ABSTRACTS OF ORAL PRESENTATIONS

WEST COAST HABS SESSION

APPROACHES TO THE DETECTION OF DOMOIC ACID IN MARINE FOOD WEBS

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Just over a decade ago in eastern Canada, the neurotoxin domoic acid was identified as the causative agent of a human intoxication syndrome known as amnesic shellfish poisoning. Since that event in 1987, domoic acid and the diatoms that produce it (i.e., *Pseudo-nitzschia* spp.) have been reported from many U.S. coastal regions, and are now recognized as a public health concern through their contamination of seafood resources. However, it has become increasingly clear that toxic *Pseudo-nitzschia* species also pose a wider threat to coastal ecosystems based on their association with unusual mortality events involving marine birds and mammals. In order to describe the process by which domoic acid is moved through marine food webs, it is imperative that robust, reliable methods of toxin detection be established. We have adopted a tiered approach to domoic acid detection in diverse sample types (e.g., plankton, seawater, invertebrates, fish, mammals) involving a high throughput receptor binding assay and tandem mass spectrometry, which provides information on toxic activity as well as the unambiguous confirmation of toxin presence. Moreover, innovative techniques for sample collection and extraction of domoic acid from specific matrices have been established that yield high quality samples and optimize our toxin detection capabilities. Finally, methods for the automated, *in-situ* collection of plankton samples for toxin analysis are under development, with the ultimate aim being remote detection of domoic acid. These various approaches to toxin detection will be discussed in the context of their application to studies of domoic acid in marine food webs.

HABS-RELATED PHYSICAL OCEANOGRAPHY OF THE U.S. WEST COAST

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The oceanography of the U.S. West coast is dominated by the California Current System, a system of currents with strong interannual, seasonal and several day scale variability. In contrast to most east coast environments, the shelf is relatively narrow, so that nutrient-rich deeper water can be effectively brought to the surface by the upwelling that occurs in the growing season along the entire coastal boundary. It is the wind-driven upwelling of nutrients from deeper layers that fuels coastal productivity, resulting in both a strong seasonal cycle and several day fluctuations that mimic changes in the wind direction and, hence, upwelling. During an upwelling event, phytoplankton respond to the infusion of nutrients near the coast and this "bloom" is moved offshore, continuing to grow while depleting the nutrient supply. When winds reverse, the bloom moves back toward shore where it can contact the coast or enter coastal estauries. In the mean, coastal currents in near surface layers at most latitudes are northward in winter and southward in spring and summer, although direction reversals occur frequently in every season.

The majority of current and nutrient fluctuations over the shelves along the coast are determined by wind forcing--sometimes by the local wind stress, but also by "remote" wind stress, which sends signals northward along the coast in the form of propagating waves. In contrast to the east coast, alongshore topography of the coastline is relatively straight and wind systems are large scale. Thus, currents and water properties (e.g., temperature, stratification etc.) are similar over relatively large (> 500 km) distances along the coast. This similarity in ocean conditions may have strong implications with respect to the generation and movement of HABS.

In the several regions where large coastal promontories occur, phytoplankton are swept offshore and southward by the meandering jets and/or eddies that form where the coastal jets detach from the shelf. At both the north and south extremities of the U.S. west coast, topography is more complex and planktonic retention areas are more likely to occur. For example, off the Washington coast a semi-permanent eddy develops seaward of the Strait of Juan de Fuca and this eddy has been shown to contain high levels of domoic acid. During some years domoic acid from this eddy appears to move onshore and also into coastal estauries during the first major storm of the fall season.

TRACE METALS AND *PSEUDO-NITZSCHIA* BLOOMS: A POSSIBLE ROLE FOR THE TOXIN DOMOIC ACID

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Toxigenic species of the pennate diatom *Pseudo-nitzschia* can produce domoic acid, an analog of the excitatory neurotransmitter glutamate and a known causative agent of the human illness amnesic shellfish poisoning (ASP). Although the trophic transfer of this phycotoxin has resulted in mass marine bird and mammal mortality, the physiological role of domoic acid to the causative organism is still unknown. The similarity in chemical structure of domoic acid to other phytosiderophores suggests a role for domoic acid as a trace metal chelator. Using a highly sensitive adsorptive cathodic stripping voltammetric technique, we found that domoic acid forms strong chelates with iron and copper, having conditional stability constants of $K^{cond}_{FeDA,Fe(III)'} = 10^{8.7 \pm 0.5}M^{-1}$ and $K^{cond}_{CuDA,Cu(II)'} = 10^{9.0 \pm 0.2}M^{-1}$. Certain species may therefore produce domoic acid to selectively bind trace metals in order to either increase the availability of an essential micronutrient such as in the case of iron, or to decrease the availability of a potentially toxic trace metal, such as in the case of copper. The combination of binding strength and the potential production/release rates of domoic acid raises the possibility that domoic acid significantly alters the chemical speciation, and thus the availability, of both iron and copper in seawater. In addition, domoic acid may be particularly important in solubilizing particulate iron suspended in these coastal waters, where *Pseudo-nitzschia* blooms tend to occur.

To further investigate why *Pseudo-nitzschia* species produce domoic acid and the possible role of trace metals in this production, *Pseudo- nitzschia* sp. isolated from Monterey Bay were grown in a chemically well defined artificial seawater media, under trace metal clean conditions. This allowed us to reproducibly induce iron-limitation (μ = 50% μmax) and copper-toxicity (μ = 30–50% μmax) in the cultures. We found that under iron and copper stressed conditions (but macronutrient replete conditions) the rates of domoic acid production/release by the cells were significantly increased. However, the particulate (non-released domoic acid) production rate of domoic acid remained essentially unchanged between these differing metal-containing culture conditions during log phase growth, resulting in the ratio of extracellular to intracellular domoic acid production rates to be 20 x higher under iron-limited conditions! These data show that domoic acid is being released to the surrounding medium under iron stress. To determine if domoic acid release affected metal acquisition by the cells, we measured iron uptake rates with and without domoic acid in solution. Iron uptake rates for *Pseudo-nizschia multiseries* (domoic acid producer) and *Pseudo-nizschia pungens* (non-domoic acid producer) were consistently faster (3 x) when domoic acid was present in solution. Our findings indicate that the physiological role of domoic acid for toxigenic *Pseudo-nizschia* species is involved with bioactive metals, and that the domoic acid production is tied closely to the acquisition (iron) or detoxification (copper) of metals in coastal waters.

FIELD STUDIES OF TOXIC PHYTOPLANKTON IN CENTRAL CALIFORNIA: 1999-2000

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Since 1991, toxic species of *Pseudo-nitzschia* have been known to be sources of domoic acid on the U.S. west coast, responsible for mortality events involving marine birds and mammals. Longer term records in the Central California region, the site of most of the mortality events, show a typical seasonal pattern of occurrence of *Nitzschia*-like species, the taxon formerly used to enumerate the morphologically similar species that include *Pseudo-nitzschia*. However, a full annual record for the toxic species has not yet been presented, nor has the spread of the toxin through the food chains been followed through the cycle. The purpose of our ECOHAB-funded research is to document the cycle in a coastal site near the epicenter of past toxic events, determining simultaneously the pattern of toxic *Pseudo-nitzschia* species abundance, domoic acid (DA) concentrations in the phytoplankton, and the spread of the toxin into benthic and pelagic populations over the cycle.

This presentation will review our findings on the occurrence of toxic *Pseudo-nitzschia* species and DA levels at a coastal station in northern Monterey Bay, a concentration site for phytoplankton in the region. A 14 month sequence of *Pseudo-nitzschia australis* and *P. multiseries* abundance will be presented, along with associated DA concentrations, and the pattern of DA levels in schooling planktivorous fish (sardines and anchovies) from the Bay. Additional data for an approximately 5 month period will show the co-occurring abundance cycles of toxic *Alexandrium* and *Dinophysis* species at the same site. The results suggest that episodic blooms of toxic microalgal species are not uncommon in this relatively "pristine" coastal system, that a wider array of species than previously suspected may be sequentially present, and that at least one of the toxins (DA) is transmitted into pelagic foodwebs by schooling fish abundantly consumed by marine birds and mammals in the region.

THE CHALLENGES OF FORECASTING AND MANAGING TOXIC *PSEUDO-NITZSCHIA* BLOOMS ON THE U.S. WEST COAST

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The food web transfer of domoic acid to shellfish, crustaceans, seabirds, finfish, and marine mammals has been recently documented on the U.S. West coast. Sub-regions of the U.S. West Coast face unique challenges during toxic episodes, including choosing the appropriate sentinel organism and understanding which *Pseudo-nitzschia* species are responsible for toxicity. Data collected during West Coast cruises in the years 1997-2000 indicate that often the highest toxin levels and greatest numbers of toxic cells are positioned in water masses associated with offshore eddies or in upwelling zones near coastal promontories. Such cruise data are essential in the characterization of offshore initiation sites that will lead to the effective placement of automated sensors, such as moored arrays. In addition, beach monitoring is a necessary component of regional species characterization, resulting in the development of specific molecular and biochemical tools needed to assist managers in each coastal area. Indeed, beach samples collected in 1998 indicated that a *P. pseudodelicatissima* bloom was responsible for razor clam toxicity on the Washington coast, whereas toxin produced by *P. australis* resulted in sea lion mortalities in central California. The challenges faced on the West Coast due to HAB-related mammal mortalities, widespread closures of shellfish harvest, and human illness can only be met by sustained beach monitoring programs such as the Olympic Region Harmful Algal Bloom (ORHAB) project and dedicated research cruises. These onshore and offshore efforts will give us a comprehensive picture of the oceanography influencing the location and intensity of domoic acid-producing HABs. Complete characterization of physical, biological and chemical conditions that favor harmful *Pseudo-nitzschia* blooms, only possible through large-scale, synergistic collaboration, is a prerequisite for forecasting of these events. A forecasting capability will substantially improve the management of valuable coastal resources and the protection of human health, both of which are affected by these toxins.

ABSTRACTS OF POSTERS

ENVIRONMENTAL CONDITIONS ASSOCIATED WITH DOMOIC ACID IN RAZOR CLAMS ON THE WASHINGTON COAST

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In October 1998, record levels of the neurotoxin domoic acid were detected in razor clams (*Siliqua patula*, Dixon) resulting in the closure of shellfish harvesting areas along the Washington coast. This toxin was measured in seawater samples collected at Kalaloch Beach and Second Beach on the central Washington coast using a receptor binding assay and liquid chromatography-tandem mass spectroscopy. Domoic acid levels ranging from 0-2700 ng/L were measured in seawater samples containing from 70-100% *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle at concentrations of 1.0-15 x 10^6 cells/L, resulting in maximum levels of cellular toxin of approximately 500 fg/cell. A cultured isolate of this species collected from Kalaloch Beach also produced DA, as determined by the receptor binding assay, during late exponential and stationary stages of growth. The toxic *P. pseudodelicatissima* bloom in the late summer and autumn of 1998 occurred 2-3 weeks after strong coastal upwelling during a period of anomalously low rainfall, typical in post-El Niño years. Higher toxin levels in seawater at Kalaloch Beach compared to Second Beach were attributed to the periodic nature of upwelling at Kalaloch Beach, demonstrated by a 175-fold increase in nitrate in seawater coincident with a 5^{o}C decrease in sea surface temperature on September 1. The upwelling event in September was followed by wind relaxation and reversal at the end of that month, resulting in the transport of toxic cells toward the coast where nutrients were already present to fuel the algal bloom. A pulse of nutrients, either from rainfall or upwelling, to coastal regions that have experienced several weeks of low nutrients, followed by wind relaxation or reversal events that transport cells to inshore regions, are suggested to be important factors in the initiation of the most toxic *Pseudo-nitzschia* spp. blooms on the Washington coast.

CAN KRILL TRANSFER DOMOIC ACID TO HIGHER TROPHIC LEVELS SUCH AS SQUID AND WHALES?

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Euphausiids (krill) are a ubiquitous prey for many consumers. The principal predators of Pacific euphausiids are squid, baleen whales, and certain seabirds (e.g., ashy storm petrel). Krill are potential vectors for domoic acid (DA) transfer from toxic *Pseudo-nitzschia* to higher trophic levels in the Monterey Bay food web. An understanding of the quantitative trophic interactions and body burden of DA in krill is required to predict whether krill can effectively vector the toxin. Nonetheless, laboratory experiments on euphausiids together with their larger predators are not currently feasible.

This poster presentation will describe the *Pseudo-nitzschia* link to whale and squid diets in Monterey Bay based on results from the examination of blue and humpback whale fecal material and squid stomach contents collected from 1993 to 2000. Scanning electron microscopy was used to identify *Pseudo-nitzschia frustules* in fecal material or stomach contents of whale and squid. Our aim was to determine whether these diatoms were transmitted to the predators through their food source, as was reported previously for a sea lion mortality event in 1998. Samples are also being tested for domoic acid activity using a receptor binding assay. Detection of *Pseudo-nitzschia frustules* in feces and stomach contents demonstrated that both baleen whales and squid obtained this diatom as part of their diet. Results of toxin analyses currently underway will be discussed in the context of potential transfer of DA via krill vectors from *Pseudo-nitzschia* to higher trophic levels.

INVOLVEMENT OF DOMOIC ACID IN MARINE MAMMAL MORBIDITIES AND MORTALITIES ON THE WEST COAST OF THE U.S. DURING THE YEAR 2000

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Domoic acid (DA) is an neuroexcitatory amino acid that has been the causative agent for amnesic shellfish poisoning in humans (Prince Edward Is., 1987), seabird mortalities (Monterey Bay, CA, 1991), and sea lion mortalities (Monterey Bay, 1998). This study summarizes DA analyses of body fluids from marine mammal stranding events that occurred along the California coast in the year 2000. For the first time, DA poisoning has had widespread impacts on several different marine mammal species with diverse feeding habits and geographic distributions. Year 2000 west coast marine mammal mortality events began early in the spring, with an abnormally high number of gray whale mortalities occurring during their northward migration from the Baja calving grounds to the Alaska feeding grounds. Over 350 gray whale mortalities were documented on the west coast this year, compared with 273 in 1999 and less than 50 in previous years. Of these, 25 mortalities were reported in the San Francisco Bay area (San Mateo to Marin counties). Unlike many of the stranded gray whales elsewhere on the west coast, most of the animals that stranded in the Bay area in during April and May were in good body condition. Their stranding followed, by approximately two weeks, a bloom of the DA producing diatom *Pseudonitzschia australis* in Monterey Bay, an area the whales would have migrated through. Body fluid samples (urine, feces) were positive for DA in two of the 11 animals tested (for the other 9 animals, only blood was available for analysis). Gray whales feed on benthic invertebrates by disturbing the sediment and then sieving disrupted sediments through their baleen. Therefore, it was reasoned that toxin exposure might be through a benthic food web that became exposed to DA as the planktonic diatom bloom declined. Urine from from 4 of 7 benthic-feeding sea otters that died during the same time frame (February – April) in this region also tested positive for DA.

In June - July, more than 90 sea lions stranded on the central coast of California, primarily in San Luis Obispo County. Most of the stranded animals were in good body condition, but displayed seisure and scratching activities documented previously in the 1998 sea lion mortality event. All animals displaying DA symptoms tested positive for DA in urine or feces. Blood samples from the same animals rarely had measurable levels of DA; thus, analysis of blood in future mortality events may be regarded as non-informative. Concurrent *Pseudo-nitzschia* bloom activity was documented in this region. Subsequent to sea lion strandings in the San Luis Obispo area, seizuring sea lions began to occur approximately 100 miles to the south, in Ventura and Los Angeles counties (mid-late July), where all symptomatic animals tested were positive for DA in urine. Sea lions most likely acquired DA toxicity through the planktonic food web, as previously described (Scholin et al. 2000 Nature 403: 80-83).

In addition to these species, two humpback whale strandings occurred during this time frame, the first in May and the second in late July. The second stranding co-occurred with a transition from *Pseudo-nitzschia* dominated algal blooms to *Alexandrium* dominated blooms. Because *Alexandrium* is a saxitoxin (STX) producer (paralytic shellfish poisoning), serum and stomach contents were analyzed for both DA and STX. Humpback whale samples were negative for both algal toxins.

ANNUAL BLOOM CYCLES OF TOXIC *PSEUDO-NITZSCHIA*, CELLULAR DOMOIC ACID AND MACRONUTRIENT DYNAMICS IN MONTEREY BAY, CALIFORNIA

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Much of our understanding of the physiology and toxin production in species of *Pseudo-nitzschia* is inferred from laboratory based studies conducted on cultures of *P. multiseries*. These studies have done much to advance our knowledge but are limited since they were conducted on clonal isolates instead of natural populations.

Here we present, for the first time, a 12 month sequence of field observations documenting several bloom cycles of *Pseudo-nitzschia australis* and *P. multiseries.* Abundances of toxic *Pseudo-nitzschia* species, together with cellular domoic acid (DA) concentrations and corresponding levels of water column macronutrients were measured. We compare these data with previously reported HABs and results of current laboratory research on DA production in cultures of *Pseudo-nitzschia sp*. to better understand bloom dynamics and DA production in natural populations. Preliminary results indicate particulate DA concentrations in the water column are strongly correlated with the abundances of the known toxin producers *Pseudo-nitzschia australis* and *P. multiseries.* In addition, cellular concentrations of DA from these field samples corroborate published values for *Pseudo-nitzschia* cultures. Peak abundances of *Pseudo-nitzschia* do not necessarily correspond to maxima in chlorophyll a concentrations but likely reflect rapid response to changing nutrient levels or other meso scale features. Likewise, cellular concentrations of DA in *P. australis* may vary due to changing ratios of macronutrients. The results and preliminary interpretations from this ongoing study will be presented.

***EMERITA ANALOGA* (STIMPSON)- POSSIBLE NEW INDICATOR SPECIES FOR THE PHYCOTOXIN DOMOIC ACID IN CALIFORNIA COASTAL WATERS**

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Documented occurrences of harmful algal blooms (HAB's) producing the neurotoxin, domoic acid (DA), has increased dramatically along the west coast of North America over the last decade. Blooms of DA synthesizing diatoms (Pseudo-nitzschia spp.) have been associated with the death and injury of hundreds of marine birds and mammals, posed serious health risks to humans, and threatened to significantly impact coastal fisheries and economies dependent on marine resources. Unlike the paralytic shellfish poisoning (PSP) toxins, also common on the west coast, a reliable intertidal indicator species for monitoring the presence of DA in the environment remains to be identified. Here we evaluate and confirm the utility of the common sand crab (*Emerita analoga*) as a DA indicator species in comparison with sea mussels (*Mytilus californianus*), the species currently used by the California Department of Health Services Shellfish Program for HAB toxin monitoring. Mussels and *Emerita* sampled from natural populations at two state beaches in Santa Cruz, California (Apr. 1999 - Feb. 2000) were tested for DA by the HPLC-UV method. Determinations of DA in mussels were below instrument detection. However, detectable levels of the toxin in *Emerita* ranged from 0.07 to 10.4 ug DA g-1 and coincided with observed trends in densities of DA producing *Pseudo-nitzschia* species neashore. The rise and fall of DA in *Emerita* in synchrony with *Pseudo-nitzschia* abundance, combined with this common intertidal species' accessibility and ease of DA extraction, recommends *Emerita* as a reliable, cost effective monitoring tool for DA.

FINE-SCALE VERTICAL DISTRIBUTIONS AND POTENTIAL MICROZOOPLANKTON GRAZING IMPACT ON TOXIC DINOFLAGELLATES IN EAST SOUND, WA

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Many microplankton taxa occur in <0.5 m thick layers in the water column of East Sound, WA. Vertical profiles of this highly structured water column were collected during June in 1997 and 1998 using a high resolution profiling package. In both years the toxic dinoflagellate *Dinophysis acuminata* was located in a layer near the surface. The toxic dinoflagellate *Alexandrium catenella* was located in a layer centered at ~5 m depth. *A. catenella* cells were plainly visible in the food vacuoles of a variety of heterotrophic protist taxa distributed within and around the *A. catanella* layer, including *Lacrymaria* sp., *Polykrikos schwartzii*, and various aloricate choreotrich ciliates. These protozoan predators exhibited maxima around, but not within, the *A. catenella* layer. The role of predation by protozooplankton in cropping and termination of HABs is not well understood. These preliminary observations demonstrate (1) that the spatial distributions of HAB organisms can be highly localized in the water column and (2) that protozooplankton are potentially important predators on HAB organisms.

OLYMPIC REGION HARMFUL ALGAL BLOOMS (ORHAB): WASHINGTON STATE HARMFUL ALGAL BLOOM MONITORING PROJECT

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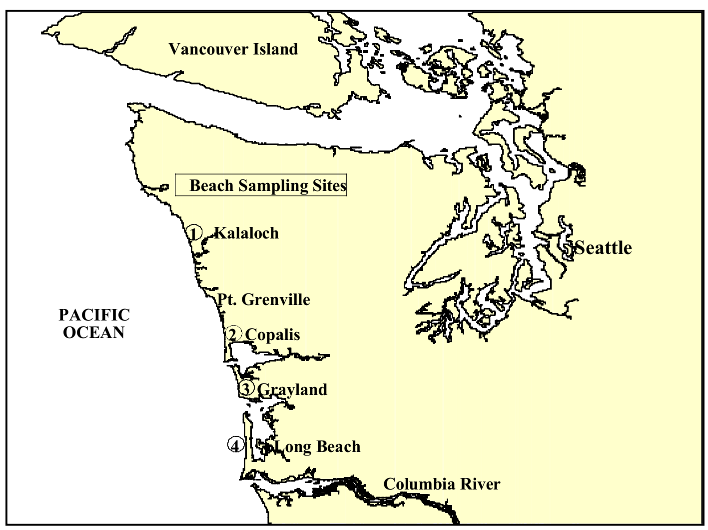
Harmful algal blooms (HABs) are a common occurrence on the Washington coast, but our knowledge of the processes that govern their timing and spatial distributions, subsequent advection to the coast, and dispersal are not sufficient to predict the possibility of shellfish contamination in the area. In response to this need, the Olympic Region Harmful Algal Bloom (ORHAB) group was organized as a forum for collaboration and cooperation among federal, state, and local government agencies, academic institutions, coastal Native tribes, marine resource-based businesses, and public interest groups. Its mission is to support applied and basic research on HABs and to build a greater local capacity to monitor and mitigate the effects of such events. The ultimate goal is to sustain a long-term monitoring program into the future without reliance on federal support.

A multi-agency, multi-disciplinary project funded by NOAA's Coastal Ocean Program is investigating the origins of open-coast toxic blooms, monitoring where and when the toxic species are present on the coast, assessing the environmental conditions under which blooms occur and are transported to intertidal shellfish populations, and exploring methods that can be used to forecast HABs. The primary focus will be on the presence of *Pseudo-nitzschia* spp. and domoic acid in razor clams (*Siliqua patula* Dixon). The monitoring sites were chosen because they have harvestable razor clam populations and several have historical phytoplankton data.



The working hypothesis is that phytoplankton blooms in water over the Washington continental shelf are the source of toxins in razor clams on coastal beaches. The precise timing of physical processes (currents, winds, upwelling, and downwelling) off the coast determines whether a toxic bloom will be advected into the nearshore region and be sustained there long enough for razor clams to become toxic. The project will determine the temporal and spatial distributions of *Pseudo-nitzschia* spp. and relate these to hydrological and meteorological parameters using standard oceanographic and biological methods. New techniques for the rapid detection of toxins and toxigenic species will be tested in the field as they become available.

The major partners/collaborators and contact persons are: Northwest Fisheries Science Center (Vera Trainer, project PI); Battelle Marine Laboratory (Dana Woodruff); Olympic Coast National Marine Sanctuary (Ed Bowlby); Pacific Shellfish Institute (Dan Cheney, Ralph Elston); Quinault Indian Nation (Joe Schumacker); Saigene Corporation (Paul Haydock); University of Washington Olympic Natural Resources Center (Miranda Wecker); University of Washington School of

Oceanography (Barbara Hickey, Rita Horner); Washington Department of Ecology (Jan Newton); Washington Department of Health (Judy Dowell); Washington Department of Fish and Wildlife (Doug Simon, Dan Ayres).

NUTRIENT REGULATION OF PHOTOSYNTHETIC PERFORMANCE AND VARIABLE FLUORESCENCE IN *PSEUDO-NITZSCHIA MULTISERIES*

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Pseudo-nitzschia spp. are known to vary domoic acid production as a function of multiple nutrients, including nitrogen, phosphorous, silicate, iron, copper, and possibly lithium. Although there has been a great deal of work done on several of these nutrients (in particular N, P, and Si), the interactions between macro- and micronutrients are further complicated by the role of light in controlling diatom production. Silicic acid transport requires ATP, a byproduct of photosynthesis. Previous studies, however, have reported that it takes > 6h for transport to degrade, and that it does so fairly slowly. The implication is that silicic acid utilization is only weakly controlled by the availability of photosynthate and is essentially uncoupled from ambient irradiance, unlike C and N assimilation, which are strongly dependent on light and can become uncoupled from one another under non-steady-state growth. Recent evidence from Monterey Bay, California demonstrates that contrary to expectations, Si assimilation demonstrates a positive uptake vs. irradiance response. Work done with non-*Pseudo-nitzschia* diatom cultures also demonstrates that Si limitation can directly affect photosynthetic performance, and acts similarly to iron and nitrogen (nitrate) limitation. This response includes variability in photosynthesis versus irradiance parameters, and more interestingly, in reversible changes in the quantum efficiency of Photosystem II as measured by variable fluorescence.

Here we present results from continuous culture experiments using *Pseudo-nitzschia multiseries* (Hasle) grown under constant temperature and irradiance with either silicic acid or nitrate as the limiting nutrient. At the termination of the experiments photosynthetic performance was assessed using ^{14}C photosynthesis versus irradiance (P vs E) curves and by off-line assessment of Photosystem II efficiency using a Walz Pulsed Amplitude Modulation (PAM) fluorometer. As expected, DA production was significantly enhanced under Si limitation. We also observed a significant decrease in photosynthetic performance under both nitrate and silicate limitation, suggesting that silicic acid availability directly affects photosynthesis in this diatom with diagnostic symptoms similar to iron or nitrogen limitation. Results and preliminary analyses of these data will be presented, emphasizing the potential role of silicic acid in controlling the photosynthetic competency of this toxigenic species.

INFLUENCE OF HARMFUL ALGAL BLOOM TOXINS ON THE FORAGING BEHAVIOR OF SEA OTTERS AND SHOREBIRDS

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We tested the general hypothesis that the foraging behavior and distribution of high level marine predators (sea otters and shorebirds) under natural conditions are mediated by benthic prey toxicity due to harmful algal blooms (HAB's). Sea otters in southeast Alaska did change their foraging behavior at sites were *Saxidomus Giganteus* (Butter Clams) were found to contain parallytic shell fish poisoning toxins (PSPT) in high concentrations. At the most toxic sites (>500 µg STX/100g prey tissue weight) sea otters shifted their diet away from their primary butter clam prey to smaller and less abundant non-toxic species. At sites of intermediate prey toxicity (200 to 400 µg STX/100g) some sea otters continued to eat butter clams while discarding the most toxic body parts of these clams. In California, changes in shorebird (Oyster Catchers and Willets) feeding behavior was correlated with seasonal changes in PSPT concentrations in their primary prey (mussels and mole crabs respectively). We conclude that predators are not excluded from areas where their primary prey become toxic due to HABs, but they do alter their foraging behavior and diet in a graded response to toxin levels. These behavioral shifts in turn, can result in spatial and temporal refuge for preferred prey due to the reduction in predation pressure.

ACCUMULATION OF DOMOIC ACID BY THE COASTAL DIATOM *PSEUDO-NITZSCHIA MULTISERIES*: A POSSBLE COPPER COMPLEXATION STRATEGY

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Domoic acid (DA) is a neurotoxic amino acid produced by several members of the diatom genus *Pseudo-nitzschia*. Trophic transfer of DA up the food chain has been implicated in the deaths of 100's of marine birds and marine mammals along the central California Coast. The physiological function of DA in *Pseudo-nitzschia* spp. has not been defined, although some evidence indicates that elevated metal concentrations can induce DA accumulation (Subba Rao and others 1998 P.S.Z.N. Mar. Ecol. 19:31). Although California coastal waters have experienced a decline in several heavy metals from 1977-1990, copper concentrations have increased by as much as 25% (Stephhenson, M. D. and Leonard, G. H. 1994 Mar. Poll. Bull. 28: 148). Many algae produce chelators, including amino acids, in response to toxic [Cu^{2+}] (Wu and others 1998 J. Phycol. 34: 113). Domoic acid, a tricarboxylic acid, has 4 functional groups that may readily form chelation complexes with transition metals like copper. Copper enrichment experiments indicate that while Cu^{2+} is toxic to *P. multiseries* at total [Cu] > 16.1µM (pCu ≈ 6.0), intracellular DA accumulation increases up to this point with no decline in growth rates relative to cultures grown in standard enriched seawater. These data suggest that intracellular accumulation of DA by *P. multiseries* may serve to mitigate the toxicity of elevated [Cu^{2+}]. Direct measurement of the impact of DA on cupric ion activity using sensitive chemiluminescent assays indicate that DA exhibits significant chelation capacity for cupric ion with a conditional stability constant, $K_{c,DACu(II)} = 10^{12.4}$ at pH 8.0. These data indicate that 1) accumulation of DA can be induced by increases in total dissolved copper and 2) that DA functions as a strong intracellular chelator. Field data from the ongoing *Pseudo-nitzschia* spp. Bloom in Monterey Bay will be presented examining the association of cellular DA content and cupric ion activitiy. Defining the Cu-DA dose response relationship in *Pseudo-nitzschia* can facilitate prediction of future toxic bloom events.

SMALL PELAGIC FISH; DANGEROUS VECTORS OF DOMOIC ACID

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Domoic acid producing *Pseudo-nitzschia* species have been responsible for mass mortality events involving seabirds and marine mammals in Monterey Bay California on several occasions since 1991. In at least three major DA-poisoning events, the northern anchovy (*Engraulis mordax*) was identified as the vector for toxin transfer from diatoms to pelicans (*Pelecanus occidentalis*), cormorants (*Phalacrocorax*), or sea lions (*Zalophus californianus*). Because of its prominent role as a DA vector in Monterey Bay, we began an ongoing study documenting DA levels in anchovies (as well as sardines) on a regular basis from freshly collected field samples. Here we present data on gut contents and DA levels detected in anchovies and/or sardines collected by fishermen from various sectors of Monterey Bay over a one year period. Over 70 fish samples were analyzed with the highest DA levels reaching 588 ppm in viscera preparations. DA levels and the presence of *Pseudo-nitzschia* in the digestive tracts of fish were correlated with diatom densities calculated from whole water samples taken from Monterey Bay at regular time intervals during the same one year period.

In the most recent west coast DA poisoning event, over 100 California sea lions were observed exhibiting seizures in the Morro Bay, California area from approximately July 23- August 6, 2000. Of eight fecal samples collected from sea lions exhibiting seizures, one contained detectable levels of DA (8.7 ug/g). Anchovies collected from Morro Bay waters during July, contained low levels of DA (23 ppm in viscera). *Pseudo-nitzschia* densities and DA levels measured in Morro Bay waters during July will also be presented.

PARALYTIC SHELLFISH POISONING IN ALASKA: A FIELD PROGRAM FOR DETECTION OF TOXIC ALGAE AND SHELLFISH

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Shellfish are one of Alaska's most abundant natural resources. Paralytic shellfish poisoning (PSP) severely limits recreational, subsistence, and commercial harvesting. To optimize shellfish utilization, the Alaska Science and Technology Foundation recommended implementation of novel monitoring procedures to detect *Alexandrium* and provided funding for the state's first beach monitoring program for this toxic alga. Kodiak Island was chosen as the study site due to its insidious history with PSP events (shellfish frequently reach 20,000 µg toxin/100 gm). The focus of our work revolves around determining the abundance of *Alexandrium* using DNA probe methodologies. PSP toxin levels are being monitored with receptor binding assays and by HPLC. Ribbed mussels (*Mytilus edulus*), known for their rapid depuration rates, were chosen as sentinel shellfish species. A primary goal is to determine if there is a correlation (both short and long term) between *Alexandrium* abundance and shellfish toxicity. Water chemistry data are also being collected to determine if the seasonal blooms of *Alexandrium* are triggered by predictable environmental/weather patterns. Cultures of *Alexandrium* are being established for detailed laboratory analysis.

Shellfish samples collected during the winter of 1998/1999 had PSP toxins at levels too low for detection. No shellfish samples were collected in April/May but by early June, PSP toxins were reaching unsafe levels. Toxin levels peaked in late June/early July at 300 - 1100 µg/100 gm, and slowly declined to very low levels by mid August. Mussels from one of the nine test site maintained PSP toxin levels below the cutoff limit (80 µg/100 gm) during the entire summer of 1999. This site was situated geographically between two of the toxic sites. We are unable to explain the differences in shellfish toxicity between sites at this time. *Alexandrium* was present at high cell densities, 200 - 1000 cells/L, from early June through mid July at most sites. Technical problems prevented collection of a robust data set using the DNA probe methodologies in 1999, however, it was clear that the decreasing levels of PSP toxins observed in shellfish during late summer were correlated with declining numbers of *Alexandrium*.

Preliminary data from summer 2000 indicate that there were two small blooms of *Alexandrium*, both of approximately two weeks duration with moderate cell densities (generally less than 500 cells/L). The first bloom was in early June, the second in late August. We are optimistic that water chemistry data will help explain why there was one large bloom in 1999 and two smaller ones in 2000. We are also anxious to determine if shellfish toxicity will show two smaller peaks in 2000, each coinciding with one of the two small *Alexandrium* blooms. Shellfish and water column toxin levels, more detailed analysis of Alexandrium abundance data, and water column chemistry data should all be available within the next few months for discussion at the December HAB meeting.

PRELIMINARY RESULTS FROM PHYTOPLANKTON AND ENVIRONMENTAL MONITORING, WESTERN WASHINGTON, USA

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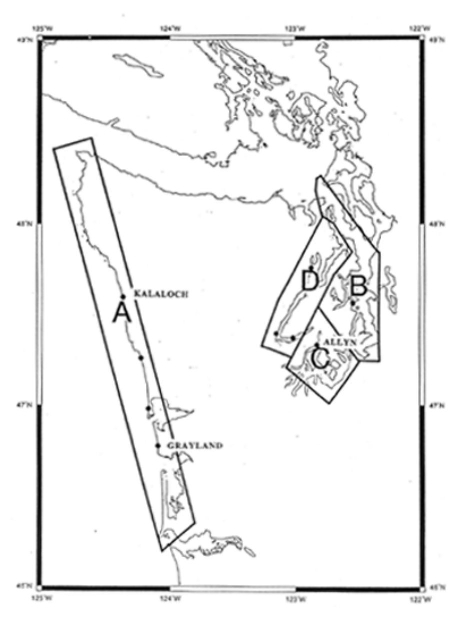
Washington state has a long history of harmful algal blooms as indicated by the presence of toxin in shellfish and mortalities of pen-reared salmonids. However, little is known about the temporal and spatial distribution of the causative algal species. Consequently, selected sites on the Washington Pacific Ocean coast and on inland waters of Puget Sound were monitored with varying frequency since 1990. Emphasis was on determining the presence and distribution of potentially harmful species: *Pseudo-nitzschia* spp., *Alexandrium* spp., *Dinophysis* spp., *Chaetoceros* spp., *and Heterosigma akashiwo*. Here we present preliminary results from five coast and five Puget Sound sites that were sampled twice monthly (weather permitting) from May 1997-January 2000(Fig. 1).

Water was collected with buckets and poured through a 20 µm mesh net to concentrate cells, directly with a net, or with water bottles. Temperature was measured with a laboratory thermometer, salinity was determined with a Gildline Autosal 8400A salinometer, and nutrients were measured with a Technicon Autoanalyzer II. Phytoplankton samples were analyzed using light and scanning electron microscopy



Our samples come from four distinct hydrographic regimes with conditions ranging from tidally-mixed estuaries and protected bays to open ocean beaches (Fig. 1). The coast beaches (A) are gently sloping with fine sand and are influenced by ocean currents and seasonal upwelling; they are prime locations for razor clams. Blooms of surf zone diatoms are common at these beaches. The Puget Sound Main Basin (B) is a fjord influenced by tidal and wind mixing and with a seasonal pycnocline that allows development of phytoplankton blooms; fish farms are present in some areas. Southern Puget Sound (C) has restricted circulation, low dissolved oxygen and the potential for non-point source pollution; shellfish farms are common. Hood Canal (D) is a fjord, with oxygen depletion throughtout the year, but especially in late summer/fall; shellfish beds are common and there is a seasonal shrimp fishery.

Potentially harmful species were present at one or more sites in all months except March 1999. Further, all potentially harmful genera occurred at all sites except that *Alexandrium* was not positively identified in Hood Canal. *Alexandrium* was found sporadically and never in high numbers at open coast beaches. In the fall of 1997, it was present at Allyn in southern Puget Sound when record high levels (to nearly 7,000 µg/100 g) of PSP toxin were reported. *Pseudo-nitzschia* spp. were common on the open coast with blooms at Grayland in July 1997 (no domoic acid reported) and at Kalaloch in September 1998 when record high levels of domoic acid (287 µg/g) occurred in razor clams. *Pseudo-nitzschia* spp. were also common at Puget Sound and Hood Canal sites, but rarely occurring in high concentrations. *Dinophysis* spp. were present primarily in spring to summer and at all sites. *Heterosigma akashiwo* was rarely present, but a small bloom occurred at Allyn in October 1999.

Environmental conditions included extended priods of < 0.5 μM nitrate on the ocean beaches with periodic replenishment by upwelling events or seasonal changes in the coastal currents. The Puget Sound Main Basin seldom had < 5 μM nitrate due to strong tidal mixing and weak stratification; Hood Canal and southern Puget Sound generally had < 0.5 μM nitrate concentrations throughout the summer periods.

DYNAMICS OF *ALEXANDRIUM CATENELLA* BLOOMS IN QUARTERMASTER HARBOR, WA

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Alexandrium catenella, that causes paralytic shellfish poison (PSP) in Puget Sound, WA, was studied in Quartermaster Harbor (Fig. 1), a small, semi-enclosed, seasonally stratified bay in central Puget Sound to determine the effects of biological, chemical, and physical factors on the formation, maintenance, and decline of blooms. A shore station was sampled near mid-bay 1-3 times per week from May to September 1998 to determine the environmental conditions of the surface layer in the bay during the optimal growing season for this species. Additionally, three cruises occupied a station at 20 m depth in 1997 and 1998 to study the diel migration pattern of the *Alexandrium* population and to estimate growth and mortality (from grazing) rates of the total dinoflagellate population from dilution experiments. Estimates of tidal flushing were made by calculating the volumes of the bay at various tide heights and the volumes of typical tidal prisms for mean, neap, and spring tide ranges.

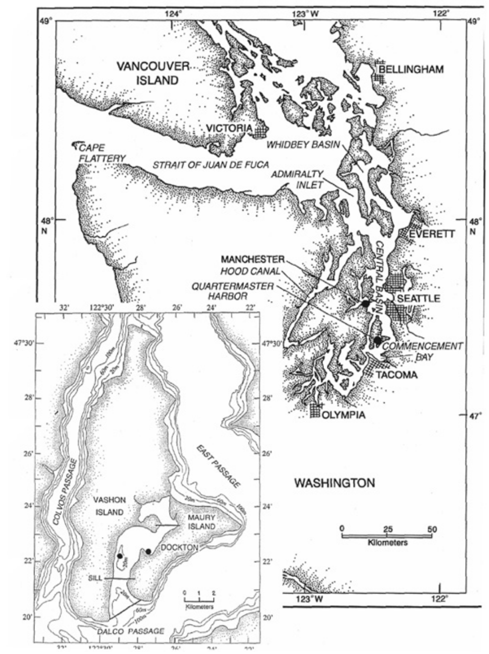

Figure 1. Quartermaster Harbor, Vashon Island, WA

PSP concentrations in caged mussels at 1 m depth exceeded 80 μg 100 g^{-1} mussel meat twice during 1997 and 1998, both times following the occurrence of *A. catenella* concentrations > 10,000 cells L^{-1}. The species was present continuously from late May through September 1998, blooming in July in surface water. Over this time interval surface temperatures varied from 14-20 °C; nitrate levels remained near-zero; phosphate and silicate were low, but measurable; and salinity ranged from 25-30 psu. Thermal stratification beginning in late spring led to the development of a shallow, low-nutrient surface layer (upper 1-3 m), a distinct pycnocline (3-5 m depth), and a deeper, nutrient-replete layer (5-20 m). During the July 1998 cruise strong spring tidal cycles eroded the pycnocline and mixed the water column within 24 h. This disrupted the distribution of *A. catenella* and contributed to bloom decline.

The diel vertical migration pattern of *A. catenella*, studied in 1997 and 1998, was limited to the upper 10 m of the water column with migration rates varying from 0.4 - 2.0 m h^{-1}. Maximal *A. catenella* cell numbers varied from < 10,000 - > 60,000 cells L^{-1}, but the phytoplankton assemblage was dominated by large diatoms which were present at > 500,000 cells L^{-1}. Dilution experiments in 1998 indicated that the total dinoflagellate population grew at about the same rate as the diatom population but was grazed more heavily by microzooplankton. If estimated loss due to tidal flushing of the population is added to grazing rate, the dinoflagellate population growth rate was less than the combined loss rate, but growth still exceeded loss for the diatoms during spring tidal exchange periods. However, during mean or neap tidal periods, population growth rate was higher than loss rate for both dinoflagellates and diatoms. Assuming that the results from the overall dinoflagellate population are applicable to *A. catenella*, it appears that when water temperature exceeds 13 °C, permitting active *A. catenella* growth, blooms are rarely generated and then only for a limited period each summer. A combination of biological, chemical, and physical factors can lead to decline of the bloom within the bay and prevent an accumulation of PSP in the shellfish.

DEVELOPMENT OF A PROTOCOL FOR DETERMINATION OF DOMOIC ACID IN MOLE CRABS (*EMERITA ANALOGA*): A POSSIBLE NEW INDICATOR SPECIES

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The transfer of algal toxins through marine food webs has important implications for not only public health, but also the health of ecosystems and their trophic structure. Impacts of algal toxin trophic transfer are manifested as contaminated commercial and recreational fishery resources, as well as mortality events involving a variety of birds and mammals. Protection of public health requires identification of an indicator or sentinal species that rapidly accumulates toxin upon its appearance in local waters. Monitoring the movement of an algal toxin through food webs requires identification of potential points of entry and thus vectors for toxin transfer. In the case of domoic acid (DA), mole crabs (*Emerita analoga*) have the potential to serve both as a sentinel species and as a vector for trophic transfer of this potent neurotoxin produced by members of the diatom genus, *Pseudo-nitzschia*.

Monterey Bay, California is a site of recurrent, toxic and non-toxic *Pseudo-nitzschia* blooms, as well as extensive mole crab populations. This location was therefore selected to begin evaluating the utility of *E. analoga* as an indicator for DA presence in local waters. Moreover, one of the current sentinel organisms, the sea mussel (*Mytilus californianus*), shows minimal or undetectable toxicity during some bloom events in Monterey Bay. It is also anticipated that data collected during this project will provide information on the efficacy of mole crabs as a vector for DA transfer to higher trophic levels.

The efficiency of extracting algal toxins from diverse sample types is highly variable and depends on the individual sample matrix. Using a conventional aqueous methanol extraction method for shellfish as a starting point, we have developed and validated a highly efficient protocol for extracting DA from whole mole crabs that yields toxin recoveries of 96.5 ± 2.9 percent. We also confirmed by LC-MS/MS that mole crabs accumulated measurable amounts of DA during toxic *Pseudo-nitzschia* bloom events (0.5-10 micrograms DA/g tissue), while the blue mussel showed no detectable toxin. In addition, an extensive comparison (n = 60) of inter-animal variability in DA content revealed values ranging over an order of magnitude (*ca*. 0.5 to 5 micrograms DA/g tissue) and no consistent trend with size class, based on either animal weight or length. These data on the toxicity of individual animals will be useful in designing an appropriate sampling strategy and, importantly, indicate that mole crabs do not appear to progressively bioaccumulate DA with age. In fact, initial observations by Ferdin et al. (this conference) revealed a rise and fall of DA toxicity in mole crabs, coinciding with similar changes in *Pseudo-nitzschia* cell concentrations. The extraction protocol developed herein will be implemented during upcoming field trials to further compare the DA toxicity of mole crabs vs. blue mussels and its correlation to the concentrations of toxic *Pseudo-nitzschia* cells in local waters. Mole crab toxicity values will also be available for reference, in the event of suspected DA-associated mortality events involving consumers of *E. analoga*.

FISH BIOASSAYS AND PILOT-SCALE APPLICATION OF CLAY TO CONTROL BLOOMS OF *HETEROSIGMA AKASHIWO* AT PACIFIC NORTHWEST SALMON NET PENS

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Fish killing microalgae are a major impediment to the increasing fish mariculture activities in many locations around the world. Fish farmers use a variety of physical methods to mitigate effects of harmful algal blooms (HABs) including towing of pens, perimeter skirts, upwelling systems and other means. But these are not without risks, such as ripping of pens when towing and inefficient displacement of blooms by upwelling due to unpredictable vertical distribution of algae. Certain types of clay flocculation appears to be a promising means of treating some fish killing HABs in or around fish farms, based on laboratory tests and reports from large-scale applications in Korea and Japan. Recurring wide-scale blooms of *Heterosigma akashiwo* have caused major fish losses in the Pacific Northwest Coast in Washington State, but more so in British Columbia, where the industry is much larger and broadly distributed. Since *Heterosigma* does not produce a persistent toxin, but apparently kills fish through the action of reactive oxygen species that quickly dissipate in seawater, the alga is a good candidate for the use of clay mitigation. (Note there is a possibility that *Heterosigma* may produce a weak brevetoxin-like compound, based on one documented case). This work reports initial laboratory results of clay bioassays with Atlantic salmon (*Salmo salar*) and plans to test the mitigation method at a commercial fish farm using perimeter skirts to retain the clay in a small area and reduce reintroduction of the alga.

Replicated laboratory bioassays were conducted with clay concentrations ranging from 30 to 100 mg/L clay, held in suspension by air lift pumps for five hours, much longer than would occur in the field. Turbidity and total suspended solids were used as indices of clay loading, and were positively correlated to a high degree. Turbidity is the preferred measurement for practical and water quality standard reasons. Fish began "coughing" immediately upon introduction of the clay in the aquaria, a natural reaction to many types of irritants, and cough frequency occurred in a dose-related fashion up to 10 times per minute. The nature of the coughing was different from that previously seen with fish exposed to harmful *Chaetoceros concavicornis*; it was shallower and less pronounced. Coughing ceased immediately upon transfer of the fish to clean seawater at the conclusion of the bioassay. Gill tissue samples of other treated fish were collected for histological analysis. There was some mucus present on the affected gills, but not an usually large amount. These results suggest that there will not be any significant or permanent effects of clay on treated fish, as high levels of turbidity occur naturally during the freshwater phase of salmonid life. Prior studies have shown that juveniles of several salmon species tolerate much higher concentrations of silts or clays than were used in our experiments, and for much longer periods.

Our null hypothesis for the sediment chemistry and infauna fieldwork is that there will be no measurable impact on the seabottom or benthos of clay application. Pacific Northwest fish farms are required to locate where there are no other significant aquatic resources, including shellfish beds. Moreover, there is little permanent deposition of the wastes from these farms, as they are without exception located in current swept areas with erosional bottoms. Although *Heterosigma* occurs frequently each spring and fall at many farm sites, concentrations are usually less than that which will cause fish mortality in about 9 out 10 years at a given farm site. A typical farm producing several million pounds of fish annually will therefore only require an annual clay application (pro rata over 10 years) of about 40 kg per farm [(0.4 ha per farm x 0.1 kg m^2)/10]. There are only 9 large, separate commercial pens in the 0.8 million hectares of Puget Sound, so cumulatively the required clay amount is trivial. The volume of treatment clay needed pales in comparison to the estimated 6 million metric tons of sediment including clay entering Puget Sound each year from riverine and shoreline erosion (Dexter et al. 1981 NOAA Tech. Memo. OMPA-13).

FREE AMINO ACID (FAA) POOLS IN SPECIES AND STRAINS OF *Pseudo-nitzschia* FROM MONTEREY BAY, CA: DYNAMICS OF FAA PROFILES DURING A *PSEUDO-NITZSCHIA* BLOOM

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The phenomenology of domoic acid (DA) production by strains of the *Pseudo-nitzschia* species complex has received considerable attention, leading to general observations that DA accumulation is stimulated by growth limiting or stress conditions. Our efforts have been guided by the hypothesis that DA, in addition to its obvious structural similarity, also serves as a functional analogue of the amino acid proline and is derived from proline catabolism. Ongoing studies have used HPLC-UV profiling of phenylthiocarbamyl amino acid (PTC-AA) derivatives to characterize the FAA composition and FAA pool dynamics in clonal isolates of several *Pseudo-nitzschia* species from Monterey Bay, CA. DA accumulation varied by 2-orders of magnitude among independent isolates of *P. multiseries* and *P. australis*, with the isolates of the latter species exhibiting consistently higher cellular yields of DA. Proline content was lower in cells accumulating high levels of DA (>1 fmole/cell) consistent with the hypothesis that DA is derived in part from proline catabolism. All *Pseudo-nitzschia* species accumulated large pools of taurine (_ 50% of total FAAs) when grown in Monterey Bay seawater (34 ppt). This amino acid was not detected in other diatom species when grown under equivalent conditions. As taurine content covaried with DA accumulation in *Pseudo-nitzschia*, it may provide a useful biomarker for potentially toxic bloom events. Monterey Bay is presently experiencing a large and persistent summer bloom initially dominated by *P. australis*. This event provides a novel opportunity to compare FAA compositions in natural populations and laboratory cultures to determine whether the unique FAA profiles characterizing *Pseudo-nitzschia* are evident in heterogenous samples. At a nearshore sampling station (Monterey Warf 2, 36^{o} 36' 18"N, 121^{o} 53' 23"W), *Pseudo-nitzschia* cell abundance has remained near 10^{5} cells L^{-1} in the surface waters (0-2 m) through mid-September. DA content of the particulate fraction (≥ 20 µ net fraction) varied between 0.7 and 3.5 nM with estimates of cell specific content ranging between 6 and 30 fmole $cell^{-1}$. FAA pool compositions will be determined for samples encompassing transitions from high to low and low to high DA content and compared to datasets from previous laboratory cultures as well as *Pseudo-nitzschia* isolates from the current bloom. It is anticipated that taurine content in the field samples will not only track *Pseudonitzschia* spp. abundance but will also covary with cellular DA content. Possible interactions between DA, proline and taurine metabolism will be discussed.

EXTRACELLULAR COMPOUNDS THAT UNBALANCE CALCIUM-MEDIATED METABOLISM AS POSSIBLE TOXINS FROM *HETEROSIGMA AKASHIWO*

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Toxin(s) from the ichthyotoxic alga *Heterosigma akashiwo* have been responsible for millions of dollars of lost aquaculture stocks around the globe. Suggested mechanisms of toxicity include the production of reactive oxygen species (ROS), the release of large quantities of mucus from mucocysts, and/or the production of an organic toxin that may be brevetoxin-like in structure (Khan et al. 1997). However, to date, none of these postulates has been confirmed and recent evidence suggests that an alternate proposal is needed.

We have taken unique steps in order to collect and test the bioactivity of a mixture of extracellular organics from *H. akashiwo* cultures on a variety of cultured carcinoma cell lines. Chronic exposure (12 to 24 hrs) of these extracellular organics to mammalian cell lines (UMR and HEK) have been shown to cause extraordinary effects on these cell lines, specifically by elevating cellular metabolism up to 10 fold basal. This was determined by monitoring mitochondrial succinate dehydrogenase activity via a tetrazolium-dye assay. With continued exposure (>36 hrs), cell death became evident which was preceded by detachment of cells from an original, confluent monolayer.

Acute exposure of *H. akashiwo* extracellular organics to the model insect cell line SF9, elevates intracellular calcium quickly and transiently by over 100 nM, followed by maintenance of elevated intracellular calcium concentrations for prolonged periods of time. However, this response can only be significantly observed by SF9 cultures that have been transfected with membrane-bound receptors that couple to G-protein specific pathways. Control cells and cells transfected with the vector alone (baculovirus) only elicit a minimal response suggesting pathway-specific effects of the bioactive compounds collected from *H. akashiwo* cultures.

Cell-assay-mediated determination of bioactive compounds from cultures of *H. akashiwo* have thus far shown that extracellular compounds from this alga can elicit biological effects towards multiple, cultured cell lines and may serve to isolate and identify a potential ichthyotoxic compound from this alga.

A FIELD TRIAL MONITORING *HETEROSIGMA AKASHIWO* IN PUGET SOUND, WASHINGTON USING THE SANDWICH HYBRIDIZATION ASSAY

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Sandwich hybridization technology has been applied to detecting *Heterosigma akashiwo*, *Fibrocapsa japonica* and *Chattonella spp*. (Tyrrell et al. in review, Scholin et al. 1999). Preliminary field testing of the *H. akashiwo* and *F. japonica* sandwich hybridization assays (SHA) in New Zealand has shown they detect target species at levels below that of concern (Rhodes et al. in review). Field testing of the SHA for *H. akashiwo* is also currently underway in Puget Sound, Washington. The objectives of that study are: 1) to determine the specificity and sensitivity of the SHA in the context of field samples; 2) to establish methodology for archiving field samples for future examination, and; 3) to determine the potential of the assay as a monitoring tool. In addition, the utility of the SHA is being examined for the detection *H. akashiwo* in clay mitigation experiments/trials.

All field samples from Puget Sound shown to contain *H. akashiwo* by light microscopy were also detected by the SHA. To date, no false positives have been returned using the SHA against a wide panel of organisms, including detritus and flocculent material. Preliminary data suggests that field samples can be preserved with acid Lugol's, or stored as frozen filters or frozen lysates for up to 30 days with no loss of signal. Also it appears that samples preserved with acid Lugol's may enhance the signal of the assay. This enhancement is currently under study. The results from preliminary trials in the laboratory show no interference to the SHA from clay at the maximum proposed application rates for clay mitigation. Results to date are extremely encouraging and together with the results from earlier trials in New Zealand indicate that the assay has potential for monitoring purposes. *H. akashiwo* is detected at levels well below the level of concern, thus allowing ample time to map the geographic boundaries of a bloom and whether is is growing or in decline. The SHA may be improved for monitoring purposes by integrating the plate reader with the robotic processor, making it more simplistic and easier to use. The SHA microtiter format can be transferred onto a solid support (filter membrane) and used on a remote *in situ* processor, which ultimately may be mounted onto a buoy, drifting arrays, ROVs, on-board ship, etc (Scholin et al. 1998). The SHA could also be applied to a dipstick format, which in comparison to the microtiter format would be cheaper, easier to use, but less quantitative.

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