THE ABILITY OF THE GASTROPOD *Crepidula fornicata* TO SUSPENSION FEED IN THE PRESENCE OF CULTURED AND WILD POPULATIONS OF THE HARMFUL BROWN TIDE ALGA, *Aureococcus anophagefferens*

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The American slipper limpet (*Crepidula fornicata*) is a protandrous, hermaphroditic gastropod native to the Atlantic coast of North America, including some estuaries on Long Island, NY. An important characteristic of *Crepidula* that may contribute to its ecological success is its ability to retain efficiently and consume particles down to 1µm in size, even under extraordinarily high particle loads. The Peconic Estuary in NY formerly hosted a multi-million dollar bay scallop fishery (*Argopecten irradians*) which collapsed with the onset of harmful brown tides (*Aureococcus anophagefferens*) during the late 1980s and early 1990s. During this collapse, populations of *C. fornicata* expanded in the Peconic Estuary and brown tides ceased to occur in this estuary after 1995. Brown tides continue, however, to plague other NY estuaries such as Great South Bay (GSB) where the northern quahog or hard clam (*Mercenaria mercenaria*) was once the largest fishery in New York, and where densities of *C. fornicata* are currently low. Landings of *M. mercenaria* in GSB have declined by 99% since the early 1980s, due to overharvesting and the sensitivity of this species to brown tides. In this study, we quantified rates of suspension feeding by *C. fornicata* and *M. mercenaria* in the presence of different monocultures of *A. anophagefferens* and wild brown tide blooms of varying densities. Our preliminary results suggest that *C. fornicata* can actively feed during dense brown tides which cause *M. mercenaria* to feed at low rates.

REGIONAL AND GLOBAL PHYLOGENETIC RELATIONSHIPS OF *Cochlodinium fulvescens* FOUND IN THE COASTAL WATERS OF CALIFORNIA

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The dinoflagellate genus *Cochlodinium* forms red tides resulting in massive fish kills and economic loss in the coastal waters of Korea, China, and Japan. The first recorded *Cochlodinium* bloom, off the Korean coast, was in 1982, and since then, blooms have expanded globally with speculation that ballast water transport facilitated their widespread distribution. *Cochlodinium* blooms along the eastern Pacific, stretching from British Columbia, Canada down to Costa Rica, were not reported until 2000. In 2004, California had its first large *Cochlodinium* bloom event and subsequently, *Cochlodinium* has been seen in elevated numbers along the central and southern California coastline during bloom and non-bloom events. Three years later, in 2007, a *Cochlodinium* red tide in Monterey Bay, California, impacted a local abalone farm resulting in the state's first reported commercial shellfish loss due to this organism. Large subunit (LSU), internal transcribed spacer (ITS) regions, and small subunit (SSU) ribosomal DNA (rDNA) sequences are all commonly used to evaluate inter- and intra-specific diversity in phytoplankton genera. *Cochlodinium polykrikoides* has distinct LSU rDNA ribotypes revealing global-scale regional populations: East Asian, Philippines, and American/Malaysian. Although *C. polykrikoides* is known to exist along the west coast of the Americas, recent work has suggested that the California strains of *Cochlodinium* belong to a distinct phylotype, *Cochlodinium fulvescens*. To complement global-scale sequencing, a similar effort, targeting LSU and ITS rDNA regions in *Cochlodinium* populations off the California coastline, is underway to elucidate phylogenetic relationships within this genus on a smaller, regional scale in addition to evaluating how these populations fit in globally. LSU rDNA sequences obtained in this study will also aid in the development of rRNA-targeted probes specifically for *Cochlodinium* found in Monterey Bay, which do not bind to currently available whole-cell probes designed for *C. polykrikoides*. This effort complements the parallel attempt to develop a qPCR-based approach for identifying and enumerating these organisms (see Howard et al., this meeting).

FIELD VALIDATION OF MOLECULAR SANDWICH HYBRIDIZATION ASSAYS TO DETECT *Karenia brevis* IN SOUTH-WEST FLORIDA COASTAL WATERS

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Sandwich hybridization assays (SHAs) for *Karenia brevis, K. mikimotoi, K. selliformis and K. papilionacea* were concurrently tested with the same whole, seawater samples collected offshore and inshore during the 5-year Monitoring and Event Response for Harmful Algal Blooms (MERHAB) Gulf of Mexico Sentinel Program and the local *red tide* monitoring program respectively. Results from testing ~900 samples (~700 from ship surveys, ~200 local samples) for the principal bloom-forming species in the region, *Karenia brevis,* were converted into cells.L^{-1} equivalents using an average standard curve (Charlotte A3 strain) to allow comparison with cell counts from microscopy. The 96-well plate version of the *K. brevis* SHA accommodates two different sensitivities. The lower limit of detection approximates near background cell concentrations (~1000 cells.L^{-1}) and the upper detection limit extends to ~400,000 cells.L^{-1} given a fixed sample volume of 250 mL, although the range can be varied by altering sample and lysis buffer volumes. Cell equivalents were binned into the same categories currently used for reporting *K. brevis* cell concentrations and the associated risk assessment to the public, spanning 4 orders of magnitude from $1x10^3$ to $>1 \times 10^6$ cells.L^{-1}. Additionally, natural and cultured samples were processed using the SHA in both the 96-well plate and printed probe array format for six different gymnodinioids including the above four species of *Karenia* using the Environmental Sample Processor (ESP). Preliminary results with matched sample lysates showed that a similarly wide dynamic detection range to $>1 \times 10^6$ cells.L^{-1} is also possible using the ESP, to allow remote, *in situ* categorical reporting of cells.L^{-1} equivalents. Both the 96-well plate and ESP array SHA techniques offer the ability to screen for multiple harmful algae including concurrently present *Karenia* species. For example, the SHA allowed the discrimination of overlapping populations of *K. brevis* and *K. mikimotoi* present during a 2005 marine mortality event, even though cells were misshapen in the hypoxic to anoxic conditions encountered.

A BEAD-BASED IMMUNOASSAY FOR DOMOIC ACID: APPLICATION OF LUMINEX XMAP® TECHNOLOGY AND TRANSITION TO A MICRO-FLOW CYTOMETER

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Among the recommendations of the U.S. National Plan for Algal Toxins and Harmful Algal Blooms (HARRNESS, 2005) is a need to develop methods for the rapid, field-based detection of HABs and toxins. A commercial, bead-based assay technology (Luminex xMAP®) is capable of rapid, multiplexed detection of target analytes via antibody- or nucleic acid-based recognition elements. Assays developed on this system can be transitioned to a micro-flow cytometer (Golden et al., 2009) that was designed with the aim of producing field-portable methods.

Domoic acid (DA) was selected for development of a model competitive immunoassay on the Luminex 100 system followed by its transition to the micro-flow cytometer. The toxin was attached covalently to polymeric microspheres, and the immobilized DA competed with DA in a sample/standard for phycoerythrin-labeled antibody. A calibration curve in PBS buffer was linear from 0.8-30 ng DA/mL. Assay performance was evaluated in several sample matrices, including natural seawater (30 salinity) and aqueous methanolic extracts of algae (culture and field material) and shellfish (mussels), all showing lower limits of quantification (in extract) from ~3-12.5 ng DA/mL. Comparisons of Luminex data with values obtained by LC-MS/MS for these sample types are underway.

Initial trials of the bead-based DA immunoassay were conducted on the micro-flow cytometer. The miniaturized flow cytometer employs a novel, groove-based flow design that allows the sheath fluid to completely envelope the core (or sample) stream, minimizing the potential for clogging and permitting recycling of sheath fluid. The device consists of a PDMS flow channel (390 µm wide) capable of measuring simultaneously both light scatter and fluorescence properties of beads (5.6 µm in diam.) passing through the optical interrogation region. Preliminary results showed that the micro-flow cytometer was capable of detecting DA in buffer. Moreover, the system could discriminate between different fluorescent bead sets, which will permit multiplexed detection of DA and other phycotoxins. Ultimately, our aim is to integrate the micro-flow cytometer into hand-held and potentially automated in-water platforms capable of providing near-real time data on HAB toxicity.

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NET COMMUNITY PRODUCTION AND DARK COMMUNITY RESPIRATION IN A Karenia brevis (Davis) BLOOM IN WEST FLORIDA COASTAL WATERS, USA

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Primary production rates in harmful algal blooms (HAB) have primarily been measured by the carbon-14 method. Oxygen-based productivity and respiration rates, in contrast, can describe the autotrophic versus heterotrophic state of pelagic communities. We determined net community production (NCP) and community dark respiration (R) in west Florida coastal waters during summer 2006 to evaluate the proportion of community respiration demands met by autotrophic production in a harmful algal bloom. The sampling program was adaptive in that surface waters were collected throughout the bloom at locations where surveys by the Florida Fish and Wildlife Research Institute indicated there were high cell abundances of *Karenia brevis*. Rates of NCP in surface waters during the 2006 bloom ranged from 10 to 42 mmol O_2 m^{-3} day^{-1} in light bottles with highest rates where *K. brevis* abundances exceeded 10^5 cells L^{-1}. Community dark respiration rates from dark bottle incubations ranged from < 10 to 70 mmol O_2 m^{-3} day^{-1} after periods of 12 h (daytime) to 24 h (diel period). Gross primary production estimated from the sum of NCP and R varied from ca. 20 to 120 mmol O_2 m^{-3} day^{-1}. Most surface communities exhibited net autotrophic production (NCP > 0) after 24 h, although heterotrophy (NCP < 0) characterized the densest bloom sample where *K. brevis* exceed 10^6 cells L^{-1}. A series of NCP and R rates were also derived from an automated productivity sampler deployed for six days in Sarasota Bay, Florida during a period when *K. brevis* varied from 10^5 to 10^6 cells L^{-1}. Rates of NCP and R from the automated sampler were comparable to those from light and dark bottle incubations at other locations, and further suggest that in surface communities with high abundances of *K. brevis* (10^6 cells L^{-1}) the community respiration demands can exceed net community production.

LET'S CREATE A 10 MINUTE OUTREACH VIDEO ON OUR HAB RESEARCH…… EASY, RIGHT?

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Abstract: As part of our ongoing research project on the environmental exposures and human health effects of aerosolized Florida red tide, we created a 10-15 minute video to recruit and educate prospective field study participants. While prospective field study volunteers were our primary audience, the web-based video could also be used to demonstrate the importance of our research to policy makers and funding agencies, as well as serve to educate both local residents and tourists about the ongoing Florida red tide research.

Methods: The content of the video covers the location of the study, the human health issues of aerosolized Florida red tide, the environmental sampling used, the epidemiologic field study, and future directions of our research. It includes interviews with researchers and study participants. Footage was taken over 4 days, and 7 interviews (total) were completed for the project. Approximately 8-10 hours of footage were edited for the 10 minute piece.

Discussion: Initially, the process of creating the video was estimated to take 6 months to complete. In reality, the script took about 20 hours to write and edit, and the video project involved 50 hours of active production and post-production over 8-9 months. The delay is due to a number of reasons including the community concerns surrounding Florida red tide and the possible impacts on tourism in southwest Florida, requiring multiple script rewrites and video re-editing. We will illuminate some of the issues, both positive and negative, that came out during the production of the video. Furthermore, we will detail the video production process, as well as lessons learned. The actual study video will be shown on a loop as part of the presentation.

Conclusion: The lessons learned from this video production may be helpful to other field scientists as they consider multimedia products to communicate their research.

QUANTITATIVE REAL-TIME PCR FOR *Cochlodinium* sp., A POTENTIALLY HARMFUL DINOFLAGELLATE FROM CALIFORNIA COASTAL WATERS

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Dinoflagellate species within the genus *Cochlodinium* have been linked to toxin production and fish kills. Blooms of these species have caused serious problems in the Pacific Ocean off the coasts of Japan, China, Korea and Canada but until recently their presence off the California coast was unremarkable. However, blooms of *Cochlodinium* have recently been documented in the coastal waters of central and southern California in 2004 and 2006. Cells belonging to the genus *Cochlodinium* are now routinely identified as members of algal assemblages at many locations along the central and southern California coastline. The increasing incidences of *Cochlodinium* blooms, the fact that these species are not readily preserved, and the difficulty of identifying these species based on morphological criteria, makes *Cochlodinium* a prime candidate for the development of a quantitative PCR approach. Sequences of the 18S ribosomal rDNA gene were obtained from a natural sample collected during the Los Angeles area *Cochlodinium* bloom in 2006, and from single cells identified as *Cochlodinium* based on morphological analyses. We exploited the unique sequence signature from local *Cochlodinium* sp. isolates to design and test a Molecular Beacon® approach to identify and quantify abundance of these species in natural water samples. We are in the process of optimizing our newly-developed qPCR approach. Analysis of archived field samples collected during ongoing monitoring efforts in Monterey Bay, Los Angeles and Orange County regions will allow us to describe the recent historical abundance of *Cochlodinium* sp., as well as provide a tool for documenting future bloom events.

INFORMATION TECHNOLOGY SUPPORT OF A HAB OBSERVATORY

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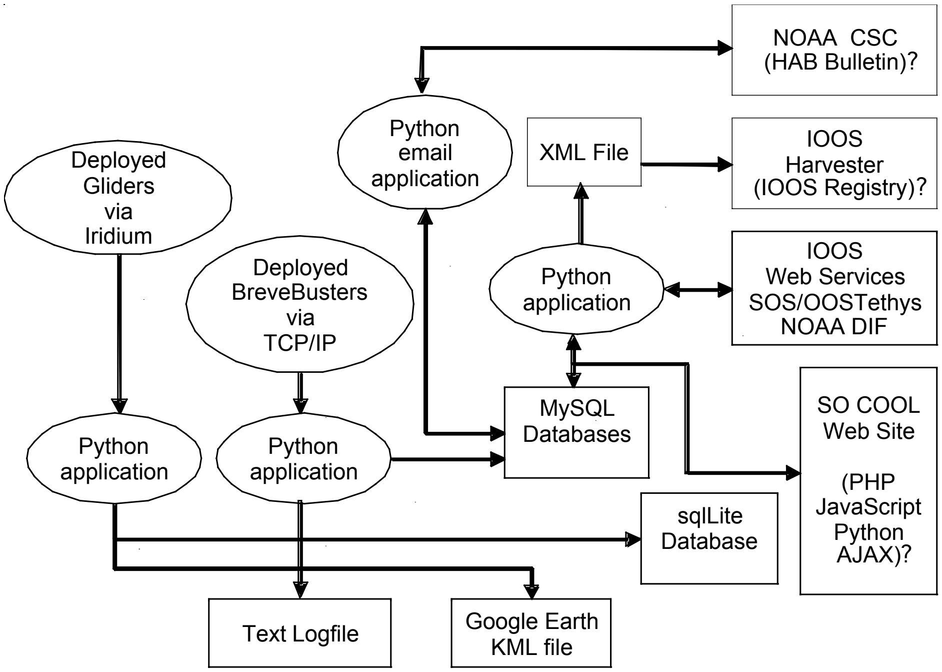
In May 2003 the Optical Phytoplankton Discriminator began making automated *in situ* observations of the presence/absence of harmful algae. At that time observations were recorded internally for collection after the return of the instrument. From that simplified beginning the information technology aspect of our HAB Observatory has grown, in many ways, faster than the instrument technology itself. Very shortly after the first *in situ* deployments of our HAB detection technology the idea of waiting until the device was recovered to obtain the findings was simply unacceptable. Thus began our effort to produce near-real-time and subsequently real-time information about the presence/absence of HABs for the research community, resource managers, government agencies and the public. Along the way, it became obvious that HAB observations did not have to come only from inanimate observers, but rather, many useful near-real-time manual observations of HABs were being made for the Beach Conditions Reporting System that needed to be incorporated in the automated information flow.

Once observations had been collected that information had to be transported, received, stored, checked, processed and disseminated. We used a variety of data transfer methods including direct cable, wireless network, VHF radio, cell phone and satellite phone depending on remoteness and available resources. Those data were initially received by desktop computer and viewed in tabular form for the instrument user. This quickly transitioned to an automated data server function that archived all received information and relayed only the salient pieces of information to the system manger or research scientist. A short but significant step from that point allowed customized data extractions to be sent to any requesting activity including the IOOS Web Service. The diagram below, with all of its IT nomenclature, is representative of our current information flow and will be central to this presentation.



MANAGING CONFOUNDING FACTORS IN THE STUDY OF DOMOIC ACID NEUROTOXICITY IN HUMANS: THE ROLE OF ANXIETY AND DEPRESSION IN THE CoASTAL COHORT

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A variety of behavioral factors are associated with abnormal performance on neuropsychological measures of cognition. These include anxiety and depression, the most common mental disorders reported in adults. Extant data suggests that subjective memory complaints are often associated with depression and anxiety. Moreover, both anxiety and depression can have detrimental effects on the hippocampus and its concomitant memory functions. Amnesic shellfish poisoning as a result of domoic acid (DA) intoxication has also been associated with hippocampal damage and memory impairment in humans, marine mammals and rodents. Therefore, the co-existence of depression or anxiety disorders could potentially confound the interpretation of memory data in studies of human domoic acid neurotoxicity. This has particular relevance to the American Indian CoASTAL cohort study of chronic, low level domoic acid exposure for two reasons: 1) memory is an important marker of neurotoxicity and 2) the base rates of depression in American Indian populations are relatively high compared to the U.S. population.

The purpose of this study was to determine whether or not there were elevated levels of anxiety or depression in the CoASTAL cohort. 533 Adult cohort members were formally studied with the Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory at baseline. Children were studied with the Children's Depression Inventory (CDI) and the Children's Behavioral Checklist (CBCL). Findings indicated that although women documented more symptoms of depression than men (BDI: mean = 11.8 v. 8.3, $p<0.01$) neither group fell into the clinically depressed range. Men and women reported similar levels of state anxiety, and women reported significantly higher trait anxiety (STAI: mean = 54.4 v. 51.9, $p=0.01$). All anxiety scores remained well within normal limits. With respect to children, both boys and girls scored in the normal range for total symptoms of depression, negative mood, interpersonal problems, ineffectiveness and anhedonia as measured by the CDI. Scores on the negative self esteem scale were slightly below average for both boys and girls. No significant differences were noted between boys and girls for any of the CDI scale scores. Similarly, scores on the Internalizing, Externalizing, and Total Behavior problem scales of the CBCL were also in the average range with no significant differences in behavior problems noted between boys and girls.

In conclusion, levels of depression and anxiety in men, women and children were well within normal limits in the CoASTAL cohort. These neurobehavioral factors should not be contributory to memory difficulties if they occur. Overall the children seem to have poorer self esteem than the general population. This may have significance for methods and models of community outreach and education for the children in the cohort.

TESTING A NEW TECHNOLOGY, SOLID-PHASE ADSORPTION TOXIN TRACKING (SPATT), FOR APPLICATION TOWARDS FIELD DETECTION AND MONITORING OF THE HYDROPHILIC PHYCOTOXIN DOMOIC ACID

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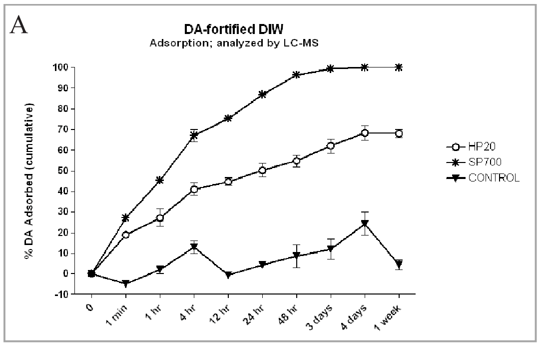
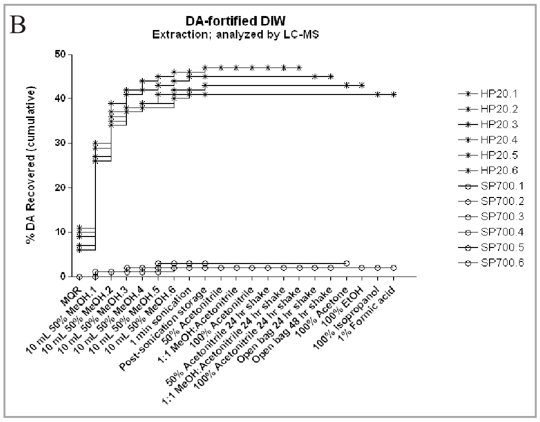
Solid Phase Adsorption Toxin Tracking (SPATT) is a phycotoxin tracking methodology in which man-made resin is used in the passive adsorption of toxins in water. First developed in New Zealand (MacKenzie et al. 2004), this technology was subsequently put into limited use in the UK (Turrell et al. 2007) and Australia (Takahashi et al. 2007), but remained untested in the US. Designed and tested for use with lipophilic toxins [e.g. dinophysis-toxin (DTX-1), okadaic acid (OA), pectenotoxin (PTX-2), yessotoxin (YTX), azaspiracid (AZA-1)], most initial laboratory trials and fieldwork exercises focused on the use of DIAION HP20, a polyaromatic adsorbent resin (styrene-divinylbenzene matrix) suitable for passive adsorption of lipophilic compounds. The development and use of SPATT technology suitable for use with hydrophilic phycotoxins [e.g. domoic acid (DA)], was pursued to a more limited extent. Here, we present results from laboratory trials and field deployments designed to develop, test, and optimize SPATT technology for use with domoic acid in both the field and laboratory setting.

A



B



Domoic acid is a hydrophilic molecule, suggesting that the SPATT resin identified as optimal for use with lipophilic toxins, HP20, may have reduced applicability in adsorption of this toxin and for its detection in the field. As a starting point, we elected to trial both the HP20 resin and a new resin recently identified as useful with domoic acid (E. Turrell, pers. comm.). This new polystyrene-based resin, SEPABEADS SP700, was reported to have demonstrated good applicability towards DA in terms of adsorption and recovery efficiency, and was suggested as an excellent candidate for our trials. As a field component, we developed a SPATT bag deployment design and maintained regular (~7d) deployments of 100µm-mesh Nitex® bags each containing 3g of HP20 and SP700 resins. These resins were deployed alongside sentinel mussels that are sampled and sent weekly to the California Department of Public Health (CDPH) for biotoxin analysis as part of the state-wide monitoring program. Results from these field trials, ongoing since July 2008, will be presented alongside laboratory trial results describing adsorption and extraction efficiencies for both resins. Our preliminary results for the adsorption of DA from DA-fortified Milli-Q by both the HP20 and SP700 resins are shown at right (Fig. A); our preliminary results from the subsequent extraction series is also shown (Fig. B). While SP700 demonstrated superior adsorption efficiency over the HP20 resin, it demonstrated a recovery efficiency that was substantially lower (~3% versus ~46%).

These preliminary results instigated an inter-laboratory comparison of SP700 extraction efficiency with E. Turrell of the Fisheries Research Services (FRS) Marine Laboratory in Aberdeen, UK, and our reconsideration of the previously described extraction protocol prescribed for use with SP700 and DA. In addition, we sought alternative resins that might demonstrate more optimal extraction and adsorption efficiencies, ultimately identifying (and evaluating) three additional resin candidates. In preliminary laboratory trials of adsorption and extraction efficiencies, one of these resins demonstrated an adsorptive efficiency that was substantially improved over the SP700 resin (SP207SS; >90% in <30 min.). These results, and our suggestions for future work, will be presented.

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HARMFUL ALGAL BLOOM ILLNESS-RELATED SURVEILLANCE SYSTEM (HABISS) – PROGRESS REPORT

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Background
The National Center for Environmental Health at the Centers for Disease Control and Prevention developed HABISS to assist public health decision-making regarding the risks of harmful algal blooms. The system securely rests on top of a CDC data collection program known as the Rapid Data Collector (RDC). The program is flexible enough to allow data collection on human and animal exposures to numerous environmental contaminants, including the known algal toxins. Ten state grantees contribute historical and current data to HABISS as part of a 5-year funded cooperative agreement while other states contribute on a volunteer basis. We conduct active case-finding in collaboration with regional Poison Information Systems, National Wildlife Health Center, NOAA's Marine Mammal Center, as well as follow-up to reported HAB events in the media. The current case definitions used in HABISS were developed by a cross-disciplinary team of public health professionals in conjunction with CDC. Geospatial data for HABISS is captured via Google Maps^{TM} and aggregate data for all states is reported to users of the system.

Results
Since inception of this project, the system has captured more than 700 reported harmful blooms, 50 confirmed human illness cases, 75 suspected human cases, and 25 suspected animal events – all occurring between September 1, 2003 and May 20, 2009. Types of human illnesses reported include suspect and confirmed cases of azaspiracid poisoning (AZP), brevetoxin poisoning from aerosols, brevetoxin poisoning from skin contact, ciguatera fish poisoning, domoic acid (amnesic shellfish) poisoning (ASP), lyngbyatoxin poisoning from skin contact, microcystin poisoning, and saxitoxin poisoning from ingestion (PSP).

Activities
Ongoing and future initiatives include: 1) Mapping vocabulary to existing medical codes, 2) Creating messages consistent with the Public Health Information Network, 3) Linking reported weather conditions and meteorological factors to HAB events, 4) Adding a simplified prediction component, 5) Collaborating with state partners to build web-based bulletins for early warnings of HAB events, 6) Providing scientific data from HABISS to aid the discussion of HABs and global climate change, 7) Expanding the HABISS network to include international partners and participants.

OLIGONUCLEOTIDE PROBES DESIGNED TO DETECT TOXIC *Pseudo-nitzschia* (BACILLARIOPHYCEAE) SPECIES IN THE GULF OF MAINE, USA

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As a result of a Marine Mammal Unusual Mortality Event (UME) involving whales and seals and potential domoic acid (DA) toxicity in the Gulf of Maine (GOM) in July of 2003 and the detection of DA at low levels in offshore scallops over the last several years, a heightened sense of concern is now focused on *Pseudo-nitzschia* populations in this region. Because *Pseudo-nitzschia* species are morphologically similar to one another, and only certain species within the genus produce DA, it is important for researchers to quickly enumerate the toxin-producing assemblages in a water sample to assess potential risk. To this end, five oligonucleotide probes were designed to quantify two toxic *Pseudo-nitzschia* species we have found in the GOM: *P. seriata,* and the *P. pseudodelicatissima* "complex". These probes were developed using ribosomal DNA sequence data from 36 *Pseudo-nitzschia* strains comprising seven species established from samples collected during GOM research cruises in 2006, 2007, and 2008. Three RNA probes (pse1, pse2 and pse3) were developed for *P. seriata* and two (pps1 and pps2) for the *P. pseudodelicatissima* "complex". All probes were labeled with 6-FAM at the 5' terminus and tested for specificity using standard whole cell FISH methods on samples from our culture collection. These probes are now being used to quantify these species in field samples collected in the study years in order to understand the distribution and density of toxic *Pseudo-nitzschia* species in the GOM region.

GLOBAL DISTRIBUTION OF CIGUATERA CAUSING DINOFLAGELLATES IN THE GENUS *Gambierdiscus*

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Ciguatera fish poisoning (CFP) results from the bioaccumulation of toxins produced by dinoflagellates species in the genus *Gambierdiscus*. Globally, CFP is the most common non-bacterial food poisoning reported. Currently, little is known about the distribution of individual *Gambierdiscus* species or their relative toxicities. This poster presents an LSU rDNA phylogeny supporting the existence of at least 12 different *Gambierdiscus* species along with a map documenting the unequal distribution of these species between the Atlantic and Pacific Oceans. Isolates of *Gambierdiscus belizeanus*, *G. carolinianus*, *G. ruetzleri*, and *Gambierdiscus* sp. ribotypes 1 and 2 all came from the Atlantic, whereas isolates of *G. pacificus*, *G. polynesiensis*, *G. toxicus*, and *G. yasumotoi* were all from the Pacific. *G. caribaeus* and *G. carpenteri* were the exceptions being widely distributed throughout both basins. Interestingly, the unequal species distributions noted in this study paralleled known structural differences in the dominant ciguatoxins recovered from Atlantic and Pacific fishes. Jointly, these data suggest that Atlantic species may produce a suite of toxins different from those synthesized by their Pacific counterparts. A literature survey further showed that the overall densities of *Gambierdiscus* in the Atlantic and Pacific were similar and that both regions contained isolates whose toxicity ranged from below detection to extremely high. Analysis of field collected cells showed variations in toxicity were equally large. These data imply that the toxin levels in a given location depend on which *Gambierdiscus* species or strains are present as well as overall cell densities. This may account for why significant increases in *Gambierdiscus* abundance sometimes fail to produce a subsequent CFP event. What these laboratory studies could not adequately resolve was whether the among isolate variation in toxicity was due more to inherent differences in the toxicity among *Gambierdiscus* species, to differences among strains, or to both. The reason is a majority of the toxicity studies on *Gambierdiscus* isolates were undertaken when only one species had been described. Data from the limited number of isolates whose identity was unambiguously established showed that among species toxicities varied by at least 100 fold and that environmental factors likely modulate toxicity to a lesser degree (~2 to 9 fold range). Only two comparable estimates for between isolate variations in toxicity were available precluding similar range estimates. Whether the variation in toxicity is due more to species or strain differences has profound implication on our ability to develop an effective early warning system for CFP.

Alexandrium catenella CYSTS, SEDIMENT ANALYSES, AND PB-210 DATING OF A LONG CORE FROM QUARTERMASTER HARBOR, PUGET SOUND, WASHINGTON

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Alexandrium catenella, a dinoflagellate known to cause paralytic shellfish poisoning, has existed in parts of Puget Sound for years. *A. catenella* spends part of its life cycle as a cyst in the sediment before germinating to become a free-swimming dinoflagellate. *A. catenella* cysts in surface sediments are used to determine the present spatial distribution of this organism, while cyst concentrations in sediment cores can be used to evaluate historical presence and temporal variability. Surface sediments in Quartermaster Harbor (QMH) have the highest abundance of *A. catenella* cysts anywhere in Puget Sound. We present the results of *A. catenella* cyst counts from a 112 cm sediment core collected in the middle of QMH during March 2005. Other down core properties presented include grain-size and total organic carbon analyses, and ^{210}Pb dating. Cyst abundance shows an exponential decrease from over 10,000 to 13 cysts/g of dry sediment in the upper 20 cm, fluctuating from 0 to 28 cysts/g 20-64 cm, below 64 cm numbers drop to 3 cysts/g of sediment, and then to 0 from 88-112 cm depth. Total organic carbon fluctuates from 3.3-9.2% for the first 64 cm of core, and beyond 64 cm, there is a steady decrease of TOC from 5.1-1.4% at the bottom of the core. For grain-size distribution, silt is the dominant grain-size to 72 cm, where sand takes over as the dominant size to the bottom of the core at 112 cm. ^{210}Pb analysis of the piston core was inconclusive because the excess activity of the ^{210}Pb isotope decreased to background level (0.5dpm/g) below 10 cm deep in the 112 cm core. Understanding the historical distribution of *A. catenella* cysts is important in tracing its spread throughout the Puget Sound.

THE IMPACT OF NUTRIENT AVAILABILITY AND HYDRODYNAMICS ON PHYTOPLANKTON COMMUNITY STRUCTURE OF THE WEST FLORIDA SHELF

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Harmful algal blooms (HABs), such as the dinoflagellate *Karenia brevis*, occur suddenly in surface waters of the Gulf of Mexico. Despite extensive research, little information is known about source populations contributing to bloom formation. A few studies have demonstrated that dinoflagellates can exhibit diel vertical migration (DVM) where they are associated with sediments but not surface waters. This study focused on the 'Benthic Dinoflagellate Migration' (BenDiM) niche where sediment nutrients are available and PAR attenuation is too low for microphytobenthic algae yet within the 12 h swimming range. We investigated the temporal and spatial occurrence of phytoplankton community composition in relation to hydrographic conditions and nutrient availability. Cross-shelf transects were conducted between the 20 and 60 m contours of the continental shelf off Panama City, FL, in October 2008, before and after a front passage. Water samples were collected at selected depths for FlowCAM and chemotaxonomic plankton identification, biomass determination, and nutrient concentration. Prior to the wind event, the thermocline was approximately 10 m above the bottom. Nitrate was highest (3.5-6 μM) in surface and near-bottom waters between the 15-35 m contours. A near-bottom chlorophyll *a* maximum occurred between the 15 and 25 m contours and based on pigment and FlowCAM analysis, dinoflagellates and diatoms were most abundant at the sediment interface. Dinoflagellates contributed 42% to chlorophyll *a*, while diatoms contributed 29%. After the wind event, the water column was well mixed and the thermocline disappeared. Nitrate, chlorophyll *a*, dinoflagellates, and diatoms decreased and were uniform throughout the water column. According to pigment and FlowCAM analysis, dinoflagellates were the dominant group.

DEVELOPMENT OF A PREDICTIVE MODEL FOR DOMOIC ACID PRODUCTION OFF THE OREGON COAST

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The ecological and economical impacts of domoic acid (DA), a neurotoxin produced by some species of the genus *Pseudo-nitzschia*, are well known, yet knowledge of the factors driving DA production and cessation is limited. Our goal is to develop a predictive logistic regression model that links the production of DA with environmental variables. The foundation of our model is a growing database, currently containing over 700 discrete particulate and dissolved DA samples from sites spanning the Oregon and southern Washington coastline. In addition to DA concentrations, the database contains the samples' co-located ancillary biological and physical data (nutrient and chlorophyll concentration, temperature and salinity) and corresponding satellite data (sea surface temperature, chlorophyll concentration and a satellite bloom detection product that is currently under development). Further physical proxies of bloom proliferation, such as upwelling intensity, based on the Bakun upwelling index, and USGS river outflow rates will be included as well. An estimated 300 more samples and their ancillary data will be collected each year through at least 2012, and used to both augment the existing database and refine and test the predictive capabilities of the model.

PREDATOR IMPACT ON THE POPULATION DYNAMICS AND VERTICAL DISTRIBUTION OF *Heterosigma akashiwo*

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We tested the hypothesis that avoidance of the raphidophyte *Heterosigma akashiwo* by potential protist predators can enhance *H. akashiwo's* survival. In the field, we observed the brief occurrence of a dense *H. akashiwo* layer in a shallow fjord in coastal Washington State. At its peak, *H. akashiwo* reached 4 million cells per liter. Photosynthetic parameters, measured using P vs. E experiments, for *H. akashiwo* dominated samples did not indicate significantly higher growth potential than for other phytoplankton. When *H. akashiwo* was at its peak density, there was no measurable grazing impact by heterotrophic protists. Grazing rate measurements, made using the dilution technique, both prior and after peak *H. akashiwo* density showed active predation by heterotrophic protists. In the laboratory, we measured heterotrophic protist behaviors and grazing rates in response to *H. akashiwo* to elucidate whether predator avoidance reduces grazing pressure on this HAB species. We exposed heterotrophic protist predators to discrete layers of *H. akashiwo* and simultaneously measured both predator and prey population distributions within a 1 L, 30 cm tank using automated, computer-controlled stereo-video and image analysis procedures. None of the predators avoided exposure to *H. akashiwo*, although a marine ciliate (*Favella ehrenbergii*) experienced increased mortality in the presence of *H. akashiwo* relative to a control and preferred prey treatment. In grazing experiments, large ciliates were more affected by *H. akashiwo* toxicity than two species of heterotrophic dinoflagellates (*Gyrodinium dominans, Oxyrrhis marina*). Toxicity was enhanced at lower salinities (<15 PSU) for some ciliate species. The presence of non-toxic algae partially alleviated the toxic effect. If predator-prey interactions in the field follow similar dynamics as observed in the laboratory, *H. akashiwo* would experience lower mortality rates due to lower grazing pressure from some predators, especially at lower salinities. This lack of mortality can lead to increased survival of *H. akashiwo* and may be a contributing factor in the formation or persistence of HABs.

Pseudo-nitzschia BLOOMS ON THE WASHINGTON COAST: AN ANALYSIS OF LONG-TERM MONITORING DATA FROM THE ORHAB PROGRAM

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Pseudo-nitzschia cell concentrations and particulate domoic acid (pDA) in seawater at six key core monitoring sites from the Olympic Region Harmful Algal Bloom (ORHAB) program on the Washington State coast are examined from 2000 to 2007. Twice-monthly observations of *Pseudo-nitzschia* cell concentrations are interpolated on a daily timescale and are used to determine interannual variations in bloom characteristics. Annual bloom duration was calculated as the number of days that cell concentrations exceeded action levels for "large" and "small" *Pseudo-nitzschia* cell types (i.e., 5×10^4 and 1×10^6 cells L^{-1}, respectively). In general, action levels were exceeded and pDA was detected at least once every year at one or more sites on the Washington coast, but cells did not always produce detectable levels of toxin during blooms. When action levels were exceeded it was mostly by the large cell types (including *P. multiseries*, *P. pungens*, *P. australis*, *P. heimii*, *P. fraudulenta*) and usually between the months of June and October at the central coast beach sites and April to October at the southern sites. Unsafe levels of domoic acid in Pacific razor clams resulted in closures at some beaches on the Washington coast during all years except 2006 and 2007. In 2006, toxic razor clams were detected at one central coast beach site but this did not interfere with a scheduled recreational dig and therefore did not result in a closure. The most toxic event occurred during October 2002 when domoic acid in razor clams peaked at 188 ppm – over 9 times the regulatory limit for human consumption. Razor clams on the Washington coast remained toxic for approximately 1 year following the October 2002 event. Linking these patterns of bloom characteristics with environmental parameters will contribute towards the development of quantitative empirical models to be used as decision-support tools for translating monitoring efforts and seasonal-to-interannual climate predictions into HAB risk assessments.

COASTAL EUTROPHICATION LAND USE CHANGES AND *Ceratium furca* BLOOMS IN PAGO PAGO HARBOR, AMERICAN SAMOA 2007-2009

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The bloom forming dinoflagellate, *Ceratium furca*, has been linked with coastal eutrophication worldwide in tropical and subtropical locations. During the summer of 2007, an unusual 6 month long bloom of *C. furca* was observed in Pago Pago Harbor, American Samoa. Incidents of dinoflagellate blooms in this area have not been previously reported. The bloom was first reported in May and dissipated in November 2007. In the February-March 2009, a similar *C. furca* bloom was observed. During both blooms, no fish mortality events were reported. Maximum cell counts were observed on 20 September 2007 at 9,300 cells/ml. At this time, total nitrogen was measured at 1.2 mg/l while total phosphate was below detection limits.

Changes in land use practices may have been the primary driver of these blooms. Over- fertilization of athletic fields is hypothesized to have a direct link to the increase in nutrients found in the Pago Pago Harbor and may have been the trigger for the initialization of these blooms. For the construction of the athletic fields, coral sand was imported to American Samoa. Along with the sand, an invasive species of fire ant (*Solenopsis geminate)* was also imported. During 2008, these fields were not used due to an infestation of these colonial ants. Once controlled, the fields were open again in 2009 and fertilizers were applied in January, a month before the bloom was observed.

THE U.S. GEOLOGICAL SURVEY NATIONAL WATER-QUALITY ASSESSMENT PROGRAM: ADVANCES IN UNDERSTANDING SOURCES, TRANSPORT, AND THE BIOLOGICAL EFFECTS OF NUTRIENTS FROM HEADWATER STREAMS TO COASTAL RIVERS

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The U.S. Geological Survey implemented the National Water-Quality Assessment (NAWQA) Program in 1991 to provide nationally consistent information on the status and long-term changes in the physical, chemical, and biological condition of streams and rivers. This information directly supports national, regional, state, and local information needs and decisions related to water-quality management and policy. The NAWQA Program uses a combination of monitoring-based observations and watershed models to assess nutrient and biological conditions in streams, nutrient transport from headwaters to coastal waters, and effects of human activities and natural processes on nutrient conditions. Agricultural and urban land uses are major sources of stream nutrients, with the highest nitrogen concentrations associated with agricultural streams and the highest phosphorus concentrations associated with agricultural and urban streams. NAWQA studies of nitrogen processing in small streams indicate that nutrient-enriched agricultural streams are ineffective at removing nitrogen from the water column because of high nutrient inputs and reduced retention time, and therefore export a large fraction of their nitrogen load to downstream waters. Although biological communities in streams are influenced by various natural and anthropogenic factors, nutrient enrichment has a strong influence on community structure. For example, NAWQA determined that the condition of algal, invertebrate, and fish communities decreased in streams nationwide as nutrient concentrations increased. Additionally, regional- and national-scale models were developed that provided information on the downstream fate of nutrients and the effect of hydrological and biogeochemical processes on delivery of nitrogen and phosphorus loads from inland watersheds to coastal estuaries. These assessments reveal that agricultural sources (cultivated crops, livestock manure) in the central and eastern Mississippi River Basin contribute a majority of the nitrogen and phosphorus to the northern Gulf of Mexico, where elevated nutrients have contributed to long-term seasonal hypoxia. New regional-scale models, based on updated geospatial and stream monitoring data, currently are under development for six major river basins of the conterminous United States. Results from these models will be used to identify the nutrient sources and watersheds that contribute elevated nutrient loads to estuaries and other receiving waters, including those in the South Atlantic and Gulf of Mexico, inland and coastal waters of the Northeast, the Upper Mississippi and Great Lakes, and Puget Sound.

FLORIDA RED TIDE PERCEPTION: RESIDENTS VERSUS TOURISTS

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The west coast of Florida experiences frequent blooms of the toxic dinoflagellate, *Karenia brevis*. The brevetoxins produced by *K. brevis* are not only harmful to humans if ingested in shellfish, but also through inhalation of the toxic aerosols. Numerous outreach materials, including Frequently Asked Question (FAQ) cards, exhibits at local museums and aquaria, public beach signs, and numerous different websites have been developed to disseminate information to the public (i.e. www.mote.org, www.floridamarine.org, www.redtideonline). In addition, during intense onshore blooms, considerable media attention, primarily via newspaper and television, is focused on red tide. However, the only measure of effectiveness of these various outreach methods has been to quantify the users, e.g. the number of people to visit a website, the number of visitors at a museum and/or aquarium, and the number of FAQ cards distributed. There has been no assessment to determine if these materials affect public knowledge about Florida red tide.

Our study addresses these issues by creating and implementing an evaluation tool for the assessment of public knowledge about Florida red tide. A focus group of Florida red tide outreach material developers was used to create the assessment tool. The evaluation was administered on the west coast of Florida, in Sarasota County during the 2005 Florida red tide bloom. Our objective was to learn what Gulf Coast residents and tourists to Sarasota know about Florida red tide and how that knowledge differs between the two groups. Also, how these two groups of people get their information about Florida red tide. The results from this evaluation will aid the various agencies developing outreach materials by recognizing what the gaps are in public knowledge regarding Florida red tides, identifying needed materials to address the information gaps, and also to determine the sources of information preferred by the public. These insights will be valuable in subsequent outreach and education programs related to all HAB research as well as its importance to public understanding Florida red tide impacts.

CHANGES IN WORK HABITS OF LIFEGUARDS IN RELATION TO FLORIDA RED TIDE

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Karenia brevis is the marine dinoflagellate responsible for Florida red tide. Brevetoxins, the neurotoxins produced by *K. brevis* blooms, can cause fish kills, contamination of shellfish, and respiratory irritation in humans. Several studies have assessed different economic impacts from Florida red tide blooms. However, no studies to date have considered the impact on beach lifeguard work performance. Sarasota County has guarded beaches 365 days a year and this study examines lifeguard and attendance records during the time periods of March 1 to September 30 in 2004 (no bloom) and March 1 to September 30 in 2005 (bloom). The lifeguard attendance data show a statistically significant difference in the amount of time taken off during these two periods, with increased absenteeism during a Florida red tide bloom. Records of the number of beach visitors show no difference between 2004 and 2005 periods. The costs associated with absenteeism during blooms results from lifeguards taking more time off when there is the same need for guards on the beaches. Lifeguard presenteeism is another economic cost of *K. brevis* blooms since the lifeguards are required to work during a bloom regardless of the health effects they experience. When surveyed, lifeguards report not only that they experience health effects of exposure to Florida red tide, but also that their attentiveness and productivity decrease when they work during a bloom. Thus, lifeguard performance, both absenteeism and presenteeism, is one of the economic impacts of Florida red tide. However, more studies are needed to accurately quantify the cost.

Pseudo-nitzschia **SPECIES IN WEST FLORIDA COASTAL WATERS; DO DIFFERENCES IN TEMPERATURE AND SALINITY CONTRIBUTE TO LOW DOMOIC ACID PRODUCTION?**

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Nine species of *Pseudo-nitzschia* have been identified from Florida coastal waters. Domoic acid (DA) is produced by members of this genus in other parts of the world. Elevated levels of DA due to *Pseudo-nitzschia* blooms have been reported to cause numerous DA poisoning events across the United States (Scholin et al., 2000; Work et al., 1993). Morphologically *Pseudo-nitzschia* species identified from Florida waters are identical to some of the major toxin-producing species in Californian and Canadian waters; however comparable levels of DA have never been recorded from Florida waters. As part of the Florida Fish and Wildlife Conservation Commission (FWC) Harmful Algal Bloom (HAB) monitoring program, surface seawater samples were collected from 8 sites on a weekly basis between 2005 and 2008. Fluctuations in cell abundances during the four-year study period revealed a pattern with peaks occurring in spring and summer of each year. In addition to an apparent seasonal periodicity, our data suggest that cell abundance increased each year between 2005 and 2008, and reached a maximum of 5.2×10^6 cells L^{-1} in May 2008. Particulate DA values ranged from <LD - 3.63×10^3 ng L^{-1}. Low levels of DA (<LD - 2.21 µg/g) were detected in shellfish samples collected during 2007 and 2008, representing the first report of DA in shellfish from the Florida waters. Temperature and salinity was measured at each site during the study period. Salinities ranged from 27.5 - 40.2. Observed elevated cell abundances and salinity values support recent findings by Thessen et al., (2005) in which *Pseudo-nitzschia* species occurred more frequently at higher salinities. Temperatures ranged from 12^{o}C to 34^{o}C and rarely dropped below 15^{o}C during the study period. Research conducted by Trainer et al., (2009) suggests that there are particular water properties favorable to high values of particulate DA, i.e. temperatures of 11^{o}C - 15^{o}C and salinities of 31.5 - 33.0. Differences in temperature and salinity between Florida and areas of high toxin production may help to explain the apparent lack of significant DA production by west Florida's *Pseudo-nitzschia* populations.

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MODELING *Karenia brevis* BLOOMS ON THE WEST FLORIDA SHELF

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Karenia brevis is present in low concentrations in many areas of the Gulf of Mexico (GoM). This toxic dinoflagellate occasionally develops blooms in many parts of the GoM. On the West Florida Shelf (WFS), however, these harmful algal blooms (HABs) are recurrent events whose frequency and intensity appear to be increasing. HABs on the WFS are usually only evident once they have achieved high enough concentrations that can be detected by observation of discolored water, which may be apparent in satellite imagery; by ecological problems such as fish kills; or human health problems. Because the early development stages of HABs are usually not detected, there is limited understanding of the environmental conditions that lead to their development. We have recently formulated a new hypothesis relating to HAB development on the WFS. This hypothesis stems from the analysis of satellite-tracked drifter trajectories that have revealed the presence of a cross-shelf transport barrier on the WFS. We have characterized such a barrier as a persistent *Lagrangian coherent structure* (LCS) by analyzing simulated surface ocean currents using dynamical systems tools. Our hypothesis is that this LCS behaves as a trap for nutrients and dinoflagellates initially positioned on its inshore side, thereby providing favorable conditions for the development of HABs.

In this work LCSs are extracted from simulated surface ocean currents to detect time evolving low mixing regions that constitute potential pathways for HAB development. Identification of such pathways allows us to trace the potential early development locations of an observed HAB. A simplified population dynamics model is used to infer the factors that could possibly lead to the development of the HAB in question. The population dynamics model determines nitrogen in two components, nutrients and phytoplankton, which are assumed to be passively advected by simulated surface ocean currents. The nutrient sources are found to be located near shore and likely due to land runoff.

Thalassiosira pseudonana PROTEOME: USING CORRA TO ANALYZE THE DIFFERENTIAL EXPRESSION OF PROTEINS UNDER DIFFERENT NUTRIENT SOURCES

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To understand the biology of nutrient requirements by a centric diatom and explore how different nutrient conditions affect the proteome of *Thalassiosira pseudonana*, we grew cultures of this organism under various nitrogen sources (replete/starved), and we also used a newly emerged liquid chromatography-mass spectrometry (LC-MS) quantification technique Corra, which takes advantage of a high mass accurate mass spectrum instrument (e.g., Orbitrap or FT) to detect features and quantify. Those features are then annotated with proteins that are identified by Trans-Proteomic Pipeline MS2 search pipeline. We are also able to detect differentially expressed proteins between NO_3 replete and starved conditions. Those differentially expressed proteins are further analyzed by Protein Information and Property Explorer and channeled into Gaggle bioinformatics platform in order to provide insight of biological process by visualization and analysis of the differentially expressed proteomic data in context of metabolic pathways, and genome annotations. We are applying this technology and analysis technique to the toxin producing diatom *Pseudo-nitzschia.*

GENOME SEQUENCING OF THE TOXIN-PRODUCING DIATOM *Pseudo-nitzschia multiseries*

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Diatoms of the genus *Pseudo-nitzschia* can produce the potent neurotoxin domoic acid (DA). *Pseudo-nitzschia* is a cosmopolitan genus with species that thrive in coastal environments, where they threaten local fisheries, and species that appear well-adapted to low productivity open-ocean gyres, dominating iron-induced blooms in these regions. In an effort to uncover a molecular basis for toxin production and to better understand the global success of this genus, *Pseudo-nitzschia multiseries* CLN-47 was chosen for whole genome sequencing. The estimated genome size is nearly 300 Mb, an order of magnitude larger than the two diatoms with published whole genome sequences (*Thalassiosira pseudonana* and *Phaeodactylum tricornutum*). The current draft is at 4x coverage with reads that have been clustered onto 8696 scaffolds. Additional sequencing of RNA extracted from *P. multiseries* grown under five different conditions (such as silicate starvation and iron limitation) yielded over 11,000 EST clusters. Some of these conditions promoted toxin production and the sequences derived from these EST clusters will be used in comparative analyses to find *Pseudo-nitzschia* specific genes that may have a role in toxin synthesis or regulation. Approximately 57% of the *P. multiseries* reads, with matches to known sequences in GenBank, have their best hits to the sequenced diatoms *T. pseudonana* and *P. tricornutum* (using BLAST). Preliminary analyses suggest *P. multiseries* carries a higher load of transposons (~10% of the genomic reads) compared to *T. pseudonana* and *P. tricornutum*. A potential consequence of such a high number of transposons is a greater genome and metabolic flexibility. However, transposons, like other repeat elements, frustrate assembly efforts using traditional Sanger sequencing. We are beginning to use new short-read deep sequencing technology (AB SOLiD, mate-pair libraries) to supplement the Sanger sequencing in the hopes of better coverage and assembly of this genome. An analysis of the current draft of the genome will be discussed, including comparative genomics with other photosynthetic eukaryotes and bacteria as well as metabolic maps of complete or near-complete pathways with emphasis on potential roles in toxin production.

IMPLICATIONS OF PHYTOPLANKTON COMMUNITY COMPOSITION ON WEST FLORIDA CONTINENTAL SHELF *Karenia brevis* BLOOMS. TIME SERIES TRANSECT DATA AND CHEMOTAXONOMIC ANALYSES, 1999 THROUGH 2008

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Blooms of *Karenia brevis* are a common occurrence on the west Florida continental shelf and impact ecology, human health and economics. A cross shelf monitoring transect has been maintained by Mote Marine Laboratory offshore of Sarasota, FL since May 1998 with HPLC pigment analyses included since July 1999. These data provide a long term view of phytoplankton community composition in relation to bloom events of *K. brevis*. Phytoplankton chemotaxonomic classifications were determined using the software package ChemTax® and community composition and environmental variables were incorporated into multivariate analyses with the program Primer-E. Along the transect phytoplankton biomass (in terms of total chlorophyll *a*) has shown a seasonal trend with pigment events typically occurring by summer and lasting through the fall. During four of the nine years monitored those seasonal pigment pulses appeared to have lead to, or coincided with, blooms of *K. brevis*. Multivariate analyses indicate the trends in community composition were related to salinity and previous studies have shown a correlation between *K. brevis* blooms and river flow. Bioassay studies conducted by Mote Marine Laboratory however, demonstrate diatoms are first to respond to pulses of nutrients within the coastal waters of Sarasota, FL so how then are *K. brevis* blooms linked to fresh water events? Time series data imply cyanophyte events precede *K. brevis* blooms in west Florida coastal waters.

MAPPING OF *Alexandrium catenella* CYSTS TO ENHANCE UNDERSTANDING OF HARMFUL ALGAL BLOOMS AND PARALYTIC SHELLFISH TOXINS IN PUGET SOUND, WASHINGTON STATE

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Alexandrium catenella is a marine dinoflagellate responsible for producing a suite of toxins collectively called paralytic shellfish toxins (PSTs) in Puget Sound, Washington State. *A. catenella* forms a dormant cyst that settles onto the sea floor when conditions are unfavorable for growth of the free-swimming, vegetative cell. When conditions are again favorable, the cyst germinates introducing *A. catenella* back into the water column. These cells provide the inoculum for harmful algal blooms (HABs) of *A. catenella* that threaten the safety of shellfish consumption and human health. Understanding the factors controlling encystment and germination of *A. catenella* cysts and their distribution in sediments is needed for predicting *A. catenella* blooms. We examined 11 sample sites from the Main, Whidbey, and Hood Canal basins of Puget Sound that were collected during the 23rd Puget Sound Regional Synthesis Model (PRISM) research cruise in February 2009. Surface sediment samples were analyzed for cyst abundance as well as total organic content (TOC) and sediment grain-size. Cyst mapping will identify areas with high concentrations of cysts that are at greater risk of developing and being impacted by HABs. Temporal changes in cyst distribution in Puget Sound will be assessed by comparing our cyst map with cyst distribution patterns of surveys from 2005 and 2006. Relationships between cysts and PST events, *A. catenella* blooms, TOC, and sediment grain-size will also be examined. Understanding the connections between cyst dynamics, bloom events, and concentrations of PSTs in shellfish will assist in efforts to understand HABs and mitigate negative impacts. These data contribute to the long-term goal of creating a forecasting model for *A. catenella* blooms in Puget Sound.

COMPARATIVE GENOMICS OF THE DIATOM *Pseudo-nitzschia* REVEALS G-PROTEIN-COUPLED- RECEPTOR CONSERVATION WITH POTENTIAL LINKS TO CELLULAR REGULATION OF DOMOIC ACID

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Pseudo-nitzschia is a cosmopolitan diatom genus with species capable of producing the potent neurotoxin, domoic acid (DA). The role of DA in *Pseudo-nitzschia* cells is unclear; but when biomagnified and consumed by higher trophic levels, DA has serious neurotoxic effects in sea birds, fish and mammals, including humans. Mechanisms involved in the regulation and functional response of *Pseudo-nitzschia* to DA have received little attention relative to studies aimed at elucidating pathways of DA production. Here we use an *in silico* comparative genomics approach to identify conserved signaling pathways and proteins that may be involved in a shared functional response in diatoms and humans to DA. Bioinformatic analyses of the draft genome (4x coverage) of *Pseudo-nitzschia multiseries*, the completed genomes of two non-toxin producing diatoms (*Thalassiosira pseudonana* and *Phaeodactylum tricornutum*), and known pathways of DA toxicity in humans revealed two *P. multiseries* protein sequences as candidates in DA sensing and/or regulation. Both proteins contain seven transmembrane domains, with significant sequence similarity to family 3 G-protein coupled receptors (GPCRs). The transmembrane domains of these two sequences are highly similar to each other but considerably diverged from the transmembrane domains of known eukaryotic GPCRs. Analyses further suggest that among the photosynthetic eukaryotes, genes encoding family 3 GPCRs may be specific to the diatoms and other heterokonts. Thus far, no member of the GPCR superfamily has been confirmed in diatoms, but if present it further suggests the presence of second messenger signaling in diatoms. Although ionotropic glutamate receptors (not in the GPCR family) are activated by domoic acid in mammalians to initiate toxicity, our study suggests domoic acid may bind GPCR-type metabotropic glutamate receptors and have a role in regulating cellular concentrations of the toxin in *Pseudo-nitzschia*.

A STUDY OF THE MECHANISMS OF TOXIC ALGAL SUCCESSION ALONG THE CALIFORNIA COAST

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Some toxic phytoplankton species produce harmful algal blooms (HABS) which can poison or kill aquatic animals and can cause human mortalities due to the consumption of shellfish exposed to toxic phytoplankton. Toxic algae may also have adverse effects on bacteria, grazers, and other algae which may alter trophic interactions and modify community structure. Scientists have observed an increase in the occurrence of HABs in coastal regions and closely monitor these events to manage marine resources and protect human health. The investigation of harmful algal ecology is important to help scientists and resource managers predict the occurrence of HABs. A little understood aspect of harmful algal ecology is the natural succession of phytoplankton community structures over time. The Southern California Coastal Ocean Observing System (SCCOOS) monitors HABs along the Southern California coast and includes a station at California Polytechnic State University's research pier. Physical, chemical, and biological data from these stations can be utilized to identify natural succession events related to toxic producing species in particular. By observing changes in phytoplankton assemblages and environmental parameters, both temporally and spatially along the coast, I hope to reveal some of the mechanisms of succession in phytoplankton assemblages containing toxic species. The results of this study will contribute to our understanding of succession of toxic phytoplankton communities and will help us predict the occurrences of HABs.

LOSS OF WATERBORNE BREVETOXIN ON EXPOSURE TO PHYTOPLANKTON COMPETITORS: INSIGHTS INTO MECHANISMS OF REMOVAL

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The increasing frequency of devastating blooms of the harmful dinoflagellate *Karenia brevis* have motivated investigations into understanding bloom dynamics and the potential for mitigation. Our group previously showed that waterborne concentrations of the most abundant brevetoxin (brevetoxin B or PbTx-2) associated with these blooms are reduced in the presence of other phytoplankton species. We are therefore exploring the mechanism of removal of brevetoxins on exposure to phytoplankton competitors. Initially we established that the ability of phytoplankton competitors to remove waterborne PbTx-2 is widespread among taxonomic groups including diatoms, cryptophytes, and dinoflagellates. Selective removal of PbTx-2 and -1 but not PbTx-3, -6, or -9 by cultured *Skeletonema costatum* revealed that brevetoxin metabolism is dependent both upon brevetoxin ring structure and the presence of an α,β-unsaturated aldehyde functionality. For maximal removal of PbTx-2, it is necessary for the competitor phytoplankton to be live, although compounds (probably proteins) exuded by the competitor species are responsible for some metabolism. Assays with marine invertebrates including the sea anemone *Aiptasia pallida* indicated that the presence of *S. costatum* ameliorated, and in some circumstances completely protected against the negative physiological consequences of exposure to waterborne brevetoxins. These findings support the concept of potentially using competitor phytoplankton species or enzymes derived from phytoplankton as biocontrol agents for waterborne toxins associated with red tide.

STEROL BIOSYNTHESIS IN THE MARINE DINOFLAGELLATE, *Karenia brevis*

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Dinoflagellates are a diverse group of photosynthetic and heterotrophic algae that regularly form blooms throughout the world's oceans and in several of its freshwater bodies. The biosynthesis of sterols, ringed lipids thought to contribute to membrane integrity in eukaryotes, has been elucidated in great detail in yeast and plant/green algae model systems, but is comparatively understudied in dinoflagellates, even though dinoflagellates have been studied for decades as producers of a number of sterols that serve as environmental biomarkers. The objective of this study is therefore to elucidate important biochemical steps in the biosynthesis of brevesterol and gymnodinosterol, the primary biomarker sterols produced by *Karenia brevis*, by determining structures of selected intermediates that accumulate during exposure to particular fungicides known to inhibit sterol biosynthesis. The fungicides utilized include econazole, an inhibitor of cytochrome P-450-dependent 14α-demethylation of lanosterol and obtusifoliol, intermediates in yeast and plant pathways, respectively, and fenpropidine, an inhibitor of Δ^{14}-reductase and $\Delta^{8(9)®7(8)}$ isomerase. This work is thus one of the first forays into understanding biochemical steps in a eukaryotic microbe that is not considered a model organism (such as the well studied yeast and green algal genera *Saccharomyces* and *Chlamydomonas*, respectively), yet which greatly impacts human activity.

To date, a small number of peridinin-containing dinoflagellates have been observed to utilize a yeast-like pathway, with lanosterol as a key intermediate (Giner *et al*. 1991); however, other key intermediates formed en route to production of their normal sterol complement are unknown (there are approximately ten intermediates between cyclization of squalene to formation of end-product sterols). Giner *et al*. also observed in *Kryptoperidinium foliaceum*, a dinoflagellate with an aberrant diatom endosymbiont, that both lanosterol and cycloartenol, a key intermediate in the plant sterol biosynthesis pathway, were present. Intermediates in the biosynthesis of brevesterol and gymnodinosterol in the non-peridinin-, aberrant-plastid-containing (potentially of red algal origin) *K. brevis* are completely unknown.

Our preliminary data suggest, like *K. foliaceum*, a combination of yeast- and plant-like pathways may be present in *K. brevis*, even though its plastid ancestry is very different. Exposure of *K. brevis* to econazole produces an intermediate that resembles lanosterol, two intermediates also observed in econazole-treated yeast and, curiously, an intermediate with a mass spectrum virtually identical to stigmasterol, a common plant sterol that wouldn't be an expected biosynthetic intermediate en route to brevesterol and gymnodinosterol. Exposure to fenpropidine produces two tri-unsaturated intermediates that appear to be consistent with 14α-demethylation of lanosterol. Characterization of these intermediates and their potential placement within a brevesterol/gymnodinosterol biosynthetic pathway will be presented.

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THE IMPACT OF LONG-TERM SHIFTS IN CO_2 AND TEMPERATURE ON THE GROWTH AND PHYSIOLOGY OF DIFFERENT ISOLATES OF *Heterosigma akashiwo*

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It is yet unknown how projected changes in future global CO_2 and temperature may influence the ecology of many microalgal species. Given these and other key anthropogenic impacts, such as eutrophication, it is important to understand how such multiple environmental factors may influence harmful algal populations. Recent work has shown that short-term exposure to elevated temperature and carbon dioxide resulted in marked differences in growth, photosynthesis and cellular nutrient quotas in the mid-Atlantic sympatric harmful algal species *Heterosigma akashiwo* and *Prorocentrum minimum* such that *Heterosigma* might have a competitive advantage under "green-house" conditions. However, it is not clear if such a positive response is ubiquitous for other raphidophytes or at the intraspecific scale among isolates of *Heterosigma* originally isolated from different regions. Assessment of the physiological response of several different isolates of *Heterosigma* from three different locations in the United States is currently underway, following long-term exposure to elevated levels of pCO_2 (750 ppm), temperature (30°C) and different nitrogen sources (NO_3- or NH_4^+). Batch cultures were initially established under ambient and elevated CO_2 and temperature, followed by semi-continuous balanced growth conditions. The results of this work to date will be presented in the context of understanding how such projected changes in global CO_2 and temperature may impact growth, photosynthetic response, and the genetic expression and activity of nitrate reductase in these harmful algae.

MAPPING THE CONCENTRATION OF *Alexandrium catenella* CYSTS IN QUARTERMASTER HARBOR, PUGET SOUND, WASHINGTON

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Alexandrium catenella can produce saxitoxins, which bio-accumulate in filter-feeders. The accumulation of toxin in shellfish can cause paralytic shellfish poisoning (PSP) in humans after consumption. During part of *A. catenella's* life cycle benthic resting cysts form which, upon germination, can seed harmful algae blooms (HABs). These blooms are responsible for periodic closures of shellfish harvesting in Puget Sound and other parts of the world. Over the past twenty years, an increase in studies of *A. catenella* cyst presence has given us insight into the distribution of this species, both globally and locally. In 2005, a study sampled 32 locations throughout Puget Sound and found Quartermaster Harbor (QMH), in central Puget Sound, to have the highest concentration of *A. catenella* cysts in surface sediments. This result was based on samples being collected at one location from each bay or section of Puget Sound. We were interested in examining how representative these samples might be of each location by mapping the "patchiness" of *A. catenella* cysts in surface sediments within one bay. Twenty-four surface sediment samples were collected in Quartermaster Harbor prior to the expected 2008 *A. catenella* spring bloom, to investigate environmental conditions in QMH that may be associated with the increased abundance of *A. catenella* in this bay. These samples were processed, stained, and counted for *A. catenella* cysts. Sediment samples were also analyzed for particle size distribution and total organic carbon (TOC). Our map illustrates the concentrations of *A. catenella* cysts in Quartermaster Harbor (QMH). We found a higher concentration of cysts in the inner and central bay of QMH than the outer bay. Lowest concentrations of cysts were in the outer bay near the mouth of the harbor. There is a weak correlation of cyst presence with silt-size grains and high TOC. Understanding the distribution of cysts can be used to focus on "at-risk" shellfish beds and potential closures for seasonal HABs, which occur regularly within some regions of Puget Sound.

TOWARDS AN UNDERSTANDING OF THE MECHANISMS CONTROLLING DOMOIC ACID RETENTION IN FISH, CRABS AND MUSSELS

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Domoic acid (DA) is an excitatory neurotoxic amino acid produced by several marine algal species and is the causative agent of amnesic shellfish poisoning in humans, seabirds and marine mammals. Profound differences in the toxicokinetics of DA have been identified in a wide variety of shellfish including several species of mussels (*Mytilus* sp.), which do not retain the toxin for significant time periods and razor clams (*Siliqua patula*), which can retain the toxin for many months. We have previously characterized the toxicokinetics of DA in razor clams, mussels (*M. galloprovincialis*), Dungeness crabs (*Cancer magister*) and rainbow trout (*Oncorhynchus mykiss*) in controlled laboratory studies. Among the findings from these studies was the identification that clams and mussels differ primarily with respect to DA elimination rates as there did not appear to be significant differences in the kinetics of tissue distribution of DA. In crabs, the hepatopancreas has an enormous capacity to retain DA after oral dosing. For example, the hepatopancreas: hemolymph partition coefficient was estimated to be approximately 1000:1 after oral dosing. This result indicates the hepatopancreas is actively concentrating DA and effectively prevents distribution to other tissues such as muscle. In fish such as rainbow trout, DA has low oral bioavailability with highest tissue levels observed in the liver, kidney and in bile. Integrating the findings from the bivalve, crab and fish studies suggests a common physiological process is occurring that sequesters DA in specific tissues (hepatopancreas in crabs) or regulates excretion from the animal. Our hypothesis is that some type of plasma membrane bound, transporter protein such as an invertebrate homolog to the organic anion transporters (OATs) are regulating the transport of DA in shellfish. To obtain experimental evidence in support of this hypothesis, we performed both in vivo and in vitro experiments. In vivo studies used shellfish that were co-exposed to DA and known inhibitors of transporters (verapamil and cyclosporine A). Verapamil is a competitive inhibitor of p-glycoprotein (pgp) type transporters, which are believed to be similar to the multi-xenobiotic transporter described in bivalves. Cyclosporin A inhibits both pgp and OAT transporters. These in vivo experiments were designed to determine whether co-exposure altered the toxicokinetic behavior of DA in crabs, clams and mussels. In vitro studies used isolated hepatopancreas tissue and measured DA uptake, subcellular distribution and protein binding. For example, when one gram of hepatopancreas tissue was incubated 12 hrs with 45 µg of DA, 68% was absorbed by the tissue. Subsequent homogenization of the tissue followed by differential centrifugation to isolate the cytosol fraction indicated 79% of the absorbed DA was in the cytosol. Ultrafiltration of the cytosol through a 10,000 MW cut-off filter indicated that 85% of the DA passed through the filter. This latter result indicates DA is not appreciably bound to cytosolic proteins. These results are consistent with DA being actively transported into hepatopancreas cells but not bound to any specific intracellular carrier protein(s). This presentation will summarize our current understanding of interspecies differences in DA retention and the physiological mechanism(s) that contribute to these differences.

A HISTORICAL RECORD OF VERTICAL DOMOIC ACID FLUXES FROM SANTA BARBARA BASIN (CA)

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Recent evidence suggests that vertically sinking particles may be a substantial source of domoic acid to bottom sediments, where it can enter the food chain of both nearshore and benthic biota (e.g. flatfish and crabs). Sediment trap measurements in the Santa Barbara and San Pedro Basins have found that DA-enriched sinking particles, comprised of phytoplankton aggregates and fecal pellets, rapidly sink (within 3-5 days) to depths of 800 m following toxic *Pseudo-nitzschia* surface blooms (Sekula-Wood *et al*. 2009). This strong surface to depth correlation further suggests that sediment traps may provide a historical record of surface water domoic acid events in the absence of surface water monitoring. Here, we present domoic acid measurements (n = 182) from bimonthly sediment trap samples (particulates and supernatants) collected from 540 m in the Santa Barbara Basin from late 1993 through May 2007. Archived sediment trap samples reveal periodic toxin events occurring in the Santa Barbara Basin as early as 1994 including four large-scale events from 2001 to 2007. In many, but not all, instances, sediment trap toxin events from the center of the Basin coincide with nearshore domoic acid events identified by shellfish monitoring.

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DO MEMBERS OF THE RAPHIDOPHYCEAN CLASS USE BREVETOXINS AS ALLELOPATHIC COMPOUNDS?

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Fish kills associated with raphidophycean blooms have occurred along both the eastern and western coasts of the United States, the western coast of Mexico, the northern coast of Egypt and in Japanese waters. The ichthyotoxic potential of the raphidophytes has been speculated to be due either to the production of brevetoxin compounds, of reactive oxygen species and polyunsaturated fatty acids. This study focuses on one possible motive behind the brevetoxin production by raphidophycean species and the potential use of brevetoxins as allelopathic compounds. Three raphidophycean species, *Chattonella marina, Fibrocapsa japonica,* and *Heterosigma akashiwo*, originally isolated from the waters of Southern California were used in investigations of brevetoxin allelopathy. Brevetoxin production was previously suggested for one of these cultures (*C. marina*), but recent analyses revealed that all three presently do not produce detectable amounts of toxin. The current lack of toxicity may be due to a number of factors, most importantly the age of the cultures. Experiments were carried out examining the allelopathic potential of the varying concentrations of PbTx-2 and PbTx-3. Raphidophyte species were co-cultured with commonly co-occurring microalgal taxa from Southern California waters. The raphidophytes and their microalgal competitors were separated by a 5 µm Nitex mesh to avoid direct contact or predator-prey interactions. Trials for the allelopathic potential of brevetoxin involved the addition of PbTx-2 or PbTx-3 to the co-cultures. Multiple concentrations were investigated in order to determine whether or not there is a critical concentration at which brevetoxin negatively affects the microalgal competitor. Cell densities of each species were monitored on a daily basis via microscope counts. Information on the effects of brevetoxins on the growth of both the raphidophytes and their microalgal competitors will be presented.

UTILIZING AN IMAGING FLOW CYTOMETER (FlowCAM®) AS A COASTAL MONITORING TOOL FOR HARMFUL ALGAL BLOOM SPECIES

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The ability to detect, identify and enumerate harmful algal species is a requirement in coastal ecosystems for monitoring programs and early detection of harmful bloom events. To date, most monitoring programs utilize microscopes for identifying bloom species in a laboratory or from field samples, which can be laborious and time consuming. FlowCAM is an imaging-flow-cytometer that combines the capabilities of a flow cytometer with a digital-imaging microscope and automates phytoplankton detection and enumeration. Previously, FlowCAM has been shown to successfully detect and enumerate harmful algal bloom species (*Alexandrium fundyense* and *Karenia brevis*) from both laboratory and field samples. Here we compare sample data from the FlowCAM with weekly volunteer monitoring and fluorescent in-situ hybridization (FISH) probe analysis for *Alexandrium* sp. from the near shore waters of Maine. By developing numerous filter sets based on morphologic and color criteria we are able to use the FlowCAM to determine cells/L for target species using duplicate fixed samples from volunteer monitors and FISH analysis. The key benefits of this technology are the ability to analyze phytoplankton continuously, calculate cells/L for individual phytoplankton, and the archival collection of color digital images for further analysis.

MARINE PHYTOPLANKTON MONITORING IN CENTRAL PUGET SOUND

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The King County Marine and Sediment Assessment Group manages a long-term marine monitoring program designed to assess water quality in the Central Puget Sound Basin. Since 1995, data are collected monthly for physical, chemical, and biological (chlorophyll *a*) parameters at various locations and depths throughout the Puget Sound Central Basin. The recent addition of a long-term phytoplankton species component to this program was deemed critical in order to predict how changes in climate and other regional stressors will impact the Sounds's trophic structure.

The goals of the phytoplankton monitoring component are 1) to assess relative abundance of major phytoplankton taxa during the bloom season, 2) to document the timing of seasonal shifts in major taxa, 3) to investigate relationships between physical/chemical parameters and species relative abundance, and 3) to detect long-term changes in community composition.

In its second year, the program is limited to three locations within the Central Basin: Point Jefferson and East Passage are long-term ambient monitoring stations representing open north and south areas of the Puget Sound Central Basin. Quartermaster Harbor is a shallow, protected embayment with poor tidal flushing; a telemetered mooring has been deployed to provide high frequency water quality data from this site. Our long-term goal is to expand the phytoplankton monitoring program and build an extensive database that could help evaluate the effects of environmental and anthropogenic changes on the Puget Sound food web. This dataset will also be a valuable resource for detecting the occurrence and environmental conditions that lead to blooms of HAB species in Puget Sound.

DETECTION OF THE PSP-CAUSING DINOFLAGELLATE *Alexandrium catenella* AND ITS RESTING CYSTS IN HENDERSON INLET, WA: FURTHER EVIDENCE FOR THE SPREAD OF *Alexandrium catenella* INTO SOUTH PUGET SOUND

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Blooms of cyst-forming dinoflagellates *Alexandrium* spp. have become a growing occurrence in many temperate coastal marine environments worldwide. Many bloom-forming species of *Alexandrium* produce saxitoxins that bioaccumulate in benthic shellfish, which when consumed by humans can result in paralytic shellfish poisoning (PSP). Although Puget Sound has experienced recurrent and increasing outbreaks of PSP, little data exists on the distributions of *Alexandrium* spp. and cyst dynamics in South Puget Sound. The South Sound is of particular interest due to its extensive commercial shellfish beds and because of the apparent migration of PSP events into that area over the last four decades. This study surveyed Henderson Inlet, one of the five major inlets in South Puget Sound during the spring of 2009 (March to May). This inlet experiences low flushing rates, aquatic pollution problems and reoccurring elevated PSP levels. Henderson Inlet contains residential shoreline, a nature conservancy area and a community shellfish farm. Samples were collected to determine the presence and distribution of *Alexandrium catenella* cysts and planktonic cells in sites where past outbreaks of PSP had been reported. A bloom of *A. catenella* occurred during March-April 2009 with a maximum cell count of 21,192 cells/L. Resting cysts were discovered in sediments underlying the bloom location with a maximum cyst density of 191 cysts/g wet sediment**.** The relationship between cyst distributions, *A. catenella* blooms and PSP events in this area will be discussed. This initial data coupled with further study over time, will contribute to an ecological profile of the inlet. Cyst distribution data could become a valuable tool in predicting future PSP outbreaks benefiting both commercial and ecological interests in South Puget Sound.

NOVEL GLOBIN-CONTAINING NITRATE REDUCTASE CHIMERIC SEQUENCES FOUND IN THE RAPHIDOPHYTES, *Heterosigma akashiwo* AND *Chattonella subsalsa*

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Novel globin-containing nitrate reductase sequences (*HaNR2* and *CsNR2*) were identified in the raphidophytes, *Heterosigma akashiwo* and *Chattonella subsalsa*. The deduced amino acid sequence for the globin domain of *HaNR2* shows structural similarity to group I truncated hemoglobins (trHbNs) and retains the conserved amino acids found to be characteristic of the trHbN group. TrHbNs are known to be involved in the detoxification of nitric oxide (NO) by pathogenic bacteria, and structural studies suggest that the trHbN active site is specifically designed for NO/O_2 chemistry. A variety of algal species have been shown to produce NO during normal growth and raphidophytes were found to produce higher concentrations of NO than other algae. *Heterosigma akashiwo* and *Chattonella subsalsa* also produce superoxide during normal growth, which can rapidly react with NO to produce highly toxic peroxynitrite. The mechanism of raphidophyte toxicity is currently unknown, but reactive nitrogen species derived from NO alone or in combination with reactive oxygen species may be involved. Data characterizing the relationship between *NR2* gene expression and NO production by *H. akashiwo* and *C. subsalsa* will be presented here, and future directions in the characterization of the *NR2* gene by recombinant protein methods will be discussed.

ENVIRONMENTAL FACTORS INFLUENCING OBSERVATIONS OF *Microcystis aeruginosa*

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The distribution and intensity of a bloom of the toxic cyanobacterium, *Microcystis aeruginosa*, in western Lake Erie was characterized using a combination of satellite ocean color imagery, field data, and meteorological observations. The bloom was first identified by satellite on August 14, 2008 and remained observable for over two months. The distribution and intensity of the bloom was estimated using a satellite algorithm that is sensitive to the near-surface concentration of *M. aeruginosa*. The variation in area and intensity correlated with variations in wind stress. The area and intensity of the surface bloom increased with wind stress < 0.05 Pa. Some decrease in area, but not in intensity, occurred for wind stress between 0.05 to 0.1 Pa, and the surface area and intensity showed a marked decrease for wind stress > 0.1 Pa. The modulation of the surface concentration of the bloom with wind indicated that the bloom mixed through the water column during strong wind, and returned to the surface during weak winds. This relationship is consistent with what would be expected from cells that are buoyant, like *Microcystis*. Cloud cover (reduced light) did not have an apparent influence on the area and intensity of blooms. While water temperature remained > 15°C, the bloom reintensified during calm conditions. For water temperature < 15°C, the bloom subsided under similar conditions. The study indicates that interpretation of satellite imagery of these blooms must incorporate wind and water temperature conditions. It also suggests a means of inferring the concentrations of *Microcystis* cells in the water column, which would be important for assessing the risk to water supply intakes, found near the bottom of the lake.

INCIDENCE OF CIGUATERA FISH POISONING AND SEA SURFACE TEMPERATURES IN THE CARIBBEAN SEA

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Ciguatera fish poisoning is a circumtropical disease caused by ingestion of a wide variety of reef fish that bioaccumulate algal toxins in marine food webs. The distribution and abundance of the organisms that cause ciguatera fish poisoning, chiefly dinoflagellates of the genus *Gambierdiscus*, are reported to be strongly correlated with temperature. Consequently, there is a growing concern about the effects of increasing temperatures on ciguatera fish poisoning in the context of global warming and the validity of its seasonal occurrence. This concern prompted experiments on the growth rates of six G*ambierdiscus* species at temperatures between 18°C and 33°C and the examination of sea surface temperatures in the Caribbean for areas that could support maximum *Gambierdiscus* growth rates. Specifically, the National Buoy Data Center's records were queried for the number of days with temperatures above 29°C, an average thermal optimum for five of the six *Gambierdiscus* species tested. In addition an active query with uniform effort was conducted in the 24 island nations and 9 mainland counties surrounding the Caribbean to determine the incidence of ciguatera fish poisoning from 1996-2006. Comparisons of the incidence rates of ciguatera fish poisoning from this study were made with incidence rates from the tropical Pacific and the Caribbean reported in the literature.

DETERMINING THE GENE EXPRESSION OF BREVETOXINS

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Karenia brevis, the major harmful algal bloom forming species in the Gulf of Mexico, produces neurotoxic polyketides responsible for large fish kills and human respiratory distress. Clones of *K. brevis* exhibit unique brevetoxin signatures, not only differing from one another, but also under different stress conditions (Baden and Tomas 1988; Brown, Dortch et al. 2006; Errera et al., 2009; Loret et al. 2002). Transcripts of polyketide synthases (PKS) have been localized to *K. brevis,* and it has been proposed that these types of transcripts are involved in the synthesis of brevetoxins (Snyder, Guerrero et al. 2005; Monroe and Van Dolah 2008). Spliced leader (SL) sequences were found on several PKS transcripts and have helped to confirm that the messenger RNA was coming from *K. brevis* and not the associated bacteria (Lidie and Van Dolah 2007; Zhang, Hou et al. 2007). If transcription regulation is involved in brevetoxin production, then the amount of PKS mRNA transcripts involved in the synthesis will correspond to brevetoxin concentrations. Reverse transcription PCR, using gene specific primers that overlap the SL junction, will be used to quantify the mRNA transcripts and provide information about potential links between the PKS genes and brevetoxin production. Preliminary data shows varying levels of messenger RNA, comparing the clones Wilson and TSP3 at salinities of 27 and 35. This data will be beneficial in identifying the genes associated with the synthesis and regulation of brevetoxins and help elucidate the hypothesized gene clusters of multi-protein enzymatic complexes involved in brevetoxin production, potentially one for each backbone. This method will also be used to characterize the clones by comparing toxin levels under different stresses to their gene expression.

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CELL MOTILITY, LIFE STAGE TRANSITIONS AND CYST DISTRIBUTION OF THE HARMFUL ALGA, *Heterosigma akashiwo*

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In many harmful algal blooming (HAB) species, transitions between a vegetative, motile phase in the water column and a dormant, non-motile cyst phase in the sediments play an important role in bloom dynamics. Emergence from cysts accumulated in bottom sediments, together with regional flow, regulate the occurrence, timing, and location of bloom events (Anderson et al. 2005). Many HAB species use motility to control their vertical position in the water column in order to locate parts of their environment that are favorable to growth. We hypothesize that HAB species use swimming behaviors, expressed during the transition between pelagic and benthic stages, to favorably affect the timing and location of cyst deposition in sediments.

Cell swimming behaviors of *H. akashiwo*, a harmful marine flagellate, during the transition into resting cells were quantified using video analysis. Cells were induced into the resting stage following the methods of Han et al. (2002), subjecting cells to low temperature (10°C) and darkness for 14 days. Cell counts verified that the conditions successfully induced cells into resting stage. Video clips of cell swimming behaviors during the transition period were obtained for the length of the induction period. Cell swimming paths were analyzed using Matlab-based software. This specialized methodology provides detailed characteristics of individual cell swimming behaviors including, overall directionality; total speed; mean vertical and oscillatory speeds; and turning rates. Incorporating observed swimming behaviors into a simple 2-dimensional water column model suggests that transitional swimming has potentially significant impacts on cyst distributions in the sediments, and consequently, on subsequent bloom dynamics.

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THE EFFECTS OF TEMPERATURE, LIGHT AND GROWTH STAGE ON THE TOXICITY OF *Dinophysis acuminata* FROM WOODS HOLE, MASSACHUSETTS

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The dinoflagellate *Dinophysis acuminata*, a toxic alga that causes diarrhetic shellfish poisoning, was isolated and established in culture from Eel Pond (Woods Hole, MA) in September 2006. The effects of temperature, light, and growth stage on the toxicity of *D.acuminata* were studied using HPLC/MS/MS. OA, PTX11 and PTX2 content increased with a decrease in temperature, whereas OA-D8 and DTX1 seemed not to change with the temperature. Similarly, DSP toxin content (DTX1, OA and PTX11) was greater under low light conditions (65 µmol photons$\cdot$m$^{-2}\cdot$sec^{-1}) than under 2 higher light levels (284 and 145 µmol photons$\cdot$m$^{-2}\cdot$sec^{-1}); the lowest toxin production was seen under no light where growth did not occur. In general, PTXs were the predominant DSP toxins with PTX2 being present at concentrations of 7.3-19.8 pg$\cdot$cell^{-1} and PTX11 found at 26.4-1078.4 fg$\cdot$cell^{-1}. Toxin production varied with growth stage in a low temperature treatment as OA, DTX1 and PTX2 toxin content was low in early exponential growth, peaked during early plateau phase and decreased in late plateau phase. In contrast, PTX11 toxin content was high in early exponential growth and decreased dramatically throughout the culture's growth cycle. These results strongly suggest that *D.acuminata* produces DSP toxins during active cell growth, and demonstrate that environmental factors influence DSP concentrations and variant composition.

MOLECULAR DETECTION AND MONITORING OF *Karenia* SPP. IN COASTAL DELAWARE WATERS

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One of the most predictable blooms is that of *Karenia brevis* off of the South Western coast of Florida. Though primarily restricted to the Gulf of Mexico, a field sampling survey by the University of Delaware Citizen Monitoring Program conducted in late summer, 2007 found *Karenia* spp. in the Delaware Bay, Delaware Inland Bays, and in coastal waters up to 11 miles offshore. To confirm visual analyses of samples collected in 2008, samples were evaluated by PCR using primers targeting *Karenia brevis* and *Karenia papilionacea*. Quantitative real-time PCR methods were also developed for enumeration of both species. Using these molecular methods, samples from 2008 were confirmed to consist of *K. papilionacea*. Samples collected in early July 2007, were also positive for *K. papilionacea*, suggesting that this species was present several weeks before they were observed by microscopy. *K. brevis*, however, was not detected in any of the samples using *K. brevis*–specific primers targeting genes encoding both rRNA and Rubisco. *K. papilionacea* reappeared in low concentrations in 2008, and were consistently detected in samples collected from August to early November when temperatures were as low as 12.7°C. A study will be conducted in summer, 2009 to evaluate the distribution of the genus along coastal New Jersey and Delaware during peak *Karenia* bloom season. Results of this study and their implications with respect to monitoring efforts will be presented.

INTERPRETING THE PUBLIC HEALTH SIGNIFICANCE OF NEUROPSYCHOLOGICAL TEST DATA IN HAB RELATED EXPOSURES: CoASTAL COHORT

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One of the challenges of neuropsychological research is evaluating the public health importance of statistically significant results. This challenge is even greater if we attempt to evaluate the significance of changes in performance that occur over time. Many physiological measures have established criteria that define impairment or lack of impairment in function. For example, a body mass index value of 20 is considered normal or healthy, while an obtained body mass index value of 27 is considered as overweight. Cognitive tests also have recommended interpretive criteria (published norms) that define degrees of impairment. However, what is left to interpretive judgment are: 1) how to interpret statistically significant group differences in the absence of impairment and 2) how to interpret the practical, public health importance of statistically significant changes in cognitive test scores in the presence or absence of impairment.

One strategy that has been suggested for evaluating the public health importance of group differences is the use of a minimally important difference (MID) measure. MID is defined as the smallest difference in a score perceived to be important—either as an indicator of harm or benefit—that would suggest notable, functional impairment and would suggest the need for intervention or remediation.

One method of determining MID is to calculate an effect size to quantify the magnitude of difference in standard units. Effect sizes can then be compared to theoretical cutoffs for small, medium and large effects. An alternative strategy is to calculate the Standard Error of Measurement. The SEM provides a sample-independent value that reflects both variability and reliability of measurement and expressed in the units of the measure. A difference greater than 1 SEM is considered of probable clinical and public health importance.

Data from the CoASTAL cohort study is used to illustrate different methods for evaluating minimally important differences and discuss the advantages and disadvantages of each method for understanding the public health importance of HAB-related exposures.

CHARACTERIZATION AND PARTIAL PURIFICATION OF A SOLUBLE PARALYTIC SHELLFISH TOXIN BINDING PROTEIN FROM THE BUTTER CLAM, *Saxidomus giganteus*

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A soluble paralytic shellfish toxin binding protein (PSTBP) was isolated from the siphon of the giant butter clam, *Saxidomus giganteus* and shows nanomolar affinity to a variety of paralytic shellfish toxins including saxitoxin, decarbamoyl saxitoxin and the gonyautoxins. Pharmacological characterization and receptor kinetics were performed using a radio-receptor assay specifically designed for the analysis of soluble protein activity. PST derivatives present in the clam siphon clearly affected the binding results but dissociation of the ligand was achieved without significant protein degradation. Protein samples were analyzed by competitive binding and association/dissociation kinetics to determine the relative affinity to the PST derivatives. Purification was initiated using affinity-based chromatography with saxitoxin, resulting in a 500 fold increase in the specific activity of the protein. The presence of a soluble PSPBP in this species may relate to it's ability to retain paralytic shellfish toxins for extended periods of time, assist in the compartmentalization of toxin from the viscera to the siphon, or simply act as a toxin resistance mechanism enabling the clams to survive in regions. These biological mechanisms and additional data will be discussed.

SAXITOXIN CONCENTRATIONS IN COASTAL OREGON SHELLFISH: THE INFLUENCE OF EL NIÑO AND THE PACIFIC DECADAL OSCILLATION.

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Shellfish on the Oregon coast have been tested for saxitoxin by the Oregon Department of Agriculture since 1979. This saxitoxin time series was analyzed for relationships to the Pacific Decadal Oscillation (PDO), and the El Niño –Southern Oscillation (ENSO), an upwelling index, and AVHRR sea surface temperature (SST). A possible link is observed between years with warmer surface ocean conditions and an increased proportion of positive saxitoxin samples. This is hypothesized to be due to an increase in the number of days conducive to *Alexandrium* growth (number of days SST > 13ºC, Nishitani & Chew 1984) during positive phases of the ENSO and the PDO. Defining relationships such as these enhances our understanding of what causes toxic blooms and aids prediction, therefore helping to protect public health.

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CONCURRENT EXPOSURE OF BOTTLENOSE DOLPHINS (*Tursiops truncatus*) TO MULTIPLE ALGAL TOXINS IN SARASOTA BAY, FLORIDA USA

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Sentinel species such as bottlenose dolphins (*Tursiops truncatus*) can be impacted by mass mortality events due to exposure to marine algal toxins. In the Sarasota Bay region (Gulf of Mexico, Florida, USA), the bottlenose dolphin population is frequently exposed to red tide blooms of *Karenia brevis* and the neurotoxic brevetoxins produced by this dinoflagellate. Although exposure to the brevetoxins may be direct, it is well-documented that algal toxin trophic transfer can also result in exposure and bioaccumulation. Dolphins caught during capture-release health assessments performed in this region were found to contain significant levels of both brevetoxin (PbTx) and domoic acid (DA). We have determined over an eight year study period (2000-2008) that bottlenose dolphins are exposed to brevetoxin (PbTx) and/or domoic acid (DA) on virtually an annual basis. Analysis of multiple dolphin tissues/fluids revealed maximum levels of 704.0 ng PbTx equiv. g^{-1} feces and 41.5 ng DA g^{-1} feces where there were several examples of both neurotoxins being present in the same individual. Although there have been no published reports of DA in Sarasota Bay marine mammals, the May 2008 live capture coincided with a *Pseudo-nitzschia pseudodelicatissima* bloom that is the likely producer of the DA observed in the dolphins. The trophic transfer of DA and PbTx appear to be via potential prey fish (mullet, pigfish, pinfish, striped mojarra, and scaled sardines). Measured clinicopathologic values in the biotoxin exposed dolphins were for the most part within normal range (i.e., within 95% reference intervals) but a few parameters were significantly correlated with measured DA values. The correlated parameters included eosinophil count ($p=0.04$) which has previously been reported to be increased in California sea lions exposed to DA. Although high levels of either PbTx or DA in marine mammals can result in mortality, the effects of long term exposure to low levels of multiple toxins are unknown. Our findings provide an impetus to examine potential impacts of both toxins on bottlenose dolphin health.

Karlodinium veneficum CCMP1609: TOXIN CONTENT AS A FUNCTION OF NUTRIENT SPECIES, ABSENCE OF MIXOTROPHY, AND IMPORT FOR TIDAL CHESAPEAKE BAY

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Adolf et al. (2009) presented a spectrum of nutrient-induced toxin profiles for a suite of *Karlodinium veneficum* strains. However, the *K. veneficum* strain (CCMP1609) originally isolated from the Choptank River, a tributary of Chesapeake Bay, was not examined. Subsequent analysis of the pigment profile and ITS sequence established this strain was indeed a *K. veneficum*. The strain was grown in a suite of medium f dilutions to three nitrogen sources and inorganic phosphorus. At early stationary phase in f/10 concentrations of ammonium, nitrate, and urea as well as P, aliquots were transferred to f/10 media of each nutrient. Growth was followed through time, with sub-samples removed for toxin analysis initially, during lag phase, and then in exponential, stationary, and senescence growth periods. Using HPLC-MS, toxin distributions were determined, with the idea that composition might potentially shift through growth phase and under different nutrient species. *K. veneficum* CCMP1609 responded as did the strains examined by Adolf et al. (2009) in that nutrient limitation increased toxin content and P-limitation was most important in maximum toxin production. However, unique to CCMP1609 was the extremely low toxin cell quota observed (<0.01 pg/cell) and the production of KmtX-2 as its dominant karlotoxin, not previously detected as the dominant toxin in any Chesapeake Bay isolates. In addition, CCMP1609 did not ingest cryptophytes under standardized grazing conditions. Considering the seasonal distribution of *Karlodinium* in Chesapeake Bay, it is likely that N, not P, will increase toxin production and further, the absence of mixotrophy in the strain indicates a wider spectrum of food-web dynamics for this ichthyotoxic species than previously believed.

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VARIATIONS IN THE LIGHT ABSORPTION COEFFICIENTS OF PHYTOPLANKTON, NONALGAL PARTICLES, AND COLOR DISSOLVED ORGANIC MATTER IN NEW YORK/NEW JERSEY HARBOR ESTUARY

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The New York/New Jersey harbor estuary is being monitored for bloom occurrence by remote sensing. Bio-optical and inverse modeling can be utilized to derive optical water quality parameters: absorption coefficients of phytoplankton, nonalgal particles, and color dissolved organic matter (CDOM). To optimize the modeling, the three *in situ* absorption coefficients were measured and evaluated from August 2008 to June 2009. The exponential slope of CDOM and nonalgal particles absorption spectrum was evaluated by comparing with that of coastal waters around the world. The phytoplankton spectra are being decomposed into Gaussians to evaluate the pigment composition. At the same time, a pigment analysis is being assessed with high performance liquid chromatography (HPLC). As a result, the taxonomic groups will be estimated. Variations in the phytoplankton specific absorption coefficients are being studied and explained in terms of cell size, intracellular pigment concentration, package effect, and the relative importance of auxiliary pigments.

A MASSIVE BLOOM OF TOXIGENIC *Pseudo-nitzschia cuspidata* OFF THE WASHINGTON STATE COAST

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In September 2004, an Ecology and Oceanography of Harmful Algal Blooms in the Pacific Northwest (ECOHAB-PNW) research cruise occurred during a large, nearly monospecific diatom bloom of *Pseudo-nitzschia cuspidata* that reached cell concentrations of 6.1×10^6 cells L^{-1} and produced maximum particulate (pDA), dissolved (dDA), and cellular domoic acid (DA) concentrations of 43 nmol L^{-1}, 4 nmol L^{-1}, and 63 pg $cell^{-1}$, respectively. This bloom co-dominated the phytoplankton assemblage with the euglenoid, *Eutreptiella* sp. in the Juan de Fuca eddy region, a known initiation site for toxigenic *Pseudo-nitzschia* blooms off the Washington State coast. Two isolates of *P. cuspidata* collected during separate cruises were shown to produce DA in culture. During the September 2004 survey, 84% of the stations ($n = 98$) had detectable *Pseudo-nitzschia* and 78% had detectable pDA. No significant correlations were observed between either pDA or cellular DA and ambient concentrations of macronutrients, however when considering only those stations where *Pseudo-nitzschia* were present, pDA was positively correlated with chlorophyll *a* and negatively correlated with temperature ($p \leq 0.01$) at both 1 and 5 m depths. No correlations were found between cellular DA concentrations and total bacteria or cyanobacteria abundances. Variable ratios of pDA:dDA in the eddy region suggested that DA release was under cellular regulation by *Pseudo-nitzschia*. Stations where dissolved Fe concentrations were limiting (< 0.5 nmol L^{-1}) had the highest *Pseudo-nitzschia* abundances, pDA, and cellular DA values. These results provide field evidence that cell toxicity is not related to macronutrient (N, P, Si) limitation but suggest a role of metal limitation in controlling cellular DA levels in this region.

IN VIVO MONITORING OF CYANOBACTERIA USING TURNER DESIGNS' C3 SUBMERSIBLE AND PHYTOFLASH ACTIVE FLUOROMETERS

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The ubiquitous nature and rapid bloom formations of toxin-producing cyanobacteria make them a risk to human health and livestock. Detection of these algae helps researchers learn about their distribution and can lead to a decrease in health-related issues by increasing public awareness. Monitoring cyanobacterial abundances within a mixed algal population can be difficult, time consuming, and expensive if techniques such as cell counting, either automated or manual, are employed. More rapid *in situ* fluorescence techniques are available to help distinguish cyanobacteria from eukaryotic algae using pigment-based fluorescence characteristics. The Turner Designs' C3 Submersible Fluorometer is an *in situ* fluorometer that can be configured with up to 3 different pigment-specific optical sensors to help characterize algal communities within an aquatic habitat. The versatility of this instrument allows it to be used in numerous applications. The ability to take several measurements simultaneously or log data reliably for long-term deployments using the C3 Submersible Fluorometer provides researchers with more insight for a given community.

In addition to characterizing distribution within aquatic systems Turner Designs' Red PhytoFlash, which is an *in situ* Active Fluorometer, was developed to help determine the physiology, namely the photosynthetic efficiency (health), of cyanobacteria, which can be used to detect the onset of blooms. When cyanobacteria have enough light, nutrients, and optimal conditions for bloom activity, their ability to photosynthesize, or photosynthetic efficiency, increases leading to bloom events. The Red PhytoFlash provides users with the yield (Fv/Fm) parameter, among others, which are direct measurements of photosynthetic activity for the community being monitored. Users can monitor the health of the algal community and anticipate the occurrence of blooms based on yield measurements. This early warning system will help facilities set up and implement proper protocols for decreasing the risks associated with toxic blooms and answer important questions related to the causes of these events.

TRENDS IN ALGAL BIOMASS AND SPECIES COMPOSITION DURING THE SPRING BLOOM IN BUDD INLET, WASHINGTON STATE, AND THEIR POSSIBLE RELATIONSHIP TO CHANGES IN DENSITY STRATIFICATION AND NUTRIENT (N AND Si) COMPOSITION AND CONCENTRATION

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South Puget Sound has experienced HAB events, and there is evidence that the frequency and severity of these events are increasing. Understanding the factors controlling phytoplankton species composition and biomass can provide useful information to understand when harmful species will become dominant. A study was conducted from April to May 2009 in Budd Inlet, the southernmost inlet in Puget Sound (Washington State) to determine environmental factors associated with changes in biomass and species composition during the spring phytoplankton bloom. Sampling was conducted once a week at two locations in the inlet representing areas with different degrees of density stratification and nutrient input. One station was near the Deschutes River and the city of Olympia and the other was near the mouth of the inlet. Surveys determined species composition (vertical tows), abundance of dominant species (surface samples), phytoplankton biomass (chl a) and nutrient concentrations (N and Si). Phytoplankton biomass was consistently greater at the stratified site, but species composition was similar for both study sites. The majority of the species detected were diatoms, and diatoms were also the most abundant phytoplankton. A bloom of the diatom *Chaetoceros* sp. in early May was the largest concentration of phytoplankton observed with 4.9×10^5 cells l^{-1} at the stratified site and 2.9×10^5 cells l^{-1} at the mixed site. Towards the end of spring, the number of dinoflagellate species and their abundance increased, but diatoms continued to dominate the phytoplankton. The presence of the harmful dinoflagellate *Alexandrium* sp. was observed. Diatoms of the genus *Pseudo-nitzschia* were also detected, but it was not possible to determine if these cells belonged to toxic species. Dinoflagellate species observed in vertical tows sometimes were not detected in surface samples. This is probably due to dinoflagellate vertical migrations and underscores the importance of sampling throughout the water column to determine the presence of dinoflagellates. Our study also observed significant variations in phytoplankton parameters over short transects indicating patchiness in the concentration of plankton. Trends in phytoplankton parameters and their relation to changes in nutrient concentration and relative composition (N:Si) will also be presented.