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

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

DETERMINING THE FATE OF SAXITOXIN IN CENTRAL CALIFORNIA MARINE FISHERIES

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

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

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

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

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

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SOLUBILIZATION OF A DOMOIC ACID BINDING SITE FROM PACIFIC RAZOR CLAM

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

NUMERICAL MODELING OF THE JUAN DE FUCA EDDY

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

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

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

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

***Heterosigma akashiwo* IN CENTRAL CALIFORNIA WATERS**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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