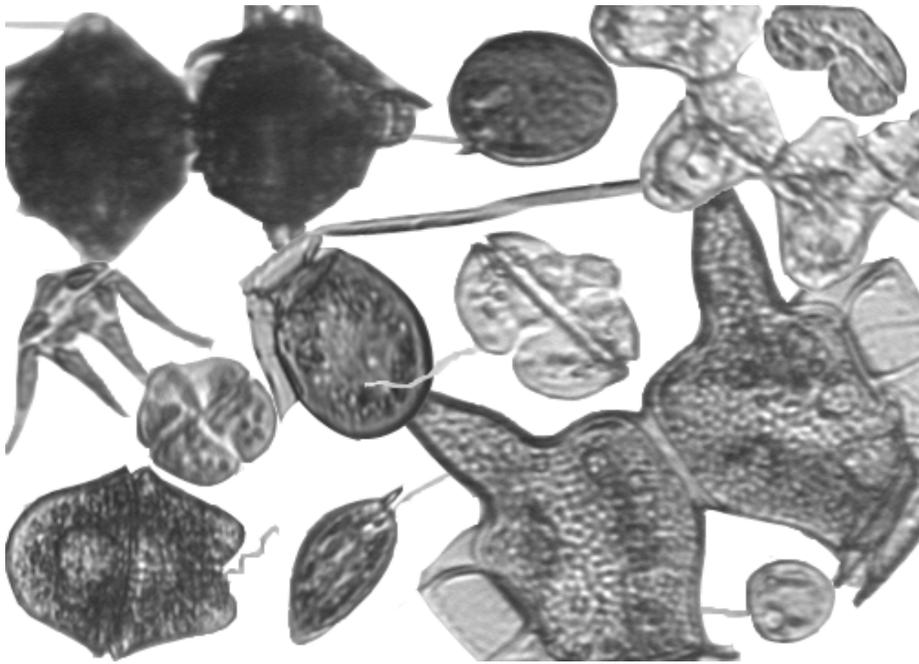


# **Sixth Symposium on Harmful Algae in the U.S.**



**November 13-18, 2011  
University of Texas, Austin TX**

**Presentation Abstracts**

## **Sixth Symposium on Harmful Algae in the U.S.**

### **Symposium Chairs:**

Lisa Campbell, Texas A&M University  
Deana Erdner, University of Texas Marine Science Institute

### **Local Organizing Committee:**

Meridith Byrd, Texas Parks and Wildlife Department  
Edward Buskey, University of Texas Marine Science Institute

### **Steering Committee:**

Sibel Bargu	Louisiana State University
Greg Boyer	SUNY College of Environmental Science and Forestry
Monica Bricelj	Rutgers University
Kathy Coyne	University of Delaware
Quay Dortch	NOAA ECOHAB Program
Greg Doucette	NOAA Marine Biotoxins Program
Dianne Greenfield	University of South Carolina, Belle Baruch Institute
Barbara Kirkpatrick	Mote Marine Laboratory
Judy Kleindinst	Woods Hole Oceanographic Institution
Kathi Lefebvre	NOAA Northwest Fisheries Science Center
Stephanie Moore	NOAA Northwest Fisheries Science Center
Steven Wilhelm	University of Tennessee

### **Session Chairs:**

Emerging Issues: HABs, and Climate Change	Clarissa Anderson
Mechanisms of Toxicity	Juliette Smith
Fisheries and Foodwebs	Hans Dam
Prevention, Control and Mitigation	Kevin Sellner
Genomics and Population Genetics	Sonya Dyhrman, Kate Hubbard
Bloom Dynamics and Ecology	Mike Brosnahan, Hugh MacIntyre, Anita McCulloch, Mike Parsons, Mindy Richlen, Mike Twiner
Communication, Outreach, and Education	Barb Kirkpatrick
Technologies for Cell and Toxin Detection	Alison Robertson
Modeling, Forecasting, and Economic Impacts	Rick Stumpf
Monitoring and Management	Cindy Heil, Raphe Kudela

**The Sixth Symposium on Harmful Algae in the U.S. would not have been possible without the generous support of our sponsors:**

NOAA Center for Sponsored Coastal Ocean Research/Coastal Ocean Program  
FDA Center for Food Safety & Applied Nutrition, Office of Food Safety, Gulf Coast  
Seafood Laboratory  
The Gordon & Betty Moore Foundation  
Texas A&M University College of Geosciences  
MBARI SURF Center  
University of Texas Marine Science Institute  
New York SeaGrant  
Belle Baruch Institute for Marine and Coastal Sciences  
Louisiana Sea Grant

CENTER FOR SPONSORED  
COASTAL OCEAN RESEARCH



**Student and Postdoctoral Travel Award Support:**

NOAA Center for Sponsored Coastal Ocean Research/Coastal Ocean Program  
FDA Center for Food Safety & Applied Nutrition, Office of Food Safety, Gulf Coast  
Seafood Laboratory  
Fluid Imaging Technologies, Inc.  
Texas Sea Grant  
Mississippi/Alabama SeaGrant

CENTER FOR SPONSORED  
COASTAL OCEAN RESEARCH



**Manager Travel Award Support:**

NOAA Center for Sponsored Coastal Ocean Research/Coastal Ocean Program

## THE ROLE OF COUPLED PHYSICAL-BIOLOGICAL MODELS FOR HAB PREDICTION IN CALIFORNIA

Anderson, C.R.<sup>1</sup>, Kudela, R.M.<sup>1</sup>, Shulman, I.<sup>2</sup>, Penta, B.<sup>3</sup>, Chao, Y.<sup>4</sup>, Siegel, D.A.<sup>5</sup>, Benitez-Nelson, C.<sup>6</sup>

<sup>1</sup>University of California Santa Cruz, CA 95064 USA [clrander@ucsc.edu](mailto:clrander@ucsc.edu), [kudela@ucsc.edu](mailto:kudela@ucsc.edu), <sup>2</sup>Naval Research Laboratory, Stennis Space Center, MS 39529 USA [igor.shulman@nrlssc.navy.mil](mailto:igor.shulman@nrlssc.navy.mil), [penta@nrlssc.navy.mil](mailto:penta@nrlssc.navy.mil), NASA Jet Propulsion Laboratory, Pasadena, CA 91109 USA [Yi.Chao@jpl.nasa.gov](mailto:Yi.Chao@jpl.nasa.gov), University of California Santa Barbara, CA 93106 USA [davey@eri.ucsc.edu](mailto:davey@eri.ucsc.edu), University of South Carolina, Columbia, SC 29201 USA [cbnelson@geol.sc.edu](mailto:cbnelson@geol.sc.edu)

The use of optical signals to detect harmful algal blooms has proven successful for a variety of species and regions but shown limited application for remotely sensing toxic blooms on the U.S. west coast where the diatom *Pseudo-nitzschia* is a common HAB-former. Statistical models that incorporate satellite data are useful for detection and tracking of blooms but show reduced skill in remote detection of toxin production. The latter generally requires knowledge of *in situ* nutrient distributions to constrain our inference of bloom physiological state. Regional ocean models accurately predict physical parameters for forcing statistical models of HAB probability and, when coupled to ecosystem models, provide simulations of chemical and biological fields that are critical for estimates of toxin production. Initial testing of previously developed statistical models parameterized with coupled physical-biological numerical model output shows promise but cannot currently replace environmental data. Regional tuning or coupled development of statistical models and numerical output may greatly aid in improving predictive skill and may be the way forward for development of short-term (weekly) forecasts and decadal analyses of forcing functions driving blooms and toxin production.

*ALEXANDRIUM* FUNDYENSE CYST DISTRIBUTIONS IN THE GULF OF MAINE:  
INTERANNUAL VARIABILITY AND LINKS TO PAST AND FUTURE BLOOM  
MAGNITUDE

Anderson, D.M.<sup>1</sup>, McGillicuddy, D.J. Jr.<sup>1</sup>, Keafer, B. A.<sup>1</sup>, He, R.<sup>2</sup>, Couture, D.<sup>3</sup>, Martin, J.L.<sup>4</sup>

<sup>1</sup>Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA danderson@whoi.edu; <sup>2</sup>North Carolina State University, Raleigh, NC 27695 USA; <sup>3</sup>Maine Department of Marine Resources, Boothbay Harbor ME 04575, USA; <sup>4</sup>Fisheries and Oceans Canada, St. Andrews Biological Station, St. Andrews, NB Canada E5B 2L9

A major goal for those conducting HAB research has been to identify the mechanisms underlying interannual variability in blooms. Here our focus is on interannual variability in *Alexandrium fundyense* blooms and the associated PSP toxicity in the Gulf of Maine - a region that is frequently impacted by blooms. Large-scale surveys of *A. fundyense* cyst abundance were conducted in the fall (late October - early November) of 1997 and 2004 - 2010. Significant interannual variability in cyst abundance is evident. 1997 had by far the lowest cyst abundance within the Gulf of Maine, nearly 15-fold lower than the highest levels, which were observed in 2009. In contrast, the Bay of Fundy cyst abundance was less variable, with a factor of approximately four between the lowest and highest levels over the eight years surveyed. A strong relationship is apparent between the total number of cysts in the top cm of sediment with the extent of the *A. fundyense* bloom the subsequent season. There is, however, no clear relationship between the size of a regional bloom (as evidenced by the extent of shellfish harvesting closures along the coast due to PSP toxins) and the abundance of cysts present in sediments later that year. Reasons for the correlation with future blooms and the lack of a correlation with past blooms will be discussed. Here we also describe a "HAB Index" that is used to characterize the severity of PSP toxicity in two regions of Maine on a yearly basis and explore linkages between that variability and the abundance of *A. fundyense* resting cysts. A relationship emerges that not only explains decadal-scale patterns of toxicity, but that also leads to a hypothesis that a regime shift recently occurred and that now the region has entered an era or interval that will have frequent, widespread, and high levels of toxicity, possibly for a decade or more into the future.

UNUSUAL POSITIVE EFFECTS OF THE PST-PRODUCING DINOFLAGELLATE  
*ALEXANDRIUM FUNDYENSE* ON THE CALANOID COPEPOD *ACARTIA*  
*HUDSONICA*

Avery, D.E., Dam, H.G.

University of Connecticut Dept. of Marine Sciences, Groton, CT 06340 USA  
davery@uconn.edu, hans.dam@uconn.edu

We report novel responses of individual grazers to a toxic alga. In laboratory experiments copepods of the species *Acartia hudsonica* responded to the toxic dinoflagellate *Alexandrium fundyense* with improved performance in some traits. In mixed-diet experiments with toxic *Alexandrium fundyense* and nontoxic food designed to control toxin dose, ingestion rate increased at low densities then decreased at higher densities of toxic *A. fundyense*. Egg production also increased significantly when toxic *A. fundyense* was added to the diet, while fecal pellet production declined. The responses were not due to food quality as generally conceived, nor were they due to food quantity. Further, some positive effects were present in both laboratory and wild populations. We hypothesize that the neurotoxins of *Alexandrium* sp. can improve copepod ingestion, assimilation, and egg production by slowing food processing in the gut. Such positive effects reveal new insights into copepod physiology. They will also influence grazing feedbacks in situ and, therefore, the fate of toxic blooms.

OCEAN, ATMOSPHERE, AND WATERSHED IMPACTS ON *ALEXANDRIUM CATENELLA* IN PUGET SOUND, WA: CLIMATE-TIMESCALE MODELING AND INTERANNUAL OBSERVATIONS

Banas, N. S.<sup>1</sup>, Moore, S.K.<sup>2</sup>, Greengrove, C.L.<sup>3</sup>, Salathé, E.P.<sup>4</sup>, Stein, J.E.<sup>2</sup>, Bill, B.D.<sup>2</sup>, Trainer, V.L.<sup>2</sup>, Anderson, D.M.<sup>5</sup>, Mantua, N.J.<sup>4</sup>, and Masura, J.E.<sup>3</sup>

<sup>1</sup>Applied Physics Laboratory, University of Washington, Seattle, WA neil@apl.washington.edu; <sup>2</sup>NOAA Northwest Fisheries Science Center, Seattle, WA; <sup>3</sup>University of Washington–Tacoma, Tacoma, WA; <sup>4</sup>Climate Impacts Group, University of Washington, Seattle, WA; <sup>5</sup>Woods Hole Oceanographic Institution, Woods Hole, MA

The PS-AHAB (Puget Sound *Alexandrium* Harmful Algal Blooms) program, part of NOAA ECOHAB, seeks to understand environmental controls on the benthic (i.e., cyst) and planktonic life stages of the toxic dinoflagellate *Alexandrium catenella*, and evaluate the effects of climate change on blooms. This includes mapping the distribution of overwintering cysts, determining environmental and endogenous controls on cyst germination, and assessing the effect of summer transport patterns on connectivity between cyst “seed beds” and shellfish-growing areas. A spatially detailed map of Puget Sound winter cyst distributions from Jan 2011, the first of three such annual mapping projects planned, found the highest cyst concentrations in Bellingham Bay, Birch Bay and Semiahmoo Bay in the north, Port Madison, Liberty Bay and Port Orchard on the west side of the Main Basin and Quartermaster Harbor in central Puget Sound. The cyst maps provide input for climate-timescale modeling. A 40-year atmospheric climate projection downscaled using WRF (Weather Research and Forecasting Model) from the CCSM3 global model was coupled to a high-resolution hydrodynamic simulation of the Salish Sea and Northwest coastal ocean (MoSSea, Modeling the Salish Sea: <http://faculty.washington.edu/pmacc/MoSSea/>). A comparison between present-day and circa-2050 conditions lets us disentangle the effects of three climate pathways on transport patterns and conditions affecting cyst germination and *A. catenella* growth. These three pathways are 1) changing ocean inputs (in the CCSM3 scenario, associated with stronger summer upwelling winds), 2) changing streamflow magnitude and timing, and 3) increased direct insolation. Running the hydrodynamic model in hindcast mode for selected years from 2005–present lets us examine the same pathways on the interannual scale.

## THE MYSTERY BEHIND ALFRED HITCHCOCK'S FILM "THE BIRDS": THE CAUSE REVEALED?

Bargu, S., Silver, M. W., Ohman, M. D., Benitez-Nelson, C., Garrison, D. L.

In August 1961, a seabird invasion of a coastal community was reported prominently in the local newspaper, the Santa Cruz Sentinel, inspiring Alfred Hitchcock to produce his thriller "The Birds" soon thereafter. The report described disoriented sooty shearwaters (*Puffinus griseus*) regurgitating anchovies, flying into objects and people. Thirty years later, a very similar event resulted in the US west coast discovery of the neurotoxic domoic acid (DA), produced by the diatom *Pseudo-nitzschia*, present in anchovies that were ingested by pelicans. In the absence of phytoplankton samples from Monterey Bay in summer of 1961, we turned to archival samples of herbivorous zooplankton collected on CalCOFI (*California Cooperative Oceanic Fisheries Investigations*) ship surveys, which provided gut "samples" (i.e. collections) of the regional flora at the time of the bird kill. Our analyses of the gut contents of zooplankton from the late summer of 1961 revealed species of *Pseudo-nitzschia* now known to be toxic were present very near the time of the shearwater deaths. Given the many similarities to the well-established domoic acid poisoning event of 1991, the encounter of southern hemisphere shearwaters in Monterey Bay to toxic prey, led to the intoxication of the birds, fancifully described in the Hitchcock's film. These results indicate the toxic diatoms have been in Monterey Bay, and hence central California, for at least 5 decades, though their toxin has only been recognized since 1991.

POTENTIAL CHANGES IN TOXIC *PSEUDO-NITZSCHIA* BLOOM DYNAMICS IN LOUISIANA COASTAL WATERS DUE TO DEEP WATER HORIZON OIL SPILL

Bargu, S.<sup>1</sup>, Smith, E.A.<sup>1</sup>, Rabalais, N. N.<sup>2</sup>

<sup>1</sup>Louisiana State University sbargu@lsu.edu, esmi122@tigers.lsu.edu; <sup>2</sup>Louisiana Universities Marine Consortium nrabalais@lumcon.edu

Phytoplankton community structure is known to shift in response to changes in environmental conditions such as temperature, nutrient availability, and lack of grazing pressure. Previous studies indicate that phytoplankton growth response also varies with respect to contaminants such as petroleum-based hydrocarbons (PH). Some studies indicate growth stimulation while others indicate growth inhibition in the presence of PH. Therefore, the phytoplankton community response in the event of an oil spill may be influenced by site/species specific conditions. An ongoing study was collecting water samples on a monthly basis to test the abundance and toxicity of *Pseudo-nitzschia* in Louisiana coastal waters, when the Deep Water Horizon oil spill occurred in April 2010. This unique opportunity has allowed us to look at the potential impacts of oil on bloom dynamics and toxicity of *Pseudo-nitzschia*, and gave the possibility to compare these results to historical records. *Pseudo-nitzschia* and its associated toxicity have been observed in moderate levels in March and early April of 2010, before the oil spill occurred. In May, after the oil spill, *Pseudo-nitzschia* numbers were higher and toxicity has increased significantly by 10 fold. Due to their dominance and abundance in spring months, any effects of oil exposure and dispersants will result in large-scale perturbation of the ecosystem structure and function.

## EFFECTS OF COPEPOD POPULATION ON PST INDUCTION IN THE DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE*

Batoh, C.D., Dam, H.G.

University of Connecticut Dept. of Marine Sciences, Groton, CT 06340 USA  
christina.senft@uconn.edu, hans.dam@uconn.edu

Grazers exhibit a wide variety of responses to toxic algae, but there may also be reciprocal effects of grazers on algal toxin production. Toxin induction may occur directly via grazing or indirectly via a chemical signal from the grazers. It has been posited, but not tested, that grazer-induced toxin production varies with co-evolutionary history between grazers and prey. Populations of the copepod *Acartia hudsonica* along the NE coast of the USA vary in historical exposure and tolerance to paralytic shellfish toxins (PSTs) produced by the dinoflagellate *Alexandrium*. In this study, we hypothesized that *A. hudsonica* from Maine (a location historically exposed to toxic *Alexandrium* blooms) induce greater toxin production by *Alexandrium fundyense* than *A. hudsonica* from New Jersey (a location unexposed to toxic *Alexandrium* blooms). To test this hypothesis, we compared PST induction in *A. fundyense* between the Maine and New Jersey *A. hudsonica* populations. Direct and indirect effects were separated by running 72-h experiments in containers with grazer cages (polycarbonate beakers fitted with a 10 $\mu$ m mesh bottom placed within holding-beakers containing *A. fundyense*). PST production was measured for cells within cages containing copepods (direct effect), for cells outside cages (indirect effect), and for cells outside cages holding starved copepods (indirect effect independent of signal from damaged cells). The control consisted of incubations without copepods. PST production of cells directly within cages was 6.75-fold (*A. hudsonica* from Maine) and 5.4-fold (*A. hudsonica* from New Jersey) greater than in controls (ANOVA,  $p < 0.0001$ ). However, direct PST induction was independent of copepod population ( $p > 0.05$ ). PST production in cells outside cages was significantly enhanced 2.5-fold relative to the control (ANOVA;  $p < 0.0001$ ) only when exposed to starved copepods from the Maine population. This phenotypically plastic response of *A. fundyense* in the presence of *A. hudsonica* from Maine may be evolutionarily advantageous in algal defense against co-evolved predators.

## ALGAL TOXINS FROM THE FLORIDA EVERGLADES AND RELEVANCE TO HEALTH AND ECOSYSTEM STRUCTURING IN THE REGION

Berry, J.P.<sup>1</sup>, Jaja, A.<sup>1</sup>, Walton, K.<sup>1</sup>, Berry, G.<sup>1</sup>, Gantar, M.<sup>2</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, Marine Science Program, Florida International University, North Miami, FL 33181 USA berryj@fiu.edu; <sup>2</sup>Department of Biological Sciences, Florida International University, Miami, FL 33199 USA gantarm@fiu.edu

The Florida Everglades, the largest wetlands in the U.S., represents a continuously flowing system from Lake Okeechobee to the Florida Bay, and as such, is a fundamental component of South Florida. This vast aquatic system is characterized, in particular, by a unique diversity of freshwater cyanobacteria and other microalgae. We have evaluated, as part of an on-going study, toxic or otherwise bioactive metabolites from Everglades microalgae, specifically in relation to those metabolites that may have impacts on human and/or environmental health, as well as possible roles in structuring microecosystems (e.g. microbial assemblages of periphyton mats) within this system. Cyanobacteria and other microalgae (e.g. chlorophytes) were isolated and cultured from representative compartments of the Florida Everglades, including water and floating periphyton mats. Extracts of algal cultures were evaluated for relevant bioactive metabolites by a broad-based bioassay screening. In particular, possible allelopathic secondary metabolites were evaluated with respect (1) antialgal, or otherwise antimicrobial metabolites, in relation to their possible role in shaping microalgal communities; and (2) toxicity to relevant invertebrates, and specifically mosquito larvae that occur sympatrically, as potential micrograzers, with algal communities. Vertebrate toxicity, as it relates to both human and environmental health, was evaluated using the zebrafish (*Danio rerio*) embryo model of vertebrate toxicity. This combined approach has led to the identification and subsequent characterization of several relevant metabolites. The results of screening efforts, as well as isolation and characterization of bioactive metabolites, will be presented, along with a discussion of the possible role of these metabolites with regards to both health and ecosystem structuring in the region.

TOWARDS PREDICTING *Alexandrium catenella* EVENTS IN PUGET SOUND:  
ASSESSING GERMINATION, GROWTH AND TOXICITY

Bill, B.D., Emenegger, J.N., Trainer, V.L., Moore, S.K.

NOAA Northwest Fisheries Science Center, Seattle, WA, 98112 USA  
brian.d.bill@noaa.gov, jennifer.emenegger@noaa.gov, vera.l.trainer@noaa.gov,  
stephanie.moore@noaa.gov

The severity of *Alexandrium catenella*-induced toxic events varies spatially and interannually within Puget Sound. *A. catenella* cells previously isolated from Quartermaster Harbor (QH) demonstrated growth rates of 0.30 and 0.28 day<sup>-1</sup>. Their germination cue could be either environmental (e.g. oxygen, light, temperature) or via another mechanism, such as an endogenous clock. Whether Puget Sound *A. catenella* cysts possess an endogenous clock that controls germination is not known. Cysts resting deep on the seafloor are exposed to less seasonal variability of environmental parameters than cysts in shallower waters, therefore their cues for germination could differ. Germination controlled by an endogenous clock will occur at specific times of the year regardless of environmental cues. In order to test whether cysts are controlled by an endogenous clock, sediment was collected from QH in February 2011, and stored in dark, 4°C, and anoxic conditions to prevent germination. These cysts were then isolated at 2-week intervals and incubated in conditions known to promote germination. Understanding the growth rates and toxicity of *A. catenella* vegetative cells as well as the mechanisms of cyst germination will inform a predictive model of *A. catenella* bloom dynamics to mitigate the effects of future harmful algal blooms. The model will benefit the Washington State Department of Health and regional shellfish growers in Puget Sound.

SAMPLING HARMFUL ALGAL BLOOMS ON THE FLY: USING AN ARRAY OF OCEAN OBSERVING TOOLS TO TRACK SUSPECTED HARMFUL ALGAL BLOOMS IN REAL-TIME.

Birch, J.<sup>1</sup>, Harvey, J.<sup>1</sup>, Ryan, J.<sup>1</sup>, Preston, C.<sup>1</sup>, Marin III, R.<sup>1</sup>, Demir, E.<sup>1</sup>, Roman, B.<sup>1</sup>, Jensen, S.<sup>1</sup>, Pargett, D.<sup>1</sup>, Doucette, G.<sup>2</sup>, Chavez, F.<sup>1</sup>, Scholin, C.<sup>1</sup>

<sup>1</sup>Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039, jbirch@mbari.org; <sup>2</sup>NOAA/National Ocean Service, Charleston, SC 29412

In October 2010 we participated in a multi-institutional, month-long experiment to identify, characterize, and predict the development and movement of phytoplankton blooms on time-scales of days to weeks using an array of fixed and mobile autonomous sensing systems ([http://www.mbari.org/news/news\\_releases/2010/canon/canon.html](http://www.mbari.org/news/news_releases/2010/canon/canon.html)). The focus of this investigation was on the northern portion of Monterey Bay, California, where highly concentrated algal blooms typically develop in autumn.

Early in the experiment a phytoplankton bloom was observed in nearshore waters of northeastern Monterey Bay. Based on remote sensing observations, the bloom was transported rapidly westward and it subsequently spread throughout much of northern Monterey Bay. As the evolution and extreme patchiness of the bloom was observed from space, the in-water assets were tasked with observing its physical, chemical and biological characteristics *in situ*. The *in situ* observing systems confirmed the presence, patchiness and trajectory of the bloom. A layer of phytoplankton that was not detected by satellite observations was also found at depth. Molecular analyses conducted *in situ* (onboard the Environmental Sample Processor) as well as on samples collected by an AUV and returned to the laboratory revealed HAB populations in the deeper water community, particularly *Pseudo-nitzschia*, with domoic acid also being present in particulate and dissolved fractions.

The October 2010 experiment heralded a number of significant technological milestones. One involved the ability to remotely direct autonomous vehicles to acquire discrete samples while drifting with targeted features, in this case a phytoplankton bloom. Such Lagrangian observations are particularly relevant to advancing understanding of the development and growth of coastal plankton. At the height of the experiment we performed a “robot ballet” involving two different classes of AUVs, *Tethys* (<http://www.mbari.org/auv/LRAUV.htm>) and *Dorado* (<http://www.mbari.org/auv>). *Tethys* autonomously mapped around a drifter that moved with the bloom and then relayed the information to *Dorado*, which also mapped the bloom and intelligently collected samples using the Gulper system (<http://www.mbari.org/auv/upperwater.htm>). A second milestone involved the first use of the Ocean Decision Support System that provided an integrated interface for coalescing data from a distributed set of sensors, which in turn informed the science and engineering participants regarding changing environmental and biological conditions, the locations of sensors, and emergent opportunities to optimize sampling strategies in real-time.

## EFFECT OF LIGHT INTENSITY AND LIGHT SHIFT ON NITRATE REDUCTASE EXPRESSION AND ENZYME ACTIVITY IN *HETEROSIGMA AKASHIWO*

Bouchard, J.N.<sup>1</sup>, Hennige, S.<sup>2</sup>, Warner, M.<sup>1</sup> & Coyne, K.J.<sup>1</sup>

<sup>1</sup>College of Earth, Ocean and Environment, University of Delaware, 700 Pilottown Rd., Lewes, Delaware 19958 USA jnb@udel.edu; <sup>2</sup>Centre for Marine Biodiversity and Biotechnology, Heriot Watt University, Riccarton Campus, Edinburgh, EH14 4AS UK.

To better understand how harmful algae can sometimes outcompete other microalgae under stressful conditions, we investigated variations in nitrate reductase (NR) enzyme activity and transcript abundance in *Heterosigma akashiwo* (CCMP 2393) after shifts in light intensity. This enzyme catalyzes the first step in reduction of nitrate to ammonium. Nitrate reduction is energetically expensive and competes with RuBisCO for reductant produced during the light reactions. Previous work by Coyne and Warner demonstrated a rapid (<15 min) increase in NR expression after shifts to high light, followed by a sharp decline in transcript abundance. Here, *H. akashiwo* was grown in continuous cultures under either low light (LL: 80  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) or high light (HL: 400  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) on a 12 h light: 12 h dark cycle and diel expression of NR was examined. Results showed that under static LL conditions, levels of NR transcripts remained fairly constant throughout the day. Under HL conditions, however, diurnal variability in NR expression was observed. For the light shift experiment, replicate cultures of *H. akashiwo* grown in LL were transferred to HL and maintained under this condition for two weeks. A reciprocal experiment was also performed with high-light grown cells being transferred and maintained in low light for two weeks. When cells were shifted from low to high light, there was a temporary decline in levels of NR transcripts 1 hour after the light shift. Levels remained low for 48 h but then recovered and remained stable until the end of the experiment. When cells were shifted from high to low light, there was a transient increase (within 1 h) in NR expression. Cells then acclimated to the lower light intensity by maintaining levels of NR transcripts which were lower than those measured under HL. These results highlight the potential of *H. akashiwo* for a fast acclimatory response to high light. They will be discussed in more detail with regard to photosynthetic parameters/NR enzyme activity.

DIFFERENTIAL EFFECT OF ELEVATED TEMPERATURE, DARKNESS AND HYDROGEN PEROXIDE ON OXIDATIVE STRESS-INDUCED CELLULAR MORTALITY OF *MICROCYSTIS AERUGINOSA*

Bouchard, J.N.<sup>1</sup>, Purdie, D.<sup>2</sup>

<sup>1</sup>College of Earth, Ocean and Environment, University of Delaware, 700 Pilottown Rd., Lewes, Delaware, 19958, USA jnb@udel.edu; <sup>2</sup>University of Southampton, National Oceanography Centre, Waterfront Campus, European Way, Southampton, United Kingdom, SO14 3ZH

This study assessed the implication of oxidative stress in the mortality of cells of *Microcystis aeruginosa* Kütz. Cultures grown at 25°C were exposed to 32°C, darkness and hydrogen peroxide (0.5 mM) for 96 h. The cellular abundance, Chl *a* concentration and content, maximum photochemical efficiency of PSII ( $F_v/F_m$  ratio), intracellular oxidative stress (determined with dihydrorhodamine 123), cell mortality (revealed by SYTOX-labelling of DNA), and activation of caspase 3-like proteins were assessed every 24 h. The presence of DNA degradation in cells of *M. aeruginosa* was also assessed using a TUNEL assay at 96 h. Transferring cultures from 25 to 32°C was generally beneficial to the cells. The cellular abundance and Chl *a* concentration increased, the mortality remained low (except for a transient burst at 72 h) as did the oxidative stress. In darkness, cells did not divide and the  $F_v/F_m$  continuously decreased with time. The slow increase in intracellular oxidative stress coincided with the activation of caspase 3-like proteins and, with a 15 and 17 % increase in mortality and TUNEL-positive cells, respectively. Exposure to hydrogen peroxide had the most detrimental effect on cells as growth ceased and the  $F_v/F_m$  declined to near zero in less than 24 h. The two-fold increase in oxidative stress matched the activation of caspase 3-like proteins and a 40 and 37 % increase in mortality and TUNEL-positive cells, respectively. These results demonstrate the implication of oxidative stress in the stress response and mortality of *M. aeruginosa*.

## GENETIC DISTRIBUTION OF *MICROCYSTIS* POPULATIONS THROUGHOUT AN AGRICULTURALLY INFLUENCED LAKE IN MARYLAND

Bowers, H.A.<sup>1</sup>, Roche, S.<sup>1</sup>, Place, A.R.<sup>1</sup>, Sellner, K.G.<sup>2</sup>

<sup>1</sup>Institute of Marine and Environmental Technology, Baltimore, MD 21202 USA  
bowers@umces.edu; sroche@umces.edu; place@umces.edu; <sup>2</sup>Chesapeake Research Consortium, Edgewater, MD 21037 USA

Blooms of *Microcystis* are common throughout the summer and into late fall in freshwater and low salinity waters of Maryland. These blooms commonly result in advisories warning residents to avoid contact with the water and to keep animals at a safe distance. Stakeholders at various levels (e.g., state and local officials, health departments, residents) are anxious to explore options for mitigation of *Microcystis* in the region. In order to do this, we need a better understanding of the species present within this genus, particularly inter- and intra-species variation associated with toxicity. While microscopy has successfully identified several species of *Microcystis* in Maryland, research to uncover variation at the genetic level has been limited. We chose to intensely sample a lake in Maryland plagued by annual *Microcystis* blooms that severely impact recreational activities. Samples were collected during the height of *Microcystis* activity as well as pre- and post-bloom periods. Because *Microcystis* species overwinter in surficial sediments we included surface sediment sampling in our study. In addition to temporal distribution, we identified several sampling sites within the lake to address spatial differences in populations. Clone libraries based on key genes were produced from representative samples and used to assess standing populations and shifts over time. Data were analyzed in relation to the presence of microcystin, other microcystin-producing species, water temperature, dissolved oxygen, rainfall, prevailing wind patterns, chlorophyll *a*, phycocyanin, pH, and phosphorus and nitrogen levels. Finally, mesocosms sampled through time for these same parameters indicate fate of the populations and toxin as the blooms are flocculated and removed from the water column, providing key background information for use of this technique in routine mitigation of these recurring blooms in regional waters.

## THE CYANOBACTERIA NEUROTOXIN BETA-METHYL AMINO ALANINE (BMAA) IS NOT A MAJOR NEW HAZARD IN THE GREAT LAKES; A COMPARISON OF LC AND LCMS/MS TECHNIQUES.

Boyer, G.L.

Department of Chemistry, SUNY-College of Environmental Science and Forestry,  
Syracuse, NY 13210

Cyanobacteria produce a wide range of natural toxins. The most recently described cyanobacterial toxin is the amino acid  $\beta$ -N-methyl amino alanine (BMAA). BMAA is a non-protein amino acid produced by a wide range of cyanobacteria (Cox et al., 2005). Originally discovered in cycad seeds from Guam, it has been associated as a possible cause of amyotrophic lateral sclerosis or Parkinsonism dementia complex. Many potential BMAA-producing genera (e.g. *Microcystis*, *Anabaena*) are common in the Great Lakes. To investigate if BMAA is an emerging threat in Great Lakes ecosystems, voucher samples from more than 1000 samples collected between 2006 and 2010 as part of the NOAA's MERHAB-LGL and OHHI cyanobacterial toxin monitoring efforts analyzed for BMAA using a highly sensitive HPLC-positive ion electrospray mass spectroscopy method. The detection limit for free BMAA contained within the cells is generally less than 0.01  $\mu\text{g/L}$  of raw lake water. These samples span a wide range of biomass, cyanobacteria species, and many contain one or more known cyanobacterial toxins. However results to date suggest that detectable levels of free BMAA contained within the cells is extremely rare, indicating that this neurotoxin is not a major emerging threat to the Great Lakes. To help understand why high levels of cyanobacteria were not correlated with high levels of BMAA – several different LC-MS/MS methods and instruments were compared using laboratory cultures. Use of an Orbitrap exact mass instrument allowed us to quantitatively separate BMAA from its structural isomers amino ethyl glycine (AEG) and diamino butyrate (DAB). Both DAB and AEG are naturally occurring amino acids with identical molecular weights to BMAA. DAB was found in significant levels in many common cyanobacteria and its presence may be responsible for some of the reports of BMAA in these species.

## AN INTERNATIONAL COLLABORATION EFFORT BETWEEN THE U.S. AND MEXICO TO IMPLEMENT A HARMFUL ALGAL BLOOM MONITORING SYSTEM

Boyes, C.N., Boyes A.J., Kirkpatrick G.

Mote Marine Laboratory, Sarasota, FL 34236 USA cory@mote.org, anamari@mote.org, gkirkpat@mote.org

Blooms of the toxic dinoflagellate, *Karenia brevis*, occur almost annually in the Gulf of Mexico, affecting coastal communities of the U.S. and Mexico.

In an effort to establish a monitoring system in Veracruz Mexico, NOAA, SAIC, Mote Marine Laboratory and the Aquarium of Veracruz established a partnership over several years with the goal to develop an integrated ocean observing system. The installation of three MX C-MAN weather stations with OPD (Optical Phytoplankton Discriminator) instruments occurred in September 2010 at Isla Sacrificios, La Mancha beach and Veracruz port. Unfortunately, hurricane Karl struck just north of Veracruz as a category 4 hurricane on September 17<sup>th</sup>. The observatory systems suffered damage that delayed data collection for a couple of months.

The observatory network relays real time data from the stations through GOES satellite transmitters to Stennis Space Center, Mississippi. The received data are re-packaged and sent back to the Aquarium of Veracruz where the results are locally monitored. The data are shared with Mote Marine Laboratory to assess the operation of the OPDs, and shared with other end users to monitor and to manage resources. These data will also be used to model and to enhance forecasting to reduce local and global economic impacts.

This project has helped to unify the Gulf of Mexico HAB community and to raise awareness of the Gulf-wide problems associated with *Karenia brevis* blooms. This collaborative, international effort was an important first step to better understand the complexity and interactions of HABs, the ecosystem and human activities across the Gulf.

## AN ART SCHOOL, A MARINE LAB, AND A TOXIC DINOFLAGELLATE: COLLABORATION FOR IMPROVED PUBLIC OUTREACH

Boyes, A.J.<sup>1,2</sup>, Hall, E.R.<sup>1,2</sup>, Nierenberg, K.<sup>1</sup>, Kirkpatrick, B.<sup>1</sup>

<sup>1</sup>Mote Marine Laboratory, Sarasota, FL 34236 USA anamari@mote.org, emily8@mote.org, knierenberg@mote.org, bkirkpat@mote.org, <sup>2</sup>Ringling College of Art and Design, Sarasota, FL 34234 USA anamari@c.ringling.edu, ehall@c.ringling.edu

Blooms of the toxic dinoflagellate, *Karenia brevis*, occur annually off the coast of Florida. Over the years, numerous outreach strategies by the science community, such as FAQ cards and website information, have been used to explain this complicated, multifactorial event of nature to the communities impacted. In spite of these efforts, feedback ranging from confusion to frustration has persisted.

In 2010, Mote Marine Laboratory partnered with the Ringling College of Art and Design to create outreach materials from an art student's point of view. Teams of students selected different areas of *K. brevis* they wanted to address, from glider technology to public health issues to adapting the messaging to elementary students. They were encouraged to use any medium of interest to them- animations, cartoons, video, etc. Students were encouraged to consult with local HAB scientists for fact checking. The projects were displayed at Mote Aquarium for the week of November 30 through December 7<sup>th</sup> for the first exhibit of the Art of Red Tide Science. Aquarium visitors provided feedback with a short survey. A panel of HAB scientists also graded the projects for effectiveness and accuracy of the information. The general response of the exhibit was very positive and lead to a second exhibit of the Art of Red Tide Science, which took place in April 2011 with a new semester of students. Examples of the winning projects will be presented as well as 'lessons learned' from the endeavor.

## PORTABLE SURFACE PLASMON RESONANCE DETECTION OF THE TOXIN-PRODUCING DINOFLAGELLATE *ALEXANDRIUM*

Bratcher, A.R., Connell, L.B.

University of Maine, School of Marine Sciences, Orono, ME 04469 USA  
amber.bratcher@umit.maine.edu, laurie.connell@umit.maine.edu

Dinoflagellates of the genus *Alexandrium* can produce dangerous amounts of paralytic shellfish toxins (PSTs) at such low cell densities that water discoloration often associated with Harmful Algal Blooms (HABs) is not always evident. Species that produce PSTs are difficult to distinguish morphologically from non-PST producing species, and current identification methods are expensive, time-consuming, and require special training. Development of a rapid, low-cost and easy-to-use device to detect and monitor *Alexandrium* in the field would be an important advancement for coastal managers, as HABs vary interannually in location, intensity, and duration.

We are developing a portable biosensing instrument based on RNA detection. This method uses peptide nucleic acid (PNA) probes and surface plasmon resonance (SPR) for detection of the *Alexandrium tamarense* species complex and *A. ostenfeldii*, two species of *Alexandrium* commonly found in the Gulf of Maine. PNAs are synthetic DNA mimics with a neutrally-charged peptide backbone that is resistant to degradation, and they have a high discrimination for mismatches, making them highly amenable to field use. SPR is a label-free, optical detection method that measures the change in refractive index after the hybridization of a target to a complimentary, target-specific probe on a surface.

Previously, we have shown that this method can reliably discriminate between synthetic oligonucleotides designed to mimic each species and negative control sequences. Using RNA extracted from cultured *Alexandrium*, we have confirmed the specificity of the PNA probes to detect only the species targeted with no cross-reactivity with other probes. To validate the performance of this custom-built instrument we have conducted comparison assays on a second, commercially available SPR instrument, the industry-standard Biacore Q. Results from these assays have confirmed the use of this method for the detection of *Alexandrium*, and we have also found our instrument to be more sensitive than the Biacore. Further, utilizing different surface chemistries for probe attachment to biosensor chips yields dramatically different detection signals, allowing us to determine the attachment chemistry that will allow for the best sensor performance over time as well as provide the lowest limit of detection for this method. These results show great promise for the use of this technology as a way to quickly and more effectively detect and monitor HABS at bloom sites.

## GENOTYPE SELECTION OF SOFTSHELL CLAMS IN RESPONSE TO NATURAL AND SIMULATED TOXIC *ALEXANDRIUM* BLOOMS

Bricelj, V.M.<sup>1</sup>, Connell, L.B.<sup>2</sup>, Hamilton, S.A.<sup>2</sup>, MacQuarrie, S.P.<sup>3</sup>

<sup>1</sup>Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ 08904 USA mbricelj@marine.rutgers.edu; <sup>2</sup>School of Marine Sciences, University of Maine, Orono, ME 04469, USA Laurie.connell@umit.maine.edu; <sup>3</sup>Institute of Marine Biosciences, National Research Council, Halifax, NS, Canada Scott.MacQuarrie@nrc-cnrc.gc.ca

Resistance to paralytic shellfish toxins (PSTs) in *Mya arenaria* is controlled by a point mutation in the pore region of the Na<sup>+</sup> channel gene. This study is the first to determine the differential responses of softshell clam juveniles of known genotype [sensitive (SS) or resistant (RR) homozygotes based on the Domain II Na<sup>+</sup> channel gene sequence], to blooms of toxic *Alexandrium*. Juveniles were generated via controlled breeding of genotyped adults (SS or RR) from two populations in Atlantic Canada that differ in their history of toxin exposure.

Marked genotypic differences in fitness, measured by motility and survival, were determined when ~ 6 mm clams were exposed in the laboratory to the highly toxic *A. tamarense* strain PR18b (~96 cells ml<sup>-1</sup>) but not when fed a volume equivalent, non-toxic diatom diet for 11 d. Survival of RR clams was comparable in control and toxic diets, whereas that of toxified SS clams was significantly reduced relative to controls. Percent motility, however, was also compromised in toxified RR clams, although to a much lesser extent than that of SS clams (36% and 99% reduction relative to controls respectively) by the end of the experiment. Motility is critical for juvenile clams to burrow and thus achieve protection from predators and non-biotic stressors in their high-energy, intertidal habitat.

Juveniles (~10 mm) of known genotype (SS and RR) were deployed in May in mesh-covered pots to allow predator protection, and recovered at the end of the red tide season, at sites in the Bay of Fundy (BoF) and Gulf of Maine differing in their PST history. Our goal was to determine whether selection for resistance would occur under ecologically relevant red tide conditions in the Northwest Atlantic. Over two years, percent recovery of clams at the end of the red tide season was greater for RR than SS clams only at sites, BoF and eastern Maine, with high PST levels, but was comparable at non-toxic sites. Shell growth rates were also significantly lower for SS relative to RR clams only at the PST-exposed sites. Selection intensity for toxin resistance in the field will thus vary depending on the severity of red tides along a latitudinal gradient on the Atlantic coast. We speculate that RR clams may exhibit a cost of resistance to PSTs at sites with low or undetectable toxin levels, thereby allowing persistence of the S allele in the region.

NEW INSIGHTS INTO MEMBERS OF THE *ALEXANDRIUM TAMARENSE* SPECIES COMPLEX THROUGH RNA-SEQ AND *DE NOVO* TRANSCRIPTOME ASSEMBLY.

Brosnahan, M.L.<sup>1</sup>, Hackett, J.D.<sup>2</sup>, Erdner, D.L.<sup>1</sup>, Anderson, D.M.<sup>3</sup>

<sup>1</sup>University of Texas Marine Science Institute, Port Aransas, TX 78373 USA  
mbrosnahan@mail.utexas.edu; <sup>2</sup>University of Arizona, Tucson, AZ 85721 USA; <sup>3</sup>Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA

Toxic dinoflagellates within the *Alexandrium tamarense* species complex cause Paralytic Shellfish Poisoning, a significant and growing human health threat worldwide. However closely related nontoxic *A. tamarense* also occur, sometimes in close geographical proximity to toxic blooms. In at least one case - the interaction of toxic Group I and nontoxic Group III - the expansion of one species into the range of another may be hindered by lethal interspecies hybridization. Such interactions have important implications for the spread of toxic species and also suggest a new method for toxic bloom remediation. In this study, genetic differences between three *A. tamarense* species are explored with the aim of discovering differences in their physiology, and also to contrast their transcriptomes to those of other eukaryotic algae. Four *A. tamarense* strains were sequenced: two from Group I, which occurs in northern sub-polar oceans and along the east and west coasts of the Americas; one from Group III, which occurs in northern Europe; and one from Group IV, another toxic species, which occurs in temperate east Asia. The approach taken was intensive short read sequencing of poly-T selected RNAs ('RNA-seq') and *de novo* assembly of mRNA contigs. The methods underlying this approach continue to undergo intensive development, and the *A. tamarense* transcriptome presents a particularly difficult challenge because of its many expanded gene families and large diversity of unique genes. Transcriptome builds from several assembly programs are compared with one another and to existing EST, MPSS, and protein mass spectra data sets. Comparisons among the *A. tamarense* transcriptomes and to other eukaryotic algae are considered in light of uncertainties in the assembly of their transcripts, and special emphasis is given to analysis of candidate genes that are shared among two or more of the *A. tamarense* transcriptomes.

## A CANDIDATE GENE APPROACH TO INVESTIGATING RESISTANCE TO HARMFUL ALGAL BLOOMS IN BOTTLENOSE DOLPHINS

Cammen, K.<sup>1</sup>, Rosel, P.<sup>2</sup>, Wells, R.<sup>3</sup>, Read, A.<sup>4</sup>

<sup>1</sup>Duke University Marine Lab, 135 Duke Marine Lab Rd, Beaufort, NC 28516 kristina.cammen@duke.edu; <sup>2</sup>NOAA Southeast Fisheries Science Center, 646 Cajundome Blvd, Lafayette, LA 70506, Patricia.Rosel@noaa.gov; <sup>3</sup>Chicago Zoological Society, c/o Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, rwells@mote.org; <sup>4</sup>Duke University Marine Lab, 135 Duke Marine Lab Rd, Beaufort, NC 28516, aread@duke.edu

Harmful algal blooms are increasing in frequency and intensity worldwide resulting in increased exposure of upper trophic level organisms, such as marine mammals. However, inter-population differences in susceptibility to harmful algal blooms are often poorly understood. For example, several large-scale mortality events of bottlenose dolphins (*Tursiops truncatus*) have been attributed to blooms of *Karenia brevis* in the Florida Panhandle, but dolphins in bays in central-west Florida appear more resistant. This difference may be due to the evolution of a genetic mechanism of resistance in dolphin populations that historically have been more frequently exposed to *K. brevis*. We describe the development of a candidate gene approach to test this hypothesis. The efficacy of this approach, which focuses on a small number of target genes, relies on previous knowledge of the toxins' effects and genetic information on the species of interest. Fortunately, previous brevetoxin exposure studies in model systems and *in vitro* have identified the toxin's binding site, described the detoxification pathway, and quantified the upregulation of certain genes. Additionally, the bottlenose dolphin genome has been sequenced, and previous studies have found differences in gene expression among dolphin populations. Using this knowledge, we selected three sets of candidate genes related to (1) brevetoxin binding, (2) brevetoxin detoxification, and (3) the immune response to brevetoxin exposure. Regions within each gene were prioritized for high variability, and PCR primers were designed using the bottlenose dolphin genome. Genetic variation will be evaluated in bottlenose dolphins that died due to brevetoxin exposure and dolphins that survived from both resistant and susceptible populations. These methods are designed to identify genetic biomarkers of susceptibility, further our understanding of brevetoxins' mechanism of action in bottlenose dolphins, and determine the potential of a marine mammal to evolutionarily adapt to a natural toxin produced by harmful algal blooms.

## THE ROLE OF PROTOZOAN GRAZERS IN HARMFUL ALGAL BLOOM DYNAMICS

Campbell, J.R., Buskey, E.J.

University of Texas Marine Science Institute, Port Aransas, TX 78373 USA  
jena23@mail.utexas.edu.com

The increasing occurrence of harmful algal blooms (HABs) has prompted studies aimed at determining biotic and abiotic factors which may promote bloom initiation, maintenance, and decline. Although many studies have tried to evaluate the effectiveness of “top-down” or “bottom-up” control of HABs, there is evidence to support both mechanisms. Protozoan grazers are likely candidates for “top-down” control of HAB species since they have the potential to increase their populations at growth rates similar to those found for many HAB species. However, it is difficult to determine if protozoan grazers are feeding on HAB species based on microscope observations alone. This study uses species- or genus-specific DNA primers to more definitively determine grazing of selected HAB species by a particular ciliate or dinoflagellate grazer. PCR amplification of genetic material, focused on a species-specific HAB gene, will more precisely determine if an individual grazer has ingested a HAB species during controlled grazing experiments; these results can then be tested in natural grazer assemblages. Long-term grazing experiments will show whether a particular grazer can be an effective “top-down” control of a HAB species. The dinoflagellates *Noctiluca scintillans* and *Protoceratium* sp. are the grazers included in this study.

A NEARLY-DECADAL PERSPECTIVE ON *PSEUDO-NITZSCHIA* BLOOMS AND DOMOIC ACID EVENTS IN SOUTHERN CALIFORNIAN WATERS.

Caron, D.A.<sup>1</sup>, Schnetzer, A.<sup>1</sup>, Seubert, E.<sup>1</sup>, Gellene, A.<sup>1</sup>, Jones, B.<sup>1</sup>, Ragan, M.<sup>1</sup>, Seegers, B.<sup>1</sup>, Sukhatme, G.<sup>2</sup>, Weisberg, S.<sup>3</sup>, Howard, M.<sup>3</sup>, Schaffner, R.<sup>3</sup>, Palmer, L.<sup>4</sup>, Evans, R.<sup>5</sup>, Kaveggia, S.<sup>6</sup>.

<sup>1</sup>Department of Biological Sciences and <sup>2</sup>Department of Computer Science, University of Southern California, Los Angeles, CA 90089-0371 USA dcaron@usc.edu; <sup>3</sup>Southern California Coastal Water Research Project, Costa Mesa, CA 92626 USA; <sup>4</sup>Marine Mammal Care Center, 3601 S. Gaffey St., San Pedro CA 90731 USA; <sup>5</sup>Pacific Marine Mammal Center, 20612 Laguna Rd., Laguna Beach, CA 92651 USA; <sup>6</sup>California Council for Wildlife Rehabilitators, P.O. Box 434, Santa Rosa, CA, 95402 USA

A conclusive link between the occurrence of toxic *Pseudo-nitzschia* blooms, domoic acid, and unusual animal mortality events along the coast of California was first demonstrated off central California during the late 1990s (with putative attribution of earlier mortality events to domoic acid poisoning as early as the 1970s in that region). Despite that knowledge, information on domoic acid outbreaks and their impact on animal populations in southern Californian waters has become available only within the last decade. The report of an unusual mortality event of marine animals in 2003, and a concomitant occurrence of *Pseudo-nitzschia* and high domoic acid concentrations, added the Southern California Bight to the list of regions affected by toxic blooms of this diatom genus. Subsequent studies conducted since that time have provided information on the frequency and severity of these events in southern Californian waters, and evidence linking these outbreaks to animal poisoning events.

HAB monitoring efforts in the region have indicated the presence of substantial abundances of *Pseudo-nitzschia* and measurable particulate domoic acid concentrations in coastal waters in the majority of years since 2003. Moreover, the highest concentrations of particulate domoic acid observed in samples has increased substantially during this period, with values in the plankton in excess of 50 µg per liter observed during 2011, more than 4-fold higher than the maximal values observed during 2003. Collaborations with animal rescue and care centers have helped establish that animals stranding during toxic blooms typically exhibit symptoms and concentrations of domoic acid in body fluids indicative of domoic acid poisoning. Collectively, these results indicate an alarming increase in both the frequency and severity of domoic acid outbreaks since 2003, posing a significant and apparently increasing threat to wildlife.

## ORIGINS OF *KARENIA BREVIS* BLOOMS ALONG THE TEXAS COAST

Chen, F., Hetland, R., Campbell, L.

Texas A & M University, College Station, TX 77843 USA feichen@tam.u.edu, hetland@tam.u.edu, lisacampbell@tam.u.edu

A suite of nested hydrodynamic models of the circulation over the Texas-Louisiana shelf was used to investigate a number of ecological problems, such as seasonal hypoxia over the Louisiana shelf and the origins of *Karenia brevis* blooms along the Texas coast. The model uses realistic river and atmospheric forcing, and is nested within a larger-scale North Atlantic model (HYCOM). A snapshot of the model surface salinity field in figure to the right, shows the distinct Mississippi and Atchafalaya plumes, and illustrates how these two fresh water sources influence the Texas coast. Multiyear animations are available online at <http://pong.tamu.edu/~zhangxq>.

Previous studies have demonstrated that convergence of flow in the surface mixed layer may lead to increases in concentration of *K. brevis* similar to expected increases based on typical growth rates. The present study sought to discover the source regions of *K. brevis* blooms and to understand environmental factors (such as downwelling wind stress) that favor or discourage bloom formation. The surface distribution of *K. brevis*, a dominant harmful algal bloom dinoflagellate along the Texas coast, was investigated using a numerical hydrodynamic model. The conceptual model for plankton distribution is that plankton remain near the surface, and changes in concentration are influenced by convergence and divergence in the surface flow field. We used hindcast numerical simulations of surface currents to quantify convergence. Surface currents are used to advect tracers representing *K. brevis* concentrations and these concentrations change in proportion to flow divergence. A series of numerical modeling tests were run and the results suggest that the buoyancy forcing from the Mississippi river plays an important role in the advection of the high concentration of *K. brevis* along the Texas-Louisiana coast. In the downwelling season, the integration of identified blooms patches shows strong impact from the buoyancy of the rivers, when compared to the model results with no river impact included. The simulated *K. brevis* concentrations were compared against field observations from 2009. Intensity and spatial extent of known blooms were estimated using in situ sampling and satellite images. Possible source regions are identified by advecting known blooms ‘backwards’ through the numerical simulation by integrating these patches backward in time and using the negative of the velocity field.

*Heterosigma akashiwo* ECOHAB ACTIVITIES IN PUGET SOUND, WA: DEFINING GROWTH AND TOXICITY LEADING TO FISH KILLS.

Cochlan, W.P.<sup>1</sup>, Trainer, V.L.<sup>2</sup>, Trick, C.G.<sup>3</sup>, Wells, M.L.<sup>4</sup>

<sup>1</sup> Romberg Tiburon Center for Environmental Studies, San Francisco State University, Tiburon, CA 94920 USA cochlan@sfsu.edu; <sup>2</sup>NOAA Northwest Fisheries Science Center, Seattle, WA 98112 USA vera.l.trainer@noaa.gov; <sup>3</sup>The University of Western Ontario, London, ON N6A 5B7 Canada trick@uwo.ca; <sup>4</sup>University of Maine, Orono, ME 04469 USA mlwells@maine.edu.

Fish farms in the Pacific Northwest are exposed to the menace of ichthyotoxic phytoflagellates. Recurring blooms of the raphidophyte, *Heterosigma akashiwo* Hada (Sournia) have caused extensive damage (\$2-6 million per episode) to wild and net-penned fish of Puget Sound, Washington, and are believed to be increasing in scope and magnitude throughout the Salish Sea and elsewhere in the world over the past two decades.

The mechanism of *H. akashiwo* toxicity is not well understood. The toxic activity of *H. akashiwo* has been attributed to the production of a combination of reactive oxygen species, neurotoxic organic molecules, excessive mucus, or hemolytic activity, however these mechanisms are not confirmed consistently in all fish-killing events or cultured strains. There is also very little definitive information of the physical and nutrient (both macro- and micro-) conditions that result in a toxic bloom.

The difficulty of conducting research with active, toxin-producing field populations of *H. akashiwo* have resulted in conflicting findings from those obtained in laboratory culture studies, thereby limiting the ability of fish farmers to respond to these episodic blooms. The overall goal of this project is to identify the primary toxic element and the specific environmental factors that stimulate fish-killing *H. akashiwo* blooms, and thereby provide managers with the fundamental tools needed to help reduce the frequency and toxic magnitude of these harmful algal events.

We provide interpretations of findings from laboratory, field and *in situ* growth experiments with regards to the project objectives: 1. identifying the element(s) of toxic activity (inorganic, organic, or synergistic) associated with blooms of *H. akashiwo* and the various cellular morphologies of this alga, and 2. determining the environmental parameters that stimulate the growth success and expression of cell toxicity in the *H. akashiwo* populations of Puget Sound.

CARBON ACQUISITION STRATEGIES OF THE TOXIC DINOFLAGELLATE, *KARLODINIUM VENEFICUM*, UNDER CLIMATE CHANGE CONDITIONS

Coyne, K. J.<sup>1</sup>, Salvitti, L.<sup>1</sup>, Tilney, C.<sup>1</sup>, Main, C.<sup>1</sup>, Warner, M.<sup>1</sup>, Kelly, D.<sup>1</sup>, Mangum, A.<sup>2</sup>, Ozbay, G.<sup>2</sup>

<sup>1</sup>University of Delaware College of Earth, Ocean, and Environment, Lewes, DE 19958 USA kcoyne@udel.edu; <sup>2</sup>Delaware State University, Dover, DE USA

Global climate change is expected to have a significant impact on phytoplankton community structure due to species-specific changes in growth and physiology. Elevated CO<sub>2</sub> and temperature may also change the balance of nutrient acquisition in mixotrophic algal species which supplement phototrophy with phagotrophy, potentially altering climate change impacts on phytoplankton communities due to interspecies interactions. Here, we investigated the growth and heterotrophic activities of the toxic dinoflagellate, *Karlodinium veneficum*, at ambient temperature (25°C) and CO<sub>2</sub> levels (375 ppm) and after acclimation to anticipated climate change conditions of elevated temperature (30°C) and/or CO<sub>2</sub> levels (750 ppm). Changes in growth, productivity, and gene expression were evaluated along with carbon and nitrogen assimilation products. Grazing activities were also investigated, using *Rhodomonas* sp. as prey. Analyses are ongoing, but our preliminary results indicate little to no effect of temperature or CO<sub>2</sub> input on the growth rate of *K. veneficum* after long-term acclimation to treatment conditions. Particulate carbon, however, increased under elevated temperature and elevated pCO<sub>2</sub>. Chlorophyll *a*, RuBisCO expression, and Chl-*a*:C ratios all increased significantly with the increase in temperature, but showed no significant changes with the increase in pCO<sub>2</sub>. The data we have so far indicate that elevated temperature alone, or in conjunction with elevated pCO<sub>2</sub> will have a positive effect on the physiological state and productivity of this species, but may not have a significant impact on its cell densities. Grazing by this species decreased with elevated temperature and/or pCO<sub>2</sub>, consistent with the apparent enhancement in physiological condition of the dinoflagellate when cultured under these conditions. Our results suggest that, under anticipated climate change conditions, *K. veneficum* will reduce dependence on grazing for carbon acquisition, potentially impacting phytoplankton community composition and dynamics.

## PNWTOX - A BIOPHYSICAL MODEL OF HARMFUL ALGAL BLOOMS IN THE PACIFIC NORTHWEST

Davis, K.A.<sup>1</sup>; Banas, N.<sup>2</sup>

<sup>1</sup>Applied Physics Laboratory – UW USA kadavis@uw.edu; <sup>2</sup>University of Washington USA neil@apl.washington.edu

As part of the effort to improve HAB forecasting in the Pacific Northwest, NOAA and NSF funded the PNWTOX (Pacific Northwest Toxins) Project, an interdisciplinary project that uses a four-box (NPZD) model of planktonic nutrient cycling coupled to a high-resolution circulation model of the Washington and Oregon coasts to advance our understanding of HAB development and transport in the region. This project focuses on the dynamics of *Pseudo-nitzschia*, which produces domoic acid, a neurotoxin that accumulates in shellfish and causes Amnesic Shellfish Poisoning in humans. Physical, chemical, and biological data from two recently completed observational studies – The Ecology and Oceanography of Harmful Algal Blooms in the Pacific Northwest (ECOHAB) and River Influences on Shelf Ecosystems (RISE) – were used in the development and validation of the PNWTOX model. Using the coupled physical-ecological model, we examine regional patterns of phytoplankton biomass and productivity and the connectivity between HAB source regions and the coast. Additionally, we will present a retrospective analysis of specific HAB events captured in the ECOHAB and RISE observations and investigate the comparative-advantage for *Pseudo-nitzschia* along particle paths, using nutrient, light, and grazer fields.

## REGULATORY IMPLICATIONS OF NEWLY DISCOVERED DIARRHETIC SHELLFISH POISONING TOXINS IN U.S. SHELLFISH.

Deeds, J.R.

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, College Park, MD 20740 USA

Diarrhetic Shellfish Poisoning toxins (DSTs) [okadaic acid (OA), dinophysistoxins (DTX), and a series of fatty acid esters of these toxins] are produced by marine dinoflagellates (*Dinophysis* and *Prorocentrum* spp.) and accumulate in commercial shellfish. Consumption of DSTs results in acute diarrhea, nausea, vomiting, and abdominal pain. The additional non-diarrhetic marine toxins yessotoxin (YTX) and pectenotoxin (PTX) can co-occur in shellfish with DSTs, complicating analysis. YTX, produced by different organisms than those that produce DST's, are regulated separately in the EU at 100 µg/100g shellfish tissue. Because they co-extract, are lethal in traditional *in-vivo* assays, and are produced by the same organisms that produce DSTs, PTX are currently included in the 16 µg OA equivalents/100 g EU action level for DSTs. FDA currently makes no recommendations for controls for either YTX or PTX for shellfish harvested in the U.S. In 2008, the DST okadaic acid was found in oysters (*Crassostrea virginica*) from the Texas Gulf coast in excess of the FDA action level of 16 µg/100g tissue, resulting in the first shellfish harvesting closures due to toxin presence in U.S. history. Every year since then, *Dinophysis* has been noted in plankton samples and low levels of DSTs have been found in oysters in Texas Gulf coast waters. PTX has not been found in either plankton samples or in shellfish from Texas, but they have been found in plankton samples from U.S. waters of the Pacific Northwest, and both Mid-Atlantic and Atlantic Northeast waters. To date, harvesting closures due to DSTs in shellfish have not occurred outside of Texas. Due to a lack of previous occurrence for these toxins in U.S. shellfish, the National Shellfish Sanitation Program currently makes no specific recommendations for monitoring or acceptable testing methods for DSTs. Around the world, *in-vivo* assays have been the preferred method for analysis, but there is currently an EU mandate to develop and validate acceptable alternatives. To date LC/MS, immunoassays, and *in-vitro* functional assays based on phosphatase inhibition are all in various stages of validation. It should be noted that many of these methods will not detect PTX, which would lead to inconsistent results if compared to *in-vivo* data under the current EU guidelines. Furthermore, for most methods, appropriate extraction and base hydrolysis of samples will be required to detect the fatty acid ester derivatives of DSTs found in high concentrations in certain shellfish species, including oysters. New FDA guidance levels for DSTs are for total toxins (free plus esterified). At FDA, efforts are underway to assess available methodologies for DST analysis and provide appropriate guidance on this issue to our state regulatory partners for shellfish management.

NOVEL MAITOTOXINS FROM *Gambierdiscus belizeanus*.

Deeds, J.R.<sup>1</sup>, Handy, S.M.<sup>1</sup>, White, K.D.<sup>1</sup>, Dickey, R.W.<sup>2</sup>.

<sup>1</sup>US FDA, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, College Park, MD 20740 USA; <sup>2</sup>US FDA, Center for Food Safety and Applied Nutrition, Office of Food Safety, Dauphin Island, AL 36528 USA.

For many years, it was believed that *Gambierdiscus toxicus* was the sole source of maitotoxins (MTX), the most potent non-protein toxin yet discovered, and ciguatoxins, the primary causative agents for ciguatera fish poisoning. Even though several families of ciguatoxins have now been identified, including “Caribbean” (C-CTX), “Pacific” (P-CTX), and “Indian Ocean” (I-CTX), for many years only a single maitotoxin (MTX) was described in the literature. Compounds termed MTX-2 and MTX-3 were described from Australian *Gambierdiscus* isolates (Lewis et al. 1994), but no structural data has been provided for these compounds to determine their relationship to MTX. It has long been one of the great unanswered questions in *Gambierdiscus* /ciguatera research as to why only certain isolates of *Gambierdiscus* produce toxins and why there is such great regional variation in specific ciguatoxin composition in fish from different geographic regions. Recently, it has been shown that at least ten species of *Gambierdiscus* exist and that much of the previous work using isolates of “*Gambierdiscus toxicus*” was actually done using multiple *Gambierdiscus* spp. (Litaker et al. 2010). Research into which toxins are produced, if any, by the various *Gambierdiscus* spp. is just beginning, and there are hopes that this research will provide information that can be used to better predict the presence of toxins in seafood and prevent seafood poisonings. Along those lines, we analyzed two isolates of *Gambierdiscus* purchased from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) culture collection that had, until recently, been identified as “*Gambierdiscus toxicus*”. It is now known that these isolates are, or are closely related to, *Gambierdiscus belezianus*. No formal studies have been performed on the toxicity of this species, but previous work has been done using these isolates as “*G. toxicus*”. We show here two novel maitotoxins, a desulfo-MTX as the primary toxin from isolate CCMP 1655 and a desulfo-hydroxy-MTX as the primary toxin from isolate CCMP 399. We further show that these isolates are genetically consistent with *Gambierdiscus belezianus* (CCMP 399) and *Gambierdiscus* “ribotype-2” (CCMP 1655), as described in Litaker et al. (2010). Both isolates originated in the Caribbean but it remains to be seen whether these maitotoxins can be considered “Caribbean MTXs” as analogous to Caribbean-CTXs and Pacific-CTXs.

Litaker RW, Vandersea MW, Faust MA, Kibler SR, Nau AW, et al. 2010. *Toxicon* 56: 711-730.

Lewis RJ, Holmes MJ, Alewood PF, Jones A. 1994. *Natural Toxins* 2:56-63.

## INTEGRATED MANAGEMENT OF THE OCEAN: HAB DETECTION AND PREDICTION AT THE NAPLES, FLORIDA NWLON STATION, 2007-2009

Derner, K.<sup>1</sup>, Kirkpatrick, G.<sup>2</sup>, Boyes, C.<sup>2, 3</sup>, and Stumpf, R.<sup>3</sup>

<sup>1</sup>NOAA/NOS Center for Operational Oceanographic Products and Services, 672 Independence Parkway, Chesapeake, VA 23320 USA, katie.derner@noaa.gov; <sup>2</sup>Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236 USA, gkirkpat@mote.org, cory@mote.org; <sup>3</sup>NOAA/NOS National Centers for Coastal Ocean Science (NCCOS), Silver Spring, MD 20910 USA, richard.stumpf@noaa.gov

Sensor technologies for harmful algae detection have been developed and employed throughout the Gulf of Mexico and other regions to aid in harmful algal bloom (HAB) identification, bloom forecasting, and early mitigation of potential public health, economic, and ecological impacts. To mitigate the impact of the toxic harmful algae *Karenia brevis*, in 2005 NOAA's National Ocean Service (NOS) formed the Global Leadership in Integrated Management of the Ocean (GLIMO) Partnership entitled "Red Tide Prediction to Benefit Public Health and Coastal Economies". Among the numerous project objectives were the integration of new technologies with existing observing systems and a cost-benefit analysis of biological sensor enhancements to NOS water level and coastal meteorological stations. To support these objectives, the NOS Center for Operational Oceanographic Products and Services (CO-OPS), in partnership with several other NOAA offices, Mote Marine Laboratory, Naval Research Laboratory, and the University of South Florida, installed the BreveBuster, an optical phytoplankton discriminator developed by Mote Marine Laboratory, at the Naples, FL water level station. The project required development of software to interface the BreveBuster with the water level station data collection platform, installation of the sensor at the Naples Pier, and transmission of the data via Geostationary Operational Environmental Satellite (GOES). Following sensor installation, completed in late February 2007, BreveBuster data was assimilated into the existing NOAA HAB Operational Forecast System. The two-year project successfully demonstrated the integration of the BreveBuster sensor at the Naples, FL water level station, illuminated benefits and challenges of sensor technologies for HAB detection, and resulted in recommendations for future implementation. In addition, the project demonstrated the vital benefits of an integrated ocean observing system and showed that improvements to coastal monitoring and forecasting can be obtained by combining current and new technologies from a variety of agencies to achieve a common goal.

## A RECENT HISTORY OF THE GULF OF MEXICO AND REGIONAL HABs

Dickey, R.W.

FDA Gulf Coast Seafood Laboratory, Dauphin Island, Alabama,  
robert.dickey@fda.hhs.gov

The Gulf of Mexico (GOM) region is vital sector of the U.S. economy, comprising about 20% of the U.S. population and national gross domestic product. The largest industries in the region are oil, tourism, fishing and shipping. Shipping ports number among the largest in the nation in traffic and tonnage, petroleum extraction and refining account for more than 30% of domestic production, and the Gulf fisheries industry produces 16% of domestic landings. The coastal and open waters of the Gulf define cultures and economies, and provide habitat to more than 15,000 estuarine and marine species inextricably linked to regional and national well-being. Perhaps more than any other U.S. coastal ocean, human activity and natural events regularly challenge the GOM region. Nutrient loading from Mississippi River Basin, population growth, artificial reef and aquaculture development, offshore construction for energy extraction, accidental oil spills, and hurricanes are among the many stressors that test the resiliency and diversity of regional culture, enterprise, and the estuarine and marine ecosystems of the Gulf. HABs are a historical feature of the GOM, and a growing body of evidence suggests that they are increasing in frequency, intensity, and diversity. *Karenia brevis* HABs have dominated public and environmental health concerns in the GOM for as long as anyone can remember, and may be shifting in distribution. Well known HAB species with lower scientific and public profiles, e.g. *Aureoumbra lagunensis*, *Prymnesium parvum*, *Prorocentrum minimum*, and *Alexandrium monilatum*, regularly and negatively impact coastal economies and inshore ecosystems. And, in recent years, other species known to be toxic but sparsely populated in the GOM have emerged from the background to present new hazards. Species of *Gambierdiscus*, progenitors of ciguatoxins, have expanded their range from the tropics into the northern Gulf. *Dinophysis* spp., progenitors of diarrhetic shellfish toxins, have appeared in blooms along Texas shores, and *Pseudo-nitzschia* spp., progenitors of amnesic shellfish toxins, may be on the rise in central Gulf coastal waters. This presentation will provide an overview of GOM HABs and emerging HAB species, and environmental factors that may influence their occurrence.

## THE ROLE OF BENTHIC NUTRIENT FLUX IN SUPPORT OF *KARENIA BREVIS* BLOOMS OFF OF WEST CENTRAL FLORIDA, USA.

Dixon, L.K.<sup>1</sup>, Murphy, P.<sup>2</sup>

<sup>1</sup> Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236 USA  
lkdixon@mote.org; <sup>2</sup> HydrO2, Inc., 285 Morton Farm Lane, Athens, Georgia 30605 USA  
hydro2pm@bellsouth.net.

The project "EcoHAB: *Karenia* Nutrient Dynamics in the Eastern Gulf of Mexico" (NOAA EcoHAB grant NA06NOS4780246) was designed to quantify and constrain a series of novel nutrient sources which can support the nearly annual blooms of *Karenia brevis* in the coastal waters of west central Florida. Estuarine outflows and water column stocks of nutrients have been previously demonstrated to be insufficient to support the observed cell densities which *K. brevis* can achieve. Nutrients released from sediments, however, are a component nutrient source common to offshore, coastal, and estuarine environments. Prior to this project, regional measurements were generally lacking, particularly in coastal waters. Nutrient fluxes from the sediments were directly measured using large (65 L, 0.27 m<sup>2</sup>), *in situ*, opaque, stirred chambers deployed over 6-9 hours. Stations were located in coastal waters, a lagoonal system, and in the lower portions of two major estuaries, Tampa Bay and Charlotte Harbor. Each was sampled during the fall of 2007, 2008, and 2009. Field procedures included triplicate chambers at each station and a water column control. Fluxes were quantified for dissolved oxygen, ammonia, nitrate-nitrite, urea, and total dissolved nitrogen, ortho- and total phosphorus, and silica. Fluxes were dominated by silicates (1,000-14,000  $\mu\text{M m}^{-2} \text{d}^{-1}$ ), ammonia (600-1600  $\mu\text{M m}^{-2} \text{d}^{-1}$ ) and ortho-phosphorus (100-250  $\mu\text{M m}^{-2} \text{d}^{-1}$ ), were minimal for remaining parameters, and were relatively consistent between years. Fluxes differed greatly from literature values which had been previously incorporated in ecological modeling efforts. Based on uptake rates and growth rates determined by others, and on cell complement data, sediment flux of nutrients can provide up to 300% of P requirements, and up to 100% of N requirements of 50,000 cells L<sup>-1</sup> of *K. brevis*, depending on water depth and cell density. Density stratifications common to upwelling coastal plumes would restrict mixing and increase the percentage of supply afforded by sediment flux. Relative ammonia and ortho-phosphorus flux were substantially less than Redfield ratios of 16:1, were consistent with the phosphorus-enriched geology of the region, and would be highly complementary of N supplied by common nitrogen fixers such as *Trichodesmium sp.* There is some evidence of an onshore-offshore gradient in nutrient flux that would be important to quantify for ecological modeling and prediction applications.

DETERMINING FACTORS THAT INFLUENCE MOLECULAR QUANTIFICATION OF THE HARMFUL RAPHDOPHYTE *HETEROSIGMA AKASHIWO* USING A SANDWICH HYBRIDIZATION ASSAY (SHA)

Doll, C.<sup>1</sup>, Greenfield, D.<sup>2</sup>

<sup>1</sup>College of Charleston, Charleston, SC 29412 USA ragnarok21386@hotmail.com; <sup>2</sup>Belle Baruch Institute for Marine & Coastal Sciences, University of South Carolina, Charleston, SC 29412 USA, dgreenfield@belle.baruch.sc.edu

Sandwich hybridization assay (SHA) is a molecular technique that directly detects target organisms using large subunit rRNA-targeted DNA probes. SHA has been adapted to detect and quantify bacteria, invertebrate larva and several HAB species. The resultant optical density represents the amount of rRNA in the sample, and in the case of HABs, this value can be used to approximate cell density. Cellular rRNA content may be influenced by a variety of factors, but the extent to which this may influence SHA results and/or be relevant for HAB research and monitoring purposes remains unclear. This study examines various factors that may affect cellular rRNA content as measured by SHA. Here we present preliminary data determining whether geographically distinct populations exhibit different SHA responses; to what extent sample preservation in Lugol's iodine affects quantification by SHA; and how SHA results change over growth and diurnal cycles. Ongoing and future work also addresses SHA results related to nutrient levels as well as various aspects of field sampling. The raphidophyte, *Heterosigma akashiwo*, was chosen as the test species because it occurs globally, produces ichthyotoxic blooms and SHA capabilities have been validated for it. Additionally, results will be compared to quantitative PCR (qPCR) results for the exact same homogenate as part of a broader four-year NOAA-MERHAB award. Both SHA and qPCR been validated for *H. akashiwo* quantification, but this project entails a rigorous methods comparison of SHA and qPCR on criteria such as cost, accuracy, range of detection, speed, etc. for HAB research and monitoring.

## MODELING *PSEUDO-NITZSCHIA* ADVECTION INTO THE INTERTIDAL IN LOFALL, HOOD CANAL, WASHINGTON

Dusek-Jennings, E.<sup>1</sup>, Stevens, A.W.<sup>2</sup>, & Simenstad, C.A.<sup>1</sup>

<sup>1</sup>University of Washington School of Aquatic & Fishery Sciences, Seattle, WA 98105 USA itseva@gmail.com; <sup>2</sup>U.S. Geological Survey, Menlo Park, CA 94025 USA

Although several models of water circulation exist for Puget Sound, Washington, these models lack the spatial resolution to predict phytoplankton advection into intertidal areas. The ability to model phytoplankton flux into the intertidal may prove especially valuable for investigating availability of toxin-producing phytoplankton to intertidal clams living at various tidal heights. In order to determine the concentrations of toxin-producing *Pseudo-nitzschia* that might become available to nearshore suspension-feeders over a tidal cycle, we combined several types of water column profile data for one location within Puget Sound: Lofall, Hood Canal, with a 3-dimensional hydrodynamic model of the region. Water column profile data were collected from six sampling stations along a transect oriented from offshore to inshore. Data were collected on two dates: August 8, 2005 and December 13-14, 2005, and include: vertical profiles of conductivity, temperature, depth and fluorescence; water samples collected from the surface and from the depth of chlorophyll maximum, and analyzed for total suspended solids, chlorophyll pigments, and microscope examination of fixed phytoplankton cells; and measurements through the water column of particle velocity and direction from an acoustic Doppler current profiler (ADCP) that was placed at one of the stations. A hydrodynamic model, Delft3D, was used to track movement of water masses and phytoplankton that they contain in order to understand spatial variability beyond the measurement sites. The model is forced with tides and includes freshwater discharge at several locations within Hood Canal. Bathymetry data for the Lofall region were collected using high definition LIDAR (light detection and ranging), and were used to accurately represent nearshore morphology for the Lofall region in the model. Model predictions of currents were validated with time-series measurements collected at the ADCP station. Through this modeling exercise, we have been able to predict the availability of phytoplankton to clams at each point in the intertidal as the tide rises and falls in Lofall, Hood Canal.

## LOW-COST COLORIMETER DEVELOPMENT FOR FIELD-BASED DETECTION OF TOXIC *ALEXANDRIUM* SPECIES

Duy, J.<sup>1</sup>, Smith, R.L.<sup>1,2</sup>, Collins, S.D.<sup>1,3</sup>, Connell, L.B.<sup>1,4</sup>

<sup>1</sup>Graduate School of Biomedical Sciences, <sup>2</sup>Electrical and Computer Engineering Department, <sup>3</sup>Department of Chemistry, <sup>4</sup>School of Marine Sciences  
University of Maine, Orono, ME 04469 USA janice.duy@umit.maine.edu

The *Alexandrium fundyense/tamarense/catanella* species complex are harmful algal bloom (HAB)-causing dinoflagellates which produce a suite of toxins responsible for paralytic shellfish poisoning (PSP) incidents. Spatial and temporal monitoring of the abundance of *Alexandrium* cells in the water is important, since even low cell densities (which may not discolor the water) can be toxic. However, current detection methods rely on microscopic cell counting, which is time-consuming and requires special training due to the almost-indistinguishable morphological similarities between toxic and non-toxic *Alexandrium* species. The use of molecular probes targeted to these organisms permits specific detection of toxic *Alexandrium* species, but detection generally involves fluorescence or polymerase chain reaction (PCR), both of which require expensive reagents and equipment.

To address this problem, we have developed a rapid and specific colorimetric assay using peptide nucleic acid (PNA) probes and the symmetric cyanine dye 3,3'-diethylthiadicarbocyanine iodide (DiSC<sub>2</sub>(5)). Short (16-base) PNA probes are used to capture target *Alexandrium* sequences. The addition of DiSC<sub>2</sub>(5) to a solution with PNA-containing hybrids causes the dye to change color from blue to purple, due to the formation of higher-order dye aggregates in the minor grooves of the duplexes. This change can be quantitatively monitored as an increase in the solution absorbance at 540 nm (from the dye aggregates) at the expense of the dye monomer peak at 650 nm, and the "hybridization signal" is calculated as the ratio  $A_{540}/A_{650}$ . The assay has been tested with synthetic DNA and RNA oligonucleotide sequences, and the addition of a short micrococcal nuclease (MNase) digestion step yields single-base mismatch resolution at room temperature (25°C) within 10 minutes, with an unoptimized detection limit of 100 nM (0.4 fmole).

A field-compatible two-wavelength colorimeter designed to quantify *Alexandrium* cell numbers using this assay is currently in development. The hybridization signal is calculated as the ratio of the absorbance values from the dye aggregate (green) and dye monomer (red) wavelengths ( $A_{\text{green}}/A_{\text{red}}$ ), which approximates the value obtained from the total absorbance spectrum ( $A_{540}/A_{650}$ ). Preliminary testing of the dye assay using this setup has yielded hybridization signals comparable to those obtained using a benchtop spectrophotometer. Instrument testing with ribosomal RNA (rRNA) extracted from cultured *Alexandrium* cells and spiked seawater samples is in progress. This integration of a simple and specific bioassay with a portable instrument capable holds promise as a tool for monitoring phytoplankton distributions in real time.

## TRANSCRIPTOME PROFILING IDENTIFIES NUTRIENT-RELATED RESPONSES IN BROWN-TIDE CAUSING *AUREOCOCCUS ANOPHAGEFFRENS*.

Dyhrman, S.T.<sup>1</sup>, Wurch, L.L.<sup>1</sup>, Gobler, C.J.<sup>2</sup>

<sup>1</sup>Biology Department, Woods Hole Oceanographic Institution, Woods Hole MA 02543 USA [sdyrman@whoi.edu](mailto:sdyrman@whoi.edu); <sup>2</sup> Stony Brook University, School of Marine and Atmospheric Sciences, Southampton, NY 11968 USA

Harmful Algal Blooms (HABs) are a global problem with widespread effects on public health, the coastal environment, and the economy. *Aureococcus anophagefferens* has emerged as an important model for studying HABs as it can reach very high densities, produces toxic (but poorly characterized) metabolites, and is well adapted for proliferation into new areas. Recent analysis of the *A. anophagefferens* strain CCMP 1984 genome suggests it is uniquely adapted to the estuaries where it occurs. For example, *A. anophagefferens* outcompetes other phytoplankton in estuaries with elevated organic matter and the genome of *A. anophagefferens* contains a large number of genes that may permit the degradation of organic nitrogen (N) and phosphorus (P) containing compounds.

To initially screen for the expression of genes associated with N and P metabolism, including the processing of organic matter, Long-SAGE (Serial Analysis of Gene Expression) was used to identify how the transcriptome of *A. anophagefferens* strain CCMP 1984 responds to N and P deficient and replete conditions. The short sequence reads (tags) were mapped to the genome of *A. anophagefferens*, to identify putative annotations. Transcripts significantly more abundant in the P deficient library included a phosphate transporter, 5' nucleotidase, alkaline phosphatase – genes involved in the metabolism of organic P. In the N deficient library an ammonium transporter and a xanthine/uracil/vitamin C permease were significantly more abundant than in the control condition. Detailed studies using quantitative, reverse transcriptase PCR have confirmed that the phosphate and the xanthine/uracil/vitamin C permease are regulated by P and N supply respectively.

We are in the process of additional, higher resolution, transcriptome profiling with *A. anophagefferens* strain CCMP 1850. The patterns identified from these transcriptome data will be compared to strain CCMP 1984 to examine responses within the species. These data will also be used to interpret metatranscriptome sequence from a 2010 brown tide in Quantuck Bay Long Island. Together, the combined culture and field efforts will provide a comprehensive view of metabolic potential in *A. anophagefferens*, and likely drivers of bloom formation and decline in this and perhaps other systems.

## POTENTIAL ROLE OF BREVETOXIN IN OSMOREGULATION BY *KARENIA BREVIS*

Errera, R.M., Campbell, L.

Department of Oceanography, Texas A&M University, 3146 TAMU, College Station, TX 77843 USA [RMErrera@tamu.edu](mailto:RMErrera@tamu.edu), [lcampbell@ocean.tamu.edu](mailto:lcampbell@ocean.tamu.edu)

The major harmful alga in the Gulf of Mexico, *Karenia brevis*, produces potent neurotoxins known as brevetoxins. Although a number of environmental conditions have been identified to produce small changes in brevetoxin concentrations, the functional role for brevetoxin has remained unknown. When *K. brevis* was exposed to hypo-osmotic stress (a rapid shift from a salinity of 35 to 27), three out of four clones of *K. brevis* (Wilson, SP3 and TXB4) were able to rapidly respond to the osmotic stress by increasing brevetoxin cell quota greater than 14-fold. During hyper-osmotic stress (a rapid shift from a salinity of 27 to 35) brevetoxin cell quotas also increased greater than 6-fold in the same three clones. Examination of live cell images to determine changes in cell volume revealed that *K. brevis* Wilson clone was able to quickly respond to hypo-osmotic stress by keeping cell volume stable and increasing brevetoxin cell quota from 10 to 160 pg brevetoxin cell<sup>-1</sup>. During hyper-osmotic stress, *K. brevis* Wilson clone was also able to quickly increase brevetoxin cell quota to 61 pg brevetoxin cell<sup>-1</sup>, and regain cell size within 24 hours after the stress was applied. However, *K. brevis* SP1, a clone that has a consistently low brevetoxin cell quota (< 1 pg cell<sup>-1</sup>), increased its cell volume during hypo-osmotic stress and decreased in cell volume during hyper-osmotic stress while brevetoxin cell quota remained low under both treatments. Our results demonstrate that brevetoxin production in *K. brevis* was triggered during osmotic stress. Furthermore, our results are the first to suggest that brevetoxins play a functional role in osmoregulation through regulation of ion concentrations during osmotic shifts.

MICROZOOPLANKTON GRAZING DURING BLOOMS OF THE TOXIC  
DINOFLAGELLATE *ALEXANDRIUM* SP. IN LONG ISLAND SOUND

Finiguerra, M.B.<sup>1</sup>, Batoh, C.S.<sup>1</sup>, Skelton-Flores, H.K.<sup>2</sup>, Avery, D.E.<sup>1</sup>, Courneyer, B.<sup>1</sup>,  
Dam, H.G.<sup>1</sup>

<sup>1</sup>University of Connecticut Department of Marine Sciences, Groton, CT 06340 USA  
michael.finiguerra@uconn.edu, christina.senft@huskymail.uconn.edu,  
david.e.avery@uconn.edu, benjamin.cournoyer@huskymail.uconn.edu,  
hans.dam@uconn.edu. <sup>2</sup>Algenol Biofuels, Ft. Meyers, FL 33912 USA  
hayley.flores@algenolbiofuels.com

Microzooplankton are recognized as dominant grazers of primary production in oceanic waters. However, there are few studies documenting microzooplankton grazing during toxic algal blooms, on both chl *a* and, importantly, the toxic cells themselves. The goal of this study was to measure microzooplankton grazing impact during blooms of toxic *Alexandrium* in Northport Harbor, Long Island Sound, USA. We conducted dilution experiments before, during and after the 2010 and 2011 toxic *Alexandrium* blooms. In conjunction with standard dilution experiment measurements (e.g. chl. *a*, microplankton biomass), we used a molecular probe and fluorescent *in situ* hybridization (FISH) to label *Alexandrium spp.* cells. Therefore, we were able to measure grazing impact separately on total phytoplankton (chl. *a*) and on *Alexandrium*. During 2010, there was no impact of microzooplankton on total phytoplankton prior to the bloom. During the bloom, microzooplankton had a strong direct effect on total phytoplankton, but consistently no effect on *Alexandrium sp.* After bloom termination, microzooplankton continued to have a strong effect on total phytoplankton. Data for the 2011 field season are still being collected and analyzed, and will be discussed at the meeting. The current results, however, demonstrate that even while microzooplankton may have a strong direct effect on total phytoplankton, they do not appear to impact *Alexandrium* cells. Microzooplankton grazing relaxation may be an important factor in bloom initiation in this system.

SAXITOXIN EXPOSURE IN ENDANGERED FISH STOCKS: ASSOCIATION OF A SHORTNOSE STURGEON (*ACIPENSER BREVIROSTRUM*) MORTALITY EVENT WITH A HARMFUL ALGAL BLOOM IN MAINE

Fire, S.E.<sup>1</sup>, Pruden, J.<sup>2</sup>, Couture, D.<sup>3</sup>, Wang, Z.<sup>1</sup>, Bottein, M-Y.<sup>1</sup>, Haynes, B.L.<sup>1</sup>, Knott, T.<sup>1</sup>, Bouchard, D.<sup>4</sup>, Lichtenwalner, A.<sup>4</sup>, Wippelhauser, G.<sup>5</sup>

<sup>1</sup>NOAA Marine Biotoxins Program, Charleston, SC 29412 USA [spencer.fire@noaa.gov](mailto:spencer.fire@noaa.gov), [zhihong.wang@noaa.gov](mailto:zhihong.wang@noaa.gov), [marieyasmine.bottein@noaa.gov](mailto:marieyasmine.bottein@noaa.gov), [bennie.haynes@noaa.gov](mailto:bennie.haynes@noaa.gov), [trey.knott@noaa.gov](mailto:trey.knott@noaa.gov), <sup>2</sup>Protected Resources Division, Northeast Region Shortnose Sturgeon Recovery Program, NOAA National Marine Fisheries Service, Gloucester, MA 01930 USA [darcie.couture@maine.gov](mailto:darcie.couture@maine.gov), <sup>3</sup>Marine Biotoxin Monitoring Program, Maine Dept. of Marine Resources, West Boothbay Harbor, ME 04575 USA <sup>4</sup>University of Maine Animal Health Laboratory, Animal and Veterinary Sciences, Orono, ME 04469 USA [dbouchard@umext.maine.edu](mailto:dbouchard@umext.maine.edu), [alicht@umext.maine.edu](mailto:alicht@umext.maine.edu), <sup>5</sup>Maine Department of Marine Resources, Augusta, ME 04333 USA [gail.wippelhauser@maine.gov](mailto:gail.wippelhauser@maine.gov)

Saxitoxin-producing blooms of the toxic dinoflagellate *Alexandrium* sp. have been responsible for devastating ecosystem-wide impacts in coastal waters of the northeastern U.S. In summer of 2009, a severe bloom of *Alexandrium* in New England coastal waters co-occurred with a mortality event of shortnose sturgeon (*Acipenser brevirostrum*) in Sagadahoc Bay, Maine. Thirteen individuals of this endangered fish species were found dead on 10 July 2009, and these mortalities were associated with extremely high *Alexandrium* cell densities, record-breaking toxin burdens in shellfish, and closures of shellfish beds affecting nearly the entire Maine coastline. Saxitoxin-like activity was detected in sturgeon (n=3) stomach contents, liver and gill tissues via the Neuro-2A cytotoxicity assay (N2A) and receptor-binding assay (RBA) at concentrations ranging between 37 and 2300 ng STX-eq/g. Stomach content analyses of the 3 sturgeon carcasses necropsied detected a large number of clams (*Gemma gemma*). Liquid chromatography-mass spectrometry (STX-LC/MS) confirmed the presence of STX and GTX-3 in sturgeon stomach contents, at concentrations between 349 and 743 ng STX/g. This study marks the first reported detection of saxitoxins in shortnose sturgeon, and provides evidence of trophic transfer of *Alexandrium* toxins as a potential cause of mortality in this event, and a potential threat to the health of this endangered population of fish.

## OPERATIONAL HAB FORECASTING: PROGRESS, CHALLENGES, AND PROSPECTS

Fisher, K.M., Stumpf, R., Tomlinson, M.C.

NOAA, National Ocean Service, Silver Spring, MD 20910 USA  
Kathleen.Fisher@noaa.gov, Richard.Stumpf@noaa.gov, Michelle.Tomlinson@noaa.gov

A successful operational forecast system for harmful algal blooms (HABs) relies upon skilled science, the development of mathematical models and algorithms, forecast products built upon user requirements, reliable observing systems, and forecast validation for continual improvement. NOAA's HAB Operational Forecast System (HAB-OFS) for the Gulf of Mexico has been an example of this success beginning with western Florida in 2004. In the fall of 2010, HAB forecasts for the western Gulf of Mexico were transitioned to operations, allowing for full operational forecasting coverage of HABs in the Gulf of Mexico and initiating a five year plan to expand operational HAB forecasting nationally. The HAB-OFS has required extensive collaboration within NOAA, with federal, state and local monitoring and public health agencies, and with local scientists. This success has not been met without challenges. In particular, the need for a structured end-to-end research to operations strategy is a difficult, but essential, challenge that needs formalization. Additionally, a national HAB Forecast System requires a robust technological infrastructure capable of addressing the regional ecological and environmental disparities that exist throughout the coastal US and allows for forecasting variations that support specific user needs. NOAA's National Ocean Service (NOS) is focused on developing this technological capability over the next several years.

## CHRONIC HEALTH EFFECTS OF AEROSOLIZED RED TIDE TOXINS (BREVETOXINS) AND ASTHMA

Fleming, L.E.<sup>1,2,3</sup>, Kirkpatrick, B.<sup>3,4</sup>, Bean, J.<sup>5</sup>, Nierenberg, K.<sup>4</sup>, Backer, L.C.<sup>6</sup>, Reich, A.<sup>7</sup>, Hollenbeck, J.<sup>2</sup>, Cheng, Y.S.<sup>8</sup>, Pierce, R.<sup>4</sup>, Naar, J.<sup>10</sup>, Wanner, A.<sup>9</sup>, Abraham, W.M.<sup>11</sup>, Benson, J.<sup>8</sup>, Baden, D.<sup>9</sup>.

<sup>1</sup>NSF NIEHS Oceans and Human Health Center, University of Miami, 4600 Rickenbacker Causeway, Miami, FL, 33149, <sup>2</sup>European Centre for Environment and Human Health, Peninsula College of Medicine and Dentistry, Truro, Cornwall, UK, <sup>3</sup>Dept of Epidemiology and Public Health, Miller School of Medicine, University of Miami, Miami, FL, 33136, <sup>4</sup>Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL, 34236, <sup>5</sup>Children's Hospital Medical Center, Cincinnati, Ohio, 45229, <sup>6</sup>National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway NE, Chamblee, Georgia 30341, <sup>7</sup>Florida Department of Health, 4052 Bald Cypress Way, Tallahassee, FL, 32399, <sup>8</sup>Lovelace Respiratory Institute, 2425 Ridgecrest, SE Albuquerque, NM, 87108, <sup>9</sup>Dept of Medicine (Division of Pulmonary Medicine), Miller School of Medicine, University of Miami, Miami, FL, 33136, <sup>10</sup>Center for Marine Science, University of N Carolina, 5600 Marvin K. Moss Lane, Wilmington, NC, 28409, <sup>11</sup>Department of Research, Mount Sinai Medical Center, Miami Beach, FL, <sup>12</sup>Dept of Pathology, Miller School of Medicine, University of Miami, FL 33136

**Introduction:** We have previously demonstrated statistically significant changes in pulmonary function testing (PFTs) and reported symptoms for asthmatics pre/post 1 hour at the beach with subsequent continued significantly increased symptoms and decreased PFTs over several days after exposure to *Karenia brevis* aerosols. In this study, we explored possible longer term health effects over 7 years from intermittent exposure to brevetoxins in an open cohort of asthmatics.

**Methods:** In addition environmental exposure measurements, 147 asthmatic subjects were assessed for PFTs and symptoms pre/post 1 hour of exposure to brevetoxin aerosols over both several days and several years of exposed and unexposed studies.

**Results:** Over the 7 study years, as a group, the asthmatics came to the studies with normal PFT values. The 38 asthmatics participating in only one exposure study reacted statistically significantly more in terms of their pre- vs post PFTs compared to the 36 asthmatics who came to >4 exposure studies. These 36 asthmatics participating in >4 exposure studies demonstrated no significant change in their standardized percent predicted pre-exposure PFT values over the 7 years of the study.

**Conclusions:** These results appear to indicate that asthmatics living in areas with intermittent Florida red tides experience acute and subacute, but not chronic, respiratory effects from intermittent environmental exposure to aerosolized brevetoxins over a 7 year period.

## FIRST CONFIRMED CANINE ILLNESSES AND MORTALITIES RELATED TO *KARENIA BREVIS* BLOOMS IN THE GULF OF MEXICO

Flewelling, L.J.<sup>1</sup>; Castle, K.T.<sup>2</sup>; Bryan, J.A.<sup>2</sup>; Nevada, C.<sup>3</sup>; Abraham, A.<sup>4</sup>; Atwood, K.A.<sup>1</sup>; Granholm, A.<sup>1</sup>; Landsberg, J.H.<sup>1</sup>

<sup>1</sup>Florida Fish and Wildlife Conservation Commission (FWC), Fish and Wildlife Research Institute, St. Petersburg, FL 33701 USA leanne.flewelling@myfwc.com

<sup>2</sup>US National Park Service (NPS), Fort Collins, CO 80525 USA; <sup>3</sup>US National Park Service, Padre Island National Seashore, Corpus Christi, TX 78480 USA; <sup>4</sup>Gulf Coast Seafood Laboratory, US Food and Drug Administration, Dauphin Island, AL 36528 USA

Well-documented effects of exposure to brevetoxins produced by the dinoflagellate *Karenia brevis* commonly include illness and mortality of marine mammals, sea turtles, sea birds, fish, and other aquatic animals. Humans are affected through ingestion of toxic filter-feeding shellfish or inhalation of toxic aerosol. Impacts of *K. brevis* blooms on terrestrial mammalian wildlife and pets, however, are relatively undocumented occurrences.

During *K. brevis* blooms along the coast of southwest Florida in 2003 and 2005, the FWC received numerous reports from a localized beach area of domestic dogs exhibiting signs suggestive of brevetoxicosis. At least two of the dogs had been observed eating red tide-killed fish. Urine samples from five dogs submitted to FWC for testing (three in 2003 and two in 2005) were positive for brevetoxins using a competitive enzyme-linked immunosorbent assay (ELISA). PbTx-3 was confirmed by LC-MS/MS in the 2005 cases. The severity of the dogs' symptoms (including heavy salivation, seizing, paralysis, and temporary blindness) varied, but all of the dogs recovered within one to several weeks.

In October 2009, during a *K. brevis* bloom along the Texas coast, millions of dead fish washed ashore along the 70-mile length of the Padre Island National Seashore (PAIS). Between November 2009-January 2010, 12 coyotes and three domestic dogs died or were euthanized due to illness believed to be related to exposure to the ongoing red tide at PAIS.

Three coyotes and one dog were necropsied by NPS veterinarians, and routine diagnostics performed (histopathology, microbiology, toxinology, and toxicology). Significant concentrations of brevetoxins were measured in stomach contents, liver, and kidney of two coyotes and the dog by ELISA. LC-MS/MS analyses confirmed PbTx-3 in ELISA-positive samples. Dead beached and buried fish collected from PAIS in late December 2009 had extremely high brevetoxin concentrations and are believed to be the primary brevetoxin vector to the canines, many of which were seen eating dried dead fish from the beach.

These are the first confirmed reports of brevetoxin-related canine illnesses and mortalities. Historical reports of *K. brevis* bloom impacts on terrestrial mammalian wildlife and pets are restricted to a small number of anecdotal and unconfirmed cases and have possibly been under-reported.

## LABORATORY EVALUATION AND OPTIMIZATION OF CHEMICAL EXTRACTION METHODS FOR THE RECOVERY OF CARIBBEAN CIGUATOXINS FROM FISH

Flores Quintana, H.A., Hooe-Rollman, J.I., Loader, J.I., Garcia, A.C., Robertson, A.

FDA, Gulf Coast Seafood Laboratory, Dauphin Island, AL 36528 USA  
Harold.FloresQuintana@fda.hhs.gov

Ciguatera is a food borne illness caused by the consumption of fish contaminated with ciguatoxins, produced by benthic dinoflagellates from the genus *Gambierdiscus*. Current methods used for the extraction of ciguatoxins can be time consuming and consist of many steps that result in reduced toxin yields. For routine analysis of field and outbreak samples, there is a great need to improve the recovery, throughput, and sensitivity of downstream analyses.

A variety of solvent extraction schemes were evaluated and compared to two published methods including Dickey (2008), and Lewis et al. (2009). All methods were evaluated using cooked, uncooked, and freeze-dried barracuda flesh (*Sphyraena barracuda*) collected in the U.S. Virgin Islands, and confirmed by LC-MS to contain Caribbean ciguatoxins (C-CTX-1 and C-CTX-2). Modifications comprised a variety of solvent substitutions for acetone and chloroform including butanol, dichloromethane, ethyl acetate, methanol, ethanol, and isopropanol. Toxin yields were assessed using a sodium channel based neuroblastoma (N2a) cytotoxicity assay.

Preliminary results showed that maximum toxin yields were obtained with freeze-dried and cooked samples extracted with methanol and partitioned with ethyl acetate. Emulsion effects were significantly reduced when butanol, ethyl acetate, and dichloromethane were used for partitioning, when compared to chloroform. In most instances, extraction times were reduced and toxin yields were improved. The best method from this work will be evaluated for toxin recovery using synthesized internal standards and multiple fish matrices in future studies.

Dickey, R.W. Ciguatera Toxins: Chemistry, Toxicology and Detection. In *Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection*, 2nd ed.; Botana, L.M., Ed.; CRC Press-Taylor & Francis: Boca Raton, FL, USA, 2008; pp. 479–500.

Lewis, R.J.; Yang, A.J.; Jones, A. Rapid extraction combined with LC-tandem mass spectrometry (CREM-LC/MS/MS) for the determination of ciguatoxins in ciguateric fish flesh. *Toxicon* 2009, 54, 62–66.

## A COMPARATIVE LOOK AT MOCHA SURFZONE CELL COUNT DATA AND ODA TISSUE TOXIN RESULTS

Forster, Z.<sup>1</sup>, White, A.<sup>2</sup>, Wood, A.M.<sup>3</sup>, Peterson, W.T.<sup>4</sup>

<sup>1</sup>Oregon Department of Fish and Wildlife, Astoria, OR 97103 USA Zachary.R.Forster@state.or.us; <sup>2</sup>Oregon State University, Corvallis, OR, 97330 USA awhite@coas.oregonstate.edu; <sup>3</sup>University of Oregon, Eugene, OR, 97401 USA m.michellewood@gmail.com; <sup>4</sup>NOAA Fisheries, Newport, OR 97395 USA Bill.Peterson@noaa.gov

The Oregon Department of Fish and Wildlife (ODFW) has routinely monitored nearshore waters along the Oregon coast for the presence of Harmful Algal Blooms (HABs) since 2005. The Oregon Department of Agriculture (ODA) has been monitoring phycotoxins in shellfish tissue since the 1970's. Closures of shellfish harvest areas due to contamination by phycotoxins are frequent, and have a considerable economic impact on coastal communities. With the continuity of sampling and a robust data set, we were able to begin comparing isolated domoic acid (DA) events from different regions of Oregon's coast. Some basic comparisons including cell count and tissue toxin data are providing insight into DA accumulation, depuration and severity of DA toxicity in the *Siliqua patula* (Pacific razor clam). Although the analysis is often confounded by the cryptic nature of *Pseudo-nitzschia* (P-n), variance of DA production and the lack of ancillary data provided by our nearshore sampling program, these comparisons help enhance an early warning system. Initial analysis of cell count and tissue toxin data loosely centered around DA events showed only weak correlations between the presence of P-n and DA toxicity. These correlations were strengthened by narrowing the time frame before and after a "peak" in DA, isolating events with a minimum toxicity level and discerning between DA accumulation/depuration periods. What was found were similarities in cell counts, tissue toxicity, community diversity, and seasonality between multiple sample sites. Comparisons will be presented from multiple sampling sites over a range of time highlighting the relationship between P-n cell counts and DA toxicity along Oregon's beaches.

## A TALE OF TWO TOXINS: DOMOIC ACID AND SAXITOXIN MEASUREMENTS IN NORTHERN SEA OTTERS AND CALIFORNIA SEA OTTERS

Frame, E.R.<sup>1</sup>, Kendrick, P.S.<sup>1</sup>, Gill, V.A.<sup>2</sup>, Miller, M.A.<sup>3</sup>, Lefebvre, K.A.<sup>1</sup>

<sup>1</sup>NOAA, Northwest Fisheries Science Center, Seattle, WA 98112 USA  
Elizabeth.Frame@noaa.gov; <sup>2</sup>US Fish and Wildlife Service, Anchorage, AK 99503 USA;  
<sup>3</sup>California Department of Fish and Game, Santa Cruz, CA 95060 USA

Paralytic shellfish poisoning (PSP) has a long history in Alaska, with written records of human illnesses dating back to 1799. Over 150 cases of PSP have been reported in Alaska since the late 1970s; however few records of PSP-related deaths or illness exist for marine mammals in Alaska. Domoic acid (DA), another prevalent algal toxin on the US West Coast, is a relatively new concern in Alaskan waters. No documented human cases have been reported yet, but domoic acid has been found with surprising frequency in marine mammal samples from the region collected over the last few years.

The Wildlife Algal toxin Research and Response Network (WARRN-West) has tested over 200 samples from northern sea otters from South Central Alaska for saxitoxin (the most potent of the PSP toxins) and domoic acid. Of these animals over 25% tested positive for domoic acid, while 10% were positive for saxitoxin. The highest domoic acid concentration measured was 162 ng/g in a urine sample from a male pup in Cold Bay. The highest saxitoxin concentration was 112 ng/g measured in the stomach contents of an adult male from Kodiak. Domoic acid has been a prominent problem for southern Californian marine mammals since the mid 1990's. California sea lions are regularly afflicted with domoic acid poisoning and this species has been frequently tested for domoic acid. Another iconic Californian marine mammal, the California sea otter, is also at risk for algal toxin exposure based on its diet. Despite this exposure risk, domoic acid has not received as much attention in this species. The WARRN-West team has tested over 310 California sea otters for domoic acid and saxitoxin. Of these, 54% were positive for domoic acid, while only 6% were positive for saxitoxin. The highest domoic acid concentration was 230,000 ng/g in the colon contents of an adult male. The highest saxitoxin concentration was 10.3 ng/g in a fecal sample from an adult male.

Overall, the California sea otter samples had higher incidence and absolute concentrations of domoic acid than Alaskan sea otters, likely due to a higher prevalence of domoic acid producing diatoms in Californian waters. The northern sea otters from Alaska had a slightly higher prevalence of saxitoxin and greater saxitoxin concentrations than the Californian otters, as expected from the long history of PSP in Alaskan waters. Despite these overall differences in prevalence and magnitude, both sea otter populations had a higher percentage of domoic acid positive animals than saxitoxin positive animals, suggesting that domoic acid poses a threat to otter populations along the entire US West Coast.

## HARMFUL ALGAL BLOOMS AND BENTHIC NUTRIENT MINERALIZATION: A POSITIVE FEEDBACK LOOP OR A DEAD END?

Fulweiler, R.W.<sup>1</sup>, Gobler, C.J.<sup>2</sup>, Wall, C.<sup>2</sup>, Heiss, E.M.<sup>1</sup>

<sup>1</sup>Boston University, Earth Sciences Department, Boston, MA 02138 USA  
rwf@bu.edu, emheiss@bu.edu; <sup>2</sup>Stony Brook University, Stony Brook, NY 11794 USA  
Christopher.Gobler@stonybrook.edu, chuckwall@gmail.com

In coastal systems water column production deposited to the benthos or directly filtered from the water is an important food source for benthic fauna – including bacteria. Once deposited, organic matter decomposes and nutrients are subsequently recycled into the water column. This organic matter also fuels sediment denitrification, the microbial process that converts bioavailable nitrogen ( $\text{NO}_3^-$ ) into biologically unavailable nitrogen ( $\text{N}_2$  gas). Denitrification can remove up to 70% of anthropogenic nitrogen entering coastal systems and helps decrease cultural eutrophication. The balance between benthic remineralization and denitrification determines future water column productivity. While the potential effects of harmful algal bloom toxicity have been documented for many organisms the effect of HABs on benthic oxygen uptake, nutrient remineralization, and denitrification remains unknown. The extent to which these processes are altered by HABs may have important implications for coastal ocean carbon fluxes, land-ocean nutrient fluxes, and our understanding of the timing, magnitude, and duration of harmful algal blooms. To examine the potential impact of HAB organic matter on these processes we conducted a series of experiments amending sediments with HAB organic matter of varying toxicity. Initial results suggest that HAB organic matter alters rates of sediment denitrification. We found that amending sediment cores with environmentally realistic levels of HAB biomass decreased rates of denitrification by ~50-70% with HABs synthesizing toxins having a stronger impact than HABs which do not produce toxins. By decreasing denitrification more bioavailable nitrogen could be retained in the system increasing the potential for future phytoplankton blooms. We will discuss these results as well as the impact of HAB biomass on sediment oxygen demand, inorganic nutrient fluxes, and greenhouse gas ( $\text{N}_2\text{O}$  and  $\text{CH}_4$ ) fluxes.

## PREVALENCE AND TOXICITY OF CARIBBEAN CIGUATOXINS IN REEF DWELLING FISHES TAKEN FROM THE U.S. VIRGIN ISLANDS

Garcia, A.C.<sup>1</sup>, Hooe-Rollman, J.I.<sup>1</sup>, Smith, T.<sup>2</sup>, Granade, H.R.<sup>1</sup>, Richlen, M.<sup>3</sup>, Anderson, D.M.<sup>3</sup>, Xu, Y.<sup>3,4</sup>, Robertson, A.<sup>1</sup>

<sup>1</sup> FDA, Gulf Coast Seafood Laboratory, Dauphin Island, AL USA; Ana.Garcia@fda.hhs.gov ; <sup>2</sup> University of the Virgin Islands, St. Thomas, U.S. Virgin Islands 00802 USA; <sup>3</sup> Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA; <sup>4</sup> Nanjing University, Nanjing, Jiangsu 210093, P.R. China

Ciguatoxins (CTXs) are cyclic polyether toxins derived from benthic marine dinoflagellates such as *Gambierdiscus* spp. Ciguatera fish poisoning (CFP) results from consumption of CTX-contaminated finfish and is characterized by neurological, gastrointestinal, and cardiovascular symptoms. The Virgin Islands represent a ciguatera “hot spot”, with a long history and high incidence of CFP. This has had a significant impact on human health and fisheries in the region. Herbivorous fish represent the primary link between epiphytic *Gambierdiscus* spp. and commercially important finfish, and this research aims to investigate the prevalence of Caribbean CTXs in herbivorous, reef-dwelling fishes in the U.S. Virgin Islands.

Several sampling sites were selected in the waters surrounding St. Thomas, USVI, based on previous surveys showing high prevalence of *Gambierdiscus* spp. A selection of herbivorous fish was collected from these areas and muscle tissue was subsequently extracted for C-CTX analysis. An optimized neuro-2A cytotoxicity assay for screening sodium channel activity was undertaken to estimate composite toxicity.

Preliminary results from this study have revealed C-CTX-like activity from multiple fish species collected over the sampling period. Highest C-CTX-1 activity (based on toxicity equivalents/gram tissue) was found in herbivorous fishes taken from sites most protected from ocean currents, and reflect *Gambierdiscus* spp. abundances. Based on these data we discuss the spatio-temporal distribution of Caribbean CTX in relation to environmental parameters including salinity, temperature, and nutrients, and discuss the potential relationships between life history characteristics of herbivorous fishes and corresponding ciguatoxin activity measured in tissues.

## PHOTOCHEMICAL PRODUCTION OF BIOAVAILABLE NITROGEN AS A SOURCE FOR *KARENIA BREVIS* BLOOMS ON THE WEST FLORIDA SHELF

Garrett, M.<sup>1</sup>, Murasko, S.<sup>1</sup>, Yunker, A.<sup>1</sup>, Bronk, D.<sup>2</sup>, Killberg-Thoreson, L.<sup>2</sup>, Heil, C.<sup>3</sup>

<sup>1</sup>Florida Fish & Wildlife Conservation Commission, St. Petersburg, FL 33701 USA  
matt.garrett@myfwc.com, sue.murasko@myfwc.com, ashley.yunker@myfwc.com;

<sup>2</sup>Virginia Institute of Marine Science, Gloucester Point, VA 23062 USA  
bronk@vims.edu, killberg@vims.edu; <sup>3</sup>Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575 USA  
cheil@bigelow.org

Multiple nutrient sources support and regulate environmentally and economically destructive *Karenia brevis* blooms in the eastern Gulf of Mexico. One potential source is the photoproduction of bioavailable forms of nitrogen (N), such as nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>), and urea, through UV degradation of colored dissolved organic matter (CDOM). Three locations with varying CDOM characteristics were sampled monthly from February 2009 to February 2011 to assess the magnitude and ecological significance of photochemical nutrient production in the southwest Florida coastal region where *K. brevis* blooms occur. Identical quartz tubes were filled with 0.2 mm filtered subsurface water and equally divided into light and dark treatments. Samples were incubated for one diel cycle in ambient daylight in flow-through incubators. Concentrations of NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and urea were measured in triplicate post incubation. Production was calculated as the difference between the light and dark treatments and was normalized to the total amount of light exposure during incubation. Ammonium dominated N production (0 – 0.11 μM 10<sup>-3</sup> μEm<sup>-2</sup>s<sup>-1</sup>), followed by urea (0 - .022 μM 10<sup>-3</sup> μEm<sup>-2</sup>s<sup>-1</sup>) then NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>. Ammonium was infrequently produced in low CDOM samples, but was significantly and consistently produced in high CDOM samples. Urea was produced in both low and high CDOM environments, but with less consistency than NH<sub>4</sub><sup>+</sup>. Some seasonality was evident in the production of different nitrogenous compounds, with greatest NH<sub>4</sub><sup>+</sup> produced during summer months. Ammonium photoproduction rates at the high CDOM site were comparable to those measured within three *K. brevis* blooms in the region in 2007, 2008, and 2009. Separate studies conducted near the high CDOM site indicate N uptake within the phytoplankton community is dominated (20% to 72%) by NH<sub>4</sub><sup>+</sup> uptake. We conclude that photochemical NH<sub>4</sub><sup>+</sup> production may serve as a significant source of bioavailable N to *K. brevis* blooms and should be included as a potential nutrient source for harmful algal blooms in high CDOM coastal environments.

## NUTRIENT LOADING AND HABs IN FLORIDA BAY: A MULTI\_YEAR SYNTHESIS

Glibert, P.M.<sup>1</sup>, Heil, C.<sup>2</sup>, Madden, C.J.<sup>3</sup>, Alexander, J.<sup>1</sup>, Murasko, S.<sup>4</sup>

<sup>1</sup>University of Maryland Center for Environmental Science, Horn Point Laboratory, PO Box 775, Cambridge MD 21613 USA [glibert@umces.edu](mailto:glibert@umces.edu); <sup>2</sup>Bigelow Laboratory for Ocean Sciences, 180 McKown Point Road, P.O. Box 475 West Boothbay Harbor, ME 04575 USA; <sup>3</sup>South Florida Water Management District, West Palm Beach, FL 33406 USA; <sup>4</sup>Florida Fish and Wildlife Research Institute, 100 Eighth Avenue SE, St. Petersburg FL 33701 USA

Nutrient loading to Florida's coastal waters is of major concern, as numerous types of HABs are common. In Florida Bay, HABs, including sustained, multi-year cyanobacterial blooms, as well as blooms of toxic dinoflagellates, have been documented in recent years. This area is of particular concern as the hydrology of the Everglades is currently being altered to move water from the east to the west and increase fresh water flow to central Florida Bay. This alteration of flow is having, and will continue to have, impacts on the nutrient form delivered and on the transport of these forms through the sloughs that feed into Florida Bay. The key question is whether such changes will alter primary production and HAB frequency downstream, and whether trophic relationships will be altered as a result. There is an east to west gradient generally transitioning from phosphorus- to nitrogen- limited conditions in the bay. Thus the alteration of flows also has the potential of altering the supply of nutrients from regions which have traditionally been phosphorus limited to those that have been traditionally nitrogen limited.

Over the past decade, fourteen field efforts have been undertaken, during both wet and dry seasons, covering stations from the Gulf of Mexico end member, to the enclosed northeastern bay, and north to the sub-embayments of the Everglades. Sampling encompassed both non-bloom periods, when chlorophyll was  $< 1 \text{ mg L}^{-1}$ , to periods and regions of sustained blooms, when chlorophyll a was  $>100 \text{ mg L}^{-1}$ . Biomass, community composition, nutrient bioassay responses, and rates of uptake of various N and P forms were characterized. The goal of this talk is to provide an overview and synthesis of the relationships between nutrients, their concentration, forms and ratios, on the phytoplankton community structure. The ultimate goal is to develop a predictive capability of the impacts of these altered flows and nutrient loads on the potential for increased harmful algal bloom development in Florida Bay.

## NICHE OF HARMFUL ALGA *AUREOCOCCUS ANOPHAGEFFERENS* REVEALED THROUGH ECOGENOMICS

Gobler, C.J.<sup>1</sup>, Berry, D.L.<sup>1</sup>, Dyhrman, S.T.<sup>2</sup>, Wilhelm, S.W.<sup>3</sup>

<sup>1</sup>Stony Brook University, School of Marine and Atmospheric Sciences, Southampton, NY 11968 USA; <sup>2</sup>Woods Hole Oceanographic Institution, Biology Department, Woods Hole, MA 02543 USA; <sup>3</sup>The University of Tennessee, Department of Microbiology, Knoxville, TN 37996 USA.

Harmful algal blooms (HABs) cause significant economic and ecological damage worldwide. Despite considerable efforts, a comprehensive understanding of the factors that promote these blooms has been lacking because the biochemical pathways that facilitate their dominance relative to other phytoplankton within specific environments have not been identified. Here, biogeochemical measurements demonstrated that the harmful alga *Aureococcus anophagefferens* outcompeted co-occurring phytoplankton in estuaries with elevated levels of dissolved organic matter and turbidity and low levels of dissolved inorganic nitrogen. We subsequently sequenced the first HAB genome (*A. anophagefferens*) and compared its gene complement to those of six competing phytoplankton species identified via metaproteomics. Using an ecogenomic approach, we specifically focused on the gene sets that may facilitate dominance within the environmental conditions present during blooms. *A. anophagefferens* possesses a larger genome (56 mbp) and more genes involved in light harvesting, organic carbon and nitrogen utilization, and encoding selenium- and metal-requiring enzymes than competing phytoplankton. Genes for the synthesis of microbial deterrents likely permit the proliferation of this species with reduced mortality losses during blooms. Collectively, these findings suggest that anthropogenic activities resulting in elevated levels of turbidity, organic matter, and metals have opened a niche within coastal ecosystems that ideally suits the unique genetic capacity of *A. anophagefferens* and thus has facilitated the proliferation of this and potentially other HABs. Sequencing of the *A. anophagefferens* genome has permitted the exploration of the molecular mechanisms involved in brown tide events via field-based transcriptional and proteomic studies that will also be discussed during this talk.

## PERCEPTIONS OF CIGUATERA FISH POISONING IN THE VIRGIN ISLANDS: IMPLICATIONS FOR OUTREACH AND EDUCATION

Grattan, L.<sup>1</sup>, Roberts, S.<sup>1</sup>, Radke, B.<sup>2</sup>, Toben, A.<sup>1</sup>, Olsen, D.<sup>3</sup>, Morris, J. G.<sup>4</sup>

<sup>1</sup>University of Maryland SOM LGrattan@som.umaryland.edu, Roberts@som.umaryland.edu , TobenAC@aol.com; <sup>2</sup>University of Florida Bethradke@ufl.edu; <sup>3</sup>St. Thomas Fishermen's Association olsen41@aol.com; <sup>4</sup>University of Florida JGMorris@epi.ufl.edu

**Background:** Fishing is an important component of the culture and subsistence living in the U.S. Virgin Islands. Extant data indicates that residents have been at risk for ciguatera fish poisoning for more than four decades. Anecdotal information suggests that local residents are aware of some risk for “fish poisoning” and numerous stories abound regarding the best way to control their risk of exposure, diagnose the illness, and treat both long and short term symptoms. However, scientifically based environmental education programs have not been systematically implemented into this community. These programs are needed to help the local communities benefit from accurate information and advances in scientific knowledge from the HAB and medical communities.

**Purpose:** To assess current levels of factual knowledge, awareness and concern, protective behaviors and treatment remedies to decrease or mitigate risk of “fish poisoning.” This information will be used as a basis for developing and implementing community based outreach and education programs.

**Method:** Risk perception surveys (Health and Coastal Environment Perceived Risk Questionnaire) were administered to adult residents and frequent visitors to St. Thomas (USVI) at local fishing tournaments, fish markets and other fishing related events. The Health and Coastal Environment Perceived Risk Questionnaire has been previously well validated in other coastal communities with HAB-related or other environmental threats. With the assistance of local experts, the questionnaire was modified to reflect the specific language, culture and traditions of this Caribbean community.

**Results:** Findings indicated that 25% of respondents reported a personal history of fish poisoning with the most frequently recognized symptoms including diarrhea and abdominal pain (82%) joint pain (71%), numbness and tingling of lips or extremities (65%) and a bad/metallic taste in their mouth (59%). Thirty-nine percent believed there were effective treatments available for the illness. The most effective interventions reported included going to the emergency room (71%), avoiding fish consumption after an episode of poisoning (55%), taking Benadryl (20%) or ingesting brown sugar, bush tea or Mauby bark (31%) for symptom relief. After taking into consideration a wide variety of methods for “testing” the catch for a ciguatoxin (e.g. touching parts of the fish to the lips, fly test), sixty-two percent of respondents feel safe eating local fish. Sixty-five percent of respondents believed that scientists and doctors will succeed in finding a remedy for fish poisoning. The most trusted sources of information for this community are local fishermen (46%) and friends/neighbors (36%).

**Implications:** Based upon these findings, initial community outreach and education efforts should target local fishermen and community members. These efforts should focus upon identifying the human risk factors for exposure and the management of acute and long term symptoms. It is anticipated that an increase in factual knowledge may help to increase protective behavior.

## NUTRIENT UPTAKE CAPABILITIES OF TYPICAL COASTAL PHYTOPLANKTON COMMUNITIES ON THE WEST-CENTRAL COAST OF FLORIDA

Hall, E.R.<sup>1</sup>, Dixon, L.K.<sup>1</sup>, Kirkpatrick, G.J.<sup>1</sup>, Lovko, V.<sup>1</sup>, Pederson, B.A.<sup>2</sup>

<sup>1</sup>Mote Marine Laboratory, Sarasota, FL 34236 USA emily8@mote.org, lovko@mote.org, gkirkpat@mote.org, lkdxon@mote.org; <sup>2</sup>Integrated Systems, Inc., Darlington, SC 29540 USA Brad@isirobots.com

Blooms of *Karenia brevis* ("red tides") are frequent and often persistent off the west central coast of Florida. Bloom initiation typically occurs in oligotrophic conditions, can be transported by physical processes into nearshore areas influenced by a variety of large estuaries and river systems, and can last more than 21 months. Identifying specific nutrient requirements and uptake capabilities of low cell densities of *K. brevis* in the presence of the non-bloom phytoplankton population has been a complex and difficult task. To approach this, a number of bioassay experiments have been conducted from 2007 to 2011 to determine the response of the coastal phytoplankton community (using water collected approximately 1 km offshore) to different nutrient (ammonia, nitrate-nitrite, urea, various amino acids, ortho-phosphorus, silica, and urea + ortho-phosphate), decomposed fish, and estuarine water (Tampa Bay, Charlotte Harbor, and the Caloosahatchee River) inputs. Estuarine waters used as treatments were collected at salinities of ~25 PSU and applied at a 1:25 dilution of estuary:coastal waters to represent the dilution and typical exposure of coastal communities. Resulting salinity reductions of 0.3 PSU were matched for all treatments. Inorganic and organic nutrients were applied in the range of 5-15  $\mu\text{M}$ , while estuarine treatments increased coastal waters on the order of 0.3 and 0.06  $\mu\text{M}$  N and P, respectively. Results show that dissolved  $\text{NO}_{2+3}$  and  $\text{NH}_4$  were rapidly taken up within the first 24 hours. Diatoms uniformly dominated the ultimate community response from nitrogen additions. Primer-e analyses indicated a shift in community structure from coastal towards the estuarine community after both 24 and 48 hours of the nitrogen treatments (inorganic nitrogen, urea, and amino acids), while phosphorus additions in this phosphorus-rich region typically had no effect. There was little to no change in either chlorophyll content or community structure with the addition of estuarine waters. Funding for this research is through the state of Florida and the Florida Fish and Wildlife Conservation Commission-Fish and Wildlife Research Institute.

## A PHYTOPLANKTON MONITORING PROGRAM FOR CENTRAL PUGET SOUND

Hannach, G.<sup>1</sup>, Stark, K.<sup>2</sup>

<sup>1</sup>Environmental Lab, King County Department of Natural Resources and Parks, Seattle, WA 98119 USA [gabriela.hannach@kingcounty.gov](mailto:gabriela.hannach@kingcounty.gov); <sup>2</sup>King County Department of Natural Resources and Parks, Seattle, WA 98104 USA [kimberle.stark@kingcounty.gov](mailto:kimberle.stark@kingcounty.gov)

The entire Puget Sound region faces challenges from a growing human population and a changing climate that will likely exacerbate already critical threats to the health of the Sound. Recent efforts to restore and protect Puget Sound highlight a need for essential information concerning biodiversity and the seasonal dynamics of its marine inhabitants. The King County Marine and Sediment Assessment Group manages a long-term marine monitoring program designed to assess water quality in the Central Puget Sound Basin. Since 1995, data are collected monthly for physical, chemical, and biological (chlorophyll *a*) parameters at various locations and depths throughout the Puget Sound Central Basin. The recent addition of a long-term phytoplankton species component to this program was deemed critical in order to predict how changes in climate and other regional stressors will impact the Sound's trophic structure. Major goals of the program are 1) to assess relative abundance of major phytoplankton taxa 2) to document the timing of seasonal shifts, 3) to investigate relationships between physical/chemical parameters and species relative abundance, 3) to detect long-term changes in community composition, 4) to create a photo library, and 5) to collect a long-term dataset to help evaluate potential changes in the Puget Sound food web. This dataset will also be a valuable resource for detecting the occurrence and environmental conditions that lead to blooms of HAB species in Puget Sound.

Sampling occurs bi-weekly from April through September or October at three locations within the Central Basin. Point Jefferson and East Passage are long-term ambient monitoring stations representing open north and south areas of the Puget Sound Central Basin. Quartermaster Harbor is a shallow, protected embayment with poor tidal flushing; a telemetered mooring has been deployed to provide high frequency water quality data from this site. A total of 109 species or genera have been identified from samples collected between 2008 and 2010, including 63 diatom and 40 dinoflagellate taxa. Diatoms were abundant throughout the sampling season, with species of *Chaetoceros* represented in over 89% of the samples from each location and large species of *Pseudo-nitzschia* present in over 77% of the samples from the open water stations. Details about the monitoring program and results to date will be presented.

## CLIMATE CHANGE: LINKS TO GLOBAL EXPANSION OF HARMFUL CYANOBACTERIA

Paerl, H.W.<sup>1</sup>, Paul, V.<sup>2</sup>

<sup>1</sup>Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell Street, Morehead City, NC 28557 USA [hpaerl@email.unc.edu](mailto:hpaerl@email.unc.edu); <sup>2</sup>Smithsonian Marine Station, Ft. Pierce, FL 34949 USA [Paul@sms.edu](mailto:Paul@sms.edu)

Cyanobacteria are the Earth's oldest (~ 3.5 bya) oxygen evolving organisms, and they have had major impacts on shaping our modern-day biosphere. Conversely, biospheric environmental perturbations, including nutrient enrichment and climatic changes (e.g. global warming, hydrologic changes, increased frequencies and intensities of tropical cyclones, more intense and persistent droughts), strongly affect cyanobacterial growth and bloom potentials in freshwater and marine ecosystems. We examined human and climatic controls on harmful (toxic, hypoxia-generating, food web disrupting) bloom-forming cyanobacteria (CyanoHABs) along the freshwater to marine continuum. These changes may act synergistically to promote cyanobacterial dominance and persistence. This synergy is a formidable challenge to water quality, water supply and fisheries managers, because bloom potentials and controls may be altered in response to contemporaneous changes in thermal and hydrologic regimes. In inland waters, hydrologic modifications, including enhanced vertical mixing and increased flushing (reducing residence time) will likely be needed in systems where nutrient input reductions are neither feasible nor possible. However, because freshwater supplies are finite and dwindling, the flushing option is limited. Successful control of CyanoHABs by grazers is unlikely except in specific cases. Overall, stricter nutrient management will be the most feasible and practical approach to long-term CyanoHAB control in a warmer, stormier and more extreme world.

## IS *MYRIONECTA RUBRA* A PREDICTOR FOR *DINOPHYSIS OVUM* BLOOMS IN TEXAS?

Harred, L.B.<sup>1</sup>, Henrichs, D.W.<sup>2</sup>, Campbell, L.<sup>1,2</sup>

<sup>1</sup> Dept. Oceanography, Texas A & M University, College Station, TX 77843 USA lbh0196@neo.tamu.edu; <sup>2</sup> Dept. Biology, Texas A & M University, College Station, TX 77843 USA

The Imaging Flow Cytobot (IFCB), a relatively new technology for capturing images of planktonic cells at high resolution, was deployed in 2007 in Port Aransas, TX at the University of Texas Marine Science Institute. The IFCB provides continuous data, creating a data file approximately every 20 minutes that records images taken from ~5 mL samples of sea water. In 2008, a large peak in *Myrionecta rubra* cells preceded the large *Dinophysis* bloom in February/March of that year. Based on this observation, we examined the three subsequent years of IFCB data to investigate the relationship between *Myrionecta rubra* and *Dinophysis ovum* cells to determine if patterns in their bloom dynamics could be used for early warning. Time series were created using the automated classifier that was developed for the 2008 bloom. Classified results were manually corrected for mistakes and the peak time, magnitude and duration of blooms of *Myrionecta rubra* and *Dinophysis ovum* were determined for 2009, 2010 and 2011. In 2008, the daily averages for *Myrionecta rubra* and *Dinophysis ovum* were extremely high, with *M. rubra* reaching ~300 cells mL<sup>-1</sup> and *D. ovum* reaching ~175 cells mL<sup>-1</sup> at their highest peak. The *M. rubra* bloom lasted ~10 days followed ~14 days later by the onset of the *D. ovum* bloom, which lasted ~20 days. In 2009, *M. rubra* occurred briefly at low abundance (~8.5 cells mL<sup>-1</sup>) and no significant *D. ovum* bloom followed. In 2010, the *M. rubra* bloom lasted ~55 days and overlapped the onset of the *D. ovum* bloom. The maximum *M. rubra* abundance was ~27 cells mL<sup>-1</sup> and *D. ovum* peaked at ~17 cells mL<sup>-1</sup>. Finally in 2011, although *M. rubra* was present for only about three days at very low abundance (<5 cells mL<sup>-1</sup>), it was followed ~40 days later by a fluctuating bloom of *D. ovum* of highest magnitude ~15 cells mL<sup>-1</sup> lasting ~14 days. In conclusion, a *Myrionecta rubra* bloom has the potential to be a predictor for an ensuing *Dinophysis ovum* bloom. However, given the variability in timing and magnitude of *Myrionecta rubra* additional years of data are needed to further establish the relationship between bloom timing and the magnitude of the subsequent *Dinophysis ovum* bloom.

THE ROLE OF HALO-TOLERANCE IN IMPACTING PREDATOR/PREY INTERACTIONS AND BLOOM FORMATION IN THE TOXIC RAPHIDOPHYTE, *HETEROSIGMA AKASHIWO*

Harvey, E.L.; Menden-Deuer, S.

Graduate School of Oceanography, University of Rhode Island, Narragansett, RI USA  
harveyel@gso.uri.edu, smenden@gso.uri.edu

The formation of a harmful algal bloom (HAB) results when population growth rates exceed loss rates of a HAB species. Predator avoidance, or other mechanisms that reduce predator prey encounter rates may be effective in decreasing HAB loss rates. By concurrently observing population distributions and 3D movement behaviors of both HAB species and their predators we investigated the role of the broad halo-tolerance of the toxic raphidophyte alga, *Heterosigma akashiwo*, in providing refuge from potential protistan predators. In a laboratory tank, *H. akashiwo* exhibited rapid broad halo-tolerance, distributing throughout a 30cm/1L tank with a 0-30 psu linear salinity gradient, without preference for a specific salinity. Of the two predators observed, *O. marina* exhibited a similar population distribution as *H. akashiwo*, whereas the ciliate *F. ehrenbergii*, was restricted to salinities >15. *Heterosigma akashiwo* exhibited avoidance behaviors in response to the presence of *F. ehrenbergii*, resulting in an increased aggregation of cells in low salinity waters. Intra-strain comparison of 7 *H. akashiwo* strains showed differences in movement behaviors and growth rates along a range of salinities, suggesting that intra-specific variation plays an important role in the population dynamics of this species. These results indicate that for some predators, the halo-tolerance of *H. akashiwo* can decrease the potential for grazing interaction. The coupled physiological/ behavioral advantage identified here may be a mechanism that leads to the enhanced survival of the species and ultimately promotes the formation of *H. akashiwo* harmful algal blooms.

A TALE OF TWO DINOFLAGELLATES: CO-OCCURRING BLOOMS OF THE PSP- AND DSP-PRODUCING DINOFLAGELLATES, *ALEXANDRIUM FUNDYENSE* AND *DINOPHYSIS ACUMINATA*, IN A NEW YORK ESTUARY.

Hattenrath-Lehmann, T.K.<sup>1</sup>, Morton, S.L.<sup>2</sup>, Gobler, C.J.<sup>1</sup>

<sup>1</sup>Stony Brook University, School of Marine and Atmospheric Sciences, Southampton, NY 11968 USA thattenr@ic.sunysb.edu, cgobler@notes.cc.sunysb.edu; <sup>2</sup>NOAA-National Ocean Service, Marine Biotoxins Program, 219 Fort Johnson Road, Charleston, SC 29412 USA Steve.morton@noaa.gov

*Alexandrium* blooms with cell densities up to  $10^6$  cells  $L^{-1}$  have become an annual occurrence in New York, causing PSP-induced closures of nearly 10,000 acres of shellfish beds in the Northport-Huntington Bay complex during five out of the last six years. In 2008, a second dinoflagellate, *Dinophysis acuminata*, began forming large, annual blooms ( $>10^5$  cells  $L^{-1}$ ) in this same system producing the toxins okadaic acid and DTX-1 ( $>10$  pg  $mL^{-1}$  as measured via LCMS) both of which are the causative agents of diarrhetic shellfish poisoning (DSP) syndrome. This is an important new development as NY does not currently monitor for DSP and shellfish are the most valuable fishery in NY. Blooms of *Alexandrium* (March through June) and *Dinophysis* (May through August) co-occurred during May 2008-2011, suggesting that *Alexandrium* and *Dinophysis* are either promoted by similar factors or are linked by more complex interactions. To assess factors promoting these events, field-based, incubation experiments investigating nutrients and trophic interactions (including allelopathy) were performed using water from Northport Bay. *Alexandrium* densities were significantly ( $p<0.05$ ) enhanced by organic compounds including high molecular weight organic matter isolated from a sewage treatment plant and by vitamin B<sub>12</sub> while *Dinophysis* densities increased (up to 600%) significantly with the addition of ammonium. Finally, the addition of *Alexandrium* cells or *Alexandrium*-culture filtrate to natural phytoplankton communities significantly decreased densities of diatoms and autotrophic nanoflagellates while significantly enhancing dinoflagellate densities. This finding suggests that allelopathic interactions not only promote *Alexandrium* blooms by inhibition of competitors but may also account for the co-occurrence of other harmful dinoflagellates, such as *Dinophysis acuminata*, during PSP events.

DISTRIBUTION AND PHYLOGENETIC RELATIONSHIPS OF *COCHLODINIUM* SPP. FOUND IN COASTAL WATERS OF THE EASTERN PACIFIC

Hayashi, K.<sup>1</sup>, Kudela, R.M.<sup>1</sup>, and Smith, G.J.<sup>2</sup>

<sup>1</sup>Department of Ocean Sciences, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, USA, khayashi@ucsc.edu; <sup>2</sup>Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039, USA

*Cochlodinium* is a red tide forming dinoflagellate genus known to cause massive fish kills in the coastal waters of the western Pacific eliciting economic loss in China, Japan, and Korea. The first documented *Cochlodinium* bloom off the coast of Korea was in 1982, and since then, bloom events have been documented worldwide. *Cochlodinium* blooms were not routinely found in the eastern Pacific until 2000, and at least two blooms resulted in a commercial fishery loss. In 2001, a *Cochlodinium* bloom in British Columbia, Canada was linked to aquacultured salmon mortalities and in 2007, abalone die-offs resulted from a *Cochlodinium* bloom in Monterey Bay, California. Two species of *Cochlodinium*, *Cochlodinium polykrikoides* and *Cochlodinium fulvescens*, are seen regularly in eastern Pacific coastal waters stretching from British Columbia, Canada to Costa Rica with evidence suggesting the ranges of the two species overlap geographically.

Large subunit ribosomal DNA (LSU rDNA) sequences reveal three global-scale regional ribotypes for *C. polykrikoides*: East Asian, Philippines, and American/Malaysian. Recent eco-physiological findings show that strains from the American/Malaysian ribotype are more cold water and low salinity tolerant, further supporting the phylogenetic differences. Preliminary LSU rDNA sequence data for *C. fulvescens*, the most recently described species in the genus, show differences between strains isolated from different regions in the eastern Pacific, suggesting multiple regional ribotypes within *C. fulvescens*.

## DEEP WATER MIXING PREVENTS HARMFUL ALGAL BLOOM FORMATION: IMPLICATIONS FOR MANAGED FISHERIES REFUGIA

Hayden, N.J.<sup>1</sup>, Roelke, D.L.<sup>1</sup>, Brooks, B.W.<sup>2</sup>, Grover, J.P.<sup>3</sup>, Neisch, M.T.<sup>1</sup>, Valenti, Jr., T.W.<sup>2</sup>, Prosser, K.N.<sup>2</sup>, Gable, G.M.<sup>4</sup>, Umphres, G.D.<sup>1</sup>, Hewitt, N.C.<sup>1</sup>

<sup>1</sup> Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843, USA droelke@tamu.edu; <sup>2</sup> Department of Environmental Science, Center for Reservoir and Aquatic Systems Research, Baylor University, One Bear Place #97266, Waco, Texas 76798, USA Bryan\_Brooks@baylor.edu; <sup>3</sup> Department of Biology and Program in Earth and Environmental Sciences, University of Texas at Arlington, Arlington, Texas 76019, USA grover@uta.edu; <sup>4</sup> Water Rights, Permitting and Availability Section, Texas Commission on Environmental Quality, 12100 Park 35 Circle, Bldg. F, Austin, TX 78753, USA George.Gable@tceq.texas.gov

Inflows affect water quality, lower food web dynamics, and even the incidence of harmful algal blooms. It may be that inflows can be manipulated to create refuge habitat for biota trying to escape poor environmental conditions, such as fish populations in lakes during times of toxic *Prymnesium parvum* blooms. Water availability can sometimes be an issue, especially in arid climates, limiting this approach to management. Utilizing source water from deeper depths to displace surface waters, however, might effectively mimic inflow events. We tested this notion by conducting in-lake mesocosm experiments with natural plankton communities where we manipulated hydraulic flushing using deep waters. We found that *P. parvum* cell density was reduced by 69% and ambient toxicity completely ameliorated during pre-bloom conditions in the lake. During conditions of bloom development, population density was reduced by 53%, toxicity by 57%, and bloom proportions were never reached. There was minimal effect of these inflows on total phytoplankton and zooplankton biomass, and little effect on water quality. Shifts towards more rapidly growing phytoplankton taxa were observed, as was enhanced copepod nauplii. In other words, while inflows using deep waters suppressed *P. parvum* bloom initiation and development, they were benign to other aspects of the lower food web and environment, an appealing outcome for a potential management approach for some harmful algal blooms.

## DETECTION OF *KARENIA BREVIS* BLOOMS: A COMPARISON OF MOLECULAR, OPTICAL AND HYBRID DETECTION METHODOLOGIES

Heil, C. A.<sup>1</sup>, Campbell, L.<sup>2</sup>, Doucette, G. P.<sup>3</sup>, Fell, J.<sup>4</sup>, Fries, D.<sup>5</sup>, Haywood, A.<sup>6</sup>, Kirkpatrick, G.<sup>7</sup>, Luther, M.<sup>5</sup>, Millie, D.<sup>8</sup>, Paul, J.<sup>5</sup>, Scholin, C.<sup>9</sup>, Petrik, K.<sup>6</sup>, Gilbert, S.<sup>5</sup>, Ivey, J.<sup>6</sup>, Mikulski, C.M.<sup>3</sup> and Scorzetti, G.<sup>4</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575; cheil@bigelow.org; <sup>2</sup>Texas A&M University, College Station, TX 77843; lcampbell@ocean.tamu.edu; <sup>3</sup>NOAA/National Ocean Service, Charleston, SC 29412; greg.doucette@noaa.gov; tina.mikulski@noaa.gov; <sup>4</sup>University of Miami, Key Biscayne, FL 33149; jfell@rsmas.miami.edu; gscorzetti@rsmas.miami.edu; <sup>5</sup>University of South Florida, St. Petersburg, FL, 33701; dfries@marine.usf.edu; mluther@marine.usf.edu; jpaul@marine.usf.edu; sgilbert@seas.marine.usf.edu; <sup>6</sup>Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL 33701; ahaywood@xnet.co.nz; kim.petrik@myfwc.com; jim.ivey@myfwc.com; <sup>7</sup>Mote Marine Laboratory, Sarasota, FL, 34236; gkirkpat@mote.org; <sup>8</sup>Florida Institute of Oceanography, St. Petersburg, FL, 33701; david.millie@myfwc.com; <sup>9</sup>Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039; scholin@mbari.org

Blooms of the toxic dinoflagellate *Karenia brevis* pose a significant human and environmental health threat in the Gulf of Mexico. Monitoring of *K. brevis* bloom stages remains the most practical method for predicting human health impacts and is accomplished by state agencies via direct microscopic counts of water samples from monitoring programs. A variety of new molecular, optical and hybrid technologies for *K. brevis* detection have been developed in recent years. Few of these techniques have been rigorously tested against samples from monitoring programs, each other, or under realistic sampling conditions however. We evaluated a range of *K. brevis* detection techniques (Carbon Fixation Gene Technology (NASBA), Fluorescence *In Situ* Hybridization (FISH) Assay, High Performance Liquid Chromatography (HPLC), Imaging Flow CytoBot, Luminex Technology, Optical Plankton Detector (OPD, BreveBuster) and Sandwich Hybridization Assay (SHA)) against the current accepted standard technique (i.e. microscopic enumeration to the species level) in laboratory and field settings under Alliance for Coastal Technologies (ACT) oversight. The optimal conditions for use of each technique, relative target specificities and sensitivities, volume requirements and through-put times, effectiveness of each technique compared with direct microscopic counts as well as critical sample collection, handling and preservation effects varied. Recommendations for the optimal uses and application conditions of each technique as well as their potential integration with monitoring programs will be presented and discussed.

## EPIPHYTIC BEHAVIOR OF FOUR SPECIES OF THE DINOFLAGELLATE *GAMBIERDISCUS* ON DIFFERENT MACROALGAE HOSTS FROM THE FLORIDA KEYS

Heine, L.K.; Parsons, M.L.; Leynse, A.

Florida Gulf Coast University, Fort Myers, FL 33965 USA  
lheine@fgcu.edu, mparsons@fgcu.edu, akleyNSE@eagle.fgcu.edu

Ciguatera fish poisoning is caused by toxins produced by dinoflagellates of the genus *Gambierdiscus*. This genus has recently been revised, and new research on the physiology and ecology of the revised species is needed. While it has been demonstrated that *Gambierdiscus* spp. are predominately epiphytic, there is also evidence that they are conditional, not obligate, epiphytes. In addition, there are conflicting results of algal host preferences, where one study reports that a host stimulates *Gambierdiscus* growth, yet another states it inhibits growth. Such differences in epiphytic behavior and host preferences may be attributed to species differences within the *Gambierdiscus* genus. The four *Gambierdiscus* cultures used in this experiment, *G. belizeanus*, *G. carolinianus*, *G. carpenteri*, and *G. caribaeus* are known to be present in the Florida Keys. Three species of macroalgae, *Dictyota* sp., *Acanthophora spicifera*, and *Halimeda* sp., were collected in the Florida Keys. Triplicate macroalgae fragments of 1 g were placed into separate petri dishes with 10 ml of media and approximately 100 *Gambierdiscus* cells were added to each dish. Cells were counted at four, eight, 24, and every 48 hours thereafter and categorized as dead, alive but unattached to host, or alive and attached to host. Attached cells were further qualified as contact with the thallus but no mucus, contact with the thallus within a mucus shroud, and tethered to the thallus via a mucus thread. Preliminary results indicate the four species of *Gambierdiscus* have different epiphytic behaviors among different hosts. Following further investigation, complete results will be presented. The purpose of this experiment is to ascertain the nature of the epiphytic behavior of the four *Gambierdiscus* species (obligate or facultative), an important factor when considering dispersal and connectivity mechanisms that likely play a role in ciguatera flare-ups.

## MARKETING SCIENCE TO THE SOCIAL MEDIA SAVVY

Hendrickson, J.L.<sup>1</sup>, Trainer, V.L.<sup>2</sup>, Trick, C. G<sup>3</sup>, Cochlan, W.P<sup>4</sup>, Wells, M.L.<sup>5</sup>

<sup>1</sup> PhytoFilms, Seattle, WA 98103 USA; <sup>2</sup>NOAA Northwest Fisheries Science Center, Seattle, WA 98112 USA vera.l.trainer@noaa.gov; <sup>3</sup>The University of Western Ontario, London, ON, N6A 5B7 Canada trick@uwo.ca; <sup>4</sup>Romberg Tiburon Center for Environmental Studies, San Francisco State University, Tiburon, CA 94920 USA cochlan@sfsu.edu; <sup>5</sup>University of Maine, Orono, ME, 04469 USA mlwells@maine.edu.

While students and the public are exposed to science in the classroom, museums, and on television, there are limited ways for injecting science into their everyday lives. As alternatives to these traditional pathways, youth and others instead use social media sites, such as facebook, twitter, youtube, etc. as their primary sources of information. News and media spread today most effectively when people share it with one other. It is imperative then that we keep up with the every changing modes of communication, and focus our message so that it is entertaining. We have created a facebook page and twitter profile in association with our current ECOHAB project “Ecology and Oceanography of *Heterosigma akashiwo* in Puget Sound” to distribute information in real-time; an important goal because people like to see things that are happening right now.

In addition to blogging on facebook about daily activities, posting pictures of research and scientist profiles, we created a series of short, digestible videos, some highlighting the different research questions and some just for fun but still relevant to the topic. Our primary goal was not to reach people already interested in the topic (HABS), but rather to incite interest from non-scientific individuals through both entertainment and informative content. A description of the facebook and twitter pages as well as example videos on the site will be presented.

## DEVELOPMENT OF AN AUTOMATED IMAGE CLASSIFIER FOR EARLY WARNING OF *KARENIA BREVIS* BLOOMS

Henrichs, D.W.<sup>1</sup>, Christopher, S.<sup>1</sup>, Sosik, H. M.<sup>2</sup>, Olson, R. J.<sup>2</sup>, and Campbell, L.<sup>1</sup>

<sup>1</sup>Texas A & M University, College Station, TX 77843 USA ; <sup>2</sup> Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA  
dhenrichs@mail.bio.tamu.edu

Monitoring programs for HABs typically rely on manual methods for identification and enumeration of phytoplankton. The time-consuming nature and level of expertise required for manual analysis make it difficult to obtain results with sufficient temporal resolution for early warning. Continuous automated imaging-in-flow by the Imaging FlowCytobot (IFCB) deployed at Port Aransas, TX has provided early warnings of several HABs, via daily inspection of the images by researchers. Here we describe progress in automating this early warning system, utilizing a bloom of *Karenia brevis* in fall 2009 that was first observed via IFCB in mid-August. At the Port Aransas site, *K. brevis* concentrations remained low (24 hr avg:  $< 4$  cells mL<sup>-1</sup>) from early August until mid-September, with the highest observed cell concentrations occurring in mid-October (24 hr avg:  $>70$  cells mL<sup>-1</sup>). During the bloom (though not in real time), the images were automatically classified with a support vector machine and the results evaluated by manual inspection of images. The dataset was divided into three time periods (pre-bloom, bloom, and post-bloom) and statistical corrections calculated to account for false positive cell identifications associated with the automated classifier. Early identification of the presence of *K. brevis* permitted us to provide confirmation to Texas Parks and Wildlife Department and the State Department of Health Services that a bloom was forming and so led to earlier monitoring of shellfish beds. No human illness (neurotoxic shellfish poisoning) resulted from this event. Automated analysis of images produces cell count estimates to which we can apply the previously determined statistical corrections. These corrected cell counts, which can be sent to researchers and managers, will provide near real-time (less than 4 hours) information about the presence of this HAB species that might be missed by routine sampling (typically less frequent) and provide early warning for an impending bloom.

## CHRONIC LOW-DOSE EXPOSURE TO DOMOIC ACID CONTRIBUTES TO ALTERATIONS IN NEURAL HEALTH AND HOMEOSTASIS

Hiolski, E.<sup>1</sup>, Frame, E.<sup>2</sup>, Kendrick, P.<sup>2</sup>, Beyer, R.<sup>3</sup>, Bammler, T.<sup>3</sup>, Farin, F.<sup>3</sup>, Smith, D.R.<sup>1</sup>, Lefebvre, K.<sup>2</sup>

<sup>1</sup>Department of Microbiology & Environmental Toxicology, University of California, Santa Cruz, CA 95064 USA ehiolski@ucsc.edu; <sup>2</sup>NOAA Fisheries, Northwest Fisheries Science Center, Seattle, WA 98112 USA; <sup>3</sup>Department of Environmental and Occupational Health Sciences, School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98105 USA

The excitotoxic effects of acute exposure to the neurotoxin domoic acid (DA) are well-known; however, the neurological consequences of low-dose exposures lacking overt symptoms remain poorly defined. It is important to elucidate the neurological consequences of chronic, low-dose exposure in order to identify human health risks that may be currently undetected. Our previous research (Lefebvre *et al.*, 2009) has shown that a single low dose of DA in zebrafish (*Danio rerio*) led to significant transcriptional changes in the absence of overt symptoms (e.g., altered swimming behavior). Here, we greatly extend these observations through analyses of transcriptional changes via microarray in zebrafish chronically exposed to a low, asymptomatic dose of DA. We hypothesize that chronic low-dose exposure may induce transcriptional changes indicative of oxidative stress, neuroinflammation, neural stress, and altered neuronal growth and nervous system development.

Zebrafish were exposed via ip injection to either a low dose of DA ( $\ll EC_{50}$ ) or vehicle (PBS) every other week over the course of 9 months. Microarray analysis was conducted on whole brains following mRNA extraction at the conclusion of the exposure series. Chronic low-dose exposure significantly altered expression of 58 genes ( $\pm 1.5$ -fold,  $p \leq 0.05$ ), including *sqstm1*, which is implicated in the activation of NF- $\kappa$ B, a transcription factor involved with immune response, and *neol*, involved in axon guidance and netrin signaling, processes that are re-activated in response to neural injury. Other biological pathways heavily impacted include response to oxidative stress and neurotransmitter transport. These data indicate that chronic low-dose exposure to DA contributes to significant genetic alterations that may have implications for neural health and homeostasis.

## LABORATORY EVALUATION AND STANDARDIZATION OF THE MOUSE NEUROBLASTOMA (N2A) CYTOTOXICITY ASSAY FOR ASSESSMENT OF CARIBBEAN CIGUATOXINS IN FISH MATRICES

Hooe-Rollman, J.I., Garcia, A.C., Jester, E.L.E., and Robertson, A.  
FDA, Gulf Coast Seafood Laboratory, Dauphin Island, AL 36528 USA

The mouse neuroblastoma (N2a) cytotoxicity assay is a cell-based microplate method, first developed by Kogure et al (1988) for detection of paralytic shellfish toxins. Since then, it has been modified and used for the detection of a variety of marine toxins including sodium channel activators (e.g., PBTXs, CTXs), sodium channel blockers (e.g., STX, TTX) and toxins with non-specific effects (e.g., OA) (Manger et al, 1993). This method has been used in our laboratory for approximately 15 years as a screening tool for the detection of ciguatoxins in fish for research and outbreak analyses. Caribbean ciguatoxins account for an estimated 3,500 human illnesses per year in the US, with the highest incidences seen in the US Virgin Islands. Increasing reports of fish contaminated with ciguatoxins has been recently observed in the Northern Gulf of Mexico.

The focus of this research was to provide an in-depth evaluation of the N2a assay to develop a standard operating procedure for technology transfer to other laboratories. We have identified several factors that are critical for the successful application of this method as a semi-quantitative tool for CTX detection. These include cell specific variables, dosing regimens, and general technique. We have determined optimal concentrations of ouabain/veratridine (O/V) for primary treatment of cell stocks and cell dosing, optimal cell density ranges for plate preparation and passage procedures, and characterized cell passage effects on sensitivity and response. Evaluation of robustness included manipulation of buffer constituents, incubation times, and media components. Reproducibility and repeatability studies (within - and between - day) were performed using extracts from barracuda caught in the U.S. Virgin Islands with various toxicity levels (i.e. 0.5, 1.2 and 2.4 ng CCTx-1 equiv/g). Primary O/V treatment of cell stocks greatly affected the sensitivity and performance of the assay and was a critical factor in cell batch comparison. Likewise, seeding levels and cell passage confluency (70-85%) were key to reproducibility. Coefficients of variation were in the range of 10-20%. Data detailing the passage specific effects, including receptor desensitization and O/V response, will be presented. This study represents part of larger laboratory evaluation moving towards a multiple laboratory validation.

K. Kogure, M.L. Tamplin, U. Simidu and R.R. Colwell, A tissue culture assay for tetrodotoxin, saxitoxin and related toxins, *Toxicon* **26** (1988) (2), pp. 191–197

Manger, R.L., Leja, L.S., Lee, S.Y., Hungerford, J.M., Hokama, Y., Dickey, R.W., Granade, H.R., Lewis, R.J., Yasumoto, T., Wekell, M.M., 1995. Detection of sodium channel effectors: directed cytotoxicity assays of purified ciguatoxins, brevetoxins, saxitoxin and seafood extracts. *J. AOAC Int.* **78**, 521–527.

THE SOUTHERN CALIFORNIA BIGHT (SCB) REGIONAL MONITORING PROGRAM: A *PSEUDO-NITZSCHIA* SPP. BUST IN A BLOOM-READY SCB

Howard, M.D.A.<sup>1</sup>, Caron, D.A.<sup>2</sup>, Chao, Y.<sup>3</sup>, Jones, B.H.<sup>2</sup>, Nezlin, N.<sup>1</sup>, Robertson, G.<sup>4</sup>, Schnetzer, A.<sup>2</sup>, Seegers, B.<sup>2</sup>, Senqupta, A.<sup>1</sup>, Seubert, E.<sup>2</sup>, Sutula, M.<sup>1</sup>

<sup>1</sup>Southern California Coastal Water Research Project, Costa Mesa, CA 92626 USA, mhoward@sccwrp.org, nikolayn@sccwrp.org, ashmitas@sccwrp.org, marthas@sccwrp.org; <sup>2</sup>University of Southern California, Los Angeles, CA 90089-0371, dcaron@usc.edu, bjones@usc.edu, astrids@usc.edu, seegers@usc.edu, seubert@usc.edu; <sup>3</sup>Jet Propulsion Laboratory, Pasadena, CA 91109 Yi.Chao@jpl.nasa.gov; <sup>4</sup>Orange County Sanitation District, Fountain Valley, CA 92728, grobertson@ocsd.com

The Southern California Bight (SCB) Regional Monitoring Program focuses on assessing the ecological condition of the Southern California coastal waters and oceanic and terrestrial factors that influence that condition. The Bight Regional Monitoring Program (RMP) is conducted in cooperation with over 70 organizations once every five years. The objectives of the 2008 cycle of the Bight RMP Offshore Water Quality study were to quantify the major nutrient sources to the Southern California Bight (e.g. upwelling, wastewater effluent, riverine inputs and atmospheric deposition) and to characterize the factors that influence the occurrence of *Pseudo-nitzschia* spp. blooms

The nitrogen and phosphorus loads of the four major nutrient sources to the SCB were quantitatively estimated. Riverine inputs were estimated via a combination of empirical measurements and modeling of coastal runoff and upwelling estimates were quantified using dynamic simulation modeling. Direct atmospheric deposition to Bight surface waters was estimated using existing National Atmospheric Deposition Program (NADP) wet deposition data and limited empirical dry deposition estimates at the coast. In addition to the nutrient loads, discrete samples (CTD casts, chlorophyll *a*, particulate domoic acid, dissolved inorganic nutrients) were collected in the spring of 2010. The nutrient loads were used to assess the timing and magnitude of nutrient delivery to the coastal ocean relative to remotely sensed and discrete field observations of the spring diatom bloom in the SCB during 5 sampling events in February – May 2010. The oceanographic conditions were monitored using satellite imagery, weekly pier-based phytoplankton samples, ocean forecasting and deployment of Webb Slocum gliders, to determine the optimal timing for ship-based sampling.

The Bight Regional Monitoring Program is the largest spatial HAB dataset collected in California as it covers most of the California coastline from Santa Barbara to San Diego. We will present the relative magnitude of nutrient loads to the SCB and highlight the importance of multi-disciplinary studies in understanding the complexity of controls on HAB occurrence.

*PSEUDO-NITZSCHIA* SPECIES DIVERSITY AND DOMOIC ACID TOXICITY IN THE MAINE COASTAL CURRENT SYSTEM DURING TWO HYDROGRAPHICALLY DISTINCT SUMMERS (2008 AND 2010)

Hubbard, K.A.<sup>1</sup>, Ellis, C.H.<sup>2</sup>, Fernandes, L.F.<sup>3</sup>, Richlen, M.<sup>1</sup>, McGillicuddy, D.J.<sup>1</sup>, Armbrust, E.V.<sup>2</sup>, and Anderson, D.M.<sup>1</sup>

<sup>1</sup>Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA, khubbard@whoi.edu; <sup>2</sup>University of Washington, Seattle WA 98195 USA; <sup>3</sup>Universidade Federal do Paraná, Brasil

Species in the marine diatom genus *Pseudo-nitzschia* are commonly observed in the Gulf of Maine (GOM), and a few GOM species have been shown to produce high levels of the neurotoxin domoic acid (DA) in culture. *Pseudo-nitzschia* species composition and DA toxicity in shellfish are not yet routinely monitored on Georges Bank or in nearshore GOM waters. To move toward a greater understanding of bloom dynamics in the region, samples for characterization of *Pseudo-nitzschia* diversity and particulate DA concentrations were collected during monthly large-scale hydrographic surveys from May to August in 2008 and 2010. A rapid and high-throughput DNA fingerprinting method, automated ribosomal intergenic spacer analysis (ARISA), was combined with environmental sequencing to provide semi-quantitative descriptions of *Pseudo-nitzschia* species distributions. Species were present in each of 134 environmental samples collected in 2008, with nearshore GOM communities primarily dominated by the nontoxic species *P. delicatissima*. Further offshore at Georges Bank, more spatially and temporally diverse assemblages were populated by up to eleven *Pseudo-nitzschia* species. Patterns of species co-existence at Georges Bank were distinct in different months, and among hydrographically distinct water masses during the same cruise. The toxigenic species *P. seriata* dominated *Pseudo-nitzschia* communities in flanking regions of Georges Bank earlier in the summer, and at the crest (concurrent with a toxigenic member of the *P. pseudodelicatissima* complex that can be distinguished with ARISA) by mid-summer, when warmer temperatures and higher overall *Pseudo-nitzschia* diversity were observed. An enzyme-linked immunosorbent assay (ELISA) was used to measure particulate DA concentrations and was positive at two Georges Bank stations dominated by *P. seriata*, with concentrations reaching  $816.8 \pm 123.5$  ng L<sup>-1</sup> and  $460.9 \pm 162.9$  ng/L during June and July, respectively. We are currently analyzing community and toxicity data from summer 2010, when hydrographic conditions were significantly different from 2008. A newly developed fluorescent in-situ hybridization (FISH) probe shows promising results for distinguishing *P. seriata* cells from other GOM species, and we are in the process of determining its utility for enumeration of this toxic species in field samples. Results from both 2008 and 2010 field surveys will be useful for assessing the potential threat of DA toxicity in the GOM, and will provide insight into regional associations of *Pseudo-nitzschia* species with environmental conditions, DA production, and hydrographic features.

A SYSTEMS APPROACH TO FRESHWATER HAB MANAGEMENT:  
ECOLOGICALLY-BASED, WITHIN-WATER-BODY MANAGEMENT  
COMPLEMENTS WATERSHED MANAGEMENT

Hudnell, H.K.

University of North Carolina at Chapel Hill, Department of Environmental Sciences & Engineering, 105 Serrano Way, Chapel Hill, NC 27517 USA [kenhud@SolarBee.com](mailto:kenhud@SolarBee.com), [khudnell@nc.rr.com](mailto:khudnell@nc.rr.com)

Will we have a sustainable supply of useable freshwater? U.S. EPA data indicate that freshwater quality is rapidly declining. Whereas the Agency estimated in 1972 that 10-20% of lakes and reservoirs were impaired due to eutrophication, approximately 50% are now eutrophic or hypereutrophic. These data indicate that watershed-management and soil-conservation policies are insufficient for preventing and reversing eutrophication.

A complementary policy of within-water-body management is needed to address the primary stressor of eutrophication, freshwater harmful algal blooms (FHABs), and to reduce nutrient levels in the near term. An impaired water body is analogous to an ill person in need of supportive therapy to reduce stress on biochemical processes. Eutrophic water bodies need enhanced circulation, such as solar-powered, long-distance circulation, to suppress FHABs. The suppression of FHABs enables nutrients to ascend the trophic levels of the food web. Additional nutrient reduction, if needed to reduce downstream eutrophication, can be achieved using other tools such as floating islands and side-stream flowways for chlorophyte culture, harvest and reuse. Satellite monitoring of FHAB intensities and phosphorus concentrations in the past and into the future assists treatment-plan development and results evaluation.

U.S. regulations require prioritization of Clean Water Act Section 303(d) impaired waters for development of total-maximum-daily loads (TMDLs). Water bodies can be delisted from Section 303(d) when TMDLs are developed or when they exhibit water quality improvements sufficient for meeting water quality standards. Successful application of within-water-body management obviates the need to develop TMDLs. Less rigorous applications of watershed management reduce the overall cost of freshwater management. A systems-approach to freshwater management combines within-water-body management for near-term improvement in water quality with the long-term approach of watershed management that ultimately may decrease the need for the supportive therapy provided by within-water-body management. This systems-approach to freshwater management can reverse the trend of increasing eutrophication and FHAB occurrence, and ensure a sustainable supply of useable freshwater.

MONITORING OREGON'S COASTAL HARMFUL ALGAE:  
LESSONS LEARNED THROUGH A MANAGERS PERSPECTIVE

Hunter, M.V.<sup>1</sup>, White, A.<sup>2</sup>, Wood, A.M.<sup>3</sup>, Peterson, W.T.<sup>4</sup>

<sup>1</sup>Oregon Department of Fish and Wildlife Astoria, OR 97103 USA, Matthew.V.Hunter@state.or.us; <sup>2</sup>Oregon State University Corvallis, OR 97330 USA, awhite@coas.oregonstate.edu; <sup>3</sup>University of Oregon Eugene, OR 97401 USA, m.michellewood@gmail.com; <sup>4</sup>NOAA Fisheries Newport, OR 97395 USA Bill.Peterson@noaa.gov

MOCHA; monitoring Oregon's coastal harmful algae formed in 2007 after receiving a MERHAB grant. This state wide partnership includes principle investigators from Oregon State University (OSU), University of Oregon (UO), Oregon Department of Fish and Wildlife (ODFW) and Newport NOAA Fisheries. The project is designed to provide the scientific data needed to understand the ecological mechanisms underlying the occurrence of HABS in Oregon. By partnering, we are able to apply a ecosystem-based approach to HAB monitoring and event response in Oregon. The knowledge gained from the combination of these data resources has significant management implications. Specifically, this project aims to allow resource managers to determine the essential components of rapid event response programs for Oregon.

With the last year of the MOCHA project beginning, we have been trying to determine aspects of the project that should be continued, then identify funding sources and the means to access them. In this process, MOCHA PIs have had to justify the necessity of a continuation of the program. In doing so, ODFW has determined what aspects of a monitoring program have worked and those that have not. This continued project has, unfortunately, not been seen as a necessity to the state of Oregon especially in these tough economic times. Hopefully, with the toughest economic times behind, renewed efforts can continue the program in the future.

## APPARENT LIPOPHILIC TOXINS FROM A *MICROCYSTIS* STRAIN ISOLATED FROM THE GREAT LAKES

Jaja, A.D., Berry, J.P.

Department of Chemistry and Biochemistry, Florida International University, North Miami, FL 33181 USA [ajaja001@fiu.edu](mailto:ajaja001@fiu.edu), [berryj@fiu.edu](mailto:berryj@fiu.edu)

There have been increasing reports of blooms of *Microcystis*, along with associated cyanobacterial toxins, in the Great Lakes. As part of on-going studies, we evaluated lipophilic metabolites from a *Microcystis* strain isolated from Lake Ontario, specifically using the zebrafish (*Danio rerio*) embryo as a model of vertebrate toxicity. Lipophilic extracts and subsequent fractions were found to inhibit or impair development in the zebrafish model. Moreover, toxicity was found to not seemingly correlate with any recognized, water-soluble toxins (e.g. microcystins, anatoxin-a, cylindrospermopsin, BMAA) previously described for the genus or other cyanoHABs. This finding suggests that apparent lipophilic toxins might represent a previously unidentified component of HABs in the Great Lakes. The details of the isolation and characterization of these metabolites, along with possible relevance to human and environmental health in the Great Lakes, will be presented.

## CHARACTERIZATION OF CELL DEATH PROCESSES IN THE TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE* IN RESPONSE TO ABIOTIC STRESS

Jauzein, C.<sup>1</sup>, Erdner, D.L.<sup>1</sup>

<sup>1</sup>University of Texas Marine Science Institute, Port Aransas, TX 78373 USA, [cjauzein@yahoo.fr](mailto:cjauzein@yahoo.fr); [derdner@mail.utexas.edu](mailto:derdner@mail.utexas.edu)

While most of the laboratory and field studies dealing with Harmful Algal Blooms (HABs) have focused on environmental and physiological factors controlling phytoplankton growth, we still know relatively little about the causes of bloom decline and termination. Among the potential sources of mortality, the occurrence of autocatalytic cell death processes in unicellular algae has received increasing attention. We conducted experiments to characterize cell death processes in the toxic dinoflagellate *Alexandrium tamarense* in response to abiotic stress, using a suite of molecular and biochemical markers of cell death. Intracellular ROS/RNS production was followed using a fluorescent dye (Carboxy-H<sub>2</sub>DCFDA). Involvement of metacaspase genes and proteolytic activities was measured using quantitative PCR and caspase-like enzymatic assays. Cell membrane status was assessed with respect to phosphatidylserine inversion (Annexin labeling) and membrane permeabilization (Sytox labeling). Photosynthetic function was estimated from  $F_v/F_m$  ratios and measurements of chlorophyll *a* concentrations. Additionally, calcofluor labeling was used to follow the ecdysis process and estimate the production of temporary cysts.

A preliminary experiment based on oxidative stress of *A. tamarense* cells revealed a process that departs from the descriptions of “classical” autocatalytic or Programmed Cell Death (PCD): graded concentrations of H<sub>2</sub>O<sub>2</sub> did not induce caspase-like enzyme activities, but a fast response in terms of ecdysis occurred and probably interfered in the detection of phosphatidylserine inversion. Two abiotic stresses were tested, nitrogen limitation and heat shock, and showed specific cascades of events during death of *A. tamarense* cells. In the case of nitrogen stress, a slight increase of caspase 3-like enzyme activity was observed when the chlorophyll *a* cell content dropped, but no specific accumulation of ROS occurred. Exposure to high temperature induced a strong production of ROS followed by ecdysis, but no activation of caspase-like enzyme activity. These results indicate that there is not a single characteristic cell death process in *Alexandrium* when exposed to abiotic stressors.

## PRELIMINARY STUDIES OF A *KARENIA BREVIS* ENDOSYMBIONT/ PARASITE

Jayroe, D.S., McLean, T.I.

The University of Southern Mississippi, Hattiesburg, MS 39406 USA  
David.Jayroe@eagles.usm.edu, timothy.mclean@usm.edu

*Karenia brevis* is a toxic marine dinoflagellate that causes harmful algal blooms (HABs), also known as red tides, in the Gulf of Mexico. These blooms are responsible for massive fish kills, shellfish bed contaminations, adverse human health effects, and vast economic loss. For these reasons, extensive research has gone into understanding the mechanisms and dynamics of bloom behavior by studying *K. brevis* in the field and in the lab. From manipulations of our in-lab cultures we have detected 1) aberrant rRNA bands on BioAnalyzer (Agilent, Inc.) and 2) the presence of small, photosynthetic, potentially flagellated cells inside and exiting *K. brevis* cells in culture. We have tentatively concluded that we discovered an unknown intracellular organism in *K. brevis*, which is capable of leaving the cell. Occasionally a bubble is formed whilst the intracellular organisms are leaving the *K. brevis* cell. Intracellular parasites are common in photosynthetic dinoflagellates although only a single parasitic species has been previously identified in *K. brevis*. To date, efforts to characterize the organism using microscopy have been inconclusive, but they are ongoing. Currently we are using molecular strategies to isolate and clone transcripts, e.g. rRNA, from our unknown organism. It is our hope that sequence information will allow us to identify the organism at some taxonomic level. If the intracellular organism is indeed a parasite that can (exit/disrupt the cell), it could potentially be used as a biocontrol agent to mitigate or terminate *K. brevis* blooms.

EVALUATION OF A COMMERCIAL ELISA KIT FOR BREVETOXINS IN NATURALLY EXPOSED OYSTER (*CRASSOSTREA VIRGINICA*) AND CLAM (*MERCENARIA SP.*) COLLECTED DURING A *KARENIA BREVIS* BLOOM

Jester, E.L.E., Abraham, A., El Said, K.R., Plakas, S.M.

FDA, Gulf Coast Seafood Laboratory, Dauphin Island, AL USA  
Edward.Jester@fda.hhs.gov

Brevetoxins (BTXs) belong to a class of cyclic polyether toxins produced by marine dinoflagellate *Karenia brevis* that are accumulated in filter feeding shellfish (oyster and clam) during blooms. Shellfish toxicity has traditionally been monitored by mouse bioassay, although there has recently been a push for alternative analytical methods and rapid assays, such as LC/MS-MS and ELISA. We evaluated a commercial ELISA kit for brevetoxins in fortified and naturally incurred oyster and clam. Incurred shellfish were collected during a *K. brevis* bloom. ELISA data was compared to that of LC-MS for selected brevetoxin metabolites. Metabolites of BTX-2 (cysteine and cysteine sulfoxide conjugates) were chosen for oysters. BTX-B2, deoxy-BTX-B2, BTX-B1, BTX-B5, and open A-ring BTX-B5 were selected as brevetoxin biomarkers in clams.

ELISA standard curves were generated from BTX-3 standards (0.01-2 ppb) supplied with the kit and with certified reference material. Overall repeatability (RSD) of multiple standard curves (n=14) generated using kit standards was 4%. Overall repeatability of the ELISA for oyster and clam fortified at 0.4, 0.8 and 1.6 ppm using certified reference materials was 18% and 32%, respectively. Recoveries of BTX-3 from fortified oyster and clam at these levels were 72-96% and 43-60%, respectively, calculated from reference material standard curves. Incurred samples (n=9) were selected to bracket the mouse bioassay regulatory level (20 mouse units/100 g), and analyzed in triplicate over three days by ELISA. Between-day repeatability for incurred residues at 1.0-12.9 ppm levels in oyster and clam ranged from 6.3 to 35.3%. ELISA data correlated well with those of the summed biomarkers by LC/MS.

NORTHERN ANCHOVIES (*ENGRAULIS MORDAX*) WERE NOT IMPACTED BY EXPOSURE TO NATURAL BLOOMS OF TOXIC *PSEUDO-NITZSCHIA*

Jester, R.<sup>1</sup>, Baugh, K.<sup>2</sup>, Adams, N.<sup>2</sup>, Plesha, P.<sup>3</sup>, Moore, S.<sup>2</sup>, Odell, A.<sup>4</sup>, Herndon, J.<sup>5</sup>, Cochlan, W.<sup>5</sup>, Foy, M.<sup>6</sup>, Frame, E.<sup>2</sup>, Lefebvre, K.<sup>2</sup>

<sup>1</sup>Edison State College, 8099 College Parkway, Fort Myers, FL 33908 USA [rjester@edison.edu](mailto:rjester@edison.edu); <sup>2</sup>NOAA Fisheries, Northwest Fisheries Science Center, 2725 Montlake Blvd. East, Seattle, WA 98112 USA; <sup>3</sup>NOAA Fisheries, Northwest Fisheries Science Center, 10 Park Ave., Bldg. 8, Mukilteo, WA 98275 USA; <sup>4</sup>University of Washington, Olympic Natural Resources Center, 113 W. Chenault St., Hoquiam, WA 98550 USA; <sup>5</sup>Romberg Tiburon Center for Environmental Studies, San Francisco State University, Tiburon, CA 94920 USA; <sup>6</sup>University of Washington, School of Oceanography, Box 357940, Seattle, WA 98195 USA

The production of domoic acid (DA) by *Pseudo-nitzschia* spp. and its subsequent transfer through marine food webs can have severe consequences for higher trophic levels. In seabirds and mammals the neurotoxic effects resulting from DA exposure via planktivorous fish can be obvious and severe. Despite the detection of high concentrations of DA in the guts of vector fish species, there has been no documented evidence of neurotoxic symptoms in fish during toxic blooms. The primary goal of this project was to determine if exposure to a naturally-occurring toxic *Pseudo-nitzschia* bloom would induce behaviors symptomatic of DA poisoning in planktivorous anchovies (*Engraulis mordax*). The study was performed off the coast of Washington and Canada onboard the R/V Melville in collaboration with ECOHAB-PNW in September 2005. Live anchovies were brought onboard the vessel and exposed to natural blooms of *Pseudo-nitzschia* spp. over a 10 day period. Fish were observed daily for behavioral symptoms and sampled every 24-48 hrs for DA analysis. Exposed fish were compared to control fish maintained in filtered seawater and a thorough analysis of biotic and abiotic factors were measured to characterize exposure conditions. After the 10-day exposure experiment, a 24-hour grazing experiment confirmed observations that anchovies were feeding on natural algal communities under the experimental conditions.

During the experiment, anchovies were exposed to *Pseudo-nitzschia* cell densities as high as  $1.1 \times 10^6$  cells/L (avg =  $5.2 \times 10^5 \pm 2.6 \times 10^5$  cells/L). Though cell densities were notably high and fish were grazing, the concentration of DA measured in fish tissues was low (viscera max = 2966 ng/g, muscle = ND) due to the low concentrations of pDA in the exposure tanks (max = 575 ng/L). No neurotoxic symptoms were observed in exposed anchovies. Although these results are inconclusive due to the low levels of DA produced by the bloom, subsequent gavage experiments and field research by Lefebvre et al. (review submitted) support the conclusion that fish do not exhibit neurotoxic effects after exposure to DA during blooms. This project was funded by an Oceans and Human Health grant from the Northwest Fisheries Science Center.

PUTATIVE CASPASE ENZYMES, ACTIVITY, AND DOWNSTREAM DEATH SUBSTRATES ASSOCIATED WITH CHRONOLOGICAL AGING IN THE DINOFLAGELLATE *KARENIA BREVIS*.

Johnson, J.G.<sup>1,2</sup> and Van Dolah, F.M.<sup>1,2</sup>

<sup>1</sup>NOAA Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC 29412 USA fran.vandolah@noaa.gov; <sup>2</sup>Marine Biomedicine and Environmental Sciences Program, Medical University of South Carolina, Charleston, SC 29412 USA jill.johnson821@gmail.com

The molecular mechanisms that enable cell survival in high density, low growth blooms, and the mechanisms leading to often rapid bloom demise in the toxic dinoflagellate, *Karenia brevis*, remain unresolved. However, caspases (cysteine aspartic acid proteases), key metazoan enzymes involved in potentiating signals through the programmed cell death pathway, have been previously implicated in regulating aging, stress acclimation, and cell demise in other unicellular organisms, including various phytoplankton species. The transcriptomic context for chronological aging, the process a cell undergoes to survive in a sub-optimal, non-dividing state, has provided evidence that a major reorganization in metabolic and signaling requirements is associated with cell survival strategies in stationary phase growth of *K. brevis*. This extensive shift has provided a framework for directing studies towards understanding the regulation associated with aging/death processes.

In the current study, a combination of biochemical cleavage analysis and *in silico* EST sequence data mining was employed to explore the role of caspase activity in the chronological aging process and identify the upstream and downstream pathway constituents of caspase-specific activities. To gain an understanding of the presence, timing, and magnitude of *K. brevis* caspase activities during chronological aging, caspase activities (caspase 1, 3, 6, 7, 8, and 9) were quantified over the growth curve. An induction of caspase activity was observed at the transition into and maintenance of stationary phase suggesting an active role for caspase enzymes in mediating the shift into quiescence, as opposed to during cell death since caspase activities decreased by the end of late stationary phase and culture demise was not observed. Targeted *in silico* bioinformatic mining for enzymes potentially responsible for the activities observed revealed general peptidase\_C14 caspase catalytic p20 domain containing proteins, subtilisins, and vacuolar processing enzymes. The enzyme activities identified by this approach are not consistent with metacaspases, which possess different cleavage specificity. Computational prediction of downstream target substrates using substrate sequence context and predicted secondary structure parameters identified proteins involved in a wide range of biological processes including regulation of protein turnover, cell cycle progression, lipid metabolism, coenzyme metabolism, apoptotic death, and autophagic death, lending new insight into the diversity of processes involved in the modulation of aging and execution of death in dinoflagellates.

## ASSESSMENT OF THE EASTERN GULF OF MEXICO HARMFUL ALGAL BLOOM OPERATIONAL FORECAST SYSTEM: A COMPARATIVE ANALYSIS OF FORECAST SKILL AND UTILIZATION, 2004-2008

Kavanaugh, K.<sup>1</sup>, Derner, K.<sup>2</sup>, and Fisher, K.<sup>1</sup>

<sup>1</sup>NOAA, National Ocean Service, Center for Operational Oceanographic Products and Services (CO-OPS), Silver Spring, MD 20910 USA Karen.Kavanaugh@noaa.gov, Kathleen.Fisher@noaa.gov; <sup>2</sup>NOAA, National Ocean Service, CO-OPS, 672 Independence Parkway, Chesapeake, VA 23320 USA Katie.Derner@noaa.gov

Blooms of the toxic dinoflagellate, *Karenia brevis*, occur nearly every year in coastal regions of the Gulf of Mexico causing potential impacts on public health, ecosystems, and regional economies. To aid early bloom identification and response efforts, in 2004 NOAA transitioned a successful demonstration forecast system for harmful algal blooms (HABs) from research to operational status with coverage along the Gulf coast of Florida. NOAA's Gulf of Mexico HAB Operational Forecast System (HAB-OFS) issues weekly bulletins that serve as decision support tools for coastal resource managers, federal and state agencies, and academic institutions. In order to continually improve the HAB-OFS, bulletin utilization and forecast skill are evaluated regularly. Utilization is measured by user responses and observed or reported mitigation actions based on bulletin information. Forecast skill, or the relative accuracy of the bulletin forecasts, is calculated for each of the following forecast components: bloom transport, changes in the spatial extent of blooms, bloom intensification, and the daily potential for respiratory impacts along the coast. In order to evaluate the development of the HAB-OFS since the first operational bulletin was issued, this comparative analysis builds upon previously presented assessment data, encompassing 398 total bulletins issued for the eastern Gulf of Mexico from October 2004 through April 2008. Of those, 328 bulletins were issued during a total of 14 confirmed HAB events. During this period, utilization was confirmed for over 70% of the bulletins. Preliminary analysis shows that forecast skill each year was highest for respiratory impact forecasts, but skill for each forecast component was high with greater than 70% confirmed correct. With the exception of spatial extent forecasts, Heidke skill scores ranged between >.30 to 1, indicating a >30% to 100% improvement in forecast accuracy when compared to random chance. Heidke skill scores for the extent forecast component were low (0) the first year, but demonstrated improvement since then (>.40). The results also highlighted the special challenges of assessing respiratory impact forecasts, which are reliant on daily reports of field observations; between 45-80% of impact forecasts could not be assessed due to lack of information. The success of the HAB-OFS in the eastern Gulf of Mexico and the lessons learned will be applied to HAB forecasts for the western Gulf of Mexico (Texas), which became operational in 2010.

## EXPERIENCE ALGAL TOXINS: EDUCATIONAL WORKSHOPS AND INTERNSHIP OPPORTUNITIES FOR TRIBAL STUDENTS IN COASTAL WASHINGTON

Kendrick, P.S.<sup>1</sup>, Frame, E.R.<sup>1</sup>, Lefebvre, K.A.<sup>1</sup>

<sup>1</sup>NOAA, Northwest Fisheries Science Center, Seattle, WA 98112 USA  
Preston.Kendrick@noaa.gov

On the Washington Coast, fish and shellfish are an important subsistence food for many Pacific Northwest tribes. Blooms of the toxic algae *Pseudo-nitzschia* off the Washington coast can produce dangerous levels of the neurotoxin domoic acid. At high enough concentrations this toxin makes seafood unsafe to eat, results in beach closures, and devastates tribal economies.

As leaders in algal toxin research on the West Coast, WARRN-West (**W**ildlife and **A**lgal-toxin **R**esearch and **R**esponse **N**etwork) at the Northwest Fisheries Science Center (NWFSC) partnered with the Northwest Indian College (NWIC) in Bellingham, WA to offer an interactive, educational experience and provided hands-on training in seafood safety monitoring through the Experience Algal Toxins (EAT) 2010 workshop. The workshop consisted of lecture material, laboratory demonstrations, and hands-on training in the use of advanced technology to measure algal toxin levels in locally collected shellfish.

Building on the success of the EAT 2010 workshop, and in an effort to reach a new suite of undergraduate students at the NWIC, WARRN-West developed the Experience Algal Toxins 2011 workshop. In addition to hands-on training, videotaped segments of the EAT 2011 workshop will be posted on the National Marine Fisheries Service's (NMFS) education website to ensure a broader distribution and long-term accessibility for students in future NWIC science courses.

A summer internship program was established at the NWFSC's Montlake Laboratory for advanced undergraduate tribal students. This program allows interns to pursue independent research projects related to the NWFSC's algal toxin research program, participate in the WARRN-West program's marine mammal monitoring network, and learn about NOAA careers through mentoring with NWFSC scientists.

This collaboration has greatly contributed to jumpstarting the NWIC's new environmental science degree program with NMFS educational resources. The summer internship program allows tribal students the novel opportunity for hands-on science training with NMFS scientists and exposure to NMFS careers. These projects provide students with an advanced scientific understanding of the toxic effects of harmful algal blooms and will potentially meet the critical need to better monitor seafood safety and protect human health in tribal communities on the Washington coast.

# FEASIBILITY OF RAPID, AUTOMATED ASSESSMENT OF PHYTOPLANKTON ABUNDANCE, BIOMASS AND GROWTH RATE: COULTER COUNTER VS. LIGHT MICROSCOPE MEASUREMENTS

Kim, H.W., Burke, A. and Menden-Deuer, S.

Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882 USA [hyewon\\_kim@my.uri.edu](mailto:hyewon_kim@my.uri.edu), [aburke@gso.uri.edu](mailto:aburke@gso.uri.edu), [smenden@gso.uri.edu](mailto:smenden@gso.uri.edu)

Microscopic assessment of phytoplankton concentration, biomass and growth rates require considerable expertise and time. The goal of our study was to determine how accurately a Coulter counter estimates the abundance, size, growth rates of 16 monospecific phytoplankton cultures including 3 harmful algal bloom causing species (*Heterosigma akashiwo*, *Lingulodinium polyedrum*, *Alexandrium spp*). Linear regression showed good agreement (average  $r^2=0.92$ ) between Coulter counter and microscope counts for 9 species. Coulter counter abundances were underestimated for *H.triquetra* (729%), *S.costatum* (266%) and overestimated for *S.trochoidea* (71%) and *I.galbana* (59%). Volume measurement with the Coulter counter corresponded well to those from the microscope, although most volumes were overestimated by the Coulter counter (average 65%). We also observed significant volume variations between Coulter counter volume and microscope volume for each species over growth phase ( $p=0.00<0.05$ , 2 factor nested ANOVA). Growth rate estimates were in excellent agreement ( $p>0.001$ , paired t-test), suggesting either method estimates abundance consistently over time and is well suited to capture relative changes in abundance. For both biomass and growth rate, HAB species were particularly well estimated ( $b=1.00$ , microscope, CC growth rate  $0.33\text{ d}^{-1}$ ,  $0.27\text{ d}^{-1}$  for *H.akashiwo (a)*,  $b=1.04$ , microscope, CC growth rate  $0.39\text{ d}^{-1}$ ,  $0.40\text{ d}^{-1}$  for *H.akashiwo (b)*) and *L.polyedrum* ( $b=0.95$ , microscope, CC growth rate  $0.15\text{ d}^{-1}$ ,  $0.15\text{ d}^{-1}$ ) at which  $b$  is the slope (microscope count/CC count) of regression equation. Based on these comparison we found conditions under which microscope is preferable or the Coulter counter is preferable, providing considerable savings on analysis time and greatly increase accuracy through increased sample size. We were also able to identify the best estimation obtained for two HAB causing species, suggesting the Coulter counter as a powerful, accurate semi-automated counting tool for detecting, investigating harmful algal blooms formed by those two species.

## THE DISTINCTION OF PHYTOPLANKTON COMMUNITY STRUCTURE BETWEEN FOUR DIFFERENT *KARENIA BREVIS* BLOOMS.

Kirkpatrick, G.J.<sup>1</sup>, Dixon, L.K.<sup>1</sup>, Hall, E.R.<sup>1</sup>, Boyes, A.J.<sup>1</sup> and Pederson, B.A.<sup>2</sup>

<sup>1</sup>Mote Marine Laboratory, Sarasota, FL 34236 USA gkirkpat@mote.org, lkdixon@mote.org, emily8@mote.org, anamari@mote.org; <sup>2</sup>Integrated Systems, Inc., Darlington, SC 29540 USA Brad@isirobots.com

The ECOHAB regional project, ‘ECOHAB: *Karenia* Nutrient Dynamics in the Eastern Gulf of Mexico’ conducted four annual research cruises off Southwest Florida between 2007 and 2010. Three of those cruises found bloom concentrations of *Karenia brevis* in the region from Charlotte Harbor to Naples, Florida. In 2010 the cruise encountered only low concentrations of *K. brevis* in a very limited area within that region. During all of these cruises water samples from surface waters, near-bottom waters and a few intermediate depths were filtered through GF/F filters that were then immediately frozen in liquid nitrogen. These filters were processed by HPLC for lipid-soluble plant pigments. The pigment complements of each filter were then evaluated by chemotaxonomic analysis (ChemTax™) to produce estimates of the taxonomic class structure of the communities sampled.

Non-metric multi-dimensional scaling (MDS, Primer-E) was applied to these community structure data in an attempt to visualize trends in the multi-class structure from year to year. This process yielded clarification of some subtle differences in the community structures between distinctly different bloom types. For example, in two of the years, when *Karenia* blooms were characterized as being in ‘initiation’ phase, the MDS plots showed that the communities smoothly transitioned from non-*Karenia* to *Karenia*-dominated class complements. In contrast, a bloom characterized as being in ‘maintenance’ phase showed distinct community separation between the non-*Karenia* and *Karenia*-dominated class complements. Further analyses were conducted to determine the level of influence of environmental factors including numerous inorganic and organic nutrient parameters and physical parameters such as temperature, salinity, density, depth, distance from tributaries, water depth and light availability had on the community structure differences. Although there were some relations detected in individual years, they explained only a small amount of the community variability and were not consistent over years.

## THE ART OF RED TIDE SCIENCE: OUTREACH PRODUCT DEVELOPMENT MERGING SCIENCE AND ART

Kirkpatrick, B.<sup>1</sup>, Hall, E.R.<sup>1,2</sup>, Boyes, A.J.<sup>1,2</sup>, Nierenberg, K.<sup>1</sup>

<sup>1</sup>Mote Marine Laboratory, Sarasota, FL 34236 USA bkirkpat@mote.org, emily8@mote.org, anamari@mote.org, knierenberg@mote.org; <sup>2</sup>Ringling College of Art and Design, Sarasota, FL 34234 USA ehall@c.ringling.edu, anamari@c.ringling.edu

Over the years, numerous outreach strategies by the science community, such as FAQ cards and website information, have been used to explain blooms of the toxic dinoflagellate, *Karenia brevis* that occur annually off the west coast of Florida to the communities impacted. Many state and federal agencies have turned to granted research groups for help in the development and testing of environmental health outreach products. In the case of Florida red tide, the Florida Wildlife Research Institute/Mote Marine Laboratory (MML) Cooperative red tide agreement has allowed Mote to develop innovative outreach products. This project consisted of a team effort between scientists from MML and students from Ringling College of Art and Design to educate people about Florida red tide. We found that there are two major components to make the project a success. We coined the project “The Art of Red Tide Science”. The first was the communication and coordination with the Ringling college students (see Boyes et al abstract). The second component was the coordination on campus to make the student exhibition a success. This included collaboration from almost all departments within Mote such as aquarium, graphic arts, PR/communications, security, and facilities management. Also included were external collaborations with Ringling's departments, such as PR, as well as coordination of external science judges, from various state and county agencies. Our lessons learned will help other people in the HAB community understand the needs for an effective outreach program.

## CATEGORIZING THE SEVERITY OF PSP OUTBREAKS IN THE GULF OF MAINE FOR FORECASTING AND MANAGEMENT

Kleindinst, J.L.<sup>1</sup>, Anderson, D.M.<sup>1</sup>, McGillicuddy, D.J.<sup>1</sup>, Stumpf, R.P.<sup>2</sup>, Fisher, K.M.<sup>2</sup>, Couture, D.<sup>3</sup>, Hickey, J.M.<sup>4</sup>, Nash, C.<sup>5</sup>

<sup>1</sup>Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA  
jkleindinst@whoi.edu, danderson@whoi.edu, dmcgillicuddy@whoi.edu

<sup>2</sup>NOAA National Ocean Service, Silver Spring, MD 20910 USA,  
Richard.Stumpf@noaa.gov, Kathleen.Fisher@noaa.gov

<sup>3</sup>Maine Department of Marine Resources, West Boothbay Harbor, ME 04575 USA,  
Darcie.Couture@maine.gov

<sup>4</sup>Massachusetts Division of Marine Fisheries, New Bedford, MA 02740 USA  
Michael.Hickey@state.ma.us

<sup>5</sup>New Hampshire Department of Environmental Services, Concord, NH 03302 USA,  
CNash@des.state.nh

Development of forecasting systems for HABs has been a long-standing goal of HAB research and management. Significant progress has been made in this regard in the Gulf of Maine, where seasonal bloom forecasts are now being issued on an annual basis using cyst abundance maps and an *Alexandrium* population dynamics model. Thus far these forecasts have had to rely on terms such as “significant”, “moderately large” or “moderate” to convey the extent of forecasted outbreaks. In this study, historical paralytic shellfish poisoning (PSP) shellfish harvesting closure data along the coast of the Gulf of Maine was used to derive a series of bloom severity categories that are analogous to the categories used to define major storms like hurricanes or tornados. Over 30 years’ of shellfish closure data for Maine, Massachusetts and New Hampshire were collected and mapped to depict the extent of closure in each year. A variety of methods were explored for measuring length of coastline closed, which is a fractal problem. Ultimately, a simple procedure was developed using straight-line segments for sections of coastline. This method was then consistently applied to each year’s closure map to calculate the total km of coastline closed. Maps were then clustered together statistically to yield distinct groups of years with similar characteristics. A series of categories or levels was defined based on ranges of km closed, which can now be used in future forecasts instead of vague descriptors. We believe this will provide much-needed information to the public and press who read and make decisions on the basis of the forecasts.

THE ROLE OF B-VITAMINS IN CONTROLLING THE DYNAMICS OF HABs CAUSED BY *AUREOCOCCUS ANOPHAGEFFERENS* (PELAGOPHYCEAE) AND *COCHLODINIUM POLYKRIKOIDES* (DINOPHYCEAE)

Koch, F.<sup>1</sup>, Tang, Y.Z.<sup>1</sup>, and Gobler, C.J.<sup>1</sup>

<sup>1</sup>School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11790, fkoch@ic.sunysb.edu, yittang@notes.cc.sunysb.edu, cgobler@notes.cc.sunysb.edu.

Although the role of B-vitamins in the cellular metabolism and growth of microalgae has been known for decades, recent revelations that vitamins such as thiamine (B<sub>1</sub>) and cobalamin (B<sub>12</sub>) can significantly alter the plankton community structure and/or enhance algal biomass suggests these co-factors may hold substantial ecological importance. Approximately 50% of previously surveyed phytoplankton species have an obligate requirement (auxotrophy) for one or more of B<sub>1</sub>, B<sub>7</sub> or B<sub>12</sub>, with B<sub>12</sub> being required by most species (51%). Recently, we have established that harmful algae are exceptionally auxotrophic with regard to vitamins as 79% and 97% require B<sub>1</sub> and B<sub>12</sub>, respectively (Tang et al 2010), an observation consistent with the well-documented mixotrophic tendencies of many HABs. *Aureococcus anophagefferens*, the brown tide forming pelagophyte, and *Cochlodinium polykrikoides*, a red tide forming dinoflagellate, are both vitamin B<sub>1</sub> and B<sub>12</sub> auxotrophs (Tang et al 2010) and form near-monospecific algal blooms in NY coastal waters. Therefore, this study sought to investigate the role of vitamins B<sub>1</sub> and B<sub>12</sub> in the dynamics of these HABs using novel radiotracer methods applied to both culture experiments and field investigations. Culture studies using *A. anophagefferens* revealed a high degree of flexibility regarding its vitamin utilization. Cultures grown under limiting concentrations of vitamin B<sub>12</sub> displayed lower half saturation constants (K<sub>s</sub>) of B<sub>12</sub> uptake but higher maximum uptake rates (V<sub>max</sub>) compared to vitamin-replete cultures suggesting that *A. anophagefferens* may adapt to low vitamin concentrations by lowering their cellular requirement and maximizing their vitamin transport capabilities. Importantly, K<sub>s</sub> of B<sub>12</sub> uptake were within the range of concentrations found during blooms. During blooms of *A. anophagefferens* and *C. polykrikoides*, the majority of primary production and vitamin B<sub>1</sub> and B<sub>12</sub> utilization occurred in the respective size classes of these algae (1 – 5 μm and >20 μm, respectively). In contrast, picoplankton (< 2 μm) were responsible for the majority of vitamin utilization under non-bloom conditions. Vitamin amendment experiments conducted with bloom-water revealed that the addition of vitamins, particularly B<sub>12</sub>, had the ability to stimulate the growth rates of both, *A. anophagefferens* and *C. polykrikoides* (p<0.05). Collectively, these findings demonstrate that vitamins are likely to play a key role in the occurrence of these and possibly other HABs.

## DEVELOPMENT OF PREDICTIVE FORECAST SYSTEMS FOR *PSEUDONITZSCHIA* IN CALIFORNIA

Kudela, R.M.<sup>1</sup>, Anderson, C.<sup>1</sup>, Caron, D.A.<sup>2</sup>, Chao, Y.<sup>3</sup>, Howard, M.<sup>4</sup>, Jones, B.<sup>5</sup>, Kerkering<sup>5</sup>, Lane, J.Q.<sup>1</sup>, Langlois, G.<sup>6</sup>, Penta, B.<sup>7</sup>, Shulman, I.<sup>7</sup>

<sup>1</sup>University of California Santa Cruz, 1156 High St., Santa Cruz, CA 95064, USA kudela@ucsc.edu; <sup>2</sup>University of Southern California, Los Angeles, CA USA; <sup>3</sup>Jet Propulsion Laboratory, Pasadena, CA USA; <sup>4</sup>Southern California Coastal Water Research Project, Costa Mesa, CA USA; <sup>5</sup>Central and Northern California Ocean Observing System, Moss Landing, CA USA; <sup>6</sup>California Department of Public Health, Richmond, CA USA; <sup>7</sup>Naval Research Laboratory, Stennis Space Center, MS, USA

California and the US west coast are somewhat unique from a modeling perspective. A tremendous advantage in modeling and predicting HAB events in California is that the successional patterns can be “reset” as a function of short-term environmental conditions (e.g. upwelling/downwelling), providing short-term (days to weeks) predictability in addition to the underlying seasonal and interannual variability that can be used to identify HAB-favorable conditions. Despite the inherent predictability (compared to other coastal regions with HAB problems) of the California Current, relatively little effort has been applied towards development of predictive forecasts.

The California Harmful Algal Bloom Monitoring and Alert Program (Cal-HABMAP) was established to address these issues by coordinating researchers, managers, and end users working on HAB issues specific to California; we are working towards the creation of a statewide HAB monitoring and alert program. One specific goal is to build on existing monitoring and modeling efforts, expanding to the entire State of California. We have just begun (December 2010) a 3-year effort to implement existing HAB models from Santa Barbara and Monterey Bay previously developed as research exercises, to test and expand these existing models in other regions, to begin developing a similar modeling effort for paralytic shellfish poisoning, and to provide consistent field monitoring and validation data to adequately assess the model results.

Initial results show great promise. Statistical HAB prediction models are being coupled to regional physical-biological models to provide consistent spatially-explicit inputs (data) and tracking (forecasts). A statistical model for Monterey Bay has successfully been running free for 4 years, and is being integrated with the NRL NCOM/COSiNE model. Analysis suggests that this coupled approach works well, but requires specific tuning of the paired output to overcome inconsistencies between the physical-biological model and direct observations. Progress has also been made in the development of a bio-optical model developed for the Santa Barbara Channel (see C. Anderson et al. abstract). We are currently initiating “common garden” experiments for these regions, and are expanding to both Northern and Southern California, to determine whether a single model system could be applied for the entirety of the State of California.

## A LARGE SCALE CHRONIC FISH KILL IN THE ST JOHNS RIVER, FLORIDA: A COMPLEX CONSEQUENCE OF CYANOBACTERIA BLOOMS

Landsberg, J.H.<sup>1</sup>; Hendrickson, J.C.<sup>2</sup>; Williams, J.<sup>1</sup>; Burluson, C.<sup>1</sup>; Wolny, J.<sup>3</sup>; Brown, C.<sup>1</sup>; Kiryu, Y.<sup>1</sup>; Bakenhaster, M.<sup>1</sup>; Tabuchi, M.<sup>1</sup>; Cody, T.<sup>1</sup>; Wilson, P.<sup>1</sup>; Flewelling, L.<sup>1</sup>; Brodie, R.<sup>4</sup>

<sup>1</sup>Florida Fish and Wildlife Conservation Commission (FWC), Fish and Wildlife Research Institute (FWRI), St. Petersburg, FL 33701 USA [jan.landsberg@myfwc.com](mailto:jan.landsberg@myfwc.com); <sup>2</sup>St. Johns River Water Management District, Palatka, FL 32177 USA; <sup>3</sup>Florida Institute of Oceanography, St. Petersburg, FL 33701 USA; <sup>4</sup>FWC-FWRI, Jacksonville, FL, 32211USA

From late May to early July 2010, an extensive multispecies but differential chronic fish kill, affecting primarily adult red drum (*Sciaenops ocellatus*), occurred in the lower St. Johns River (LSJR) from Shands Bridge to downtown Jacksonville, Florida. Low numbers of dying fish were observed throughout the 30 mile stretch of river, or as physically concentrated patches of hundreds of dead fish. Co-occurring cyanobacteria blooms, primarily dominated by *Aphanizomenon flos-aquae*, and initiated in mid-May by rainfall and nutrient loading, were present in the freshwater reaches prior to and at the start of the fish kill. The kill was triggered by: 1) a significant reverse flow event and sudden influx of high salinity water in late May which contributed to the crash of the high biomass bloom further upstream and, 2) movement of euryhaline fish into the vicinity of the senescing bloom or to its associated by-products downstream. The decomposing bloom led to a sequence of events, including the release of low concentrations of cyanotoxins, bacterial lysis of cyanobacteria cells, high organic loading, and changes in the diversity and dominance of the plankton community to include *Microcystis*, *Leptolyngbya*, *Planktolyngbya*, and low concentrations of hemolytic *Heterosigma akashiwo*. All of these consequences resulted in complex pathological effects on fish. Dissolved oxygen levels and other water quality parameters were within normal ranges in the reach of the fish kill, although high ammonia levels were present near the senescing bloom. Low concentrations of microcystins, cylindrospermopsins, saxitoxins, and anatoxin-like derivatives were detected in water samples, and the first two toxins in fish tissues. Pathological characteristics of moribund fish were not consistent with acute cyanotoxin exposure or with poor water quality, but in part with chronic hemolysis. The presence of *A. flos aquae* in the LSJR has the potential for significant environmental repercussions if the causal factors contributing to bloom growth and maintenance are not fully understood and managed.

STEROLS OF THE GREEN-PIGMENTED, FRESHWATER RAPHDOPHYTE, *GONYOSTOMUM SEMEN* (RAPHDOPHYCEAE): A COMPARISON TO BROWN-PIGMENTED, MARINE TAXA AND CLUSTERING OF RAPHDOPHYTES ACCORDING TO STEROL COMPOSITION

Leblond, J.D.<sup>1</sup>, Roche, S.A.<sup>2</sup>, Lasiter, A.D.<sup>3</sup>, Li, C.<sup>4</sup>

<sup>1</sup>Middle Tennessee State University, Murfreesboro, TN 37132 jleblond@mtsu.edu;

<sup>2</sup>University of Maryland Center of Environmental Science, Baltimore, MD 21202

sroche@umces.edu; <sup>3</sup>University of Tennessee Health Science Center, Memphis, TN

38163 alasiter@uthsc.edu; <sup>4</sup>Middle Tennessee State University, Murfreesboro, TN 37132

cli@mtsu.edu

Sterols are a class of membrane-reinforcing, ringed lipids which have a long history of examination in algae as a means of deriving chemotaxonomic relationships and as potential lipidic biomarkers. The Raphidophyceae represent a class of harmful, bloom-forming marine and freshwater algae. To date, there have been three published examinations of their sterol composition, focusing primarily on brown-pigmented, marine species within the genera, *Chattonella*, *Fibrocapsa*, and *Heterosigma*. Lacking in these examinations has been the raphidophyte, *Gonyostomum semen* Ehrenb., which is a green-pigmented, freshwater raphidophyte with a world-wide distribution. In this study, we have examined two isolates of *G. semen*, and have found that it produces two predominant sterols, 24-ethylcholesta-5,22E-dien-3 $\beta$ -ol and 24-ethylcholest-5-en-3 $\beta$ -ol, with only the latter being a component of brown-pigmented genera. As part of this study, we have also generated a clustering algorithm-based chemotaxonomy of brown- and green-pigmented raphidophytes using our data and those of other published studies. We have found that the Raphidophyceae form four readily distinguishable clusters based on sterol composition, and that *G. semen* occupies its own cluster.

## REPETITIVE LOW LEVEL DOMOIC ACID EXPOSURES IN ZEBRAFISH REVEAL POTENTIAL BIOMARKERS USEFUL FOR WILDLIFE AND HUMAN HEALTH ASSESSMENTS

Lefebvre, K. A.<sup>1</sup>, Frame, E. R.<sup>1</sup>, Kendrick, P.<sup>1</sup>, Farin, F. M.<sup>2</sup>, Bammler, T. K.<sup>2</sup>, Beyer, R. P.<sup>2</sup>

<sup>1</sup>NOAA Fisheries, Northwest Fisheries Science Center, Seattle, WA 98112 USA [Kathi.Lefebvre@noaa.gov](mailto:Kathi.Lefebvre@noaa.gov); <sup>2</sup>Department of Environmental and Occupational Health Sciences, School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98105 USA

In an unprecedented long-term low-level exposure study, zebrafish (*Danio rerio*) were intracoelomically injected with domoic acid (DA) once a week for six weeks, followed by every other week over the course of nine months. Toxin doses were well below the EC50 and were designed to avoid overt signs of neuroexcitotoxicity. Control (PBS injected) and exposed fish were sacrificed at 13 time points; 6, 24, and 48 hours, and 1, 2, 6, 12, 18, 24, and 36 weeks, followed by two recovery time points (1 week and 5 weeks). Whole brains were taken from ten exposed and ten control fish at each time point and the transcriptome examined via microarray analyses. Additionally, blood was drawn from the caudal vein from multiple fish at each time point and the serum analyzed via Biosense ELISA for DA quantification.

Significant DA-modulated gene expression was observed at all time points including recovery. Approximately 50 to 240 genes were significantly differentially expressed ( $\pm 1.5$ -fold,  $p \leq 0.05$ ) in DA exposed fish at each of the 13 time points. In general, genes involved in immune processes, apoptosis, and nervous system function were impacted throughout the 11-month experiment. Additionally, four phases with unique DA-modulated gene expression patterns were identified as acute, early chronic, late chronic, and recovery. A detailed discussion of gene expression findings will be presented. Blood serum levels measured in experimental zebrafish matched blood serum levels commonly found in naturally exposed California sea lion populations, verifying the ecological relevancy of the exposure levels.

Collectively, these data provide candidate biomarkers of DA exposure that are currently being validated in field exposed California sea lions. The ultimate goal is to translate the diagnostic tools developed in these sentinel species to platforms for human health diagnostics

## PARTNERSHIPS IN HARMFUL ALGAL BLOOMS MONITORING AND RESEARCH: AN INSTITUTIONAL PERSPECTIVE

Leschine, T.M., Gregory, A., Chadsey, M., Trainer, V.

Harmful algal blooms are typically geographically widespread and recurrent phenomena that, by their nature engage a diverse set of local, state, tribal and federal authorities in response. In two cases we examined in Washington State, partnerships consisting of state, federal, tribal and local governments, together with a variety of non-governmental stakeholder groups, formed to develop programs of research and monitoring in response to HABs outbreaks affecting shellfish populations of economic and cultural importance. In the case of domoic acid affecting razor clams on the state's outer coast, the Olympic Region Harmful Algal Blooms Partnership (ORHAB), an ad hoc voluntary partnership, proved instrumental both in advancing research and monitoring and in securing state funding to replace seed monies provided through the national HABs monitoring and event response program MERHAB. Demonstrable improvements in management capacity emerged. In the case of SoundToxins however, a program organized to address a variety of HABs-related problems within Puget Sound, efforts to replicate the ORHAB partnership's success have proved more difficult. Among other things, shellfish growers have not contributed monitoring data to an emerging Puget Sound wide HABs monitoring network to the extent hoped for by the program's originators. These differences in outcome are explainable through institutional analysis that elucidates the conditions under which voluntary partnerships are likely to emerge to undertake and sustain attention to HABs, their associated societal impacts, and the research and monitoring needed to minimize social and economic as well as human health consequences.

TOXICITY OF *PSEUDO-NITZSCHIA* SPP. BLOOMS ASSOCIATED WITH SUBMARINE GROUNDWATER DISCHARGE AND TROPHIC TRANSFER OF DOMOIC ACID.

Liefer J.D.<sup>1</sup>, Robertson, A.<sup>2</sup>, MacIntyre, H.L.<sup>3</sup>

<sup>1</sup>Dauphin Island Sea Lab, Dauphin Island, AL 36528 USA [jliefer@disl.org](mailto:jliefer@disl.org); <sup>2</sup>FDA Gulf Coast Seafood Laboratory, Dauphin Island, AL 36528 USA [alison.robertson@fda.hhs.gov](mailto:alison.robertson@fda.hhs.gov); <sup>3</sup>Dalhousie University, Halifax, NS B3H 4J1 Canada [hugh.macintyre@dal.ca](mailto:hugh.macintyre@dal.ca)

The potentially-toxic diatom *Pseudo-nitzschia* is common in the northern Gulf of Mexico (NGOM), including the coastal waters of Alabama. The NGOM shoreline near Little Lagoon, AL, is a hot spot for blooms of *Pseudo-nitzschia* spp. and their population density is correlated with discharge from the aquifer. Salinity and nitrogen concentrations in Little Lagoon are driven by submarine groundwater discharge. Monitoring within Little Lagoon from 2008 to 2010 showed that the greatest *Pseudo-nitzschia* spp. abundances were highly correlated with increases in groundwater elevation.

A toxic bloom of *Pseudo-nitzschia subfraudulenta* occurred throughout most of Alabama's coastal waters in June 2009, following a large discharge event and rapid rise in groundwater elevation. *Pseudo-nitzschia* spp. densities above  $10^6$  cells L<sup>-1</sup> only occurred at salinities above 32 during the course of the bloom. Particulate and dissolved domoic acid (DA) was analyzed by ELISA and LC-MS/MS to determine cell toxin quotas, which ranged from 0.04 - 0.32 pg DA cell<sup>-1</sup>. Toxin cell quotas were correlated with total phosphorus concentration, suggesting that phosphorus starvation may be an important trigger of toxicity. Because groundwater typically has very high molar ratios of DIN:DIP and TN:TP, phosphorus is likely to be exhausted before nitrogen following bloom initiation by groundwater discharge. DA was also confirmed in seven species of small planktivorous fish collected during the 2009 bloom and ranged from 0.005 - 0.06 µg DA g<sup>-1</sup> with the highest levels in Gulf Kingfish and White Mullet. Fish toxicity was correlated with *Pseudo-nitzschia* abundance despite low toxin cell quotas, indicating that planktonic fish are an important vector for the trophic transfer of DA in AL waters.

EFFECTS OF DEPTH, GRAZING AND NUTRIENT ENRICHMENT ON THE ABUNDANCE AND DISTRIBUTION OF *GAMBIERDISCUS SPP.*

Loeffler, C.R.<sup>1</sup>, Richlen, M.L.<sup>2</sup>, Smith, T.B.<sup>1,3</sup>

<sup>1</sup>University of the Virgin Islands, St. Thomas, USVI 00802 USA  
Christopher.r.Loeffler@gmail.com; <sup>2</sup>Biology Department, Woods Hole Oceanographic Institution, MS 32, Woods Hole, Massachusetts 02543, USA  
mrichlen@whoi.edu; <sup>3</sup>tsmith@uvi.edu

*Gambierdiscus* spp. are benthic marine dinoflagellates that produce a suite of toxins responsible for the occurrence of Ciguatera Fish Poisoning (CFP). In ciguatera endemic areas, this syndrome significantly impacts human health and local economies, and is particularly detrimental to island communities dependent on subsistence fishing. Efforts to prevent CFP have been slow due to the lack of rapid screening methods for ciguatoxin; however, progress has been made in characterizing the ecology of *Gambierdiscus* spp. in reef ecosystems, culminating in the recent development of numerical models to simulate *Gambierdiscus* spp. dynamics.

Using standardized methodologies, this study investigated how grazing, nutrient enrichment, wave energy, and depth (light attenuation) influence the abundance and distribution of *Gambierdiscus* spp. in St. Thomas, USVI. Sampling took place on the southwest side of St. Thomas at three locations, each with adjacent reefs at depths of 10 and 20 m, for a total of six sampling sites. The paired sites at each location were oriented in a natural onshore to offshore gradient to examine differences in depth and wave energy. Artificial settlement surfaces (tiles) were deployed at each site, and subjected to several different treatments: full caging (to exclude grazers), full caging with nutrient enrichment, partial caging, partial caging with nutrient enrichment, and no caging/nutrient enrichment (control). Results of these field surveys will be discussed; however, preliminary data collected from one location, Perseverance Bay, showed that eliminating grazing pressure resulted in a five-fold increase in the abundance of *Gambierdiscus* spp. per cm<sup>2</sup> (p=0.015). The results of this study will help characterize potential controls on *Gambierdiscus* populations, and may lead to new insights regarding the potential impact of overfishing and eutrophication on *Gambierdiscus* spp. dynamics.

## GROWTH, TOXIN CONCENTRATION AND PARTICULATE C, N AND P COMPARISON OF NATURAL AND LAB-CULTURED *KARENIA BREVIS*

Lovko, V.<sup>1</sup>, Hall, E.R.<sup>1</sup>, Dixon, L.K.<sup>1</sup>, Henry, M.S.<sup>1</sup>, Kirkpatrick, G.J.<sup>1</sup>, Palubok, V.<sup>1</sup>

<sup>1</sup>Mote Marine Laboratory, Sarasota, FL 34236 USA lovko@mote.org, emily8@mote.org, lkdxon@mote.org, mhenry@mote.org, gkirkpat@mote.org, palubok@mote.org

Numerous questions persist regarding the bloom dynamics of the red tide dinoflagellate *Karenia brevis*, which forms recurring toxic blooms over the West Florida Shelf. For example, determination of growth rate of natural populations is complicated by physical forcing mechanisms and 3-dimensional circulation. Nutrient dynamics are complicated by bloom movement and multiple nutrient sources which vary spatially in relative importance. Studies involving laboratory cultured strains of *K. brevis* have frequently been used to elucidate aspects of growth, nutrient uptake and toxicity that are difficult to ascertain from natural populations. However, laboratory-reared cultures are typically grown in media with concentrations of nitrogen, phosphorous and other nutrients that exceed *in situ* levels by several orders of magnitude. These populations can demonstrate high growth rates and cellular N, C and P ratios that differ markedly from natural populations. The potential effects of long-term culture in high-nutrient media on the physiology of cultured populations is typically not considered when interpreting results of laboratory-based experiments.

We compared the growth rates, toxicity and cellular C, N and P complement between natural *K. brevis* population and cultured strains grown in high-nutrient (L) and low nutrient (L1/100) media. The results of two separate bioassays, one containing a natural population of *K. brevis* collected ~1mi. off Sarasota, FL and one spiked with a cultured strain of *K. brevis* (“New Pass”) grown in low nutrient media (L1/100) was examined along with additional studies comparing *K. brevis* strains cultured in high and low nutrient media under varying cell densities. Preliminary data suggests potential variability in the cellular complement between cultures and natural populations and between different population densities. Additionally, *K. brevis* initially cultured in nutrient rich media (L1) and then dropped to oligotrophic conditions (L1/100, offshore water), demonstrated a maximum growth rate of ~ 0.4/day, while cultures that were gradually stepped down to the same low-nutrient media exhibited significantly lower growth rates when cultured in L1/50 or below (0.6/day when dropped from L1 to L1/10, 0.25/day in L1/50 from L1/10, negative growth in L1/100 from L1/50). Additional results from on-going studies will be presented.

Funding for this research is provided by the state of Florida and the Florida Fish and Wildlife Research Institute.

## DISCHARGE-DRIVEN BLOOMS AND NICHE DEFINITION OF HAB SPECIES IN A SHALLOW-WATER SUBTROPICAL ECOSYSTEM

MacIntyre, H.<sup>1</sup>, Liefer, J.<sup>2</sup>, Novoveska, L.<sup>2</sup>, Goldman, E.<sup>3</sup>, Smith, W.<sup>4</sup>, Dorsey, C.<sup>4</sup>, Burnett, W.<sup>5</sup>, Canion, A.<sup>5</sup>, Su, N.<sup>5</sup>, Eller, K.<sup>5</sup>, Mortazavi, B.<sup>6</sup>, Bernard, R.<sup>6</sup>, Park, K.<sup>7</sup>, Peterson, R.<sup>8</sup>, Viso, R.<sup>8</sup>

<sup>1</sup>Dalhousie University hugh.macintyre@dal.ca; <sup>2</sup>Dauphin Island Sea Lab jdl602@jaguar1.usouthal.edu, ln601@jaguar1.usouthal.edu; <sup>3</sup>University of South Carolina egoldman08@gmail.com; <sup>4</sup>Alabama Department of Public Health billandelinor72@yahoo.com, Carol.Dorsey@adph.state.al.us; <sup>5</sup>Florida State University wburnett@fsu.edu, acanion@gmail.com, nisu111@gmail.com, eller@ocean.fsu.edu; <sup>6</sup>University of Alabama bmortazavi@as.ua.edu, rjbernard@ua.edu; <sup>7</sup>University of South Alabama kpark@jaguar1.usouthal.edu <sup>8</sup>Coastal Carolina University rpeters2@coastal.edu, rviso@coastal.edu

The state of Alabama (USA) has only 100 km of coastline but has had recurring harmful algal blooms (HABs). The causative organisms include dinoflagellates (*Karenia brevis*, *Prorocentrum minimum*, and *Karlodinium veneficum*), diatoms (*Pseudo-nitzschia* spp.) and raphidophytes (*Heterosigma* sp. and *Chattonella* sp.), which have caused hypoxic and toxic fish-kills.

Blooms of *K. brevis* appear to be allochthonous, advecting westward from Florida. In contrast, blooms of *P. minimum*, *K. veneficum* and *Pseudo-nitzschia* spp. develop locally and are associated with two hot-spots. The first, Weeks Bay, a sub-estuary on the eastern margin of Mobile Bay, is the site of the dinoflagellate blooms. The second, the Gulf of Mexico shoreline adjacent to Little Lagoon, a shallow and saline lagoon, is the site of *Pseudo-nitzschia* spp blooms. Initiation of both dinoflagellate and diatom blooms is correlated with discharge from the aquifer that borders the eastern side of Mobile Bay and the Gulf of Mexico. The dinoflagellates bloom in Weeks Bay during periods of low discharge in both winter (*P. minimum*) and summer (*K. veneficum*). *Pseudo-nitzschia* spp. bloom predominantly in the spring after periods of high discharge, as part of a cohort of diatoms that includes other bloom-forming taxa, *Chaetoceros* sp., *Skeletonema* sp. and *Pseudoguinaradia* sp.

Microalgal community composition appears to be driven by the interplay between discharge of groundwater vs. surface runoff and by benthic coupling. The inference is based on relationships between temperature, groundwater elevation (based on well data and subsurface resistivity), aquifer discharge, water 'age' (as the proxy Ex <sup>224</sup>Ra.<sup>222</sup>Ra), salinity, nutrient concentrations and community composition. The niches occupied by the bloom-forming dinoflagellates and diatoms can be inferred by ordination of abundance on plots of temperature vs discharge. These are proxies for stress and disturbance in the CSR model proposed by Grime (1977; Amer. Natur. 111: 1169-1194). Because global climate change is predicted to alter seasonal patterns of precipitation, hence discharge, it is likely that the niches for these HAB taxa will expand.

## THE *VIBRIO* HABS CONNECTION: INVESTIGATING INTERACTIONS BETWEEN HARMFUL ALGAL BLOOM SPECIES AND PATHOGENIC *VIBRIO*

Main, C.R., Coyne, K.J.

University of Delaware School of Marine Science and Policy, Lewes, DE 19958  
cmain@udel.edu; kcoyne@udel.edu

Bacteria within the genus *Vibrio* are an abundant, naturally occurring and diverse group found throughout coastal and marine environments. *Vibrio* spp. are often found in association with eukaryotic organisms, forming relationships that range from mutualistic to pathogenic in nature. Several species are known pathogens to humans, causing illness and possible death after ingestion or by wound infection. *Vibrio* exhibits two alternative growth strategies in the marine environment: (i) association with particles by forming a biofilm or (ii) as free-living bacterioplankton. Rising temperature and eutrophication of the marine environment has been shown to increase the abundance of *Vibrio* as well as promote algal blooms. Association between *Vibrio* spp. and algal blooms, by direct contact as a biofilm, has also been noted, and it has been suggested that this association may provide *Vibrio* with a refuge from predation. Here, we investigated species-specific associations between *Vibrio* and HAB species. The presence and abundance of pathogenic *Vibrio* spp. were examined in size fractionated water samples collected from the Delaware Inland Bays (DIB) from 2009 till present. Abundance of *Vibrio* and harmful algal species were initially determined using quantitative real-time PCR (qPCR) using the single copy gene *rpoA* for *Vibrio* abundance and group specific primers for algal species. Correlations between particle-attached and bacterioplankton *Vibrio* abundance were also examined. Preliminary results of this project indicate a significant correlation between diatoms and raphidophyte abundance with particle-attached *Vibrio*. Species specific interactions with HAB species will also be investigated by qPCR. Grazing experiments during raphidophyte and dinoflagellate blooms during summer, 2011, will be conducted to evaluate the hypothesis that particle attachment may provide a refuge from predation. With the expected increase of temperatures and increased eutrophication, the abundance and frequency of harmful algal blooms and associated *Vibrio* spp. are expected to increase occurrences in the Delaware Inland Bays. Investigation of links between both HABs and pathogenic *Vibrio* can lead to predictions of potential outbreaks and inform future management practices in this region.

# MULTIPLEXED DETECTION OF PARALYTIC SHELLFISH TOXINS AND DOMOIC ACID ON A PORTABLE SURFACE PLASMON RESONANCE PLATFORM

Marshall, N.M.<sup>1</sup>, Campbell, K.<sup>2</sup>, Elliott, C.T.<sup>2</sup>, Doucette, G.J.<sup>1</sup>

<sup>1</sup>Marine Biotoxins Program, NOAA/National Ocean Service, Charleston, SC 29412 USA [nicholas.marshall@noaa.gov](mailto:nicholas.marshall@noaa.gov), [greg.doucette@noaa.gov](mailto:greg.doucette@noaa.gov); <sup>2</sup>Inst. of Agri-Food & Land Use, Queen's University Belfast, Belfast, N. Ireland, U.K. [katrina.campbell@qub.ac.uk](mailto:katrina.campbell@qub.ac.uk), [chris.elliott@qub.ac.uk](mailto:chris.elliott@qub.ac.uk)

The effective monitoring and management of harmful algal blooms (HABs) and their adverse public health and economic impacts requires an ability to respond rapidly to a bloom event. Early detection of HAB species and their toxins is thus a critical component of monitoring programs, and field-portable detection can provide the real or near-real time information needed to minimize response time. Moreover, given the wide-ranging variation in toxicity of some HABs, an accurate assessment of the public health risk requires information on both organism abundance and toxin levels. We have begun to address this need through the application of a novel, field-portable surface plasmon resonance (SPR) platform called the Spirit (Seattle Sensor Systems, Inc.), which can accommodate both nucleic acid- and antibody-based methods. The initial focus is on multiplexed detection of paralytic shellfish toxins (PST) and domoic acid (DA). Our aim is to detect both of these toxin classes using a single sample extract, and ultimately to detect concurrently these toxins and the corresponding HAB species for determination of bloom toxicity.

The Spirit platform uses four miniature 'Spreeta' SPR chips (ICx Nomadics, Inc.), each with three addressable channels, for a total of 12 independent sensing nodes. We have developed Spreeta surfaces for the PST and DA immunoassays by adapting the methods of Yakes et al. (2011) and Campbell et al. (2011) respectively, both designed for laboratory-based SPR instruments. The assays are performed in a competitive format, with toxin immobilized on a carboxymethyl-dextran surface through a short polyethylene glycol linker. Initial assay results have yielded EC<sub>50</sub> values of 9.7 ng STX/mL and 8.0 ng DA/mL for standard reference solutions. Our next steps will include assay optimization, design of a single extraction solvent with recovery optimized for PSTs and DA, and testing of laboratory cultures and field samples containing PST- and/or DA-producing HAB species.

## References

- Campbell, K., et al. 2011. *Biosens. Bioelectr.* 26:3029-3036.  
Yakes, B.J. et al. 2011. *Sens. Actuators B: Chem.*, doi:10.1016/j.snb.2011.02.043

## PARASITIC INFECTIONS IN BLOOM-FORMING DINOFLAGELLATES FROM THE CALIFORNIA CURRENT SYSTEM

Mazzillo, F.F.M.

Department of Ocean Sciences, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95064 USA mazzillo@gmail.com

*Amoebophrya* is an endoparasitic dinoflagellate that infects and kills free-living dinoflagellates. This study reports for the first time the presence of *Amoebophrya* infections in 6 bloom-forming dinoflagellate species, including some that are toxic and harmful in Monterey Bay, an open coastal embayment on the U.S.A. west coast that is influenced by the upwelling dynamics of the California Current System (CCS). Infections were detected with 3 different methodologies (i.e., parasite green autofluorescence, DAPI staining, and rRNA-based fluorescent in situ hybridization assays) in weekly samples collected from 3 Aug 2005 to 28 July 2010 at the Santa Cruz Wharf, and from 21 and 28 October 2008 on an inshore transect in the northeastern shelf of Monterey Bay. Confirmation of the parasite presence was obtained using transmission electron microscopy. Unique parasite morphological structures and the effects of infection in host cell morphology are hypothesized to be adaptations that allow the parasite to produce hundreds of infective dinospores. The presence of *Amoebophrya* infections in bloom-forming dinoflagellate species that occur in Monterey Bay, and likely the broader CCS, and its ability to kill the host while producing hundreds of new infective dinospores suggest that this parasite may prevent the occurrence and/or facilitate the end of toxic dinoflagellate blooms and “red tides” that occur in pelagic ecosystems influenced by the CCS. Additionally, *Amoebophrya* may also influence dinoflagellate species diversity, contribute in the ascendancy of diatoms versus dinoflagellates and promote temporary changes in trophic web structure of pelagic ecosystems during epidemic parasitic outbreaks.

## COMMUNITY BLOOM DYNAMICS AND RELATED TOXINS ALONG THE OREGON COAST

McCulloch, A.<sup>1</sup>; Wood, M.<sup>2</sup>; Peterson, W.T.<sup>3</sup>; White, A.E.<sup>4</sup>; Hunter, M.<sup>5</sup>; Forster, Z.<sup>5</sup>, Smith, D.<sup>6</sup>; McKibben, M.<sup>4</sup>, and Strutton, P.<sup>7</sup>

<sup>1</sup>Cooperative Institute of Marine Resources Studies, Oregon State University/NWFSC NOAA, Newport, OR 97365 USA anita.mcculloch@noaa.gov; <sup>2</sup>Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR 97401 USA; <sup>3</sup>Northwest Fisheries Science Center, NOAA, Newport, OR 97365 USA; <sup>4</sup>College of Ocean and Atmospheric Sciences, Oregon State University, Corvallis, OR, 97331; <sup>5</sup>Oregon Department of Fish & Wildlife, Astoria, OR 97103 USA; <sup>6</sup>Oregon Department of Agriculture, Salem, OR 97301 USA; <sup>7</sup>Institute for Marine and Antarctic Studies, University of Tasmania

Blooms of harmful algae, including *Pseudo-nitzschia*, and *Alexandrium*, and associated phycotoxins, such as domoic acid and saxitoxin, often occur along the Oregon coast and threaten wildlife and recreationally important shellfish. In 2007, a monitoring and event response project (MOCHA - Monitoring of Oregon's Coastal Harmful Algae) was established to better understand the ecological mechanisms underlying the occurrence of HABs and minimize their impact on coastal communities. The dominant bloom genera and community bloom dynamics vary both spatially and temporally. Yet, little information is known about why certain genera are dominant over others. We examined the phytoplankton community distributions and nearshore toxin data over a two year period (2009 – 2010) focusing on the dominant bloom genera and associated toxin. Dinoflagellates dominated the bloom dynamics in 2009 while diatoms, specifically *Pseudo-nitzschia*, dominated coastal waters in 2010. In 2009, *Alexandrium* were the dominant genera with elevated saxitoxin concentrations as high as 900 µg/100g from August to November. Saxitoxin levels were highest and occurred slightly earlier in northern Oregon compared to central and southern Oregon. *Pseudo-nitzschia* cell counts reached elevated levels from May to early August. However, domoic acid concentrations did not exceed detection limits. The bloom transitioned to an *Akashiwo sanguinea* bloom in late 2009 (August – November) resulting in thousands of seabird deaths. During 2010, *Pseudo-nitzschia* was the dominant bloom genera with domoic acid levels exceeding detection limits from June to August. Future efforts will address the contrasting oceanographic conditions between 2009 and 2010.

SUPPRESSION OF THE 2010 *ALEXANDRIUM FUNDYENSE* BLOOM BY CHANGES IN PHYSICAL, BIOLOGICAL, AND CHEMICAL PROPERTIES OF THE GULF OF MAINE

McGillicuddy, D.J.<sup>1</sup>; Townsend, D.W.<sup>2</sup>; He, R.<sup>3</sup>; Keafer, B.A.<sup>1</sup>; Kleindinst, J.L.<sup>1</sup>; Li, Y.<sup>3</sup>; Manning, J.P.<sup>4</sup>; Mountain, D.G.<sup>5</sup>; Thomas, M.A.<sup>6</sup>; Anderson, D.M.<sup>1</sup>

<sup>1</sup>Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA  
dmcgillicuddy@whoi.edu; <sup>2</sup>School of Marine Sciences, University of Maine, Orono, ME 04469 USA; <sup>3</sup>Department of Marine, Earth, and Atmospheric Sciences, North Carolina State University, Raleigh, NC 27695 USA; <sup>4</sup>National Oceanic Atmospheric Administration, Northeast Fisheries Science Center, Woods Hole, MA 02543 USA; <sup>5</sup>2707 N. Orchard Ave., Tucson, AZ 85712 USA; <sup>6</sup>School of Marine Sciences, University of Maine, Orono, ME 04469 USA

For the period 2005-2009, the abundance of resting cysts in bottom sediments from the preceding fall was a first-order predictor of the overall severity of spring/summer blooms of *Alexandrium fundyense* in the western Gulf of Maine and southern New England. Observations in the fall of 2009 indicate cyst abundance off mid-coast Maine was significantly higher than it was preceding a major regional bloom in 2005. A seasonal ensemble forecast was computed using the fall 2009 cyst abundance and the range of forcing conditions for the period 2004-2009, suggesting a large bloom was likely in the western Gulf of Maine in 2010. Real-time forecasts of *A. fundyense* abundance made during the bloom season were generally within the range of variation predicted by the seasonal ensemble forecast. However, a major bloom did not materialize in 2010. Hydrographic survey data indicate that conditions in spring/summer 2010 were outside the envelope of prior observations used to construct the ensemble forecast. Water mass anomalies indicate a regional-scale change in circulation with direct influence on *A. fundyense*'s niche (near-surface waters were warmer, fresher, more stratified, and had lower nutrients than prior years). Moreover, a weaker-than-normal coastal current lessened *A. fundyense* transport into the western Gulf of Maine and Massachusetts Bay. Satellite ocean color observations indicate the 2010 spring phytoplankton bloom was more intense and crashed earlier than usual. Thus it appears that early-season nutrient depletion caused a temporal mismatch with *A. fundyense*'s endogenous clock that regulates the timing of cyst germination. These findings highlight the difficulties of ecological forecasting in a changing oceanographic environment, and underscore the need for a sustained observational network to drive such forecasts.

## DEVELOPMENT AND VALIDATION OF SATELLITE BLOOM DETECTION PRODUCTS FOR COASTAL OREGON

McKibben, S.M.<sup>1</sup>, White, A.E.<sup>1</sup>, Strutton, P.G.<sup>2</sup>, Foley, D.<sup>3</sup>, Wood, A.M.<sup>4</sup>, Hunter, M.V.<sup>5</sup>, Forster, Z.R.<sup>5</sup>

<sup>1</sup>College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, OR, 97331 USA, first author's e-mail: morgaine@coas.oregonstate.edu; <sup>2</sup>Institute for Marine and Antarctic Studies, University of Tasmania; <sup>3</sup>NOAA Coast Watch, West Coast Regional Node, Pacific Grove, CA; <sup>4</sup>Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR; <sup>5</sup>Oregon Department of Fish & Wildlife, Astoria, OR

Harmful algal blooms (HABs) are increasing in intensity and frequency, adversely affecting coastal ecosystems and economies worldwide. Federal and state monitoring programs are in place to protect the public in regions where toxic HABs threaten human health, and one of the primary goals in the HAB research community is development of tools to augment these programs. Satellite ocean color data offer a way to view bulk chlorophyll-a concentrations in the upper ocean at regional scales, making them an ideal foundation for such tools. Development of custom products based on satellite data, coupled with *in situ* HAB sampling, can be used to elucidate the factors leading to HAB formation, as well as the spatial coverage of HABs, their persistence and movement over time. In this regard, two custom "chlorophyll deviation" satellite products were developed for the Oregon coast using MODIS-Aqua chlorophyll-a and fluorescence line height algorithms. The products were then matched with *in situ* HAB data collected between 2006-2010 in order to evaluate how effective they are as a tool in HAB research, detection and monitoring. Together, these products and ground-truthing efforts have enhanced our understanding of HAB events in the coastal Oregon region.

## MICROBIAL COMMUNITY DYNAMICS SURROUNDING *KARENIA BREVIS* BLOOMS ALONG THE WEST FLORDIA SHELF

Meyer, K.A.<sup>1</sup>; O'Neil, J. M.<sup>1</sup>; Mulholland, M.R.<sup>2</sup>; Heil, C.A.<sup>3</sup>

<sup>1</sup>University of Maryland Center for Environmental Science, Cambridge, MD 21613 USA kmeyer@umces.edu, joneil@umces.edu; <sup>2</sup>Old Dominion University, Norfolk, VA 23529 USA mmulholl@odu.edu; <sup>3</sup>Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575 USA cheil@bigelow.org

Measures of bacterial growth and production were taken during the ECOHAB: *Karenia* Nutrient Dynamics project in the Eastern Gulf of Mexico (October 2007-2010) to determine if the size and magnitude of the microbial loop changes in response to the magnitude of *K. brevis* blooms. Bacterial abundance, leucine and thymidine incorporation, growth rate, and growth efficiency were all measured and compared to environmental parameters and abundance of *K. brevis*. In 2007 and 2009 *K. brevis* blooms were in maintenance phase, in 2008 bloom initiation was sampled, and there was no bloom in 2010.

Bacterial production was significantly lower ( $p < 0.05$ ) within blooms in the maintenance phase and significantly higher during bloom initiation. However, there was not a significant correlation between *K. brevis* abundance and bacterial production. Bacterial production exhibited significant correlations with nutrient pools measured during blooms, but bacterial growth differed by year depending on bloom stage. *K. brevis* blooms in maintenance phase had higher leucine incorporation and low bacterial growth rates, indicating high metabolic costs of bacterial growth. In an initiating bloom thymidine incorporation was equal to leucine incorporation and there were high bacterial growth rates, which indicate balanced growth with high biomass production. Bacterial growth rates were significantly higher when *K. brevis* was present, suggesting bacterial communities are undergoing physiological changes when presented with a bloom of *K. brevis*.

These data demonstrate multiple parameters of bacterial growth and production are needed to characterize microbial responses to community composition and energetic constraints within the environment. The microbial loop appears to have less energetic constraints when *K. brevis* blooms are initiating compared to established blooms. This may have an impact on the nutrient pools available to support *K. brevis* bloom growth or maintenance as well as the composition of the microbial community associated with blooms.

## THE RESPONSE OF *KARENIA BREVIS* AND ASSOCIATED MICROBIAL COMMUNITIES TO VIRAL ATTACK

Meyer, K.A.<sup>1</sup>; O'Neil, J.M.<sup>1</sup>; Heil, C.A.<sup>2</sup>

<sup>1</sup>University of Maryland Center for Environmental Science, Cambridge, MD 21613 USA kmeyer@umces.edu, joneil@umces.edu; <sup>2</sup>Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575 USA cheil@bigelow.org

Viral concentrates were obtained from Gulf of Mexico seawater collected from the west Florida shelf to investigate the effects of viruses on the red tide dinoflagellate *Karenia brevis* and associated bacterial communities. Abundance of live *K. brevis* cells and several parameters of bacterial growth and production (abundance, thymidine incorporation, leucine incorporation, bacterial growth rate, and bacterial growth efficiency) were assessed in addition to viral abundance.

To obtain viral concentrates seawater was pre-filtered through 0.2µm filters before being concentrated via ultrafiltration with a 30kDa tangential flow filter. A portion of the viral concentrate was exposed to ultraviolet radiation to serve as negative controls. This experiment was conducted twice, once each in field and laboratory settings, both time using cultures of *K. brevis* provided by Florida Fish and Wildlife Research Institute. Viral concentrates (1mL) were added to 250mL of *K. brevis* culture diluted in 750mL 0.2µm filtered seawater. Culture water was either whole (*K. brevis* and associated microbes) or had *K. brevis* removed (associated microbes only). Field experiments used surface seawater collected ~22km south of Sanibel Island, FL (salinity 35.53) and then incubated ~24h at ambient light (691.6-1682.1µE m<sup>-2</sup> s<sup>-1</sup>) and temperatures (24.89-25.82 °C). Laboratory experiments used artificial seawater (salinity 38-39) and were incubated 72h in an environmental chamber at 24°C with cool white fluorescent light bulbs (5.1-33.0 µE m<sup>-2</sup> s<sup>-1</sup>) set on a 16:8 h light:dark cycle.

Addition of viral concentrates led to significant reductions in abundance of *K. brevis* when compared to treatments with addition of ultraviolet radiated viral concentrate or no addition of viral concentrate (p < 0.0001). Bacterial abundance significantly increased during the 24 hour incubation, but was not significantly different between treatments with viral concentrates and UV radiated viruses. Bacterial growth and production exhibited similar patterns with and without *K. brevis* present, suggesting that viruses are acting upon bacterial communities found within *K. brevis* blooms, and not *K. brevis* itself. This further suggests that the microbial community composition and metabolism during *K. brevis* blooms may have an influence on the size, magnitude, and status of naturally occurring *K. brevis* blooms.

## FUTURE SCENARIOS FOR ENVIRONMENTAL CONDITIONS FAVORING THE ACCUMULATION OF PARALYTIC SHELLFISH TOXINS IN PUGET SOUND SHELLFISH

Moore, S.K.<sup>1</sup>, Mantua, N.J.<sup>2,4</sup>, and Salathé Jr., E.P.<sup>3,4</sup>

<sup>1</sup>NOAA, Northwest Fisheries Science Center, West Coast Center for Oceans and Human Health, 2725 Montlake Blvd. E., Seattle, WA 98112 USA [stephanie.moore@noaa.gov](mailto:stephanie.moore@noaa.gov);

<sup>2</sup>School of Aquatic and Fishery Sciences, University of Washington, Box 355672, Seattle, WA 98195 USA [nmantua@u.washington.edu](mailto:nmantua@u.washington.edu); <sup>3</sup>Science and Technology Program, University of Washington-Bothell, 18115 Campus Way NE, Bothell, WA 98011-8246 USA [salathe@u.washington.edu](mailto:salathe@u.washington.edu); <sup>4</sup>Climate Impacts Group, University of Washington, Box 354235, Seattle, WA 98195 USA.

The marine dinoflagellate *Alexandrium catenella* produce potent neurotoxins called paralytic shellfish toxins usually when they bloom. These toxins can accumulate in shellfish and cause human illness or even death if contaminated shellfish are consumed. A specific combination of environmental conditions creates a window of opportunity for these harmful algal blooms (HAB-WOO) and can significantly increase the risk for toxic events in Puget Sound (Moore et al. 2009). HAB-WOOs of long duration indicate long periods of time when conditions in the marine environment are favorable for the development of toxic blooms that threaten shellfish safety.

Here we evaluate past trends and future scenarios for the HAB-WOO for the 2020s, 2040s, and 2080s using an innovative modeling approach. Specifically, we simultaneously calculate time periods when multiple environmental parameters are within a range that has been determined to be favorable for the development of toxic events. Model results show that the HAB-WOO duration increased since 1978, as did the frequency and geographic extent of toxic events.

Climate change projections for the Pacific Northwest are used to evaluate scenarios for the future HAB-WOO. Under a moderate greenhouse gas emissions scenario (i.e., A1B), the annual HAB-WOO is projected to increase by an average of 13 days by the end of the 21<sup>st</sup> century. Furthermore, the annual HAB-WOO may begin up to 2 months earlier in the year and persist for up to 1 month later in the year compared to the present day typical annual HAB-WOO time period.

The extended lead time offered by these projections will allow managers to put mitigation measures in place faster and more effectively to protect human health against these toxic outbreaks. This study demonstrates for the first time how a changing climate alters the marine environment in a way that may increase the risk of human exposure to HAB toxins.

## CHARACTERIZATION OF NATURAL ANTISENSE TRANSCRIPTS IN *KARENIA BREVIS* AND ASSESSING THEIR POTENTIAL ROLE IN REGULATING GENE EXPRESSION

Namataka, H.<sup>1</sup>, McLean, T.I.<sup>2</sup>

University of Southern Mississippi, Hattiesburg, MS 39406 USA  
<sup>1</sup>masawie@hotmail.com; <sup>2</sup>timothy.mclean@usm.edu

Multiple aspects concerning the molecular genetics of dinoflagellates make these unicellular organisms unique among all eukaryotes. For this reason, it is hypothesized that gene regulation in dinoflagellates is very different from that in other eukaryotes—the emerging hypothesis is that gene expression is primarily controlled at the post-transcriptional level rather than at the transcriptional level. The recently described spliced leader found at the 5' end of all dinoflagellate mRNAs suggests a possible mechanism by which post-transcriptional regulation may occur, but its role (if any) has not been established. An expressed sequence tag library created from cultured cells of *Karenia brevis* has uncovered a relatively large number of natural antisense transcripts (NATs). NATs have been observed in a wide variety of organisms, and some are known to play a role in regulating post-transcriptional gene expression.

We will describe the initial experiments that have led to the working hypothesis in our laboratory that NATs play a role in post-transcriptional regulation of gene expression in *K. brevis*. The existence of several NATs and their cognate sense mRNAs, representing genes from all three genomes, has been verified by strand-specific PCR. The sequences of NATs and mRNAs have been captured via RACE experiments, and the size and positioning of the regions of complementarity between each pair of transcripts varies. Double-stranded RNA, presumably formed through the binding of NATs and mRNAs, can be detected inside *K. brevis* cells. Real-time PCR and/or microarray experiments are being performed to determine the expression dynamics of NATs relative to mRNAs over a time course or under various conditions to assess if a positive or negative correlation of expression exists between these two types of transcripts. A positive correlation would suggest that NATs enhance gene expression, e.g. increases translation of an mRNA, and a negative correlation would suggest the opposite.

## BLOOM CHARACTERIZATION OF CYANOBACTERIA USING RADIOMETRY

Neff, C.<sup>1</sup>, Tomlinson, M.C.<sup>2</sup>, Stumpf, R.P.<sup>2</sup>, Wynne, T.T.<sup>2</sup>, Burks, R.<sup>3</sup>, Fahnenstiel, G.<sup>4</sup>

<sup>1</sup>Consolidated Safety Services, Inc., Fairfax, VA 22030, USA Cordelia.neff@noaa.gov;

<sup>2</sup>Center for Coastal Monitoring and Assessment, NOS/NOAA, Silver Spring, MD 20910 USA  
Michelle.Tomlinson@noaa.gov; Richard.Stumpf@noaa.gov;

Timothy.Wynne@noaa.gov; <sup>3</sup>Johns River Water Management District, Palatka, FL 32178 USA RBurks@sjrwmd.com; <sup>4</sup>OAR/GLERL, NOAA, Muskegon, MI 49441 USA, Gary.Fahnenstiel@noaa.gov

Different species of HABs have different optical reflectance. Radiometers provide reflectance from nominally 300 to 1000 nm, at a bandwidth of 1nm. Using algorithms it may be possible to identify specific species of HABs with spectra collected from field radiometry. The precision of the radiometer makes determination of pigments such as phycocyanin, zeaxanthin, and phycoerythrin possible. Much of the data shown here was collected in the western basin of Lake Erie which had an interesting bloom season in 2010. The bloom season started in June with a chlorophyte from the genera of *Pandorina* which quickly dissipated. In July there was a cyanobacterial bloom dominated by *Microcystis aeruginosa*. By September the bloom composition changed again and was dominated by *Anabaena* spp. *Anabaena*, a nitrogen fixer, may have had a competitive advantage over the non-nitrogen fixer, *Microcystis aeruginosa* as it is hypothesized that the bloom succession was brought on by decreased nutrient levels. *Microcystis aeruginosa* has a dominant accessory pigment of phycocyanin, which has an absorption peak at approximately 620 nm. *Anabaena* spp., however, lacks this pigment and has an alternative pigment of phycoerythrin, which has an absorption peak at approximately 500 nm. It therefore should be possible to differentiate these two cyanobacterium with field spectra. Additional assemblages of cyanobacteria will be considered. Sandusky Bay has persistent blooms dominated by *Planktothrix* spp. Other areas that will be discussed include the St. John's River in Florida and the Chesapeake Bay.

STIMULATING EFFECT OF *ANABAENA* SP. EXUDATE ON *PRYMNESIUM PARVUM*

Neisch, M.T.<sup>1</sup>, Roelke, D.L.<sup>1</sup>, Masser, M.P.<sup>1</sup>; Brooks, B.W.<sup>2</sup>; Grover, J.P.<sup>3</sup>

<sup>1</sup>Texas A&M University, College Station, Texas 77843 USA mneisch@tamu.edu;

<sup>2</sup>Baylor University, One Bear Place #97266, Waco, Texas 76798 USA; <sup>3</sup>University of Texas at Arlington, Arlington, Texas 76019 USA

*Prymnesium parvum* blooms have become more frequent in the south-central USA, leading to significant ecological and economic impacts. Allelopathic effects between cyanobacteria and *P. parvum* were suggested and previous studies showed that a cyanobacteria-rich community suppressed the development of *P. parvum* blooms. This research focused on the effects of cultured cyanobacteria, *Anabaena* sp., on *P. parvum*. Over a 6-day period, daily additions of filtrate from the *Anabaena* culture during a period of senescence were made to *P. parvum* cultures growing in log phase. All treatments, including several types of controls, showed reductions in biomass over the course of the experiment, but the treatments receiving *Anabaena* filtrate exchanges were reduced to a lesser degree, suggesting that filtrate from the senescent cyanobacteria culture was beneficial to *P. parvum* in some way. This unexpected result may have resulted from stimulation of heterotrophic bacteria by the addition of *Anabaena* filtrate, which likely contained exudates rich in dissolved organic carbon compounds. *P. parvum* was then able to supplement its nutritional requirements for growth by feeding on the elevated bacteria population. This research suggests that interactions between cyanobacteria and *P. parvum* in natural environments are complex, where both allelopathic and growth-stimulating interactions are possible.

STABLE ISOTOPE ANALYSIS AND UNDERWATER VIDEO SHOW  
IMPORTANCE OF HIGHLY LOCALIZED FACTORS IN CONTRIBUTING TO  
EXCESSIVE GROWTH OF HARMFUL MACROALGAL BLOOMS

Nelson, T.A.

Department of Biology; Seattle Pacific University; Seattle, WA 98119-1950 USA  
tnelson@spu.edu

Blooms of *Ulva* spp. and related genera have garnered increasing attention worldwide, outcompeting other primary producers, causing anoxia, and releasing toxic hydrogen sulfide fumes as they decay. Extensive underwater video analysis in Washington State, USA indicates that ulvoid algal abundance varies interactively between sites and from year to year. E.g., abundance may be high on average state-wide in a given year, yet many sites will show declines during that year. We examined nitrogen availability and sources using stable isotope analysis at a focus site and two control sites. Most ulvoid algae in Puget Sound are not nitrogen limited; rather light and desiccation on steeply-pitched shorelines control maximal abundance. However, at our focus location in Dumas Bay, we found that algae appeared nitrogen-limited. This location has a relatively large intertidal flat that is well-watered by 3 streams and groundwater flow. Nitrogen likely limits growth more than desiccation or light availability during summer months. Algae growing in most of Dumas Bay had an isotopic signature similar to that seen at control sites and consistent with upwelling-driven nitrogen supply. At the mouth of one creek discrepancies in this ration suggest that an anthropogenic source of nitrogen may exacerbate the bloom. We conclude that differences in upwelling and summer weather are not expected to uniformly affect all sites across the state accounting for the interaction effect between sites and year of study seen in underwater video analysis.

## WHEN *KARENIA BREVIS* IS OUT THERE, HOW DO PEOPLE DECIDE IF THEY SHOULD GO TO THE BEACH OR EAT SEAFOOD?

Nierenberg, K.<sup>1</sup>, Byrne, M.M.<sup>2</sup>, Studts, J.L.<sup>3</sup>, Bauza, C.<sup>2</sup>, Kirkpatrick, B.<sup>1</sup>

<sup>1</sup> Mote Marine Laboratory, Sarasota, FL 34236 USA knierenberg@mote.org, bkirkpat@mote.org; <sup>2</sup>University of Miami, Miami, FL 33136 mbyrne2@med.miami.edu, CBauza@med.miami.edu; <sup>3</sup>University of Kentucky College of Medicine, Lexington, KY 40536 USA jamie.studts@uky.edu

**Purpose:** To collect information regarding attitudes toward: 1) knowledge of Florida red tide, 2) going to the beach, and 3) eating shellfish during a Florida red tide bloom. Due to aerosolized neurotoxins produced by the algae, going to the beach during a Florida red tide can cause respiratory distress, especially among individuals with asthma. Eating shellfish can cause neurotoxic shellfish poisoning if the shellfish are contaminated with the toxins; however because of monitoring programs, this is an infrequent occurrence.

**Methods:** To develop a conjoint valuation instrument for decision making regarding Florida red tide, we conducted semi-structured interviews with 20 individuals in Sarasota, FL. Participants included residents and snowbirds, and people with and without asthma. Through these interviews, we collected information on what type of knowledge the individuals had about Florida red tide, and on whether individuals would either go to the beach or eat shellfish during a Florida red tide, and what factors would affect these decisions.

**Results:** The most common aspects of the knowledge people had of Florida red tide were that it caused fish to die, has a respiratory irritation associated with it, causes a bad odor and was manmade. Seven individuals stated definitively that they would not eat shellfish during a bloom. The most commonly cited factors affecting decisions about buying and eating shellfish during a Florida red tide were: trust in the restaurant or grocery store, trust in the inspection of seafood, where the seafood comes from (Gulf of Mexico or not), past bad experiences with seafood, and price. Six individuals said that they would never go to the beach during a Florida red tide, but 2 stated that they would not ever give up the beach. The most common factors affecting decisions to go to the beach were: presence of dead fish and noxious odor, influence of family and friends, wind speed and direction, official warnings, respiratory effects, and parking availability.

**Conclusions:** In general, participants indicated an aversion to eating shellfish or going to the beach during a Florida red tide bloom. Because a Florida red tide can last for many months, it can have detrimental economic consequences if individuals avoid buying seafood or participating in beach activities.

## NUTRIENT STRATEGIES OF *KARENIA BREVIS* AT ELEVATED CELL DENSITIES ALONG THE WEST CENTRAL FLORIDA COAST.

Nissanka, A.<sup>1</sup>, Dixon, L.K.<sup>1</sup>, and Hall, E.R.<sup>1</sup>

<sup>1</sup> Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236 USA  
ari@mote.org, lkdixon@mote.org, emily8@mote.org.

Red tides (*Karenia brevis*) occur nearly annually in Gulf of Mexico waters near the west central coast of Florida. Monitoring, conducted for early detection and public warning and for ecological model development, has included physical and nutrient analyses, forming a detailed database spanning 1996 – 2011. The ranges of nutrient regimes associated with *K. brevis* blooms and the ranges in cellular complements of C, N, and P provides insight as to the diverse nutrient strategies of the organism in non-laboratory settings over multiple years. With over 5500 samples to date and with counts as high as >3,000,000 cells L<sup>-1</sup>, expected drawdowns of both N and P inorganic nutrients have been observed with elevated cell counts. While in some years, urea does not vary with cell count, in other years, urea drawdown is evident and supports the reported utilization of urea by *K. brevis*. Water column supplies indicate that N-limited conditions are typical, although Redfield proportions of N and P are generally maintained regardless of cell count, and cellular carbon. Patterns of dissolved organic nitrogen have increased minima and decreased maxima under elevated cell counts, and could be a result of either DON release from *K. brevis* or an increase in microbial biomass and a consequent increase in DON remineralization. Asymptotic values of approximately 58, 5, and 0.3 pg cell<sup>-1</sup> for C, N, and P, respectively, with elevated cell counts persist between years and blooms and indicate a physiological minimum. Data were also categorized and analyzed statistically by density category, distance from shore, and latitude to examine the influence of bloom stage or density and differences in estuarine loadings and influence on nutrient utilization. Funding support is through the State of Florida (in conjunction with the Florida Fish and Wildlife Conservation Commission) and NOAA "EcoHAB: *Karenia* Nutrient Dynamics in the Eastern Gulf of Mexico".

## FLUOROMETRIC DISCRIMINATION OF PHYTOPLANKTON FUNCTIONAL TYPES TO MONITOR FOR HARMFUL ALGAE USING THE TURNER DESIGNS PHYTOFIND

Palacios, S.L.<sup>1</sup>, Kudela, R.M.<sup>1</sup>, Hayashi, K.H.<sup>1</sup>, Crawford, J.<sup>2</sup>, Mayerfeld, P.<sup>2</sup>, Younan, L.<sup>2</sup>

<sup>1</sup>University of California – Santa Cruz, 1156 High St., Santa Cruz, CA 95064 USA sherry.palacios@gmail.com; <sup>2</sup>Turner Designs, Inc., 845 W Maude Ave, Sunnyvale, CA 94085 USA

Monitoring of harmful algal blooms (HABs) using traditional water collection techniques is labor intensive, costly, and episodic. Each sampling event represents only a snapshot of the phytoplankton community present at a particular time and can miss variability in taxon composition that may portend a HAB event. While intermittent water collection is necessary to enumerate algal communities to phytoplankton species, continuous detection of algal taxa using field-deployed instruments may enhance existing monitoring efforts.

Fluorescence spectroscopy is a powerful tool for the in-situ analysis of key aquatic constituents. With appropriate excitation-emission wavelength pairings, chlorophyll, algal color groups, and colored dissolved organic matter (CDOM) can be discriminated even in optically complex waters. Micro-algae can be divided into five basic color groups based on the characteristic fluorescence spectra of their peripheral antennae pigments. A generation of fluorescence-based tools has been developed to probe natural waters for diagnostic signals of particular algal color groups (e.g. 'blue', 'brown', 'green', 'red', and 'mixed'). The 'brown' color-group is broad, and discrimination between some taxa within this group, e.g. dinoflagellates and diatoms, is limited.

Turner Designs configured its popular C6 Multi-Sensor Platform with fluorescence probes selected to discriminate among algal color groups (the PhytoFind). Selection of five wavelength pairs was determined by 3-dimensional excitation-emission fluorescence spectra measured on monospecific algal cultures to arrive at the most effective wavelength pairings to diagnose particular algal groups. The wavelength pairs included excitation wavelengths centered at 430 nm, 460 nm, 530 nm, 590 nm, and 635 nm and emission detected at 685 nm. The sixth probe was configured for CDOM (ex/em: 365/470 nm) and is not used for detecting algae. The goals of this study were: 1) to develop an algorithm for the PhytoFind that discriminates among phytoplankton taxa (including among some members of the 'brown' group) and 2) apportion the biomass of those algal taxa using the fluorescence data. The instrument and algorithm were first tested using large-volume, monospecific algal cultures; then tested using whole-water field samples collected weekly from the Santa Cruz, CA Municipal Wharf (October 2010 to June 2011) and enumerated to genus. The algorithm consistently and accurately estimated algal composition of field samples. The PhytoFind may be a useful and accessible tool for the continuous monitoring of HAB taxa in marine and freshwater systems.

## TRANSCRIPTOME ANALYSIS OF THE TOXIN-PRODUCING DIATOM *PSEUDO-NITZSCHIA MULTISERIES*

Parker, M.S.<sup>1</sup>, Schruth, D.<sup>1</sup>, Berthiaume, C.<sup>1</sup>, Morales, R.<sup>1</sup>, Rocap, G.<sup>1</sup>, Jenkins, B.<sup>2</sup>, Bates, S.S.<sup>3</sup>, Marchetti, A.<sup>4</sup>, Armbrust, E.V.<sup>1</sup>

<sup>1</sup>School of Oceanography, University of Washington, Seattle, WA USA micaela@uw.edu, acgt@uw.edu, chrisbee@uw.edu, rmarohl3@uw.edu, rocap@ocean.washington.edu, armbrust@uw.edu; <sup>2</sup>Department of Cell and Molecular Biology and Graduate School of Oceanography, University of Rhode Island, RI USA bdjenkins@mail.uri.edu; <sup>3</sup>Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, New Brunswick, Canada Stephen.Bates@dfp-mpo.gc.ca; <sup>4</sup>Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC USA amarchet@email.unc.edu

Diatoms of the cosmopolitan genus *Pseudo-nitzschia* thrive in coastal environments and can produce the potent neurotoxin domoic acid (DA). DA can bioaccumulate in shellfish that are consumed by humans and marine mammals, posing a health risk with the potential to induce illness and even death. Previous studies have demonstrated that not all species of *Pseudo-nitzschia* (or even strains within a species) produce the same amount of DA. Different environmental triggers also appear to modulate DA levels in cells. To uncover the molecular basis for toxin production, *Pseudo-nitzschia multiseries* CLN-47 was chosen for whole genome sequencing by the Joint Genome Institute (JGI). JGI has completed draft assembly and automated gene modeling and annotation are underway. To supplement sequencing efforts and facilitate development of gene models, additional cultures of *P. multiseries* were harvested for transcriptome analyses. Cells were grown under five different conditions, which yielded varying amounts of DA per cell: exponential phase with inorganic nitrogen (0.2 pg DA/cell); exponential phase with urea (3.6 pg DA/cell); iron-limited (0.4 pg DA/cell); silicate-starved (6.2 pg DA/cell); and phosphate-starved (9.4 pg DA/cell). Total RNA was extracted from each culture and divided between the Armbrust lab and JGI. JGI sequenced the cDNA using Sanger-based technology to generate over 74,544 expressed sequence tags (ESTs). Using the same RNA, we are sequencing the whole transcriptomes of these cultures with an AB SOLiD, bar-coding the individual libraries for a single run to generate a depth of coverage previously shown to yield reliable differential gene expression in a related species, *Pseudo-nitzschia granii*. In a separate experiment, *Pseudo-nitzschia multiseries* CLNN-17 was grown under three different conditions (exponential phase with nitrate; nitrate-starved; silicate-starved) and also harvested for transcriptome analyses using the AB SOLiD. These sequences will be analyzed together with our CLN-47 sequences and allow us to examine differences in gene expression patterns between two strains of the same species. By focusing on the subset of genes that are expressed similarly across toxin-producing conditions, we hope to gain a better understanding of how and why *Pseudo-nitzschia* produce DA.

# THE EFFECTS OF ANNUAL SEEDING ON THE DISTRIBUTION OF A SAXITOXIN-RESISTANT MUTATION IN *MYA ARENARIA*

Phillips, J., Connell, L.B.

School of Marine Sciences, University of Maine, Orono, ME 04469

Harmful algal blooms (HABs) of *Alexandrium* spp., producers of paralytic shellfish toxins (PSTs), occur worldwide. Prior work has identified a sodium ( $\text{Na}^+$ ) channel mutation present in softshell clam *Mya arenaria* populations that causes a 1,000-fold decrease in binding affinity at the saxitoxin-binding site in the  $\text{Na}^+$  channel pore and thus confers greater resistance to PSTs. This leads to improved fitness (growth, motility and survival advantage of resistant individuals) during toxic blooms, as well as higher toxin accumulation rates. Therefore, PSTs may act as a strong natural selection agent in nature, leading to spread of toxin resistance in *M. arenaria* populations. Since the prevalence and intensity of *Alexandrium* spp. blooms varies greatly along the east coast of North America, selection for resistance may only occur in regions/sites experiencing severe red tides. An important factor to consider when attempting to relate the prevalence of the resistant (R) allele with the frequency of HAB events is the practice of *M. arenaria* seeding in the Gulf of Maine. The seeding program at the Downeast Institute in Beals, ME delivers millions of juvenile *M. arenaria* to communities in the Gulf of Maine each year. The broodstock for this program was developed by taking clams from the surrounding area, which has a very high incidence of the R allele, and selectively breeding for desired traits. This study examined the effects of annual seeding (>20yr) on the genetic composition of clams in targeted communities with respect to toxin resistance.

## COMPARISON OF THE POLYKETIDE SYNTHASE GENES IN *KARLODINIUM VENEFICUM*, *KARENIA BREVIS*, AND *CHRYSOCHROMULINA POLYLEPSIS*

Place, A.R.

Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Sciences, Baltimore, MD USA

The polyketide synthases (PKSs) are large multi-domain enzymes or enzyme complexes closely related to fatty acid synthases (FASs). They are composed of the identical ancestral set of functional modules: ketoacyl synthase (KS), acyl transferase (AT), ketoacyl reductase (KR), dehydratase (DH), enoyl reductase (ER), acyl carrier protein (ACP) [or phosphopantetheine attachment site (PP)], and thioesterase (TE) domains. Whereas all units are needed for fatty acid production by FASs, the minimal structure of PKSs requires only ACP, KS and AT for the condensation reaction of acetate units. The other domains, if present, can catalyse the stepwise reduction of the initial carbonyl units. Monroe & van Dolah (2008) managed to isolate full-length sequences of single catalytic domains from PKS genes from *Karenia brevis* by screening cDNA libraries. The sequences obtained were most similar to type I modular PKS, but the structure is most similar to type II PKS. Recently, John et al. (2010) have characterized the the PKS genes of *Chrysochromulina polylepsis* and found they belong to the type I group, as is the case for *Karenia brevis*, but whereas the structure of the dinoflagellate PKS, with its discrete catalytic domains, suggests a novel type I-like PKS gene, *C. polylepsis* seems to exhibit the conventional modular type I structure. Given these observations we screened our *Karlodinium veneficum* and *Amphidinium carterae* full length cDNA libraries to find homologs. Some but not all of the genes were found. A strategy for deciphering this mosaic nature for the dinoflagellate PKS machinery will be discussed.

John, Uwe , Beszteri, Sara , Glöckner, Gernot , Singh, Rama , Medlin, Linda and Cembella, Allan D.(2010) 'Genomic characterisation of the ichthyotoxic prymnesiophyte *Chrysochromulina polylepsis*, and the expression of polyketide synthase genes in synchronized cultures', *European Journal of Phycology*, 45: 3, 215 — 229

## INDUCTION OF PROGRAMMED CELL DEATH IN DINOFLAGELLATES BY BACTERIAL ALGICIDE IRI-160AA

Pokrzywinski, K.L., Tilney, C.L., Warner, M.E., Coyne, K.J.

University of Delaware College of Earth, Ocean, and Environment, Lewes, DE 19958  
USA Kaytee@udel.edu, ctiley@udel.edu, mwarner@udel.edu, kcoyne@udel.edu

In metazoans, programmed cell death (PCD) is initiated by a cascade of proteolytic activity, due to expression and activation of cysteine-arginine/lysine-specific proteases (caspases). This process induces changes in cell morphology, DNA fragmentation and degradation, membrane blebbing, and production of reactive oxygen species such as H<sub>2</sub>O<sub>2</sub>. Mechanisms regulating PCD in phytoplankton are not well understood but PCD-like activities have been documented in phytoplankton under stressful conditions. Previous work also suggests that metacaspases in phytoplankton species may function in a process similar to caspases in multicellular organisms. Induction of PCD in phytoplankton by algicidal bacteria has never been investigated. Hare et al. (2005) recently described a bacterium, *Shewanella* sp. strain IRI-160 that has algicidal activity specific to dinoflagellates. Further work in our laboratory demonstrated that direct cell contact was not necessary and that algicidal activity was due to a compound (designated IRI-160AA) secreted into the medium. Preliminary results indicated that IRI-160AA induced changes consistent with PCD. Here, we examined caspase-like activity on cell extracts using a fluorogenic assay, and by epifluorescence microscopy after exposure to IRI-160AA. Our data shows that caspase-like activity was enhanced in several species of HAB dinoflagellates 24 hours after addition of the algicide. A significant increase in H<sub>2</sub>O<sub>2</sub> production was also observed in cultures exposed to IRI-160AA. Together, these data provide strong evidence that PCD or a PCD-like process is occurring in these dinoflagellates after induction with the algicidal compound IRI-160AA.

## CHEMICAL CHARACTERIZATION OF ALLELOPATHIC COMPOUNDS PRODUCED BY THE RED TIDE DINOFLAGELLATE, *KARENIA BREVIS*

Poulson-Ellestad, K.,<sup>1</sup> Prince, E.K.,<sup>1</sup> Myers, T.L.,<sup>1</sup> Sieg, R.D.<sup>1</sup> and Kubanek, J.<sup>1,2</sup>

<sup>1</sup>School of Biology and <sup>2</sup>School of Chemistry and Biochemistry, Georgia Institute of Technology Atlanta, GA USA

Many species of phytoplankton produce and release compounds that inhibit the growth of competitor species, a process called allelopathy. Allelopathy may also be important for bloom-forming species, such as the dinoflagellate *Karenia brevis*, as a way to maintain monospecific blooms. Both blooms and cultures of *K. brevis* exude multiple compounds that inhibit the growth of competitors. Although the identities of these compounds are still unknown, we were successful in partially characterizing allelopathic compounds produced by *K. brevis* cultures. Allelopathic compounds from *K. brevis* are unstable, polar, organic molecules that are either neutral or positively charged, produced and released at low concentrations. *Karenia brevis* also produces a suite of less polar, but more stable compounds that are moderately allelopathic towards the model competitor *A. glacialis*. Allelopathic compounds displayed molecular weights between 500 and 1000 Da and possessed aromatic functional groups as determined by size exclusion chromatography and <sup>1</sup>H NMR spectroscopy. Brevetoxins, potent neurotoxins produced by *K. brevis*, had no allelopathic effects on competitors. We also investigated the species-specificity of the less polar allelopathic compounds produced by *K. brevis* and found that these compounds inhibit the growth of competitors *A. glacialis*, *Skeletonema grethae*, *Prorocentrum minimum*, and *Akashiwo sanguinea*, although competitors were each susceptible to different *K. brevis* compounds among this mixture. Our results indicate that the ability of *K. brevis* to out-compete co-occurring phytoplankton may be facilitated by the production of multiple allelopathic compounds, which provides a large chemical arsenal against taxonomically-diverse competitors.

## ALLELOPATHY OF A RED TIDE DINOFLAGELLATE HAS CONTEXT-DEPENDENT IMPACTS ON PHYTOPLANKTON

Poulson-Ellestad, K.,<sup>1</sup> Sieg, R.D.<sup>1</sup> and Kubanek, J.<sup>1,2</sup>

<sup>1</sup>School of Biology and <sup>2</sup>School of Chemistry and Biochemistry, Georgia Institute of Technology Atlanta, Georgia, USA

Allelopathy has been hypothesized to play an important role in shaping planktonic communities by affecting competitive outcomes between phytoplankton species. Allelopathic interactions have also been hypothesized to play a role in the ecology of bloom-forming species. We studied how a variety of ecological conditions affected the efficacy of allelopathic compounds produced by the red tide dinoflagellate, *Karenia brevis*. To determine how complex ecological communities respond to *K. brevis* allelopathy, natural, mixed plankton assemblages were exposed to allelopathic *K. brevis* extracellular extracts. Although these extracts were allelopathic in lab studies, some taxa were not susceptible to allelopathic compounds in the presence of a complex community, whereas the growth of other phytoplankton groups was stimulated by extract addition. In lab-based studies, cell physiological state and cell concentration were both important in dictating the susceptibility of competitors to allelopathy. We found that *Skeletonema grethae* was most susceptible to *K. brevis* allelopathy when in earlier growth stages (lag stage) rather than later growth stages. Additionally, cell concentration and growth stage of *K. brevis* were also found to effect allelopathic potency towards the diatom *Asterionellopsis glacialis*. There were also differences in allelopathy among five *K. brevis* strains. Specifically, strains isolated from coastal Texas, USA were less allelopathic than strains isolated from coastal Florida, USA. These results demonstrate that *K. brevis* allelopathy is highly context-dependent.

## LOWERING PH MITIGATES HARMFUL BLOOM FORMATION OF PRYMNESIUM PARVUM DURING A LIMNOCORRAL STUDY IN LAKE GRANBURY, TX, USA

Prosser, P.<sup>1</sup>; Valenti, T. Jr.<sup>1</sup>, Hayden, N.<sup>2</sup>; Neisch, M.<sup>2</sup>; Hewitt, N.; Gable, G.<sup>3</sup>; Grover, J.<sup>2</sup>; Roelke, D.<sup>2</sup>, Brooks, B.

<sup>1</sup>Baylor University Krista\_Prosser@baylor.edu, Valenti.Ted@epamail.epa.gov; Bryan\_Brooks@baylor.edu; <sup>2</sup>Texas A&M University natanyahayden@yahoo.com, mneisch@neo.tamu.edu, hewitt@neo.tamu.edu, droelke@tamu.edu; <sup>3</sup>Texas Commission on Environmental Quality George.Gable@tceq.texas.gov; grover@uta.edu

*Prymnesium parvum* (golden algae) is an invasive harmful algal bloom species that releases a mixture of toxins resulting in severe fish kills in inland and coastal ecosystems. The frequency and severity of inland *P. parvum* blooms have increased over the past decade, and have shown to be particularly severe in the Brazos River basin, which includes Lake Granbury, Texas, during late Winter/early Spring months. Previous research from our team has identified the importance of pH on determining the magnitude of aquatic toxicity associated with *P. parvum* blooms in inland waters. Ambient toxicity to fish and cladocerans resulting from harmful *P. parvum* blooms were reduced at lower pH levels, suggesting that *P. parvum* toxins exhibit similar toxicity characteristics to weak bases. In this study, we performed two 21 d limnocorral mesocosm experiments in Lake Granbury during Spring 2010. Experiment 1 consisted of two pH treatment level manipulations (7, 7.5) and assessed pre-bloom conditions, whereas Experiment 2 consisted of three pH treatment levels (7, 7.5, 8) to understand bloom development and decline. Limnocorral samples were collected weekly to assess ambient toxicity to *P. promelas* survival and *Daphnia magna* reproduction, and to assess zooplankton and phytoplankton biomass. During Experiment 1, *P. parvum* density and ambient toxicity in both pH treatment levels (7, 7.5) were significantly reduced ( $p < 0.05$ ) relative to controls, which exceeded harmful algal bloom (HAB) threshold levels and resulted in high toxicity to *P. promelas*. During Experiment 2, *P. parvum* density increased and exceeded HAB thresholds in control and pH 8 treatment level; the lower two pH treatment levels did not exceed HAB thresholds (7, 7.5). Over the 21 d study, control limnocorrals became increasingly acutely toxic to *P. promelas*. Similar to Experiment 1, a statistically significant difference was observed between *P. promelas* LC<sub>50</sub> values and pH treatment level ( $p < 0.05$ ). Acute toxicity to fish was significantly ameliorated by the pH 7 treatment level, but not the 7.5 or 8 treatment levels, on d=7, d=14 and d=21. We subsequently performed a Toxicity Identification Evaluation (TIE) pH manipulation approach to further examine the effect of a lower pH level on the limnocorrals exhibiting higher ambient toxicity. Results showed that the toxicity response to *P. promelas* was completely ameliorated in samples titrated to a pH of 6.5. In both experiments, no apparent adverse effects of pH manipulations were observed on zooplankton or phytoplankton biomass. The observations from these experiments, which examined pre-bloom conditions and bloom development, are consistent with the previous reports by our research team and thus highlight the importance of considering site-specific pH in the environmental assessment and management of *P. parvum* harmful algal bloom toxicity.

## WATCH WHAT YOU WATCH FOR: DEGRADATION OF PARTICULATE DOMOIC ACID (PDA) FIELD SAMPLES

Quay, J.Q.<sup>1</sup>, Hayashi, K.<sup>1</sup>, Kudela, R.M.<sup>1</sup>

<sup>1</sup>University of California Santa Cruz, Santa Cruz, CA 95064 USA, jqlane@gmail.com

At US HAB 2007 we presented work which assessed the degradation of particulate domoic acid (pDA), harvested from a mono-clonal batch culture, under common storage conditions (4, -20, -80 °C and liquid nitrogen). From that study, we determined that (1) pDA degrades under common storage conditions, with degradation exceeding measurement error (by ELISA) after about 2 months of storage, and (2) while increasing storage temperature results in increasing variability of replicates, this variability was minor over short (< 1 month) periods compared to the analytical variability in pDA measurement (by ELISA).

These results were compelling, and suggested the need for a more rigorous and comprehensive evaluation of pDA degradation. We revisit the subject here using field-collected pDA samples from the Santa Cruz Municipal Wharf (SCMW) in Monterey Bay, California. Unless specified otherwise, all pDA analyses were by LC-MS. We address (1) short-term and long-term degradation of field-collected pDA under multiple storage temperatures, (2) a multi-year evaluation of the degradative effects of archiving pDA samples at -80°C, and (3) pDA degradation in archived post-processing extract.

A short-term (24 hours, at 4, -20, and -80 °C) pDA degradation study was conducted as 3 successive weekly trials during a significant bloom event at SCMW. Results from the three trials indicate that degradation can be up to 20% within 24 hours, but that this rate is inconstant from week-to-week. A long-term (-20 and -80°C storage for 1 year; 4°C storage treatment for 2 months only) pDA degradation study was conducted using filters collected during a significant *Pseudo-nitzschia* bloom event at SCMW. Results indicate that pDA degradation is rapid within the first several weeks of storage, with a decline in the rate of degradation over several months, across all storage treatments; degradation is 50% after one year of storage, irrespective of storage treatment. Our evaluation of the effects of pDA archiving at -80°C confirms this result: we observe about 50% pDA degradation in our archived replicates, consistently across filters which have been archived for one year or longer. Finally, we observed pDA degradation in post-processing extract stored in glass at 4°C.

Based on these results, our conclusions and recommendations are as follows:

- \* It is necessary to process and analyze pDA samples the same day they are collected same day as collection.
- \* pDA is more stable when stored as post-processing extract (versus filters), but degradation is observed in both cases.
- \* The results of this study (obtained by LC-MS) are consistent with the results obtained in our previous studies (obtained by HPLC and/or ELISA).
- \* We observe no increase in isomers as a result of degradation -- the pDA signal simply gets smaller.
- \* It is not possible to determine whether the loss we observe is true degradation or is adsorption, etc., but the consistent loss across all weeks suggests that it's not related to environmental conditions at the time of collection, or to minor processing changes between samples
- \* There is considerable variability in actual degradation, so the development and application of a correction factor would be inappropriate and ineffective.

THE EFFECT OF EUGLENOPHYCIN ON COPEPOD SPECIES *SCHIZOPERA KNABENI*

Rafalski, A.V.<sup>1</sup>, Savage, M.L.<sup>2</sup>, Withers, K.<sup>3</sup>, Zimba, P.V.<sup>4</sup>

<sup>1</sup>Texas A & M University – Corpus Christi, Corpus Christi, TX 78412 USA  
arafalski@islander.tamucc.com; <sup>2</sup>msavage@islander.tamucc.edu;  
<sup>3</sup>kim.withers@tamucc.edu ; <sup>4</sup>paul.zimba@tamucc.edu

At least six divisions of algae are known to produce bioactive metabolites that result in animal malaise or even mortality. Euglenophycin from freshwater *Euglena* species has been shown to be toxic to fish in four states (North Carolina, Texas, Mississippi, South Carolina) and from Argentina and Ecuador. The NIVA culture collection *E. sanguinea* clones also produce euglenophycin toxin. While euglenophycin has been demonstrated to be toxic to fish and mammalian cancer cells, its effect on other animals is not characterized. Euglenophycin was collected from *E. sanguinea* cultures using HPLC-MS, and a 96 hour survival and fecundity toxicity test was completed with the marine copepod species *Schizopera knabeni*. Results indicated that the LD<sub>50</sub> of Euglenophycin for adult female copepods was less than 250 ppb. As *Euglena* grow well in eutrophic environments and can withstand dessication, high temperatures, and salinities to 6 ppt, it is expected that the prevalence of blooms will only increase in both freshwater and estuarine systems. As such, it is necessary to thoroughly understand the ecologic mechanisms of toxicity and the impacts on all aquatic biota.

## DOMOIC ACID DAMAGES OLFACTORY PATHWAYS FOLLOWING STATUS EPILEPTICUS LEADING TO AGGRESSIVE BEHAVIOR AND RECURRENT SPONTANEOUS SEIZURES IN A RAT EPILEPSY MODEL

Ramsdell, J.S., Tiedeken, J.A., Muha N., Maucher Fuquay, J.

Marine Biotoxins Program, NOAA – National Ocean Service, 219 Fort Johnson Rd, Charleston, SC 29412 USA john.ramsdell@noaa.gov

Domoic acid (DA) is a tricarboxylic acid produced by certain species of the cosmopolitan diatom *Pseudonitzschia sp.* that poisons humans and marine animals. Along with one human case study, an epidemiological report of hundreds of stranded sea lions found a portion of individuals presenting months later with a syndrome similar to temporal lobe epilepsy. To establish and better predict how chronic disease results from DA poisoning, we developed a rat model with an hourly “kindling” regime of subsymptomatic DA doses (1 mg/kg ip) to induce an acute period of seizure behavior known as status epilepticus (SE). A single episode of SE, lasting a minimum of 3 hrs, led to a permanent state of spontaneous recurrent seizures (SRS), a hallmark of epilepsy, in 94% of rats within six months.

Increased stress response to handling tests and atypical aggressive behavior towards conspecifics also developed in certain rats after SE. Detailed behavioral analysis shows an overall increase in aggressive behaviors in DA kindled rats, distinguished by an initial latent period of little aggression, followed by the more aggressive behaviors (wrestling, biting, and wrestling with biting) that transitioned to the lesser aggressive behaviors (dominance, sidling, pinning) before tapering off. Additionally, there is no correlation of aggression with SRS activity, but the aggressive behaviors themselves can be evidence of DA induced neurological disease regardless of motor seizure activity.

Brain mapping of neurodegeneration, using cupric-silver histochemistry was used to determine where brain damage occurred after SE and prior to development of neurological disease. Extensive damage was found in all animals in the olfactory bulb and related olfactory pathways, including the anterior olfactory cortex, endopiriform nucleus and entorhinal cortex. Surprisingly there was limited damage to the hippocampal formation. This finding is contrary to the existing literature for DA-induced histopathology, where few studies examine brain damage in regions more rostral to the hippocampus. This multi-aspect study provides insight into SE related damage induced by DA, specifically as it relates to the California sea lion, where many DA-poisoned animals appear to progress to a chronic disease characterized by SRS and aggressive behaviors.

## CIGUATERA FISH POISONING: A NEW REPORTING TOOL FOR PUBLIC HEALTH

Reich, A.<sup>1</sup>, Lazensky, B.<sup>1</sup>, Blackmore, C.<sup>1</sup>, Eisenstein, L.<sup>1</sup>, Hollenbeck, J.<sup>2</sup>, Fleming, L.<sup>3</sup>

<sup>1</sup>Florida Department of Health, andy\_reich@doh.state.fl.us; Becky\_Lazensky@doh.state.fl.us; Carina\_Blackmore@doh.state.fl.us; leah\_eisenstein@doh.state.fl.us; <sup>2</sup>University of Miami Rosenstiel School of Marine and Atmospheric Science, jhollenbeck@rsmas.miami.edu; <sup>3</sup>European Centre for Environment and Human Health, lora.fleming@pcmd.ac.uk

Ciguatera Fish Poisoning (CFP) is the most frequently reported marine poisoning world-wide. Exposures occur through ingestion of seafood, especially large tropical carnivorous finfish. Depending on the geographic location of exposure, symptomologies can vary with the suite of toxins produced by the organism(s).

Florida is the only state in the continental US with tropical coral reef systems. It has been proposed that the causative organism, *Gambierdiscus toxicus*, is a colonizing organism on these habitats, especially in areas with mechanisms for environmental perturbations, such as hurricanes. In Florida, CFP is a reportable illness (FS 381.0031(1, 2)) which requires both single case and outbreak reporting. Between 2000 and 2011, two hundred and fifty CFP cases/outbreaks were reported in the Florida Department of Health's (FDOH) disease tracking system, "Merlin".

By conducting routine surveillance using the Florida Poison Information Network call center data, additional cases of CFP are captured. Both clinical practitioners and private citizens can obtain basic poison information, status reports on occurrences, and for health care professionals (such as emergency department staff), technical support related to diagnosis and treatment through calling these information centers. Since 2003, there have been over 400 calls to the Poison Centers related to ciguatera exposure, with the majority handled by the Miami Center, which hosts the Aquatic Toxins Hotline.

Prior to 2011, the reporting form utilized by the FDOH County Health Departments was an outdated instrument known as the "cigua-file." In an effort to develop a modern, health-based surveillance tool, the FDOH Aquatic Toxins Disease Prevention Program collaborated with public health and marine scientists to include the latest CFP symptomology, clinical epidemiology and exposure assessments available. These include an improved demographics section, greater number of symptoms, better description of symptom characteristics, and more robust health care provider information. In addition, the format used mimicked that of the Merlin platform to facilitate long-term incorporation into this reportable disease system.

This new reporting form is currently in use by FDOH County Health Departments, Division of Environmental Health Food and Waterborne Illness Program and the Aquatic Toxins Disease Prevention Program. It is also available to users of Merlin as an attachment. The form is also being incorporated, with some minor changes, into the authorized Merlin Reportable Disease system as an extended data screen. It is anticipated that with greater utilization of this new and improved surveillance tool, the result will be a better understanding of the risk factors of ciguatera and ultimately greater protection of the public's health.

POPULATION DYNAMICS, DIVERSITY, AND PHYSIOLOGY OF CIGUATERA ASSOCIATED DINOFLAGELLATES FROM ST. THOMAS, U.S. VIRGIN ISLANDS.

Richlen, M.L.<sup>1</sup>, Smith, T.<sup>2</sup>, Robertson, A.<sup>3</sup>, Xu, Y.<sup>1,4</sup>, Kulis, D.<sup>1</sup>, Flores Quintana, H.A., Garcia, A.C.<sup>3</sup>, Hooe-Rollman, J.I.<sup>3</sup>, Anderson, D.M.<sup>1</sup>

<sup>1</sup> Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543 USA mrichlen@whoi.edu; <sup>2</sup> University of the Virgin Islands, St. Thomas, U.S. Virgin Islands 00802 USA.; <sup>3</sup> FDA, Gulf Coast Seafood Laboratory, Dauphin Island, Alabama 36528 USA; <sup>4</sup> Nanjing University, Nanjing, Jiangsu 210093, P.R.China

Ciguatera fish poisoning (CFP) is the most common form of finfish-associated illness in the United States, with international data suggesting that it is the most common of all marine food poisonings. The U.S. Virgin Islands (USVI) has a well-documented and significant annual incidence of ciguatera, and has been identified as an area of high risk in the Caribbean. As part of ongoing studies of the ecology and epidemiology of ciguatera in the USVI, monthly environmental data were collected from four sampling locations in St. Thomas, including dinoflagellate abundance (*Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* spp.), as well as water quality and reef health parameters. These field studies were coupled with laboratory analyses to document the genetic diversity, toxicity, and physiology of *Gambierdiscus* spp. present in these locations.

Over a three year period of sampling, seasonal trends in dinoflagellate abundance were apparent in which cell densities were highest in the summer-fall months, coinciding with highest seawater temperatures. This seasonal trend was most evident at the two locations on the southeastern side of the island. Patterns of abundance also corresponded to toxicity in dinoflagellate field samples and fish collected from these locations. In addition to dinoflagellate enumeration, *Gambierdiscus* cultures were established from field samples on a monthly basis and used to assess the taxonomy and physiology of *Gambierdiscus* spp. at the study sites. These studies showed that *Gambierdiscus* populations in St. Thomas are comprised of at least five species; *Gambierdiscus caribaeus* and *Gambierdiscus belizeanus* were most frequently observed. A subset of these isolates was subjected to multiple growth experiments to determine how growth and toxicity responds to changing temperature, salinity, and light levels. Both intra- and inter-specific variability in maximum growth rates and tolerances was observed among our isolates, and also when these results were compared to growth rate data for these species from the scientific literature. Results of these studies will be discussed, including how the physiological and species diversity as well as local environmental conditions may contribute to toxicity in fish.

## CIGUATOXICITY OF INVASIVE LIONFISH IN THE U.S. VIRGIN ISLANDS AND PUERTO RICO

Robertson, A.<sup>1</sup> Smith, T.<sup>2</sup>, Garcia, A.C.<sup>1</sup>, Flores Quintana, H.A.<sup>1</sup>, Hooe-Rollman, J.I.<sup>1</sup>, Granade, H.R.<sup>1</sup>, Olsen, D.<sup>3</sup>

<sup>1</sup> FDA, Gulf Coast Seafood Laboratory, Dauphin Island, AL USA; <sup>2</sup> University of the Virgin Islands, St. Thomas, USVI; <sup>3</sup> St. Thomas Fishermen's Association, St. Thomas, USVI USA

Lionfish (*Pterois volitans* and *P. miles*) are native to tropical and sub-tropical reef ecosystems in the southern Indian Ocean, South Pacific, and Red Sea. As opportunistic predators with few native predators, lionfish populations have been able to expand rapidly following introduction into Florida waters. Local population densities are now rapidly increasing throughout the western Atlantic and Caribbean Sea. In the U.S. Virgin Islands, lionfish were first reported off St. Croix in June 2008, and in St. Thomas and St. John, in 2010. In an effort to reduce their proliferation and geographical expansion, lionfish have been identified as a fisheries resource by NOAA, promoted in cookbooks, diving magazines, and popular media. While this could represent a great opportunity in local communities as an artisanal fishery, lionfish also pose a potential human health hazard based on the presence of venomous spines, and as a potential vector of ciguatera. Ciguatera is a severe poisoning syndrome that can occur following consumption of fish containing ciguatoxins, a suite of neurotoxins that activate ion channels on nerve cell membranes. Ciguatoxin precursors (gambiertoxins) are produced by benthic and epiphytic dinoflagellates of the genus *Gambierdiscus*, which are transferred to herbivorous fishes, and undergo bioconversion in predatory species to produce ciguatoxins.

In this study, lionfish (n=57) were collected from waters surrounding the U.S. Virgin Islands and Puerto Rico throughout 2010 and 2011. All samples were analyzed using a sodium channel-based neuroblastoma cytotoxicity assay to determine total composite toxicity as a Caribbean ciguatoxin-1 (C-CTX-1) equivalent, and a new LC-MS/MS method for molecular confirmation in tissue extracts. To date, 58% of the lionfish samples tested contained C-CTX-1 and C-CTX-2. Of these, more than 40% were above the current FDA guidance levels of 0.1 ppb C-CTX-1 equivalents. Health risks associated with lionfish consumption and spatial variation of toxicity as it relates to other reef dwelling fish species were assessed. Further sampling of lionfish is underway to examine changes in toxicity between habitats (i.e. patch reefs, artificial structure, coral reefs), depth, and on a temporal scale to further evaluate and generate a risk profile that may provide guidance for fisheries and health management.

## HUMAN POPULATION AND CLIMATE CHANGE, ANTICIPATED EFFECTS TO HARMFUL ALGAL BLOOMS OF SOUTHCENTRAL USA

Roelke, D.L.<sup>1</sup>, Brooks, B.W.<sup>2</sup>, Grover, J.P.<sup>3</sup>

<sup>1</sup>Texas A&M University, College Station, Texas 77843 USA droelke@tamu.edu; <sup>2</sup>Baylor University, One Bear Place #97266, Waco, Texas 76798 USA; <sup>3</sup>University of Texas at Arlington, Arlington, Texas 76019 USA

The effects of inflow on phytoplankton dynamics and assemblage structure have long been an interest of ecologists and resource managers, especially when they are linked to the incidence of harmful algal blooms. In the southcentral USA, *Prymnesium parvum* blooms were linked to diminished inflows. Unfortunately, predictions anticipate drier conditions in this region with climate change and human population growth, where inflows could be reduced by up to 60%. This research employed data collected over several years from four lake systems (three impacted by *P. parvum* blooms), and our findings show that such reductions in flow will decrease the number of inflow events above bloom thresholds between 25-65%. Furthermore, the occurrences of long duration inflow events, critical to lake flushing, will nearly disappear, e.g., inflow events lasting longer than 20 days were predicted to decrease from 40 to 1. These changes are predicted to increase the frequency of *P. parvum* blooms and fish-kill events. Multivariate analyses of monitoring data from these lakes suggest that other factors might be harnessed to mitigate *P. parvum* blooms, which will likely be necessary for this region in a drier climate. These include surface water flushing with deep waters, pH reductions, nutrient enrichment and altered ratio, and manipulation of foodweb structure.

## IDENTIFYING ION CHANNEL SEQUENCES IN DINOFLAGELLATES

Ryan, D.E.<sup>1</sup>, Thompson, N.<sup>2</sup>, Campbell, L.<sup>3</sup>

<sup>1</sup>Texas A & M University, College Station, TX 77843 USA

deryan@geos.tamu.edu; <sup>2</sup>Texas A & M University, College Station, TX 77843 USA;

<sup>3</sup>Texas A & M University, College Station, TX 77843 USA lcampbell@ocean.tamu.edu

Dinoflagellate ion channels may participate in cellular osmoregulation. A 14-fold rise in *Karenia brevis* Wilson clone brevetoxin production has been measured during hypoosmotic and hyperosmotic shock treatments, which suggests that the sodium channel-binding molecule and toxic *K. brevis* osmoregulation are linked (Errera and Campbell 2011). To identify anion and cation channels in *K. brevis* and other dinoflagellate species, we amplified six ion channel nucleotide sequences via polymerase chain reaction in *K. brevis*, *Karenia mikimotoi*, and *Prorocentrum* sp. cDNA with a dinoflagellate-specific spliced leader sequence forward primer and sequence-specific reverse primers. The targeted sequences were chosen from a pool encompassing 65,266 expressed sequence tags (EST) from four *K. brevis* EST libraries. Each targeted sequence significantly matched an EST from one of three ion channel libraries, as determined by seed alignment comparison, and was designated a likely ion channel protein coder after additional library abundance, duplicate identification, and amino acid sequence analysis. Among the six ion channel sequences, sequences homologous to both cation and anion channel protein coders from alveolate and bacteria species are present. All six sequences within dinoflagellate cDNA were successfully amplified and separated into bands during gel electrophoresis. Some ion channel sequences demonstrated similar length and abundance across species, though others varied, suggesting differential splicing and different transcription levels, respectively. Multiple PCR and gel reactions produced consistent results.

The identification of sodium channel protein coding nucleotide sequences in *K. brevis* cDNA supports the hypothesis that brevetoxins are associated with osmoregulation. Further research into dinoflagellate ion channel transcripts may provide amino acid sequence information elucidating anion and cation channel structures, transcription rate variations over different species and environmental conditions, and the role of ion channels in osmoregulation, particularly in toxic dinoflagellates.

Errera, R. M. and L. Campbell. 2011. **Osmotic stress triggers toxin production by the dinoflagellate *Karenia brevis***. *PNAS*. 108: 10597-10601

## IDENTIFYING INITIAL REACTIONS IN THE BIOSYNTHESIS OF DOMOIC ACID.

Savage, T.J.<sup>1</sup>, Clark, A.T.<sup>1</sup>, Smith, G.J.<sup>2</sup>

<sup>1</sup>California State University Sacramento, Sacramento, CA 95819 USA  
tjsavage@csus.edu; <sup>2</sup>Moss Landing Marine Laboratories, Moss Landing, CA 95039 USA  
jsmith@mlml.calstate.edu

Domoic acid (DA) production by marine diatoms varies markedly in response to different environmental stimuli, but the molecular events that regulate DA biosynthesis are poorly understood. Understanding the molecular regulation of DA production first requires a description of its biosynthetic pathway. Early feeding experiments with <sup>13</sup>C acetate indicate DA is likely formed from the condensation of a C<sub>10</sub> isoprenoid with an activated TCA cycle intermediate to generate a pyrrolidine ring skeleton that is subsequently converted to DA. However, the mechanism of pyrrolidine ring formation and the identity of the TCA cycle-derived precursor have not been elucidated. Here, we incubated *Pseudo-nitzschia* cultures with [1-<sup>2</sup>H<sub>2</sub>]geraniol and measured incorporation of label into DA using GC-MS after derivatization with propyl chloroformate to differentiate between ring formation pathways that involve hydrolysis of geranyl diphosphate and oxidation to the aldehyde prior to condensation with an amino nitrogen, and pathways that involve direct nucleophilic substitution of the pyrophosphate of geranyl diphosphate by the amino group. Both <sup>2</sup>H from <sup>2</sup>H<sub>2</sub> geraniol are incorporated into DA, which is consistent with a pathway that involves nuclear displacement of the pyrophosphate by an amino group. In addition, we incubated cultures with either <sup>13</sup>C- or <sup>15</sup>N-labeled glutamate then measured incorporation of label into DA and other amino acids to identify the activated derivative of the TCA cycle that is incorporated into DA. These experiments provide a biochemical foundation for identifying DA biosynthetic genes in bioinformatic searches of *Pseudo-nitzschia* sequence databases.

# STRAIN DIFFERENTIATION OF *EUGLENA SANGUINEA* BY GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY OF CELLULAR FATTY ACIDS

Savage, M.L., Zimba, P.V.

<sup>1</sup>Texas A&M University - Corpus Christi, 6300 Ocean Dr., Corpus Christi, TX 78412 USA msavage@islander.tamucc.edu, paul.zimba@tamucc.edu

Chemo-taxonomy has been used for the identification of bacteria by their cellular fatty acid (FA) profile for the last twenty years. This approach, if applicable to eukaryotic algae, could help to save time and money and provide unambiguous results in labs not equipped for toxin identification. Euglenoid algae have recently been shown to produce a potent neurotoxin-this toxin was in samples from several USA states, Denmark, Ecuador, and Argentina. We studied the difference in the fatty acid composition of four different strains of *Euglena sanguinea* cultured under maximal growth rate conditions (non-axenic). Maximal growth rates were obtained using 28°C, 18:8 L:D cycles – these conditions also resulted in maximal toxin production. *E. sanguinea* has approximately 14-20% lipid, of which 70% is  $\gamma$ -linolenic acid (C18:3n6) and palmitoleic acid (C16:1), and 20% is  $\alpha$ -linolenic acid (C18:3n3), oleic acid (C18:1n9c), and stearic acid (C18:0). EHA and DHA levels appear to be comparable to other green plant line algae such as Chlorophyta, Eustigmatophyta, and Bacilliarophyta. Comparison of strains appears not to have great utility at the sub-species level.

## MONITORING COASTAL OCEAN DYNAMICS WITH GLIDERS AND REMOTE SENSING TO UNDERSTAND THE ROLE OF VERTICAL AND HORIZONTAL ADVECTION ON HARMFUL ALGAL BLOOM INITIATION.

Seegers, B.N.<sup>1</sup>, Caron, D.A.<sup>1</sup>, Howard, M.D.A.<sup>2</sup>, Jones, B.H.<sup>1</sup>, Seubert, E.L.<sup>1</sup>

<sup>1</sup>University of Southern California, Los Angeles, CA 90089-0371 USA seegers@usc.edu, dcaron@usc.edu, bjones@usc.edu, seubert@usc.edu; <sup>2</sup>California Coastal Water Research Project, Costa Mesa, CA 92626 USA mhoward@sccwrp.org

A spring observational study was completed in the Southern California Bight near San Pedro Bay in 2009 and 2010 to better understand the spring seasonal variation in phytoplankton blooms related to a variety of factors including nutrients from upwelling events, rivers, and effluent plumes, and coastal dynamics. The HAB populations often outlast the original conditions supporting the blooms. Multi-month glider deployments combined with ocean color data allow for *in situ* and remote sensing monitoring of conditions before, during, and after harmful algal blooms (HABs). The gliders provide information about local subsurface ocean biology and physical dynamics, while satellites give regional large-scale physical and biological data. Webb Slocum gliders were deployed as the primary tools to gather *in situ* data for coastal assessment. The gliders were equipped with a CTD and optical instruments. The optical sensors included three fluorometers that measured chlorophyll *a*, colored dissolved organic matter (CDOM) and phycoerythrin/rhodamine, and a 3-wavelength optical backscatter sensor.

A primary objective of the research includes determining if seeding populations of HAB species are either advecting from depth or horizontally into the region and subsequently contributing to HAB initiation. The gliders are capable of monitoring many physical dynamics of the ocean including integrated subsurface currents, fronts, and eddies, which allow the oceanographic conditions that lead to HAB conditions to be observed. The glider observations were also used to target boat sampling where a larger set of variables and species specific information could be obtained from direct water samples.

Results from the glider observations show that often blooms of toxic *Pseudo-nitzschia* sp. developed offshore and subsurface prior to their manifestation in the surface layer and/or near the coast. By sampling tissue from barnacles that attach to the gliders during the deployment we have observed that low levels of domoic acid, indicative of the presence of the *Pseudo-nitzschia*, are present throughout much of the year and in most areas where the gliders were deployed. Episodic coastal transport events suggest that at least some occurrences of toxic *Pseudo-nitzschia* events could result from transport from other regions along the coast.

## MONITORING OF HARMFUL ALGAL BLOOM SPECIES AT TWO PIERS IN THE SOUTHERN CALIFORNIA BIGHT FROM 2008 TO 2011

Seubert, E.L.<sup>1</sup>, Gellene, A.G.<sup>1</sup>, Howard, M.D.A.<sup>2</sup>, Ragan, M.<sup>1</sup>, Jones, B.H.<sup>1</sup>, and Caron, D.A.<sup>1</sup>

<sup>1</sup>University of Southern California, Los Angeles, CA 90089 USA seubert@usc.edu, gellene@usc.edu, mragan@usc.edu, bjones@usc.edu, dcaron@usc.edu; <sup>2</sup>Southern California Coastal Water Research Project, Costa Mesa, CA 92626 mhoward@sccwrp.org

Monitoring of algal toxins in shellfish has been conducted by the California Department of Health (CDPH) and the Marine Biotoxin Monitoring Program (MBMP) since 1991, but the first report of a toxic *Pseudo-nitzschia* spp. bloom in San Pedro Channel occurred following an event in 2003 (Schnitzer et al. 2007). Seasonal monitoring of *Pseudo-nitzschia* spp. populations and particulate domoic acid concentrations in the San Pedro Channel and Los Angeles Harbor area has continued since 2003. The monitoring effort has demonstrated an increase in the toxicity of *Pseudo-nitzschia* spp. events in southern California over the past eight years.

We have conducted a weekly monitoring program at two coastal locations in the Southern California Bight as a means of increasing temporal resolution of the timing and location of blooms. The abundance of HAB species and environmental parameters such as temperature, salinity, nutrients and particulate algal toxin concentrations are monitored. Monitoring was begun in June 2008 at Newport Pier, Newport Beach, CA, as a part of the Southern California Coastal Ocean Observing System (SCCOOS) HAB and Red Tide Regional Monitoring Program. In January 2010, the weekly HAB species monitoring at coastal sites was expanded to include Redondo Beach Pier, Redondo Beach, CA. Analysis and comparison of the data collected at these two sites offer insight into the different HAB issues facing the Santa Monica Bay and Orange County areas, as well as insight into the parameters driving HAB events in southern California. Data will be presented on the discrete information collected from these sites, with a focus on *Pseudo-nitzschia* spp. and particulate domoic acid concentrations.

Schnitzer, A., P. Miller, R. Schaffner, B. Stauffer, B. Jones, S. Weisberg, P. DiGiacomo, W. Berelson, and D. Caron. 2007. Blooms of *Pseudo-nitzschia* and domoic acid in the San Pedro Channel and Los Angeles harbor areas of the Southern California Bight, 2003-2004. *Harmful Algae* 6:372-387.

## SPATIAL AND TEMPORAL TRENDS OF THE TOXIC DIATOM *PSEUDO-NITZSCHIA* IN THE SOUTHEASTERN UNITED STATES

Shuler, A.J.<sup>1</sup>, Paternoster, J.<sup>1</sup>, Brim, M.<sup>1</sup>, Nowocin, K.<sup>1</sup>, Tisdale, T.<sup>2</sup>, Neller, K.<sup>3</sup>, Cahill, J.A.,<sup>4</sup> Leighfield, T.<sup>1</sup>, Fire, S.<sup>1</sup>, Wang, Z.<sup>1</sup>, Morton, S.L.<sup>1</sup>

<sup>1</sup>NOAA/NOS Marine Biotoxins Program, 219 Fort Johnson Road, Charleston, SC USA; steve.morton@noaa.gov; <sup>2</sup>South Carolina State University, 300 College Street NE, Orangeburg SC 29117 USA; <sup>3</sup> First Flight High School, 100 Veterans Drive Kill Devil Hills, NC 27948 USA, <sup>4</sup> North Carolina Ecosystem Enhancement Program, 5 Ravenscroft Drive, Asheville, NC 28801

The Phytoplankton Monitoring Network (PMN) is a NOAA research-based program utilizing volunteers to monitor harmful algal blooms (HABs) and phytoplankton composition throughout the United States. The network is currently composed of over 150 sites located in 16 coastal states. Data generated by volunteers are utilized to alert state resource managers on the extent and distribution of HABs. The PMN offers an ideal pedagogic vehicle to explore the interrelationships between humans, the coastal environment and economically valuable resources, such as shellfish, while at the same time providing volunteers with meaningful opportunities for hands-on engagement in science.

Data collected by PMN volunteers, from the beginning of the program (2001) through 2010, was used to assess the spatial and temporal trends of *Pseudo-nitzschia* spp. across the Southeastern US coastline. *Pseudo-nitzschia* spp. was found to be present from North Carolina to Florida, though it was most common in North and South Carolina. Across the majority of the Southeast the highest rates of occurrence were observed in late summer, early fall, with most areas experiencing the lowest rate of occurrence in the spring. The Outer Banks of North Carolina, however, experienced a peak of occurrence in the late winter early spring in addition to a late summer, early fall peak. *Pseudo-nitzschia* was found in temperatures ranging from less than 5°C to 35°C and salinities from 5 psu to 37 psu. Six unique bloom events were documented during this time frame three of which contained detectable levels of domoic acid. The majority of these bloom events and all of the toxic events occurred in the Outer Banks of North Carolina. Given the extent and intensity of coverage afforded by the NOAA PMN this program provides the optimal approach to not only assess past trends but to monitor environmental changes and emerging trends in the dynamics of this toxigenic species.

## BIOFOULING TUNICATES ON AQUACULTURE GEAR AS POTENTIAL VECTORS OF HARMFUL ALGAL INTRODUCTIONS

Shumway, S.E.<sup>1</sup>, Rosa, M.<sup>1</sup>, Bullard, S.<sup>2</sup>, Holohan, B.A.<sup>1</sup>., Wikfors, G.H.<sup>3</sup>

<sup>1</sup>Department of Marine Sciences, University of Connecticut, 1080 Shennecosset Road, Groton, CT, USA maria.rosa@uconn.edu; <sup>2</sup>University of Hartford, Hillyer College, 200 Bloomfield Ave., West Hartford, CT 06117 USA; <sup>3</sup>NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, 212 Rogers Avenue, Milford, CT, USA.

Biofouling tunicates are ubiquitous in coastal ecosystems and are among the main overgrowers of aquaculture gear. Our study tests the hypothesis that the transport, removal, and transfer by aquaculturists of fouling tunicate species provide mechanisms for concentration and distribution of harmful-algal cells to new areas. Wild-caught specimens of common, biofouling ascidians species (*Styela clava*, *Ciona intestinalis*, *Molgula manhattensis*, *Botryloides violaceus*, *Didemnum sp.*, and *Botryllus schlosseri*) were exposed individually to cultured strains of co-occurring harmful algae (*Prorocentrum minimum*, *Alexandrium fundyense*, *Alexandrium monilatum*, *Karenia mikimotoi*, *Aureococcus anophagefferens*, or *Heterosigma akashiwo*) at simulated bloom cell densities of each HAB. After feeding, ascidians were transferred to ultrafiltered seawater. Immediately after exposure, and after 24 and 48 h in ultrafiltered seawater, biodeposits were collected and observed microscopically for the presence of intact, possibly-viable cells. Subsamples of biodeposits were transferred into ultrafiltered seawater and monitored for algal growth. Thus far, cells of three HAB species have been found to pass intact through the ascidian digestive system and remain viable. Potential mitigation strategies to prevent transport of harmful-algal species through movement of fouled shellfish-aquaculture gear and disposal of biofouling material are being investigated.

## IDENTIFICATION AND ENUMERATION OF ALEXANDRIUM USING AN IMAGING FLOW CYTOMETER (FlowCAM<sup>®</sup>) AND DNA PROBES

Sirois, A.<sup>1</sup>, Spaulding B.<sup>2</sup>

<sup>1</sup>Maine Department of Marine Resources, West Boothbay Harbor, ME 04575 USA;

<sup>2</sup>Fluid Imaging Technology, Yarmouth ME USA

The ability to detect, identify and enumerate harmful algal species is a requirement in coastal ecosystems for monitoring programs and early detection of harmful algal bloom (HAB) events. Current research and monitoring programs utilize microscopes and chemically altered samples for fluorescence identification of HAB bloom species which can be both laborious and time consuming. FlowCAM is an imaging-flow-cytometer that combines the capabilities of a flow cytometer with a digital-imaging microscope and automates phytoplankton detection and enumeration. Here sample data are processed using fluorescent in-situ hybridization (FISH) for Alexandrium with FlowCAM. Statistical analysis of enumeration results will be presented. Comparisons will be made between FlowCAM and the fluorescent light microscope in regard to throughput and error reduction for use as a coastal monitoring tool.

DOES WINTERTIME JOY OF SNOWFALL IN THE NORTH TRANSLATE TO MISERY OF TOXIC CYANOBACTERIA FOR THE SOUTH? EFFECTS OF FRESHWATER INPUT FROM THE 2011 BONNET CARRÉ SPILLWAY OPENING ON BIOMASS, DIVERSITY AND TOXICITY OF CYANOBACTERIA IN LAKE PONTCHARTRAIN

Smith, E.A., Bargu, S., Roy, E., White, J.R.

The Bonnet Carré Spillway is a managed river diversion that can be used to redirect a significant amount of Mississippi River water into Lake Pontchartrain, Louisiana, USA to protect the city of New Orleans from catastrophic flooding. Pulsed river water events such as the Bonnet Carré Spillway opening in May 2011 can increase nutrient levels, potentially causing enhanced primary production, phytoplankton community shifts, and toxic bloom formation. After the 1997 and 2008 Bonnet Carré Spillway openings, both non-toxic and toxic phytoplankton blooms occurred in Lake Pontchartrain. Phytoplankton species composition varied over time and space and the dominant species in late summer were toxic cyanobacteria associated with negative ecosystem impacts. The goals of this study are to evaluate nutrient enrichment and consequent responses in toxic cyanobacteria biomass, diversity, and toxicity in Lake Pontchartrain after the Bonne Carré Spillway closes early this summer. Additionally phosphorus limitation will be studied using alkaline phosphatase activity at the community and cyanobacteria-species level and its impact on cyanobacterial toxin production. An understanding of these effects may facilitate the control of toxic cyanobacteria blooms in the Lake Pontchartrain through nutrient management and increase understanding on how freshwater diversions of the Mississippi River influence harmful algal blooms.

## TOXIC *PSEUDO-NITZSCHIA* IN THE GULF OF MAINE – COMPARISON OF FOUR DETECTION METHODS: ELISA, FMOC-LC-FLD, LC-MS AND LC-UVD

Smith, J.L.<sup>1</sup>, Mafra Jr., L.L.<sup>2</sup>, Kulis, D.M.<sup>1</sup>, Fernandes, L.F.<sup>1</sup>, Quilliam, M.A.<sup>2</sup>, <sup>3</sup>Bates, S.S.<sup>3</sup>, Léger, C.<sup>3</sup>, Anderson, D.M.<sup>1</sup>

<sup>1</sup>Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA Jlsmith@whoi.edu, Danderson@whoi.edu, Lucianofelicio1966@gmail.com, Dkulis@whoi.edu; <sup>2</sup>National Research Council of Canada, Halifax N.S., B3H 3Z1, Canada Michael.Quilliam@nrc-cnrc.gc.ca, Mafrajr@gmail.com; <sup>3</sup>Fisheries & Oceans Canada, Moncton, NB E1C 9B6, Canada Stephen.Bates@dfo-mpo.gc.ca, Claude.Leger@dfo-mpo.gc.ca

With the goals of identifying toxic *Pseudo-nitzschia* species in the Gulf of Maine (GOM) and comparing quantitation methods, 63 cultures of *Pseudo-nitzschia* spp. from the GOM were screened for domoic acid (DA) production using the Biosense ASP ELISA assay (enzyme-linked immunosorbent assay, Biosense, Norway). Toxin-producing cultures were then analyzed using FMOC-LC-FLD (9-fluorenylmethylchloroformate derivatization-liquid chromatography-fluorescence detection), and a subset of these cultures was also analyzed using LC-MS (liquid chromatography-mass spectrometry) and LC-UVD (liquid chromatography-ultraviolet detection). Most of the cultures were classified as non-toxic (i.e., below detection limit) or produced only trace quantities of DA. Twelve of the cultures, however, produced moderate-to-high levels of DA. DA-producing species from the GOM included: *P. seriata* (five strains), *P. pseudodelicatissima*-complex spp. (seven strains), and *P. multiseriata* (one strain). DA concentrations of *P. pseudodelicatissima* complex strains ranged from 5-145 ng ml<sup>-1</sup>, as quantified by FMOC-LC-FLD, and in general these levels were higher than DA concentrations reported using the other methods. Toxicity of *P. seriata* ranged from 4-80 ng ml<sup>-1</sup> (FMOC-LC-FLD); these levels were lower than DA concentrations reported using the ELISA assay. The *P. multiseriata* culture produced low concentrations of DA, only 4 ng ml<sup>-1</sup> as quantified by FMOC-LC-FLD.

Comparison of DA concentrations between ELISA and FMOC-LC-FLD showed good quantitative agreement between the two methods ( $r^2 = 0.70$ ) and an excellent correlation was observed between DA concentrations using ELISA and LC-MS ( $r^2 = 0.99$ ). Several samples were also analyzed using a new high-sensitivity LC-UVD method. LC-UVD is a simple and inexpensive alternative to labor-intensive protocols such as FMOC-LC-FLD. These analyses showed strong correlation between LC-UVD and FMOC-LC-FLD methods over a wide range of concentrations. Together, these data suggest that the ELISA method for DA analysis is a very reliable alternative to more complex and expensive HPLC and LC-MS methodologies typically used to assay DA in cultures. More importantly, however, they prove that the GOM and Georges Bank harbor DA-producing *Pseudo-nitzschia* species, which may be an emerging HAB threat to these waters.

## NUTRIENT CONTENT OF *KARENIA BREVIS* CELLS AS A FUNCTION OF CELL DENSITY

Solomon, B.M.<sup>1</sup>, Dixon, L.K.<sup>2</sup>, Hall, E.R.<sup>2</sup>, Laber, C.<sup>3</sup>

<sup>1</sup>High Point University, High Point, NC 27262 USA solomb08@highpoint.edu; <sup>2</sup> Mote Marine Laboratory, Sarasota, FL 34236 USA lkdixon@mote.org, emily8@mote.org;

<sup>3</sup>Rider University, Princeton, NJ 08540 USA laber@rider.edu

*Karenia brevis* is an unarmored, photosynthetic dinoflagellate 18-45 µm in diameter that is responsible for red tides in the Gulf of Mexico. Current computer models that predict red tides take into account variables such as distribution, growth, primary production, nutrient requirements and utilization (Vargo, 2009), as well as cell count and toxicity. Toxicity is estimated based on cell count, and cell count is modeled from available nutrients, nutrient uptake and cell growth rates. If these models use only one value for nutrient (carbon, nitrogen, and phosphorus) per cell when the value is actually variable, the models may not accurately predict cell counts and, by inference, the presumed toxicity. Our findings on nutrient-replete cultures indicate that the amount of carbon and nitrogen per cell significantly changed from day to day and that phosphorus per cell did not. Patterns of cellular complements were complex, but overall decreased with time. These findings increase our understanding of bloom dynamics and can help make red tide predictions more accurate.

EFFECTS OF OXIDATIVE COMPOUNDS ON GOLDEN ALGA *PRYMNESIUM PARVUM* (HAPTOPHYTA) CELL DENSITY AND TOXICITY

Southard, G.M.<sup>1</sup>, Fries, L.T.<sup>1</sup>, Barkoh, A.<sup>2</sup>

<sup>1</sup>Texas Parks and Wildlife Department, A.E. Wood Laboratory, San Marcos, TX 78666 USA Greg.Southard@tpwd.state.tx.us, Loraine.Fries@tpwd.state.tx.us

<sup>2</sup>Texas Parks and Wildlife Department, Heart of the Hills Fisheries Science Center, Mountain Home, TX 78058 USA Aaron.Barkoh@tpwd.state.tx.us

The golden alga *Prymnesium parvum* has caused frequent massive fish kills in Texas inland waters since 1985, resulting in an estimated loss of over 33.4M fishes valued at US\$13M. Ammonium sulfate and copper algacides are effective in controlling this alga; however, they present adverse effects on the environment and copper compounds are under increasing regulatory scrutiny. We evaluated four oxidative compounds (potassium permanganate, hydrogen peroxide, StorOx<sup>®</sup>, and GreenClean<sup>®</sup> PRO) for efficacy in controlling *P. parvum* cell densities and ichthyotoxicity. The tests were conducted under laboratory conditions on water samples from laboratory-grown cultures. GreenClean<sup>®</sup> PRO was also tested on water samples from two west Texas reservoirs (Colorado City and E.V. Spence reservoirs) with active golden alga blooms and fish kills. Potassium permanganate ( $\geq 2$  mg/L), hydrogen peroxide ( $\geq 58$  mg/L), StorOx<sup>®</sup> ( $\geq 105$  mg/L), and GreenClean<sup>®</sup> PRO (GCP  $\geq 30$  mg/L) were all effective in significantly reducing *P. parvum* densities in laboratory-grown culture samples after 48 h post-treatment. GreenClean<sup>®</sup> PRO (20 mg/L) was also effective in reducing densities of *P. parvum* and toxicity levels in reservoir water samples within 24 h of application. Field trials have further demonstrated the effectiveness of GreenClean<sup>®</sup> PRO in controlling *P. parvum* blooms in small impoundments. Detailed results will be discussed.

## PRESENCE AND TOXICITY OF FRESHWATER CYANOBACTERIA IN PINTO LAKE, MONTEREY BAY AREA, CALIFORNIA.

Stanfield, E.R.<sup>1</sup>, Los Huertos, M.<sup>2</sup>, Sreenivasan A.

<sup>1</sup>California State University Monterey Bay Coastal Watershed Science & Policy, Seaside CA 93055 USA [estanfield@csumb.edu](mailto:estanfield@csumb.edu); <sup>2</sup>California State University Monterey Bay Environmental Science & Policy, Seaside CA 93055 USA [mloshuertos@csumb.edu](mailto:mloshuertos@csumb.edu); <sup>3</sup>California State University Monterey Bay Biology, Seaside CA 93055 USA [asreenivasan@csumb.edu](mailto:asreenivasan@csumb.edu)

Freshwater cyanobacterial harmful algal blooms (CHABs) threaten humans and other mammals through an array of potent cyanotoxins via direct ingestion or bioaccumulation. The family of hepatotoxic microcystins, produced by several cyanobacteria taxa, has been associated with liver failure at acute exposure and liver cancer at chronic low level exposure. Recently, microcystins have been implicated in the deaths of endangered sea otters in the Monterey Bay National Marine Sanctuary (MBNMS). Pinto Lake, a Monterey Bay Area freshwater lake with seasonal toxic CHABs, is a potential source of cyanobacteria biomass and microcystins for the MBNMS. Pinto Lake is also an important community recreational resource for thousands of children who visit the lake each year.

In this project, we combined freshwater ecology and molecular biology approaches to characterize the abundance of potentially toxic cyanobacteria, levels of intracellular microcystins, and the presence of microcystin synthesis genes in association with environmental factors in Pinto Lake throughout 2009 and 2010. We applied microscopy to identify and enumerate cyanobacteria samples, the polymerase chain reaction (PCR) to evaluate the presence of toxin synthesis genes and cyanobacteria-specific genes, and enzyme-linked immunosorbent assays (ELISA) to estimate intracellular cyanotoxin concentrations. We described the presence of microcystin synthesis genes and intracellular microcystin levels through the summer and autumn, when cyanobacteria abundance increased in association with increased water and air temperature, solar radiation, and dissolved phosphorus. This research constitutes the first documentation of microcystin synthesis genes in a lake draining to the MBNMS. This baseline data concerning the ecological dynamics and proliferation of toxic CHABs in the Monterey Bay area will also help the Central Coast Regional Water Quality Control Board and the City of Watsonville evaluate the risks associated with toxic CHABs and formulate the most effective management strategy for the reduction, remediation, and prevention of toxic CHABs.

## WHAT MAKES *MICROCYSTIS* BLOOM: PROGRESS IN METAGENOMIC AND METAPROTEOMIC EXAMINATION OF BLOOM EVENTS

Steffen, M.M.<sup>1</sup>, Li, Z.<sup>1</sup>, Dill B.D.<sup>2</sup>, Farnsley, S.E.<sup>1</sup>, Hauser, L.J.<sup>2</sup>, Shah, M.<sup>2</sup>, Zhu, G.<sup>3</sup>, Boyer, G.L.<sup>4</sup>, Bullerjahn, G.S.<sup>5</sup>, VerBerkmoes, N.C.<sup>2</sup>, Wilhelm, S.W.<sup>1</sup>.

<sup>1</sup>University of Tennessee, Knoxville, TN 37996 USA; <sup>2</sup>Oak Ridge National Lab, Oak Ridge, TN 37830 USA; <sup>3</sup>Chinese Academy of Sciences, Nanjing Institute of Geography and Limnology, Nanning 210008 China; <sup>4</sup>State University of New York, Syracuse NY 13210 USA; <sup>5</sup>Bowling Green State University, Bowling Green OH 43403 USA. Correspondence: wilhelm@utk.edu

Toxic cyanobacterial blooms are increasing in both frequency and intensity in freshwater systems around the world. *Microcystis* spp. are often associated with toxic blooms, produce powerful hepatotoxins and can degrade freshwater ecosystems. Using 454 titanium-based pyrosequencing, we employed shotgun metagenomics to uncover microbial community structure for three global toxic blooms. Bloom sites were sampled in Lake Tai (*Taihu*, China), Lake Erie (USA/Canada), and Grand Lake St. Marys (USA). At all three sites we observed the most dominant phyla to be *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes*. The cyanobacterial communities within these blooms were dominated by members of *Oscillatoriales* and *Chroococcales*, both of which include toxic genera such as *Microcystis* and *Planktothrix*. Interestingly, the Grand Lake St. Marys (GLSM) community was largely identified as *Lyngbya* and *Arthrospira*, two relatively innocuous genera, despite being classified as a *Planktothrix* bloom by the Ohio EPA in 2010. And although there was a general conservation of taxa at the level of phyla, we observed variation in the composition of heterotrophic bacteria associated with each bloom. This leads us to the hypothesis that a subset of the heterotrophic bacteria associated with toxic blooms, or the “companion” community, may be lake-specific. Using both COG and SEED approaches, we determined that the functional profile of each community is conserved across bloom sites despite phylogenetic divergence. In addition to examining the microbial communities at these bloom sites, we also identified a core set of conserved *Microcystis* genes, as well as a set of genes conserved between the bloom-associated *Microcystis* genome assemblies. Based on the greater *Microcystis* pan-genome, we have complemented this work with shotgun metaproteomic examinations of these blooms and cultures maintained under nutrient conditions constrained to mimic growth limitations in these lakes. An assemblage of this data is now providing insight on the physiological and biogeochemical activities of *Microcystis* during bloom events, providing new insight into answering the question “what makes *Microcystis* bloom?”

## THE GULF OF MEXICO HABSOS WEBSITE AND ITS POTENTIAL FOR COMMUNICATION, DATA DISSEMINATION, AND OUTREACH

Steidinger, K.A.<sup>1</sup>, Parsons, A.R.<sup>2</sup>, Cross, S.L.<sup>3</sup>, Allen, J.<sup>4</sup>

<sup>1</sup>Florida Institute of Oceanography, St. Petersburg, FL 33701 USA karen.steidinger@myFWC.com; <sup>2</sup>NOAA, Stennis Space Center, MS 39529 USA rost.parsons@noaa.gov; <sup>3</sup>NOAA, Charleston, SC 29412 USA scott.cross@noaa.gov; <sup>4</sup>EPA, Stennis Space Center, MS 39529 USA allen.jeanne@epamail.epa.gov

The Gulf of Mexico Harmful Algal BloomS Observing System (HABSOS) is a web-based information and data storage, integration, visualization, and dissemination platform capable of being a resource manager and outreach coordinator toolbox. It is a joint effort between EPA and NOAA and now encompasses BiNational HABSOS which involves US and Mexican federal and state agencies as well as universities. HABSOS was built to be able to input *Karenia brevis* data on a regional basis by States all along the Gulf and to graphically display that data in relation to various geo-referenced map products such as surface currents, sea surface height, winds, and other oceanographic or meteorological variables. Data products are available from buoy systems and other platforms such as satellites and can be visualized as modeled outputs or point measurements. In addition to these data there are links to the NOAA HAB Bulletins by area and to other websites. The HABSOS website was created by NOAA's National Coastal Data Development Center for data entry and integration and also houses Navy ocean nowcast/forecast models and forecasts from the National Weather Service for selected time periods. Currently the website is being reassessed for user friendliness as well as products. At this time it is projected that HABSOS will poll potential end users for input as to what they would like to access at this website. The end users are considered to be natural resource managers, aquaculture managers, public health officials, county managers, local municipalities, scientists, and students. One aspect of HABSOS as it currently is configured is that it only has *Karenia brevis* data when there are other harmful and noxious algae in the northern and southern Gulf of Mexico that warrant monitoring and recording, e.g., *Dinophysis* species, other *Karenia* species, *Pyrodinium bahamense* var. *bahamense*, *Alexandrium monilatum* and other *Alexandrium*, *Pseudo-nitzschia* species, *Karlodinium veneficum*, *Cochlodinium polykrikoides*, *Akashiwo sanguinea*, benthic microalgae such as *Prorocentrum* and *Gambierdiscus*, and others. The eventual updated site will require discussion and support.

## FORECASTING HARMFUL ALGAL BLOOMS: DIFFERENCES IN ECOLOGY, COMMONALITIES IN EXECUTION.

Stumpf, R.P., Fisher, K. , Dortch, Q. , Tomlinson, T.

NOAA, National Ocean Service, Silver Spring, MD 20910 USA

richard.stumpf@noaa.gov; kathleen.fisher@noaa.gov; quay.dortch@noaa.gov;

michelle.tomlinson@noaa.gov

The major harmful algal blooms (HABs) of concern in the U.S. are *Alexandrium* spp., *Karenia brevis*, domoic acid producing *Pseudo-nitzschia* spp., and toxic cyanobacteria, especially *Microcystis* spp. Among other less common, but problematic, blooming organisms are *Akashiwo*, *Dinophysis*, and *Cochlodinium*. Although these HABs are ecologically different, they have commonalities in forecasting requirements that support the need for a national framework for forecasts. Fundamentally, short-term forecasts require location and transport as inputs, and analysis and interpretation as outputs. Seasonal predictions involve models of the probability that a bloom will appear, based on ecological behavior.

The similarities can be seen in the way several HABs are forecasted. *Microcystis* in Lake Erie and *Alexandrium* in the Gulf of Maine differ in most ecological characteristics. Although predictive models for these HABs are initiated differently (cyst beds for *Alexandrium*; satellite imagery for *Microcystis*) the transport in both cases uses an initial field with subsequent prediction using a hydrodynamic model. In other cases, detection methods range from time-series at single points from fixed “automated” instruments (e.g., ESP, Flow-CytoBot) to mobile manual deployments which provide greater spatial coverage but less temporal data (cell counts, test kits, lifeguard reports, etc.). All can initiate a forecast or tune a model. Some regions use sentinel sites to predict the transport of blooms to coastal regions (such as retention areas in the case of *Pseudo-nitzschia* blooms along the Washington coast; HABs in the California Current System; Aransas Pass in south Texas).

Seasonal predictions involve probabilistic, statistical, or heuristic models to indicate the likelihood of bloom initiation. These models require a variety of environmental data as input. Coastal upwelling and downwelling appear to be factors in modeling initiation of *Pseudo-nitzschia* on the Washington coast or the likelihood of *Karenia* on the Florida and Texas coasts. Some predictions are based on the appropriate ecological niche for a particular species to outcompete another.

The research into these problems is specific to each organism. The execution, however, has commonality, such as switching an *Alexandrium* model for a cyanobacteria model, or monitoring with an ESP or manual sampling. These commonalities mean that advances in detection and forecasting of any specific HAB will lead to improvements in the forecasting capabilities for all HABs. These improvements will be realized more quickly if the various regions communicate effectively with one another, and if HAB forecasting efforts are coordinated to realize economies of scale within a national framework.

## USING MACROALGAE TO PREVENT, CONTROL, AND MITIGATE HABS IN THE US

Tang Y.Z., Gobler C.J.

School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794-5000 USA. yittang@notes.cc.sunysb.edu, christopher.gobler@stonybrook.edu

The prevention, control, and mitigation (PCM) of harmful algal blooms (HABs) has become a primary objective of agencies, managers, stakeholders, and scientists in the United States. Recent studies, largely in Asia, have demonstrated that macroalgae can have strong growth-inhibiting effects on phytoplankton in general, and multiple HAB species, in particular.

Many macroalgae including species of *Ulva*, *Porphyra*, and *Gracilaria* are broadly distributed along the US coastlines and may provide an environmentally friendly, ecologically safe, and commercially viable solution for the PCM of HABs in the US. We will present the results of experiments examining the effects of macroalgae, *Ulva lactuca*, *Porphyra umbilicalis*, and *Gracilaria sp.* collected from New York estuaries, on the growth of seven HAB species commonly observed on the east and west coasts of the US including *Aureococcus anophagefferens*, *Pseudo-Nitzschia multiseries*, *Chattonella marina*, *Karenia brevis*, *Cochlodinium polykrikoides*, *Karlodinium veneficum*, and *Prorocentrum minimum*. Fresh thalli of the *U. lactuca* added at environmentally realistic levels ( $\text{mg L}^{-1}$ ) were capable of lysing or strongly inhibiting the growth of all species in a dose-dependent manner within controlled laboratory experiments during which high nutrient levels and common pH levels among treatments and controls were maintained. The dramatic allelopathic effects of the extracts of dried and powdered *U. lactuca* with and without post-extraction heat treatment on HAB species demonstrated unambiguously that *U. lactuca* produces heat-stable allelochemicals that played a major role in the observed allelopathic effects. The addition of live *U. lactuca* thalli in bottle and mesocosm experiments conducted in the field during the blooms of *A. anophagefferens* ('brown tide';  $> 10^5$  cells  $\text{ml}^{-1}$ ) consistently yielded a significant ( $p < 0.05$ ) and often large ( $> 50\%$ ) reduction in cell densities in  $\sim 48$  h.

In experiments using the red macroalgae *Porphyra umbilicalis* and *Gracilaria sp.*, both algae were also capable of significantly inhibiting the growth of multiple HAB species tested by both nutrient assimilation and allelopathy.

Our findings combined with well-known nutrient removal capacity of seaweeds collectively suggest that use of macroalgae may be a promising mitigation strategy for HABs in coastal ecosystems.

## OCEAN ACIDIFICATION AND SILICATE LIMITATION SYNERGISTICALLY CONTROL DOMOIC ACID PRODUCTION

Tatters, A.O., Fu, F.X., Hutchins, D.A.

University of Southern California, Los Angeles, CA 90089 USA  
tatters@usc.edu, ffu@usc.edu, dahutch@usc.edu

Anthropogenic CO<sub>2</sub> is progressively acidifying the ocean, but the responses of harmful algal bloom species that produce potentially dangerous phycotoxins remain virtually unknown. Although production of the neurotoxin domoic acid (DA) by members of the globally-distributed diatom genus *Pseudo-nitzschia* is known to be regulated by nutrient availability, potential interactions with increasing seawater CO<sub>2</sub> concentrations are not well understood.

We present experiments measuring DA production by acclimatized cultures of *Pseudo-nitzschia fraudulenta* demonstrating a strong synergism between projected future CO<sub>2</sub> levels (750 ppm) and silicate-limited growth, which elevates cellular toxin content by >200% relative to current atmospheric (390 ppm) or pre-industrial (200 ppm) CO<sub>2</sub> conditions. The coastal California upwelling system where this species was isolated currently exhibits rapidly increasing levels of anthropogenic acidification, as well as widespread silicate limitation of diatom growth. Our results suggest that ecosystem and human health impacts of toxic *Pseudo-nitzschia* blooms could be greatly exacerbated by ocean acidification.

## ENVIRONMENTAL IMPACTS ON THE FORMATION OF HARMFUL MACROALGAL BLOOMS IN A HIGHLY EUTROPHIED ESTUARY

Thornber, C.S.<sup>1</sup>, Guidone, M.<sup>1</sup>, Deacutis, C.<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881 USA thornber@uri.edu, mguidone@my.uri.edu, <sup>2</sup>Narragansett Bay Estuary Program, URI Bay Campus – Box 27, South Ferry Road, Narragansett RI, 02882 USA deacutis@gso.uri.edu

Harmful macroalgal blooms are frequent occurrences in estuarine systems worldwide and can have numerous negative impacts on coastal ecosystems. In Narragansett Bay, RI, USA, blooms have been present since at least the early 1900s but have recently increased in duration and frequency. Over the past several decades, anthropogenic inputs of nitrogen into this system have been large (~50-60 million moles N/year); however, recent updates in sewage treatment plants are resulting in substantial decreases (>35%) in nitrogen inputs. In order to understand the causes of harmful macroalgal blooms in this system, and assess their potential responses to nitrogen inputs, we have assessed bloom formation, duration, and taxonomic diversity over the past five years in this system. Through a combination of repeated intertidal and subtidal quantitative surveys, as well as larger-scale aerial (helicopter) photography, we have determined the distribution, density, and composition of macroalgal blooms in this system. Blooms typically occur in mid-to-late summer, but we found significant spatial and temporal (both seasonal and yearly) variation in bloom composition and abundance ( $p < 0.001$  for all), with strong local variation (<2km) in bloom density. The most common bloom species included 3 species of blade-forming *Ulva* species, at least five tubular, branched *Ulva* species, the red algae *Gracilaria tikvahiae* and *G. vermiculophylla*, and *Agardhiella subulata*; more than 30 other species also occurred in these blooms. The highest density recorded was 400g/m<sup>2</sup> (wet weight) for intertidal habitats. We have found that congeneric, morphologically similar bloom-forming *Ulva* species can differ in their distribution, growth rate, and relative palatability. In addition, through the use of multivariate statistical techniques, we have assessed the likely physical and biological factors that correlate with bloom abundance, including temperature, nutrient levels (i.e. DIN), wind direction/speed, tidal height, rainfall/river flow, and the abundance of potential herbivores. Our data indicate the likelihood of rapid shifts in bloom location and composition in estuarine systems, and provide a benchmark for understanding macroalgal bloom dynamics.

## IMPACT OF THE BACTERIAL ALGICIDE IRI-160AA ON THE PHOTOBIOLOGY OF HARMFUL DINOFLAGELLATES

Tilney, C.L., Pokrzywinski, K.L., Coyne, K.J. and Warner, M.E.

University of Delaware, College of Earth Ocean and Environment, Lewes, DE 19958 USA [ctilney@gmail.com](mailto:ctilney@gmail.com), [kaytee@udel.edu](mailto:kaytee@udel.edu), [kcoyne@udel.edu](mailto:kcoyne@udel.edu), [mwarner@udel.edu](mailto:mwarner@udel.edu)

Many techniques proposed for the mitigation of harmful algal blooms continue to elude managerial use due to insufficient knowledge of both their mode of action and potential undesirable side effects. Of these methods, algicidal agents remain particularly promising due to their high species specificity and effectiveness. The effects of a bacterial filtrate from an algicidal bacteria (*Shewanella* sp. IRI-160AA) on the photosynthetic physiology of *Karlodinium veneficum* was assessed *in vitro* by active chlorophyll *a* fluorescence. Initial results under log-phase batch culture (12:12 L:D cycle,  $\sim 150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) indicated a dose-dependent, significant drop in the maximum quantum yield (Fv/Fm) of photosystem II (PSII) followed by rapid recovery within 24-42 hours. Cell densities mimicked this response but exhibited a slower recovery rate relative to PSII photochemistry. Sub-samples from the top and bottom of the experimental vessels suggest that the drop in Fv/Fm may be driven in part by permeated/dead cells, while cells at the surface remained significantly more efficient at directing PSII photochemistry. In contrast, cultures exposed to the algicide under dark conditions exhibited a significantly larger decrease in maximum quantum yield of PSII and a larger drop in cell density. The loss in photochemical activity was coupled to a significant decline in both the connectivity of PSII reaction centers in the chloroplast as well as the electron transport rate out of PSII. Recovery of photochemistry after an 18-h dark incubation with the algicide was considerably slower than cells exposed to the normal 12:12 light:dark cycle. Further tests with other harmful and non-harmful dinoflagellates, as well as other dominant phytoplankton classes typically found in the Delaware Inland Bays will be explored, and changes in chloroplast function will be tied into the wider context of the cell death pathway to address more completely the overall impact of this algicide on cellular function.

## MONITORING CYANOBACTERIAL BLOOMS WITH FULL-RESOLUTION MERIS IMAGERY TO SUPPORT STATE MANAGEMENT ACTIVITIES

Tomlinson, M.C.<sup>1</sup>, Briggs, T.<sup>2</sup>, Neff, C.<sup>2</sup>, Burks, R.<sup>3</sup>, Lazensky, R.<sup>4</sup>, Hart, C.<sup>3</sup>, Reich, A.<sup>6</sup>, Raymond, H.<sup>5</sup>, Wazniak, C.<sup>7</sup>, Stumpf, R.P.<sup>1</sup>, Wynne T.T.<sup>1</sup>

<sup>1</sup>Center for Coastal Monitoring and Assessment, NOS/NOAA, Silver Spring, MD 20910 USA Michelle.Tomlinson@noaa.gov; Richard.Stumpf@noaa.gov, Timothy.Wynne@noaa.gov; <sup>2</sup> Consolidated Safety Services, Fairfax, VA 22030 USA Travis.Briggs@noaa.gov; Cordelia.Neff@noaa.gov; <sup>3</sup> St. Johns River Water Management District, Palatka, FL 32178 USA CHart@sjrwmd.com; RBurks@sjrwmd.com; <sup>4</sup> Florida Department of Health, Gainesville, FL 32601 USA Becky\_Lazensky@doh.state.fl.us; <sup>5</sup>Ohio EPA, Columbus, OH 43215 USA Heather.Raymond@epa.state.oh.us; <sup>6</sup> Florida Department of Health, Tallahassee, FL 32399 USA Andy\_Reich@doh.state.fl.us; <sup>7</sup>Maryland Department of Natural Resources, Annapolis, MD 21401 USA cwazniak@dnr.state.md.us

Blooms of toxic cyanobacteria in estuaries, lakes and reservoirs have become an increasing concern in the U.S. and worldwide. Cyanotoxin exposures and health effects have been attributed to drinking contaminated water, consuming fish and bioaccumulated toxins, or through recreational water contact. With the availability of 300 m ocean color imagery from the medium-spectral resolution, imaging spectrometer (MERIS) onboard the European Space Agencies' ENVISAT satellite, it is now possible to monitor smaller water bodies on a semi-daily basis. The spectral resolution of this sensor provides information for a Cyanobacterial Index (CI) algorithm that identifies high biomass algal blooms that are optically consistent with cyanobacteria. Currently, experimental image products are being provided to the Florida St. John's Water Management District, Ohio EPA, and the state of Maryland in near real-time. Field validation data were collected in the summers of 2010 and 2011 to assess the accuracy of the CI for detecting cyanobacteria blooms. Initial results from the 2010 summer season in the St. John's River identified the progression of a bloom which caused an extensive multi-species fish kill. In addition, CI images accurately determined the beginning of a massive cyanobacteria bloom in Grand Lake St. Mary's, Ohio, which resulted in domestic animal deaths, adverse human health reports, and local economic impacts. Further work is being conducted to better refine the algorithm and to develop tools to assist local environmental managers in responding to cyanobacteria blooms. This proactive approach integrates early bloom detection through satellite imagery and pairs it with on the ground field response that is needed to validate the experimental satellite data. Early detection aids local public health and environmental scientists in targeting health messaging to susceptible populations, planning coordination activities pertaining to bloom management, and in directing their field sampling efforts to identify algal species present in the bloom.

## SYNTHETIC ANALOGS OF OKADAIC ACID AND OTHER DSP TOXINS ELUCIDATE PHOSPHATASE STRUCTURE-ACTIVITY RELATIONSHIPS

Twiner, M.J.<sup>1</sup>, Pang, Y.<sup>2</sup>, Fang, C.<sup>2</sup>, Bulter, S.<sup>1</sup>, Miles, C.O.<sup>3</sup>, and Forsyth, C.J.<sup>2</sup>

<sup>1</sup>The University of Michigan-Dearborn, Dearborn, MI 48128 USA  
mtwiner@umd.umich.edu, suzacoll@umd.umich.edu; <sup>2</sup>The Ohio State University,  
Columbus, OH 43210 USA pang.44@osu.edu, fang.76@osu.edu,  
forsyth@chemistry.ohio-state.edu; <sup>3</sup>Norwegian Veterinary Institute, Oslo, Norway  
chris.miles@vetinst.no

Okadaic acid (OA) and the closely related dinophysistoxins (DTXs) and belizeanic acid (BA) are polyether toxins produced by dinoflagellate species of the genera *Dinophysis* and *Prorocentrum*. These algal toxins, collectively called the diarrhetic shellfish poisons (DSPs), accumulate in shellfish and are known tumor promoters and inhibitors of serine/threonine protein phosphatases (ser/thr PPs). PPs are extremely important modulators of enzyme activity and cell signaling pathways. Although the interactions between the OA/DTX/BA toxins and phosphatases are poorly understood it is currently believed that there are two important molecular recognition sites – a bimetallic well that accommodates the carboxylate-bearing C3-C12 spiroketal of OA/DTX, and a distal hydrophobic groove that hosts the terminal C30-C38 spiroketal. Through the use of naturally isolated OA, DTX1, and DTX2 that vary in methylation about the terminal C30-C38 spiroketal region, in addition to a synthetic OA/DTX analog that varies in stereochemistry and functionalization about the C3-C12 spiroketal, we are able to assess DSP-PP structure-activity relationships. Fluorescent phosphatase activity assays have shown that OA and DTX2 potently inhibit PP2a and PP1 with slight differences in potency (OA > DTX2) related to C31 and C35 methylation. Respective IC<sub>50</sub> values (nM) for PP2A are 0.47 and 0.99, and for PP1 are 25.2 and 76.4. On the other hand, strategically designed synthetic 2-epi-DTX2 (a C2 stereoisomer of DTX2) indicate a 1 to 2 orders of magnitude reduction in potency towards both PPs (IC<sub>50</sub> values (nM) of 137 and 3114 for PP2a and PP1; respectively) further corroborating modeling studies that suggest the C1-C13 head region of the molecule is the primary pharmacophore for both PPs and that PP2a is differentially sensitive to C2 molecular modification. Preliminary studies suggest that synthetic BA has only minimal inhibitory potential towards either PP. These data continue to facilitate molecular modeling efforts and development of “minimal pharmacophores” for highly selective phosphatase inhibition.

## A CHEMICAL APPROACH FOR THE MITIGATION OF *Prymnesium parvum* BLOOMS

Umphres, G.<sup>1</sup>, Roelke, D.L.<sup>1</sup>, Netherland, M.D.<sup>2</sup>

<sup>1</sup>Texas A&M University, College Station, TX, 77843 USA georgeu@tamu.edu; <sup>2</sup>U.S. Army ERDC Gainesville, FL 32653 USA mdnether@ufl.edu

Known as Golden Algae, the harmful algal bloom causing organism *Prymnesium parvum* secretes toxic chemicals called *prymnesins* when stressed, resulting in major fish kills in Texas. Although many options exist for mitigation of blooms, a feasible protocol for control of Golden Algae blooms on large-scale impoundments has yet to be identified. Chemical control of *P. parvum* using six different enzyme inhibiting aquatic herbicides was explored in laboratory experiments. Of the six chemicals screened, one (Flumioxazin) was selected due to a significant decrease in *P. parvum* cell numbers with increasing chemical concentration. It was then applied to natural plankton communities during *in-situ* experiments (Lake Granbury, Texas). The first experiment was conducted during a period of *P. parvum* bloom initiation (March) and the second experiment conducted during a post bloom period (April). Experiments were carried out in 25L polycarbonate carboys covered in 30% shade cloth to simulate natural light, temperature and turbulence conditions. Through cell counts via light-microscopy, the chemical Flumioxazin was found to cause significant decreases in *P. parvum*, but no significant differences in zooplankton abundance during the period of bloom initiation. However, significant decreases in adult copepods were observed during the post bloom period, with no significant decreases in *P. parvum*. Experimental findings show optimistic results for the ability of Flumioxazin to mitigate a *P. parvum* bloom in mesocosm experiments, meriting a larger scale demonstration project.

## THE CHEMICAL ECOLOGY OF HARMFUL SEAWEED BLOOMS IN THE SOUTH SALISH SEA OF WASHINGTON STATE

Van Alstyne, K.L.

Shannon Point Marine Center, Western Washington University, 1900 Shannon Point Road, Anacortes, WA 98221 USA kathy.vanalstyne@wwu.edu

Ulvoid green algae (Phylum Chlorophyta, Order Ulvales) frequently form large, problematic blooms in the marine inland waters of western Washington State. These blooms are typically comprised of a number of species in the genus *Ulva* (including species in the genus formerly known as *Enteromorpha*), but can also include high biomasses of a related species, *Ulvaria obscura*. The detrimental effects of these blooms are frequently attributed to their ability to overgrow other algae, eelgrasses, and invertebrates. The blooms can also deplete nutrients, reduce light levels, and cause oxygen depletion when the algae in them die and decompose.

An additional mechanism by which the algae comprising the blooms impact other species and create environmental problems is through the production of unusual natural products that can be toxic to marine organisms or noxious smelling to humans. All of the algae that comprise these blooms produce the tertiary sulfonium compound dimethylsulfoniopropionate (DMSP). DMSP breaks down to form dimethyl sulfide (DMS) and acrylic acid or acrylate. DMS is a volatile, noxious-smelling sulfur compound that, in addition to hydrogen sulfide, is believed to be the cause of complaints about odors associated with ulvoid algal blooms. *Ulvaria obscura* also produces copious amounts of the catecholamine dopamine, which is known to be cytotoxic and genotoxic. When *Ulvaria* growing in the intertidal or shallow subtidal zones is desiccated on a low tide then re-immersed, it can release the dopamine in its tissues. Estimates of the resulting dopamine concentrations in the surrounding seawater are as high as 500-1,000  $\mu\text{M}$ . At these concentrations, dopamine can inhibit the germination of zygotes of other macroalgae, reduce the growth of co-occurring macroalgal species, and decrease survival rates of some invertebrate larvae.

## MITIGATING MICROCYSTIS IN THE CHESAPEAKE: USING HUMAN DIMENSIONS ANALYSIS TO STRUCTURE EFFECTIVE COMMUNITY OUTREACH STRATEGIES

Van Dolah, E. R.<sup>1</sup>, Paolisso, M.<sup>2</sup>

<sup>1</sup>University of Maryland Department of Anthropology, College Park, MD 20742 USA evandolah@gmail.com; <sup>2</sup>University of Maryland Department of Anthropology, College Park, MD 20742 USA mpaolisso@anth.umd.edu

In the tributaries of the Chesapeake Bay, where *Microcystis aeruginosa* has grown increasingly more pervasive and aggressive, researchers at the University of Maryland, the Chesapeake Research Consortium, and the Maryland Department of Natural Resources are testing the viability of clay flocculation to mitigate these blooms through the three-year NOAA CSCOR funded project, *Mitigating Microcystis in the Chesapeake* (MMIC). The success of MMIC, however, requires understanding the wide range of stakeholders, and regional and local communities that are impacted by *M. aeruginosa*, all of whom identify with the problem through complex socio-ecological linkages. Human dimensions research provides the necessary tools to define communities and stakeholder groups, assess the cultural knowledge that they employ to understand the causes, consequences, risks, and responses to HABs, and survey the socioeconomic impacts of HABs on communities, households, and individuals. The human dimensions analysis conducted through MMIC will enable project leaders to assess stakeholder cultural understandings of blooms as well as the cultural and socioeconomic opportunities to involve stakeholders and communities in *M. aeruginosa* mitigation efforts.

Using methods and concepts from anthropology, we will develop maps to identify impacted communities and stakeholder groups, conduct interviews with key informants, and distribute surveys to extract key social, economic, and cultural factors of *M. aeruginosa*. Data will be used to build cultural models for each community based on observed patterns of agreement and disagreement. These models will be linked to sociocultural theories on human responses to environmental change, including how humans draw upon different cultural understandings of nature to understand risk, uncertainty, and vulnerability, as well as develop strategies for adapting to environmental change. Analysis will also inform the development of an integrated matrix of factors that will guide the creation of trial-based outreach material, focus groups, and collaborative learning workshops. Input from MMIC's HAB experts throughout the project will be integral to ensuring clear articulation of biological/toxicological HAB research in sociocultural contexts. This presentation highlights the project's conceptual approach and includes some preliminary findings from the first year of research.

## WHAT DO WE CURRENTLY KNOW ABOUT DINOFLAGELLATE POLYKETIDE SYNTHASES? INSIGHTS FROM *KARENIA BREVIS*

Van Dolah, F.M.<sup>1</sup>, Rein, K.S.<sup>2</sup>, Zippay, M.L.<sup>1,3</sup>

<sup>1</sup>NOAA Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC 29412 USA [fran.vandolah@noaa.gov](mailto:fran.vandolah@noaa.gov); <sup>2</sup>Florida International University, Miami, FL [reink@fiu.edu](mailto:reink@fiu.edu); <sup>3</sup>Medical University of South Carolina, Charleston, SC USA 29425, [zippay@musc.edu](mailto:zippay@musc.edu)

Marine dinoflagellates produce some of the most potent toxins on Earth, the majority of which are polyketides, a diverse class of secondary metabolites produced by polyketide synthases (PKS). PKSs evolved from fatty acid synthases (FAS) and build the carbon backbone of polyketides in a manner analogous to fatty acid biosynthesis, through the sequential condensation of carboxylic acid subunits. However, unlike FASs, which make only saturated carbon chains, PKSs produce unsaturated products. Brevetoxins, produced by *Karenia brevis*, are known by stable isotope incorporation experiments to be of polyketide origin. PKS sequences identified in *K. brevis* by degenerate PCR showed homology to Type I PKSs, complex enzymes in which multiple catalytic domains are located on a single polypeptide. Analysis of full-length transcripts confirmed their homology to Type I PKSs, but found that each encoded only a single catalytic domain. Thus *K. brevis* PKSs have a unique structure similar in sequence to Type I PKSs but similar in architecture to Type II PKSs, where each catalytic domain resides on a separate peptide.

Several lines of evidence suggest that the *K. brevis* PKSs may function in a role other than or in addition to brevetoxin biosynthesis. Firstly, amongst ~65,000 *K. brevis* cDNA sequences, we have found no transcripts identified as FASs. Secondly, *K. brevis* PKS proteins are localized to the chloroplast; however, brevetoxin is not found in the chloroplast by either ELISA or LC/MS. Using <sup>14</sup>C-acetate labeling studies, we found that fatty acid synthesis occurs in the chloroplast of *K. brevis*. However, unlike typical FAS, the products of these labeling studies included unsaturated fatty acids. We propose that the PKSs identified in *K. brevis* may have both FAS and PKS activity. If this is true, polyketide precursors to brevetoxin are synthesized in the chloroplast, but like fatty acids, are exported to other cellular compartment(s) for synthesis into mature toxin molecules.

## FORECASTING HABs IN THE PACIFIC NORTHWEST: SENSITIVITY TO WIND FORCING UNCERTAINTIES

Wakamatsu, T.<sup>1</sup>, Masson, D.<sup>1</sup>, Foreman, M.<sup>1</sup>, Hickey, B.<sup>2</sup>, MacCready, P.<sup>2</sup>, Giddings, S.<sup>2</sup>

<sup>1</sup>Institute of Ocean Sciences, Fisheries and Oceans Canada, P.O. Box 6000, Sidney BC, V8L 4B2, Canada tsuyoshi.wakamatsu@dfo-mpo.gc.ca, diane.masson@dfo-mpo.gc.ca, mike.foreman@dfo-mpo.gc.ca; <sup>2</sup>School of Oceanography, Box 355351, University of Washington, Seattle WA USA bhickey@u.washington.edu, parker@ocean.washington.edu, sarahgid@uw.edu

Regional field studies have demonstrated that blooms of toxic *Pseudo-nitzschia* observed on Washington coastal beaches in summer and fall generally originate in the Juan de Fuca Eddy, a semi-permanent feature located northwest of the Washington coast. Toxic cells escape from the eddy during periods of upwelling-favorable winds and move southward along the Washington coast, subsequently moving onto coastal beaches when the upwelling-favorable winds are interrupted by downwelling-favorable storms. The accuracy of wind predictions provided by atmospheric models is therefore critical in providing accurate forecasts of HAB events along the Washington/Oregon coast. In this study, we use 4 years of model results to perform an error analysis of 48-hr wind predictions by the University of Washington MM5 atmospheric model to determine biases and low order statistics. An ensemble of regional ocean model runs using the regional ROMS model developed for the PNWTOX (Pacific Northwest Toxin) project is carried out using perturbations to the actual winds in 2005. Lagrangian drifters are inserted around the eddy to determine the sensitivity of their trajectories to errors in the 48-hr predicted wind field. Implications to HAB predictions in the region and future work will be discussed.

## QUANTITATIVE EXPRESSION PATTERNS OF NITROGEN METABOLISM GENES IN THE BROWN TIDE ALGA, *AUREOCOCCUS ANOPHAGEFFERENS*

Walker, E.A.<sup>1,2</sup>, Wurch, L.L.<sup>3</sup>, Dyhrman, S.T.<sup>3</sup>, Gobler, C.J.<sup>1,2,\*</sup>

<sup>1</sup>School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, NY 11968; <sup>2</sup>School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794-5000; <sup>3</sup>Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543; \*Corresponding author: christopher.gobler@stonybrook.edu

*Aureococcus anophagefferens* is a picoplanktonic (~2µm) alga that has caused harmful brown tides on the US East Coast for more than 25 years. Nitrogen (N) has been shown to play a key role in the development of brown tides as blooms typically develop when inorganic N concentrations are low, likely because of *A. anophagefferens*' ability to utilize a variety of organic N compounds. The recent sequencing of the genome of *A. anophagefferens* (strain CCMP 1984) has resulted in the discovery of genes putatively involved in the use and metabolism of a variety of organic and inorganic N sources (Gobler et al. 2011). In this study, we are seeking to determine what organic and inorganic N sources are preferentially transported and metabolized by *A. anophagefferens* during blooms and how N transport and metabolism may change as a function of ambient conditions as brown tides initiate, are sustained, and decline. We designed species-specific qRT-PCR assays for gene targets associated with the use of multiple forms of inorganic N (nitrate and nitrite reductase) and organic N (cyanase, amidase, and guanine deaminase). Culture experiments were performed to establish quantitative patterns of gene expression for *A. anophagefferens* grown on different sources and concentrations of N. During laboratory experiments, cultures grown on low levels of ammonium became N-stressed and displayed significant down-regulation of a cyanase gene compared to exponential growth, while two nitrite reductases, a nitrate reductase, and a guanine deaminase genes were up-regulated. Compared to replete ammonium-grown cells, nitrate-grown cells had more transcripts of nitrate and nitrite reductases, while urea-grown cells displayed higher transcript abundance of nitrate and nitrite reductases, cyanase, amidase, and guanine deaminase genes. These results will be used to interpret N metabolism gene expression patterns from bloom populations surveyed from 2007-2010.

Gobler CJ, Berry DL, Dyhrman ST, Wilhelm SW, Salamov A, Lobanov AV, Zhang Y, Collier JL, Wurch LL, Kustka AB, Dill BD, Shah M, VerBerkmoes NC, Kuo A, Terry A, Lindquist JPEA, Lucas S, Paulsen IT, Hattenrath-Lehmann TK, Talmage SC, Walker EA, Koch F, Burson AM, Marcoval MA, Tang Y-Z, LeClerc GR, Coyne KJ, Berg GM, Bertrand EM, Saito MA, Gladyshev VN, Grigoriev IV (2011) Niche of harmful alga *Aureococcus anophagefferens* revealed through ecogenomics. *Proceedings of the National Academy of Sciences* 108: 4352-4357

THE RISE OF *PSEUDO-NITZCHIA* CONCENTRATIONS IN THE COASTAL OREGON SURFZONE AND THE ROLE OF COASTAL UPWELLING AS A CONTROL OF THE SEVERITY AND EXTENT OF DOMOIC ACID EVENTS

White, A.E.<sup>1</sup>, Wood, A.M.<sup>2,3</sup>, Peterson, W.T.<sup>4</sup>, McCulloch, A.<sup>4</sup>; Hunter, M.<sup>5</sup>, Forster, Z.<sup>5</sup>, Smith, D.<sup>6</sup>; McKibben, M.<sup>1</sup>, and Strutton, P.<sup>7</sup>

<sup>1</sup>College of Ocean and Atmospheric Sciences, Oregon State University, Corvallis, OR, 97331 USA [awhite@coas.oregonstate.edu](mailto:awhite@coas.oregonstate.edu); <sup>2</sup>Institute for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR; <sup>3</sup>Atlantic Oceanographic and Meteorological Laboratory, NOAA, Miami, FL, <sup>4</sup>Northwest Fisheries Science Center, NOAA, Newport, OR; Cooperative Institute of Marine Resources Studies, Oregon State University/NWFSC NOAA, Newport, OR; <sup>5</sup>Oregon Department of Fish & Wildlife, Astoria, OR; <sup>6</sup>Oregon Department of Agriculture, Salem, OR; <sup>7</sup>Institute for Marine and Antarctic Studies, University of Tasmania

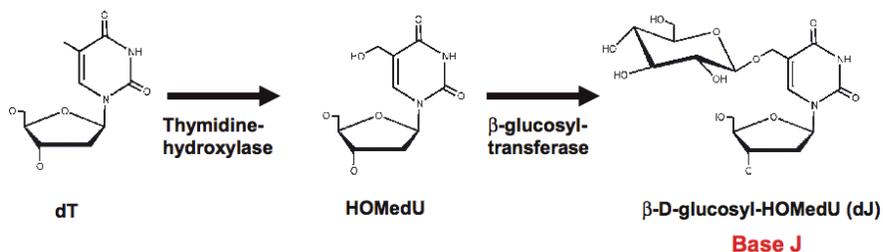
Phycotoxins such as saxitoxin and domoic acid have had a significant impact on Oregon coastal communities and their economy for decades. In recent years, particularly 2003, 2005, and 2010, domoic acid contamination has resulted in spatially extensive and prolonged closures of razor clam and mussel beds to harvesting. With initial funding from NOAA's Oceans and Human Health Initiative, and now from MERHAB, a collaborative team has been established to monitor and understand the genesis of harmful algal blooms (HABs) for coastal Oregon. This effort combines an oceanographic research component (extensive offshore sampling and remote sensing) with ongoing phytoplankton and shellfish monitoring programs to develop a streamlined ecosystem-based HAB monitoring and event response program for Oregon. Offshore cruises are conducted throughout the year with an emphasis on spring-fall seasons. Cell counts of HAB forming species are analyzed on a weekly basis at 12 nearshore sampling stations along the Oregon coastal margin by Oregon Departments of Fish and Wildlife (ODFW) personnel whereas phycotoxins levels in shellfish are assessed monthly via the Oregon Department of Agriculture (ODA). The combination of these sampling efforts have revealed that total *Pseudo-nitzchia* concentrations in Oregon surfzone samples have been steadily increasing in abundance, with the annual maximum cell counts increasing at a rate of  $4 \cdot 10^5$  cells L<sup>-1</sup> per year since sampling efforts were initiated in 2005. Coinciding with record *Pseudo-nitzchia* concentrations, domoic acid levels in shellfish tissue collected in 2010 exceeded closure levels at a scale not seen since an extensive 2005 event. Initial findings suggest that this enhanced toxicity is related to late onset of upwelling conditions.

## 5-HYDROXYMETHYLURACIL IS SYNAPOMORPHIC TO THE DINOKARYOTES ONLY

Williams, E., Place, A.R.

Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Sciences, Baltimore, MD USA

Since the original description of 5-hydroxymethyluracil (hmdU) in the genomic DNA of dinoflagellates by Rae in 1973 there has been little further research in extending these findings within the family. Accordingly, we re-examined the genomic DNA from members of various dinoflagellate genera and found that between 10 and 60% of the thymine bases were substituted by 5-hydroxymethyluracil. We also observed elevated levels of 5-methylcytosine and hydroxymethyl cytosine in some species. When we examined sister taxa to the dinoflagellates we did not find these modified bases in large quantities. This included DNA from *Chromera velia*, *Perkinsus marinus*, *Oxyrrhis marina*, and three species of *Amoebophrya* (*Amoebophyra ex sanguinem*, *Amoebophyra ex instriatum*, *Amoebophyra ex K. veneficum*). The replacement of thymidine by hmdU appears to be restricted to the species with a nuclear organization lacking nucleosomes e.g a dinokaryon. Rae proposed four possibilities for this replacement: (a) hmdU is involved in chromosome structure; (b) hmdU is involved in a modification- restriction system; (c) hmdU in DNA is required for nuclear metabolism in dinoflagellates; and (d) hmdU in DNA is a vestige, and it performs no contemporary role. We were able to identify a thymine dioxygenase gene in an EST library from *K. veneficum* that lacked a DNA binding domain and likewise have found that hmdU is present in the nucleotide pool. Thus, hmdU appears to be synthesized



prior to incorporation.

Another modified base, Base J, is found in trypanosomes and has recently been shown in dinoflagellates. Two key proteins involved in regulation of J synthesis in trypanosomes have been identified; JBP1 (J binding protein 1) and JBP2. However, these were not found in the EST library from *K. veneficum*. As opposed to most eukaryotes, transcription in trypanosomes (and all kinetoplastids) is primarily polycistronic, with long arrays of genes assembled into a PTU (polycistronic transcriptional unit). All mRNAs corresponding to a PTU are processed post-transcriptionally by a splicing reaction, which adds a 39-nt leader to the 5'-end of every mRNA, and polyadenylation at the 3'-end of the mRNA. This is very reminiscent of transcription in dinoflagellates and base J may be involved with a similar function in both cases. This finding may have direct implications for understanding the evolution of this model of post-transcriptionally regulated gene expression in kinetoplastids and dinoflagellates as well as the evolution of the dinokaryon.

## SHOTGUN PROTEOMICS IDENTIFIES THE PHOSPHORUS PHYSIOLOGY OF THE BROWN TIDE-FORMING ALGA, *AUREOCOCCUS ANOPHAGEFFERENS*

Wurch, L.L.<sup>1</sup>, Bertrand, E.M.<sup>1</sup>, Saito, M.A.<sup>1</sup>, Dyhrman, S.T.<sup>1</sup>

<sup>1</sup>Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA  
lwurch@whoi.edu

*Aureococcus anophagefferens* is the harmful alga responsible for brown tide events that have caused extensive damage to a number of coastal habitats in the Eastern U.S. Recent genomic analysis revealed that *A. anophagefferens* contains genes for the transport of phosphate and the hydrolysis of organic phosphorus (P). Additionally, transcriptome analysis showed that *A. anophagefferens* up-regulates a suite of genes involved in P metabolism under P-deficient (-P) conditions. This, combined with field data reporting a drawdown of dissolved organic P during brown tide-formation, suggests that P may play an unexpected role in *A. anophagefferens* bloom dynamics.

Advances in tandem mass spectrometry now allow analysis of mixed protein samples at increased resolution, but as yet are infrequently applied to studies of algal physiology. Proteomics offers the advantage of directly measuring changes in an organism's protein pool, thus providing a high-resolution representation of the mechanisms by which organisms adapt to varying environmental conditions. In this study, shotgun mass spectrometry was used to assess the response of *A. anophagefferens* to -P, and recovery from -P, conditions. Spectral counting techniques demonstrated that *A. anophagefferens* increased abundances of proteins involved in both inorganic and organic P metabolism, including a phosphate transporter, 5'-nucleotidase, and alkaline phosphatase under -P conditions. Additionally, an increase in abundance of a sulfolipid biosynthesis protein was detected in -P and recovery conditions, suggesting that *A. anophagefferens* may substitute phospholipids for sulfolipids when P is unavailable. Comparison of protein abundances between the -P and recovery conditions identified differences in the timing of protein degradation and turnover among individual proteins. An evaluation between the -P transcriptome and -P proteome showed that transcript and protein abundance generally appeared to be more tightly coupled for gene products involved in P metabolism compared to those involved in a range of other metabolic functions. These data provide an important first step towards using protein biomarkers to track the physiological ecology of *A. anophagefferens* in field populations.

USE OF A MICROBIAL FLOW-THROUGH TOXICITY TEST TO EXAMINE  
PESTICIDE-INDUCED ALLELOPATHY IN *PRYMNESIUM PARVUM*

Yates, B., Rogers, J., Barbee, G.

West Texas A&M University    briansyates@gmail.com,    jrogers@wtamu.edu,  
gbarbee@wtamu.edu

*P. parvum* is a toxigenic haptophyte that has invaded, bloomed and caused massive fish kills in the southern United States and around the world. Due to the rapidly evolving nature of this species and changing climactic conditions, there is concern that *P. parvum* will expand its range to other inland water bodies. Many blooms have occurred in catchments receiving agricultural runoff, leading the authors to examine the effects of atrazine and glyphosate on a natural community containing *P. parvum*. This research indicated that atrazine depopulates much of the natural phytoplanktonic community and selects for *P. parvum*, thus allowing the alga to gain a competitive edge. A microbial flow-through toxicity test system was designed to examine growth, phagotrophy, and toxicity of *P. parvum* as a function of herbicide concentration. Use of a flow-through system prevented biodegradation of the herbicide during chronic (>28 day) tests, while allowing us to simulate ambient aquatic conditions representative of the late winter pre-bloom period. The system is being used to test the effects of commonly-used cotton defoliant and pre-emergent herbicides in order to model the effects of mixtures of herbicidal contaminants for comparison with concentrations in situ. The results have major management implications for a species that threatens the health of inland fisheries and freshwater aquatic ecosystems in general.

CELL CYCLE, SAXITOXIN, AND PROTON PUMP RHODOPSIN: INSIGHTS FROM GENE EXPRESSION PROFILING FOR AN *ALEXANDRIUM FUNDYENSE* CULTURE AND A NATURAL BLOOM

Zhuang, Y.<sup>1</sup>, Zhang, H.<sup>1</sup>, Hannick, L.<sup>2</sup>, Lin, S.<sup>1</sup>

<sup>1</sup> Department of Marine Sciences, University of Connecticut, Groton, CT 06340 USA, senjie.lin@uconn.edu; <sup>2</sup> J. Craig Venter Institute, Rockville, MD 20850 USA

To understand intracellular and environmental factors regulating cell proliferation and toxin production in *Alexandrium* spp., we used 454 pyrosequencing to investigate the global gene expression pattern in cultured *Alexandrium* strain GTCA28 (originated from Gulf of Maine) and a natural PSP bloom in Long Island Sound. 18S rDNA analysis showed that the dominant strain in the LIS bloom was genetically the same population as GTCA28. Samples were collected every 2-3 hours over a 24h period to capture cell cycle phases G1, S, and G2+M and light/dark transition. More than  $7.7 \times 10^6$  and  $8.2 \times 10^6$  spliced leader-based 5' expressed sequence tags were generated from the cultures and the natural bloom, respectively. Within each library, each identified gene family was represented by 0 to over 2000 sequence tags, revealing a remarkable range of gene expression levels. In contrast, between different conditions, the expression level of each gene family varied only within 2 folds in most cases (excluding those that were not expressed in one of the conditions). A small number of gene families varied their expression levels between the conditions by over 5 folds, with the highest being 84 folds (luciferin-binding protein). Among the highly expressed genes were those related to protein translation, cytoskeleton, signal transduction, stress response, and bioluminescence. Cell cycle related genes such as proliferating cell nuclear antigen, cyclin-dependent kinases, were also expressed at relatively high levels, but expression dynamics was not dramatic, suggesting that regulation of these genes lie at posttranscriptional levels. Genes related to toxin production (e.g. Saxitoxin A, S-adenosylmethionine synthetase, S-adenosylhomocysteine hydrolase) were expressed in the field samples at different levels at different times of the day. Strikingly, proton-pump rhodopsin similar to proteorhodopsin was expressed at high levels from both the cultures and the natural bloom, suggesting the potential of directly converting solar energy to ATP in this species to fuel cellular activities even when photosynthesis is compromised by environmental conditions. As the first effort to study *in situ* gene expression of a toxic dinoflagellate bloom, the results will facilitate design of target studies to investigate intracellular and environmental mechanisms by which *Alexandrium* population and toxin dynamics is regulated.

STRAIN DIFFERENCES IN EUGLENOPHYCIN TOXIN ACCUMULATION,  
CHLOROPHYLL AND CAROTENOID PIGMENTS, AND CELL NUMBERS IN  
FOUR STRAINS OF *EUGLENA SANGUINEA*

Zimba, P.V., Ordner, P., Savage, M., Rafalski, A.

Texas A&M University-Corpus Christi, Corpus Christi, TX 78412 USA

Paul.zimba@tamucc.edu; pordner@gmail.com; msavage@islander@tamucc.edu;  
arafalski@islander.tamucc.edu

In 2004, the euglenophyte *Euglena sanguinea* was implicated in fish kill events in a North Carolina striped bass aquaculture farm. During the next five years, additional fish kills were observed in southeastern US states including South Carolina, Arkansas, Mississippi and Texas. In 2010 the structure of the toxin was determined to be a unique compound similar to solenopsin-fire ant venom. A total of seven clones from three continents produce the toxin. Four clonal isolates of *E. sanguinea* were grown in batch culture at 28°C using a 14:10 L:D cycle to evaluate growth, pigment, and toxin relationships over a 16-day period. Cell doubling ranged from 1.49-3.37 doublings/day. Chlorophyll *a* ranged from 81-977 pg/cell. Toxin content ranged from 2.86-27.6 pg/cell and increased during late exponential phase. The Ecuador strain exhibited maximal cell densities (>2900 cells/mL) whereas the Texas strain had the lowest total cell biomass (<1900 cells/mL). Strain variation was extremely high in terms of carotenoid content and toxin production.

## Index of Authors

### A

Abraham, A., 44, 75  
Abraham, W.M., 43  
Adams, N., 76  
Alexander, J., 51  
Allen, J., 138  
Anderson, C.R., 4, 85  
Anderson, D.M., 5, 7, 22, 49, 69, 83, 98,  
121, 133  
Armbrust, E.V., 69, 110  
Atwood, K.A., 44  
Avery, D.E., 6, 40

### B

Backer, L.C., 43  
Baden, D., 43  
Bakenhaster, M., 86  
Bammler, T., 66, 88  
Banas, N., 7, 29  
Barbee, G., 155  
Bargu, S., 8, 9, 132  
Barkoh, A., 135  
Bates, S.S., 110, 133  
Batoh, C.D., 10, 40  
Baugh, K., 76  
Bauza, C., 107  
Bean, J., 43  
Benitez-Nelson, C., 4, 8  
Benson, J., 43  
Bernard, R., 93  
Berry, D.L., 52  
Berry, G., 11  
Berry, J.P., 11, 72  
Berthiaume, C., 110  
Bertrand, E.M., 154  
Beyer, R., 66, 88  
Bill, B.D., 7, 12  
Birch, J., 13  
Blackmore, C., 120  
Bottein, M-Y., 41  
Bouchard, D., 41  
Bouchard, J.N., 14, 15  
Bowers, H.A., 16

Boyer, G.L., 17, 137  
Boyes, A.J., 18, 19, 81, 82  
Boyes, C.N., 18, 32  
Bratcher, A.R., 20  
Bricelj, V.M., 21  
Briggs, T., 144  
Brim, M., 129  
Brodie, R., 86  
Bronk, D., 50  
Brooks, B.W., 61, 105, 116, 123  
Brosnahan, M.L., 22  
Brown, C., 86  
Bryan, J.A., 44  
Bullard, S., 130  
Bullerjahn, G.S., 137  
Bulter, S., 145  
Burke, A., 80  
Burks, R., 104, 144  
Burleson, C., 86  
Burnett, W., 93  
Buskey, E.J., 24  
Byrne, M.M., 107

### C

Cahill, J.A., 129  
Cammen, K., 23  
Campbell, J.R., 24  
Campbell, K., 95  
Campbell, L., 26, 39, 57, 62, 65, 124  
Canion, A., 93  
Caron, D.A., 25, 68, 85, 127, 128  
Castle, K.T., 44  
Chadsey, M., 89  
Chao, Y., 4, 68, 85  
Chavez, F., 13  
Chen, F., 26  
Cheng, Y.S., 43  
Christopher, S., 65  
Clark, A.T., 125  
Cochlan, W., 27, 64, 76  
Cody, T., 86  
Collins, S.D., 37  
Connell, L.B., 20, 21, 37, 111  
Couture, D., 5, 41, 83

Coyne, K.J., 14, **28**, 94, 113, 143  
Crawford, J., 109  
Cross, S.L., 138

## D

Dam, H.G., **6**, 10, **40**  
Davis, K.A., **29**  
Deacutis, C., 142  
Deeds, J.R., **30**, 31  
Demir, E., 13  
Derner, K., **32**, **78**  
Dickey, R.W., 31, **33**  
Dill B.D., 137  
Dixon, L.K., **34**, 54, 81, 92, 108, 134  
Doll, C., **35**  
Dorsey, C., 93  
Dortch, Q., 139  
Doucette, G., 13, 62, **95**  
Dusek-Jennings, E., **36**  
Duy, J., **37**  
Dyhrman, S.T., **38**, 52, 151, 154

## E

Eisenstein, L., 120  
El Said, K.R., 75  
Eller, K., 93  
Elliott, C.T., 95  
Ellis, C.H., 69  
Emenegger, J.N., 12  
Erdner, D.L., 22, 73  
Errera, R.M., **39**  
Evans, R., 25

## F

Fahnenstiel, G., 104  
Fang, C., 145  
Farin, F., 66, 88  
Farnsley, S.E., 137  
Fell, J., 62  
Fernandes, L.F., 69, 133  
Finiguerra, M.B., **40**  
Fire, S.E., **41**, 129  
Fisher, K., **42**, 78, 83, 139  
Fleming, L.E., 43, 120  
Flewelling, L.J., **44**, 86  
Flores Quintana, H.A., **45**, **121**, 122

Foley, D., 99  
Foreman, M., 150  
Forster, Z., **46**, 97, 99, 152  
Forsyth, C.J., 145  
Foy, M., 76  
Frame, E., **47**, 66, 76, 79, 88  
Fries, D., 62  
Fries, L.T., 135  
Fu, F.X., 141  
Fulweiler, R.W., **48**

## G

Gable, G.M., 61, 116  
Gantar, M., 11  
Garcia, A.C., 45, **49**, 67, 121, 122  
Garrett, M., **50**  
Garrison, D. L., 8  
Gellene, A., 25, 128  
Giddings, S., 150  
Gilbert, S., 62  
Gill, V.A., 47  
Glibert, P.M., **51**  
Gobler, C.J., 38, 48, **52**, 59, 84, 140, 151  
Goldman, E., 93  
Granade, H.R., 49, 67, 122  
Granholt, A., 44  
Grattan, L., **53**  
Greenfield, D., 35  
Greengrove, C.L., 7  
Gregory, A., 89  
Grover, J.P., 61, 105, 116, 123  
Guidone, M., 142

## H

Hackett, J.D., 22  
Hall, E.R., 19, **54**, 81, **82**, 92, 108, 134  
Hamilton, S.A., 21  
Handy, S.M., **31**  
Hannach, G., **55**  
Hannick, L., 156  
Harred, L.B., **57**  
Hart, C., 144  
Harvey, E.L., **58**  
Harvey, J., 13  
Hattenrath-Lehmann, T.K., **59**  
Hauser, L.J., 137

Hayashi, K., **60**, 109, 117  
Hayden, N.J., **61**, 116  
Haynes, B.L., 41  
Haywood, A., 62  
He, R., 5, 98  
Heil, C., 50, 51, **62**, 100, 101  
Heine, L.K., **63**  
Heiss, E.M., 48  
Hendrickson, J.C., 86  
Hendrickson, J.L., **64**  
Hennige, S., 14  
Henrichs, D.W., 57, **65**  
Henry, M.S., 92  
Herndon, J., 76  
Hetland, R., **26**  
Hewitt, N.C., 61, 116  
Hickey, B., 150  
Hickey, J.M., 83  
Hiolski, E., **66**  
Hollenbeck, J., 43, 120  
Holohan, B.A., 130  
Hooe-Rollman, J.I., 45, 49, **67**, 121, 122  
Howard, M., 25, 85  
Howard, M.D.A., **68**, 127, 128  
Hubbard, K.A., **69**  
Hudnell, H.K., **70**  
Hunter, M.V., **71**, 97, 99, 152  
Hutchins, D.A., 141

## I

Ivey, J., 62

## J

Jaja, A., 11  
Jaja, A.D., **72**  
Jauzein, C., **73**  
Jayroe, D.S., **74**  
Jenkins, B., 110  
Jensen, S., 13  
Jester, E.L.E., 67, **75**  
Jester, R., **76**  
Johnson, J.G., **77**  
Jones, B., 25, 85  
Jones, B.H., 68, 127, 128

## K

Kavanaugh, K., **78**  
Kaveggia, S., 25  
Keafer, B. A., 5, 98  
Kelly, D., 28  
Kendrick, P., 66, 88  
Kendrick, P.S., 47, **79**  
Killberg-Thoreson, L., 50  
Kim, H.W., **80**  
Kirkpatrick, B., 19, **43**, 82, 107  
Kirkpatrick, G.J., 18, 32, 54, 62, **81**, 92  
Kiryu, Y., 86  
Kleindinst, J.L., **83**, 98  
Knott, T., 41  
Koch, F., **84**  
Kubanek, J., 114, 115  
Kudela, R.M., 4, 60, **85**, 109, 117  
Kulis, D., 121, 133

## L

Laber, C., 134  
Landsberg, J.H., 44, **86**  
Lane, J.Q., 85  
Langlois, G., 85  
Lasiter, A.D., 87  
Lazensky, B., 120  
Lazensky, R., 144  
Leblond, J.D., **87**  
Lefebvre, K., 44, 66, 76, 79, **88**  
Léger, C., 133  
Leighfield, T., 129  
Leschine, T.M., 89  
Leynse, A., 63  
Li, C., 87  
Li, Y., 98  
Li, Z., 137  
Lichtenwalner, A., 41  
Liefer J.D., **90**, 93  
Lin, S., 156  
Loader, J.I., 45  
Loeffler, C.R., **91**  
Los Huertos, M., 136  
Lovko, V., 54, **92**  
Luther, M., 62

## M

MacCready, P., 150  
MacIntyre, H.L., 90, **93**  
MacQuarrie, S.P., 21  
Madden, C.J., 51  
Mafra Jr., L.L., 133  
Main, C., 28  
Main, C.R., **94**  
Mangum, A., 28  
Manning, J.P., 98  
Mantua, N.J., 7, 102  
Marchetti, A., 110  
Marin III, R., 13  
Marshall, N.M., 95  
Martin, J.L., 5  
Masser, M.P., 105  
Masson, D., 150  
Masura, J.E., 7  
Maucher Fuquay, J., 119  
Mayerfeld, P., 109  
Mazzillo, F.F.M., **96**  
McCulloch, A., **97**, 152  
McGillicuddy, D.J., 5, 69, 83, **98**  
McKibben, M., 97, 152  
McKibben, S.M., **99**  
McLean, T.I., 74, **103**  
Menden-Deuer, S., 58, 80  
Meyer, K.A., **100**, **101**  
Mikulski, C.M., 62  
Miles, C.O., 145  
Miller, M.A., 47  
Millie, D., 62  
Moore, S.K., 7, 12, 76, **102**  
Morales, R., 110  
Morris, J. G., 53  
Mortazavi, B., 93  
Morton, S.L., 59, **129**  
Mountain, D.G., 98  
Muha N., 119  
Mulholland, M.R., 100  
Murasko, S., 50, 51  
Murphy, P., 34  
Myers, T.L., 114

## N

Naar, J., 43

Namataka, H., 103  
Nash, C., 83  
Neff, C., **104**, 144  
Neisch, M.T., 61, **105**, 116  
Neller, K., 129  
Nelson, T.A., **106**  
Netherland, M.D., 146  
Nevada, C., 44  
Nezlin, N., 68  
Nierenberg, K., 19, 43, 82, **107**  
Nissanka, A., **108**  
Novoveska, L., 93  
Nowocin, K., 129

## O

O'Neil, J. M., 100, 101  
Odell, A., 76  
Ohman, M. D., 8  
Olsen, D., 53, 122  
Olson, R. J., 65  
Ordner, P., 157  
Ozbay, G., 28

## P

Paerl, H.W., **56**  
Palacios, S.L., **109**  
Palmer, L., 25  
Palubok, V., 92  
Pang, Y., 145  
Paolisso, M., 148  
Pargett, D., 13  
Park, K., 93  
Parker, M.S., **110**  
Parsons, A.R., 138  
Parsons, M.L., 63  
Paternoster, J., 129  
Paul, J., 62  
Paul, V., **56**  
Pederson, B.A., 54, 81  
Penta, B., 4, 85  
Peterson, R., 93  
Peterson, W.T., 46, 71, 97, 152  
Petrik, K., 62  
Phillips, J., **111**  
Pierce, R., 43  
Place, A.R., 16, **112**, 153

Plakas, S.M., 75  
Plesha, P., 76  
Pokrzywinski, K.L., **113**, 143  
Poulson-Ellestad, K., **114**, **115**  
Preston, C., 13  
Prince, E.K., 114  
Prosser, K.N., 61  
Prosser, P., **116**  
Pruden, J., 41  
Purdie, D., 15

## Q

Quay, J.Q., **117**  
Quilliam, M.A., 133

## R

Rabalais, N. N., 9  
Radke, B., 53  
Rafalski, A.V., **118**, 157  
Ragan, M., 25, 128  
Ramsdell, J.S., **119**  
Raymond, H., 144  
Read, A., 23  
Reich, A., 43, **120**, 144  
Rein, K.S., 149  
Richlen, M., 49, 69, 91, 121  
Roberts, S., 53  
Robertson, A., 45, 49, 67, 90, 121, **122**  
Robertson, G., 68  
Rocap, G., 110  
Roche, S., **16**, 87  
Roelke, D.L., 61, 105, 116, **123**, 146  
Rogers, J., 155  
Roman, B., 13  
Rosa, M., **130**  
Rosel, P., 23  
Roy, E., 132  
Ryan, D.E., **124**  
Ryan, J., 13

## S

Saito, M.A., 154  
Salathé, E.P., 7, 102  
Salvitti, L., 28  
Savage, M.L., 118, **126**, 157  
Savage, T.J., **125**

Schaffner, R., 25  
Schnitzer, A., 25, 68  
Scholin, C., 13, 62  
Schruth, D., 110  
Scorzetti, G., 62  
Seegers, B., 25, 68, **127**  
Sellner, K.G., 16  
Senqupta, A., 68  
Seubert, E., 25, 68, 127, **128**  
Shah, M., 137  
Shuler, A.J., 129  
Shulman, I., 4, 85  
Shumway, S.E., 130  
Sieg, R.D., 114, 115  
Siegel, D.A., 4  
Silver, M. W., 8  
Simenstad, C.A., 36  
Sirois, A., **131**  
Skelton-Flores, H.K., 40  
Smith, D., 97, 152  
Smith, D.R., 66  
Smith, E.A., 9, **132**  
Smith, G.J., 60, 125  
Smith, J.L., **133**  
Smith, R.L., 37  
Smith, T., 49, 121, 122  
Smith, T.B., 91  
Smith, W., 93  
Solomon, B.M., **134**  
Sosik, H. M., 65  
Southard, G.M., **135**  
Spaulding B., 131  
Sreenivasan A., 136  
Stanfield, E.R., **136**  
Stark, K., 55  
Steffen, M.M., **137**  
Steidinger, K.A., **138**  
Stein, J.E., 7  
Stevens, A.W., 36  
Strutton, P., 97, 99, 152  
Studts, J.L., 107  
Stumpf, R, 32, 42, 83, 104, **139**, 144  
Su, N., 93  
Sukhatme, G., 25  
Sutula, M., 68

## T

Tabuchi, M., 86  
Tang, Y.Z., 84, **140**  
Tatters, A.O., **141**  
Thomas, M.A., 98  
Thompson, N., 124  
Thornber, C.S., **142**  
Tiedeken, J.A., 119  
Tilney, C.L., 28, 113, **143**  
Tisdale, T., 129  
Toben, A., 53  
Tomlinson, M.C., 42, 104, **144**  
Tomlinson, T., 139  
Townsend, D.W., 98  
Trainer, V.L., 7, 12, 27, 64, **89**  
Trick, C.G., 27, 64  
Twiner, M.J., **145**

## U

Umphres, G.D., 61, **146**

## V

Valenti, T. Jr., 61, 116  
Van Alstyne, K.L., **147**  
Van Dolah, E. R., **148**  
Van Dolah, F.M., 77, **149**  
VerBerkmoes, N.C., 137  
Viso, R., 93

## W

Wakamatsu, T., **150**  
Walker, E.A., **151**  
Wall, C., 48  
Walton, K., 11  
Wang, Z., 41, 129  
Wanner, A., 43

Warner, M., 14, 28, 113, 143  
Wazniak, C., 144  
Weisberg, S., 25  
Wells, M.L., 27, 64  
Wells, R., 23  
White, A., 46, 71  
White, A.E., 97, 99, **152**  
White, J.R., 132  
White, K.D., 31  
Wikfors, G.H., 130  
Wilhelm, S.W., 52, 137  
Williams, E., **153**  
Williams, J., 86  
Wilson, P., 86  
Wippelhauser, G., 41  
Withers, K., 118  
Wolny, J., 86  
Wood, A.M., 46, 71, 99, 152  
Wood, M., 97  
Wurch, L.L., 38, 151, **154**  
Wynne, T.T., 104, 144

## X

Xu, Y., 49, 121

## Y

Yates, B., **155**  
Younan, L., 109  
Yunker, A., 50

## Z

Zhang, H., 156  
Zhu, G., 137  
Zhuang, Y., 156  
Zimba, P.V., 118, 126, **157**  
Zippay, M.L., 149