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Abstract Book

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Monday Presentations

AN INTEGRATED LOOK AT KARENIA BREVIS PHOTOPHYSIOLOGY

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Karenia brevis is a prolific toxic dinoflagellate that is biogeographically constrained to the high-light environments of the Gulf of Mexico and Caribbean. Yet relatively little is known about how photophysiology affects *K. brevis* bloom dynamics. In a series of lab-based experiments, we first generated growth-irradiance curves (μ-E) for three *K. brevis* strains incubated at nine ecologically-relevant irradiance levels (10-1340 µmol photons m^{-2} s^{-1}). Initial slopes of the μ-E curves suggested that *K. brevis* is not low-light adapted, and photoinhibition beyond μ_{max} was not evident even at the highest irradiances. For two strains grown at irradiances representing 1/4, 1, and 4X the levels needed to saturate growth, we also measured toxin composition and expression of two plastid-associated genes (*rbcL, ftsH*) related to C-fixation and membrane-bound protein replacement, during exponential and stationary phase. Initial results indicated that cellular brevetoxin quotas increased in stationary phase, but were unaffected by irradiance. In a second experiment, cultures retained maximal growth rates and PSII quantum yield (F_v/F_m) after three weeks of semi-continuous culture at 1500 µmol photons m^{-2} s^{-1}. Interestingly, a significant negative relationship between cell volume and irradiance was noted as cells moved into stationary phase. Finally, we evaluated physiology across a diel cycle at 2X growth-saturating light, wherein we observed decreases in F_v/F_m around midday, and a coupling of cell size with photosynthesis and mitosis. Our results expand what is known about *K. brevis* physiology and will inform models of *K. brevis* growth to help elucidate geographical areas important for bloom initiation.

THE EFFECT OF VARIOUS SPECIES OF MACROALGAE ON THE GROWTH, SURVIVAL, AND TOXICITY OF *KARENIA BREVIS*

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Harmful algal blooms (HABs) caused by the dinoflagellate *Karenia brevis* produce toxins that result in negative impacts to both humans and the environment. Little is known about the termination stages of these blooms, and few viable control mechanisms have been suggested. Natural, algae derived compounds have been proposed as a way to limit bloom growth and reduce water column brevetoxins. The work presented here examines the ability of macroalgae to inhibit the growth or survival of *K. brevis*, similar to what has been demonstrated with other red tide species. Additionally, we attempted to determine if macroalgae decreases water column brevetoxins which, to our knowledge, has not been tested with macroalgae but has been demonstrated in other studies with microalgal species. This was accomplished via Indirect Bioassays, Methanol and aqueous extract studies, and LCMS. The macroalgae *Dictyota sp.* and *Gracilaria sp.* caused 100% mortality of *K. brevis* in under 24 hours. Compared to the control, 7 other species significantly decreased the growth rate of *K. brevis.* The *Dictyota* treatments showed significant toxin reduction and increase of the antitoxin brevanol. These results indicate that some combination of compounds produced by macroalgae inhibit growth and survival of *K. brevis* and possibly limit their toxin production. Future studies will attempt to isolate and identify these compounds and test their effects on other marine organisms such as diatoms. Determining the interactions between HAB species *K. brevis* and macroalgal species will provide insights on the mechanism of bloom termination and a potential control method.

NUTRITIONAL AND PHYSIOLOGICAL ADAPTATIONS OF *KARLODINIUM VENEFICUM*; IMPLICATIONS FOR CHESAPEAKE BAY

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Mixotrophy as a significant nutritional strategy for HABs in eutrophic environments is becoming apparent. Increased mixotrophy and toxicity allow HABs to acquire necessary nutrients and capture prey not only when nutrients are limiting, but also when nutrients may be in excess, but not in balanced (Redfield N:P of 16) proportion. Laboratory experiments were conducted with the common Chesapeake Bay toxigenic dinoflagellate *Karlodinium veneficum* to elucidate how the nutritional condition of predator and prey affects mixotrophy and toxicity. The results indicated physiological or nutritional conditions of the predator, and that of its prey, affect the feeding behavior of *K. veneficum*. Feeding rates were highest for low-NP *K. veneficum* initially growing exponentially and mixed with N-rich prey. Maximum feeding rates of low-NP *K. veneficum* on N-rich prey during exponential growth were ~ 4 fold higher than the rates of high-NP *K. veneficum* on N-rich prey. The nutritionally different *K. veneficum* were tested with the eastern oyster *Crassostrea virginica* larvae to determine putative toxicity. The negative effects on larval survival appeared to be highest when they consumed prey with higher N:P content. Using a new multi-color pulse amplitude modulation (PAM) flourometer, it appeared that even though feeding was dose-dependent and growth rate responded accordingly, there was no significant difference between phototrophy (without prey addition) and mixotrophy (with prey addition) for photosynthetic activities of *K. veneficum*. These results suggested that *K. veneficum* may show enhanced success in the Chesapeake Bay subregions where have excessive N inputs and dense prey abundance.

DETERMINING THE DIVISION TIME (T_D) OF *KARLODINIUM VENEFICUM* USING AN ASYNCHRONOUS CULTURE GROWTH METHOD

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Bloom toxicity of the HAB dinoflagellate *Karlodinium veneficum* varies widely in the field. Culture studies indicate an inverse relationship between toxicity and cell proliferation rate. If true for blooms, then slowed/halted growth could explain observed variability in bloom toxicity. Proliferation rates in *K. veneficum* blooms can be determined using a microscopy-based mitotic index technique. However, this method requires knowledge of division time (t_d), which is typically determined as the time interval between frequency peaks of two morphologically distinct division stages in synchronized populations. *K. veneficum* lacks such distinct stages, requiring that t_d be calculated from an asynchronously growing culture following McDuff and Chisholm (1982), wherein growth rate (μ) and fraction of cells in division (f) are measured constants:

$$t_d = \frac{1}{n\mu} \sum_{i=1}^{n} ln(1 + f_i)$$

Division asynchrony was induced in *K. veneficum* cultures by continuous illumination, and μ and f were determined in log phase every 1 h for 27 h. Asynchronicity was confirmed by flow cytometric cell cycle analysis. Based on this analysis, t_d was calculated to be ~3.4 h, and estimated *in situ* growth rate and gross growth rate were comparable among cultures on a 12 h light:dark cycle (~0.3 divisions day^{-1}). The average frequency of G2+M phase cells was 10.3 ± 0.5 %, indicating a duration of G2+M of ~ 2.5 h. This value should serve as an upper boundary for t_d, suggesting some overestimation. This may have been caused by sexual stages (gamete fusions) in the culture populations, which are difficult to distinguish from division stages by microscopy or flow cytometry.

RECENT DISCOVERIES BROADEN THE ECOLOGICAL NICHE OF BROWN TIDES CAUSED BY THE PELAGOPHYTES, *AUREOCOCCUS ANOPHAGEFFERENS* AND *AUREOUMBRA LAGUNESIS*

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Brown tides caused by the pelagophytes *Aureococcus anophagefferens* and *Aureoumbra lagunesis* have plagued estuaries across the globe for more than thirty years. Recent, extended brown tides in Florida, Cuba, and China have damaged habitats and fisheries and emphasized the ability of these algae to expand to new ecosystems. The niche of these algae has been well-defined by an ability to exploit organic forms of nitrogen and deter predators. Here, a series of new findings affirm this established niche of brown tides as well as reveal newly discovered ecological strategies. *Aureoumbra* has the ability to form resting cells that can survive more than seven months in darkness, an adaptation that may permit the expansion into new ecosystems. *Aureococcus* and *Aureoumbra* produce allelochemicals that detered a broad array of phytoplankton in the lab and in field experiments. The allelochemically-mediated lysis of competing algae releases cellular nitrogen that is subsequently utilized by brown tides, potentially facilitating the long-lasting nature (months) of these events. A novel method combining sorting flow cytometry and N-15 labeled compounds revealed the ability of *Aureococcus* to assimilate urea at a rate faster than cyanobacteria, heterotrophic bacteria, and co-occurring eukaryotes. The use of meta-transcriptomics during a bloom event indicated that *Aureococcus* actively transcribes the urease gene during brown tides in some cases making it nitrogen-sufficient and potentially phosphorus-limited. Collectively, these findings reveal a broader than suspected ecological niche for brown tides and provide mechanisms that may account for their expansion in the past decade.

THE INTERACTIVE ROLES OF NITROGEN LOADING AND WARMING IN CONTROLLING MICROCYSTIN PRODUCTION IN *MICROCYSTIS*

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Climate change and eutrophication promote the dominance of cyanobacteria in freshwater phytoplankton communities. Anthropogenic activities that enrich freshwater ecosystems with nitrogen (N) have been linked to the emergence of massive cyanobacterial blooms which deleteriously impact human and animal health. The microcystin-producing cyanobacterium *Microcystis aeruginosa*, for instance, exhibits maximal growth and toxin production rates at elevated temperatures, yet the reverse is true when cultures are subjected to N-limitation. Here we report the synergistic effect of N-limitation and elevated temperature on *M. aeruginosa* (LE-3). Cultures were grown in BG11 media amended with either high (1 mM) or low (50 μM) nitrate (NO_3^-) at 22°C or 28°C for ~2 weeks. Results indicated that reduced temperature and NO_3^- availability suppressed growth during early logarithmic and late logarithmic/early stationary growth phases, respectively. Furthermore, while the photosynthetic efficiencies (F_v/F_m) of N-replete cultures were consistently the highest for the latter half of the experiment, N-deficient cultures grown at 22°C exhibited higher F_v/F_m values relative to N-deficient cultures grown at 28°C. This suggests that the stress of elevated temperature further exacerbates the effect nutrient limitation has on photosynthetic efficiency. Microcystin analysis using an ELISA showed that in late logarithmic/early stationary phase, toxin content (pg cell^{-1}) was higher in low temperature, N-replete cultures relative to all other treatments, suggesting that temperature may be more important in regulating toxin production than N-availability in future climate conditions. Future work will determine whether natural populations of *Microcystis* spp. exhibit similar trends, particularly in terms of total and dissolved microcystin concentrations.

TRADE-OFF AND PUTATIVE TAX BETWEEN GRAZER-INDUCED TOXIN PRODUCTION AND CELL GROWTH IN THE MARINE DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE*

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In toxigenic prey, a trade-off between toxin production and growth implies a tax (direct cost) to defense. We tested this hypothesis using the marine dinoflagellate *Alexandrium fundyense*, which bears paralytic shellfish toxin (PST) in both constitutive (always present) and inducible (increased in the presence of grazers) forms. We measured relative gene expression (RGE) of saxitoxin, STX (*sxtA* and *sxtG*), and cell growth (*Afcyc*) by qPCR in 96h experiments. Differential (grazer treatment - control) RGE was positive for STX genes and negative for cell growth, suggesting a tradeoff, which was also demonstrated by a negative relationship between cell toxin content measured by HPLC and population growth rate calculated by changes in cell concentration. RGE of *Afcyc* in the grazer treatment measured the putative tax of induced toxin production whereas in the control it measured the constitutive tax. The former is higher by 17-40%, which indicates that the traditional equations to calculate grazing produce similar overestimates. Thus, the traditional concept of predator-toxic prey interaction needs to consider the tax of chemical defense. Otherwise, grazing losses are overestimated because the assumption of equal cell growth in grazer-free control and treatments with grazers is violated.

BENEFIT AND COST OF GRAZER-INDUCED TOXIN PRODUCTION IN THE DINOFLAGELLATE *ALEXANDRIUM TAMARENSE* COMPLEX

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There is still controversy as to whether or not saxitoxin (STX) production by prey is an efficient form of defense against grazing. We tested for benefit and cost of STX production (both constitutive and inducible) in the marine dinoflagellate *Alexandrium tamarense* complex in response to copepod grazing. We used a modification of the model of Simms & Rauscher (1987, Am. Natur. 130:570-581), which relates fitness to grazing pressure for different genotypes, as the theoretical framework for our tests. In controlled laboratory studies, we compared net cell growth rates of three *Alexandrium* strains that differed in STX production (no production, moderate and high STX production, respectively) as a function of grazing pressure (given by a gradient in copepod concentration). The most toxigenic strain grew faster in the absence of grazers, suggesting that the constitutive cost of toxin production is low. However, there were differences in the slopes of net growth rate versus grazer concentration among strains, with the non-toxigenic strain having the steepest slope (greatest loss rate), and the moderate toxin strain having the lowest slope (lowest grazing loss, higher benefit), and the highly toxigenic strain falling in the middle. This suggests that toxic production is an effective defense against grazing, but that moderate toxin production may be the evolutionarily stable strategy.

THE ROLE OF DORMANCY-BREAKING BY CHILLING IN DETERMINING *ALEXANDRIUM CATENELLA* BLOOM PHENOLOGY

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The germination of *Alexandrium catenella* cysts is critical for the initiation of new blooms and recurrence of paralytic shellfish poisoning. *A. catenella* cysts were recently discovered to require a quantifiable amount of cold conditioning (or 'chilling') to break dormancy and achieve quiescence (i.e. become germinable). The ecological consequences of this chilling requirement and temperature-dependent germination were investigated in the context of Nauset estuary on Cape Cod (MA), where *A. catenella* blooms annually. Germination rates of naturally-formed cysts were compared through lab- and field-based approaches, including germling capture from raw sediment using plankton emergence traps. All experiments produced similar, linear relationships between temperature and germination rate. Germination data were combined with dormancy and vegetative cell division relationships to formulate a model of Nauset bloom phenology from 2009–2016. The model accurately predicts the January onset of quiescence and the timing of bloom initiation over this period. It also demonstrates the importance of vernal warming for bloom initiation in Nauset. Although colder winters expedite the dormancy period, cysts always achieve quiescence prior to vernal warming, which is needed for germination to occur. We conclude that dormancy-breaking by chilling may play a more significant role in determining the phenology of *A. catenella* within habitats with milder winters. Ecologically, this mechanism is advantageous because it suppresses germination until seasonal conditions are likely to promote the formation of blooms. Given this species' extensive global distribution, there is likely plasticity in the chilling responses of different populations, reflecting differences in the temperature regimes of their habitats.

INTERPLAY OF TEMPERATURE AND GENETIC COMPOSITION ON THE SEASONAL SIZE DISTRIUTIONS OF THE MIXOTROPHIC CILIATE, MESODINIUM, ON THE NEW ENGLAND SHELF

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The cosmopolitan marine planktonic ciliate, *Mesodinium rubrum,* is an important phototrophic organism as it represents a unique metabolic lifestyle of obligate mixotrophy and can remarkably cause red tides in high abundances. While it was historically believed that all *M. rubrum* belonged to a single species, recent assessments of genetic diversity within the *Mesodinium* genus provide confirmation that *M. rubrum* is part of a species complex (*M. rubrum/major* complex) of subclades. The *M. rubrum/major* subclades have also been associated with various cell sizes. Despite numerous observations of genetic diversity and size structure plasticity, the response of these two characteristics to seasonal environmental variation has not been rigorously characterized. A 10-year time series at the Martha's Vineyard Coastal Observatory (MVCO) provides a unique opportunity to explore ciliate genotypic and physiological variations over time. At MVCO, an autonomous, submersible imaging-in-flow cytometer, Imaging FlowCytobot (IFCB), allows for morphological identification and observations of cell size of *Mesodinium* in situ. With the use of primers designed to specifically amplify *M. rubrum/major* subclades, we explored seasonal patterns of correspondence between genetic variants and cell size distributions at MVCO. We found data supporting temperature regulation on size. Peaks in abundance of large *Mesodinium* corresponded inter-annually with the temperature cycle. The *M. rubrum/major* subclades were associated with seasonal temperature niches, but not always unique size distributions. These results suggest the interplay of genetic and physiological factors regulating size structure/temperature relationships for the *M. rubrum/major* species complex.

EFFECTS OF THE DINOFLAGELLATE PARASITE AMOEBOPHRYA ON KARENIA BREVIS

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Blooms of the neurotoxin-producing dinoflagellate, *Karenia brevis*, negatively affect both humans and natural resources. Little is known about the termination stages of these blooms, and few viable control mechanisms have been suggested. *Amoebophrya* is a syndinian parasite found to infect and kill various bloom-forming dinoflagellates, including toxin-producing species, potentially serving a role in host population dynamics. *Amoebophrya* is generally considered to have high host specificity, although infection across different host species has been observed. Due to its host-specificity, *Amoebophrya* may be suitable as a potential biocontrol for some HAB species. Little work has been conducted examining the natural presence of *Amoebophrya* in *Karenia brevis* or the susceptibility of *K. brevis* to *Amoebophrya* from non-*Karenia* hosts. We examined the effect of *Amoebophrya* isolated from 3 different host species (*Alexandrium* sp., *Scrippsiella trochoidea*, *Akashiwo sanguinea*) on growth and survival of *K. brevis*. Exposure to *Amoebophrya* ex. *Alexandrium* sp. consistently resulted in decreased *K. brevis* abundance over 72-96 hours by 50-70% versus controls. *Amoebophrya* zoospores increased concurrently with decreasing *K. brevis* abundance, while controls consisting of *Amoebophrya* zoospores with no host decreased over the same period. Results with *Amoebophrya* ex. *Scrippsiella trochoidea* were similar, but inconsistent. *Amoebophrya* ex. *Akashiwo sanguinea* reduced growth in *K. brevis* after more than 96 hours, also concurrent with an increase in *Amoebophrya* zoospores. Additional data from ongoing studies, including application of CARD-FISH using an Amoebophrydae-specific HRP-modified ALV01 oligonucletide probe to visualize infection, and use of *Amoebophrya* isolated from other species (including *Karlodinium veneficum*), will be presented.

The influence of the environmental factors on the allelopathic effect of domoic acid on natural phytoplankton communities

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Domoic acid (DA), a neurotoxin produced by toxic species of the diatom genus *Pseudo-nitzschia*, is recognized as a threat to wildlife and human health worldwide. The reason for the release of DA by toxic species is still unknown. It has been hypothesized that DA is an allelochemical allowing *Pseudo-nitzschia* to outcompete other phytoplankton species by inhibiting their growth. Although the allelochemical effects of DA have been the focus of a few culture-based studies, the allelopathic effects on natural species assemblages is unknown. The purpose of this research was to examine phytoplankton responses to DA exposure of an estuarine phytoplankton community from North Inlet, South Carolina. We examined the phytoplankton responses for two different DA concentrations (40 and 400 ng/mL) and the influence of environmental factors such as temperature, salinity, precipitation, pH and macronutrient concentrations. Phytoplankton responses to DA varied between groups and differed for the two estuaries. Macronutrient concentrations and salinity played a significant role in the allelopathic effect of DA on the dominant phytoplankton groups. These results highlight the effects of DA on the different groups in combination with environmental factors. DA could result in alterations of the phytoplankton community composition, which change the structure of the entire food web with cascading effects on primary productivity and biogeochemical cycling.

DRAMATIC CHANGES IN PROTEIN SYNTHETIC RATE OF AXENIC *AMPHIDINIUM CARTERAE* IN RESPONSE TO INCREASING TEMPERATURES, DENSITY OF CULTURE AND OVER THE DIEL CYCLE

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In dinoflagellates many physiological processes appear to be under post-transcriptional control. However, the extent to which this reflects translational regulation remains unresolved. While most dinoflagellate cultures are uni-algal, usually the cultures are not axenic, making it difficult to quantitate protein synthesis by ^{35}S-methionine incorporation or to optimize cycloheximide concentrations for the stabilization of polysomes. In bacterized *Amphidinium carterae*, a significant proportion of ^{35}S-methionine incorporation appears to be bacterial. We have maintained an axenic culture over several months by maintenance in the antibiotic mix, kanamycin/carbenicillin/ streptomycin. Inclusion of the antibiotic mix gives an initial drop in ^{35}S-methionine incorporation as the bacterial count falls. However, once the bacterial community is removed, ^{35}S-methionine incorporation rises. This has allowed us to measure protein synthetic rates under different physiological conditions and study changes over the diel cycle. With increasing temperatures, ^{35}S-methionine incorporation increases to a maximum at 25 °C, falling to less than 50 % of the maximum at 35 °C. During growth of a culture, ^{35}S-methionine incorporation per cell increases to a maximum at ~9 x 10^5 cells/ml, falling swiftly in advance of the crash in cell density. Dramatic changes in ^{35}S-methionine incorporation can also be seen over the diel cycle. Using a zeitgeber regime of 14-h light/10-h dark, ^{35}S-methionine incorporation increases exponentially during the light phase up to 12 h and begins to fall in anticipation of the dark phase. ^{35}S-methionine incorporation decreases steadily during the dark period, reaching a minimum approximately 7-fold lower than the peak at 22 h in zeitgeber time.

IMAGE CYTOMETRY (ICM) AS A METHOD FOR CELL CYCLE ANALYSIS OF MIXOTROPHIC CULTURES AND FIELD BLOOM SAMPLES OF *KARLODINIUM VENEFICUM*

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The cellular toxin quotient of *Karlodinium veneficum* varies widely in blooms and laboratory strains for poorly understood reasons. However, recent studies indicate that toxin biosynthesis may be synchronized to the cell cycle in a light-dependent fashion that is related to rates of cell proliferation. In order to better understand this relationship, cellular toxin and cell cycle must be studied under natural conditions of mixotrophic growth and in blooms. Such studies are complicated when using flow cytometry by phagotrophy (i.e. endogenous prey nuclei/DNA) and by the multi-species nature of field samples. To solve this we developed a microscope-based image cytometry (ICM) method that allows user discrimination of non-target nuclei/DNA within *K. veneficum* cells as well as non-target cells in bloom samples. This technique has previously been used in the clinical analysis of mammalian cancer cells but not on phytoplankton cells. During development and optimization we tested 1) precision of ICM analysis of DAPI-stained *K. veneficum* using 40x, 20x, and 10x microscope objectives, 2) effect of ambient light on precision of those analyses, and 3) effect of different resuspension buffers on *K. veneficum* cultures and field bloom samples. The coefficient of variation for the 40x objective was nearly twice that of the 20x objective (11.4% and 6.5%, respectively), indicating a necessary compromise between focal plane depth and resolving power, while also limiting the impact of extraneous light. Specimen preparation methods such as optimal resuspension buffer and cell staining and mounting considerations are also discussed.

HOW DOES CLIMATE CHANGE AFFECT THE PHYSIOLOGICAL ECOLOGY OF *KARLODINIUM VENEFICUM* AND ITS CONSEQUENCES ON TROPHIC TRANSFER?

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Shifts in global temperature as well as carbon dioxide are expected to have potentially profound effects on phytoplankton communities, including harmful algal species, with possible cascading consequences for marine food webs. We studied the combined effects of elevated temperature and CO_2 on the physiological response of the dinoflagellate *Karlodinium veneficum*, originally collected from the Delaware Inland Bays. Algae were first acclimated to the ambient (25 °C / 400 ppm CO_2) and climate change conditions (29 °C / 1000 ppm CO_2) predicted for year 2100, using automated pH stated continuous cultures. When grown under high temperature and CO_2, cell growth improved and Primary production increased significantly (x 1.6 times), as compared to ambient conditions. Similarly, high temperature and CO_2 resulted in the cellular quota for carbohydrate, protein and total lipids also increasing by 3.1, 1.8 and 1.1-fold, respectively. Further study of how climate change conditions may affect trophic interactions is currently underway, by investigating how temperature and CO_2 influence the toxicity (as measured by karlotoxin content, hemolytic, and fish gill cell activity) as well as fatty acid profiles of *K. veneficum*. This work is in tandem to grazing analyses with the common estuarine copepod *Acartia tonsa*, while grown under the same temperature and CO_2 conditions. Our current studies will also be presented in the context of further work designed to include nutrient limitation, as well as tandem experiments with harmful raphidophytes that are common to DE waters.

GENERALIST VS. SPECIALIST PARASITIC CONTROL OF DINOFLAGELLATE BLOMS: INSIGHTS FROM NUMERIC SIMULATIONS

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Parasitic control of dinoflagellate blooms is usually focused in the interaction between a eukaryotic parasite and its dinoflagellate host. Less frequent is the perception that parasites are in turn affected by other species besides their focal hosts. A previous study based on numerical simulations indicated that parasitic control of dinoflagellate blooms is likely determined by the presence/absence as well as the relative abundance of other members of the plankton community (i.e, diatoms, grazers) which would exert a dilution effect on host-parasite dynamics. Here, we extend this model approach to evaluate how infection rates of a specialist parasite could be affected by the presence of a generalist parasite sharing the same dinoflagellate host. Considering similar population parameters for both parasites, simulations suggest that generalist and specialist parasites are expected to coexist when only one host species is present, with the addition of an second dinoflagellate species (additional host for the generalist) resulting in the extinction of the specialist. Under this scenario, the coexistence of both parasites is only possible when the specialist performance is improved by lower generation times and/or higher number of infective stages produced per infection. Similarly, higher growth rates in the shared dinoflagellate host would also help to improve the specialist performance. These model outcomes are further discussed in light of data obtained from field studies.

SALINITY TOLERANCE AND TOXIN CONCENTRATION IN *MICROCYSTIS AERUGINOSA* IN THE CHESAPEAKE BAY REGION

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The cyanobacterium *Microcystis aeruginosa* forms harmful blooms in fresh and oligohaline waters and thrives at temperatures above 25°C. *M. aeruginosa* produces microcystin, a hepatotoxin known to have detrimental effects on mammals, and also has been associated with the death of birds, turtles, and other vertebrates. As such, there is keen interest in the occurrence of these blooms in terms of both human and ecosystem health and management of these blooms is a growing concern. In the Chesapeake Bay, cyanobacteria blooms have been occurring with increasing frequency and at increased locations. This study conducted laboratory studies on the effect of salinity on proliferation and toxin concentration of *M. aeruginosa* sampled from a bloom that occurred in "ponds" within a restored wetland in the Chesapeake Bay region. The halotolerant strain was found in the field at salinities from 8-12. During salinity experiments, *M. aeruginosa* cell numbers increased in salinity treatments from 0-15 with a sharp decrease in abundance at a salinity of 18, and zero abundance above 22. Microcystin concentration, determined by a phosphatase inhibition assay, was highest at salinities between 6-14, and dropped off dramatically at salinities above 18. Toxin was detectable in water samples for approximately 30 days after *M. aeruginosa* cells were no longer present. This suggests that *M. aeruginosa* has the ability to occur throughout the oligohaline and mesohaline portions of the Chesapeake Bay, and that toxin transport both up and down estuary may be possible.

INSIGHTS INTO THE CELL DEATH OF DINOFLAGELLATE *KARENIA BREVIS* TRIGGERED BY ENVIRONMENTAL STRESS

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Historically, marine phytoplankton were presumed to replicate indefinitely by binary fission. However, drastic changes in environmental conditions have been shown to induce algal cell death, and can greatly affect development of algal populations. The Gulf of Mexico (GoM), a vast region with extensive shellfish resources, is subject to frequent toxic blooms of the dinoflagellate *Karenia brevis*. Although the behavior and physiology of *K.brevis* have been extensively investigated in both laboratory and field contexts, the mechanisms of bloom decline and termination within the GoM are not well known, hampering our ability to forecast their impact on coastal waters and human health. In this study we will present results of studies of cell death due to oxidative stress, heat shock, cold stress, high irradiance and nitrogen starvation. These cell death pathways are characterized by using stress indicators including photosynthetic efficiency, production of reactive oxygen species (ROS), caspase-like activity, and morphological changes. We expect to identify environmental stressors that can cause cell death in *K. brevis,* define stress-related death processes, and elucidate potential mechanisms of *K. brevis* bloom decline in the GoM, while adding to our ecological understanding of algal cell death in variable environments.

USE OF ANTIBIOTICS FOR MAINTENANCE OF AXENIC CULTURES OF *AMPHIDINIUM CARTERAE* FOR THE ANALYSIS OF TRANSLATION

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Most dinoflagellates in culture are bacterized, complicating the quantification of protein synthesis, as well as the analysis of its regulation. In bacterized cultures of *Amphidinium carterae* Hulbert, up to 80 % of protein synthetic activity appears to be predominantly bacterial based on responses to inhibitors of protein synthesis. To circumvent this, axenic cultures of *A. carterae* were obtained and shown to respond to inhibitors of protein synthesis in a manner characteristic of eukaryotes. However, these responses changed with time in culture correlating with the reappearance of bacteria. Here we show that culture with kanamycin (50 µg/mL), carbenicillin (100 µg/mL), and streptomycin sulfate (50 µg/mL) (KCS), but not 100 units/mL of penicillin and streptomycin (PS), prevents the reappearance of bacteria over several months and allows *A. carterae* protein synthesis to be quantified without the contribution of an associated bacterial community. We demonstrate that *A. carterae* can grow in the absence of a bacterial community. Furthermore, maintenance in KCS does not inhibit the growth of *A. carterae* cultures but slightly extends the growth phase and allows accumulation to somewhat higher saturation densities. We also show that cultures of *A. carterae* maintained in KCS respond to the eukaryotic protein synthesis inhibitors cycloheximide, emetine, and harringtonine. Establishment of these culture conditions will facilitate our ability to use polysome fractionation and ribosome profiling to study mRNA recruitment. Furthermore, this study shows that a simple and fast appraisal of the presence of a bacterial community can be made by comparing responses to cycloheximide and chloramphenicol.

A LESS-TOXIC CROSS-LINKER FOR USE IN SEM STUDIES OF PHYTOPLANKTON, INCLUDING HAB SPECIES

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Scanning electron microscopy (SEM) has many uses in marine science, including proper discrimination between toxic and non-toxic species of phytoplankton as some key morphological features are too small to identify without the aid of SEM. The quality of SEM images of phytoplankton depends heavily on the preservation and fixation technique used. Most methods, although efficacious, involve the use of hazardous toxic chemicals. The objective of this study was to test the suitability of a new chemical fixative method using a natural crosslinking compound known as genipin for phytoplankton cells by comparing with established techniques. Four phytoplankton species were used to qualitatively compare various treatments in order to determine which fixation technique would result in the highest quality of cell preservation for SEM analysis. The results indicated that no singular treatment proved most successful for all of the species tested. However, this study shows for the first time the potential of less-toxic genipin as a successful alternative chemical fixative to utilize for processing phytoplankton for SEM analysis.

GENETIC DIVERSITY WITHIN THE HARMFUL ALGAL SPECIES, *HETEROSIGMA AKASHIWO*: ENVIRONMENTAL DRIVERS AND STRAIN TOXICITY

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Heterosigma akashiwo (Class Raphidophyceae) is one of the most prevalent harmful algal bloom (HAB) forming species. Distributed mainly in temperate coastal waters and present all over the world, it has been reported to form dense, fish-killing blooms since the 1970s. However, the underlying toxicity mechanism remains controversial and little is known about its intraspecific diversity. The temperate waters of the Delaware Inland Bays (DIB) is particularly vulnerable to HABs due to increasing eutrophication caused by low freshwater input and little exchange with oceanic waters. Among many environmental drivers, salinity appears to be exceptionally important to *H. akashiwo* bloom initiation in the DIB and elsewhere. Previous research showed that geographically isolated toxic and non-toxic *H. akashiwo* strains were identical in their gene sequences of the Internal Transcribed Spacer (ITS). However, recent fragment analyses on the same region revealed three distinguishable strains associated with salinity gradients in the DIB. Phylogenic analyses of the two other independently evolving genomes of the chloroplast (transfer RNA-Leu gene (*trnL*) upstream) and the mitochondrion (cytochrome c oxidase subunit 1(COI)) showed additional diversity among isolates over local and global scales, and suggest higher intraspecific diversity among *H. akashiwo* populations. The ecological significance of this diversity was further explored through microcosm experiments investigating the effects of salinity on strain selection and toxicity of *H. akashiwo* during bloom development in the DIB. This research will help to link physiological and genetic variances together and to establish environmental condition models to predict the formation, diversity structure, and toxicity of *H. akashiwo* blooms.

EFFECTS OF THE DINOFLAGELLATE PARASITE AMOEBOPHRYA ON KARENIA BREVIS

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Blooms of the neurotoxin-producing dinoflagellate, *Karenia brevis*, negatively affect both humans and natural resources. Little is known about the termination stages of these blooms, and few viable control mechanisms have been suggested. *Amoebophrya* is a syndinian parasite found to infect and kill various bloom-forming dinoflagellates, including toxin-producing species, potentially serving a role in host population dynamics. *Amoebophrya* is generally considered to have high host specificity, although infection across different host species has been observed. Due to its host-specificity, *Amoebophrya* may be suitable as a potential biocontrol for some HAB species. Little work has been conducted examining the natural presence of *Amoebophrya* in *Karenia brevis* or the susceptibility of *K. brevis* to *Amoebophrya* from non-*Karenia* hosts. We examined the effect of *Amoebophrya* isolated from 3 different host species (*Alexandrium* sp., *Scrippsiella trochoidea*, *Akashiwo sanguinea*) on growth and survival of *K. brevis*. Exposure to *Amoebophrya* ex. *Alexandrium* sp. consistently resulted in decreased *K. brevis* abundance over 72-96 hours by 50-70% versus controls. *Amoebophrya* zoospores increased concurrently with decreasing *K. brevis* abundance, while controls consisting of *Amoebophrya* zoospores with no host decreased over the same period. Results with *Amoebophrya* ex. *Scrippsiella trochoidea* were similar, but inconsistent. *Amoebophrya* ex. *Akashiwo sanguinea* reduced growth in *K. brevis* after more than 96 hours, also concurrent with an increase in *Amoebophrya* zoospores. Additional data from ongoing studies, including application of CARD-FISH using an Amoebophrydae-specific HRP-modified ALV01 oligonucletide probe to visualize infection, and use of *Amoebophrya* isolated from other species (including *Karlodinium veneficum*), will be presented.

DRAMATIC CHANGES IN PROTEIN SYNTHETIC RATE OF AXENIC *AMPHIDINIUM CARTERAE* IN RESPONSE TO INCREASING TEMPERATURES, DENSITY OF CULTURE AND OVER THE DIEL CYCLE

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In dinoflagellates many physiological processes appear to be under post-transcriptional control. However, the extent to which this reflects translational regulation remains unresolved. While most dinoflagellate cultures are uni-algal, usually the cultures are not axenic, making it difficult to quantitate protein synthesis by ^{35}S-methionine incorporation or to optimize cycloheximide concentrations for the stabilization of polysomes. In bacterized *Amphidinium carterae*, a significant proportion of ^{35}S-methionine incorporation appears to be bacterial. We have maintained an axenic culture over several months by maintenance in the antibiotic mix, kanamycin/carbenicillin/ streptomycin. Inclusion of the antibiotic mix gives an initial drop in ^{35}S-methionine incorporation as the bacterial count falls. However, once the bacterial community is removed, ^{35}S-methionine incorporation rises. This has allowed us to measure protein synthetic rates under different physiological conditions and study changes over the diel cycle. With increasing temperatures, ^{35}S-methionine incorporation increases to a maximum at 25 °C, falling to less than 50 % of the maximum at 35 °C. During growth of a culture, ^{35}S-methionine incorporation per cell increases to a maximum at ~9 x 10^5 cells/ml, falling swiftly in advance of the crash in cell density. Dramatic changes in ^{35}S-methionine incorporation can also be seen over the diel cycle. Using a zeitgeber regime of 14-h light/10-h dark, ^{35}S-methionine incorporation increases exponentially during the light phase up to 12 h and begins to fall in anticipation of the dark phase. ^{35}S-methionine incorporation decreases steadily during the dark period, reaching a minimum approximately 7-fold lower than the peak at 22 h in zeitgeber time.

Fisheries and Foodwebs and Benthic HABS with a Focus on Ciguatera

Monday Presentations

INFLUENCE OF *KARENIA BREVIS* BLOOMS ON FISH, SHRIMP AND CRAB COMMUNITY DIVERSITY: THE APALACHICOLA BAY EXAMPLE

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Apalachicola Bay, a northwest Florida estuary traditionally responsible for ~10% of the U.S. oyster supply, is subjected to *Karenia brevis* blooms on average every 3 to 4 years. We examined the history of *K. brevis* occurrence in the Bay from 2002 to 2012 in relation to coincident monthly fish, shrimp and crab abundances and population diversity in the Bay during this period. *Karenia brevis* blooms in Apalachicola Bay occur in late fall and are introduced from established southwestern blooms. Especially large *K. brevis* blooms were present in Apalachicola Bay in 1996 and 2005 when large *K. brevis* blooms dominated most of the west Florida shelf. Overall community diversity (as measured by the Shannon-Weaver Diversity Index, H) of the 25 most abundant species harvested by trawl (18 fish, 6 shrimp and 1 crab species) was negatively correlated ($r^2 = 0.60$) with *K. brevis* abundance in the central Bay region, but was not significantly related to *K. brevis* concentration in circulation restricted areas of the Bay. Conversely, both shrimp and crab population diversity increased with increasing *K. brevis* concentrations to 50,000 cells L^{-1} in the central Bay region. These data suggest that while *K. brevis* blooms directly and negatively impact fish population diversity through toxin exposure, detrital based organisms and populations may indirectly benefit from *K. brevis* blooms.

PRESENCE AND PERSISTENCE OF THE CYANOTOXIN MICROCYSTIN IN AQUATIC AND RIPARAIN CONSUMERS

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We undertook a comparative study of the James River Estuary, a sub-estuary of Chesapeake Bay, and the Curonian Lagoon, a sub-estuary of the Baltic Sea, to better understand how sources of organic matter affect the presence of an algal toxin (microcystin; MC) in food webs. Both sites exhibit high phytoplankton production and cyanobacteria blooms known to produce MC, but differ in the relative importance of terrestrial inputs. We found that high phytoplankton production in the lagoon, coupled with low terrestrial inputs, resulted in higher levels of MC in sediments and in fishes, which were dominated by benthivores. In the James, terrestrial inputs diluted phytodetritus contributions to sediment resulting in low MC concentrations in sediment, and among benthivorous fishes. We also show that MC is present in emerging aquatic insects (*Hexagenia* Mayfly) from the James River Estuary and their riparian consumers (*Tetragnathidae* Spider, Prothonotary Warbler; *Protonotaria citrea*). Warbler body condition and growth rate were not related to liver MC levels suggesting that aquatic prey (i.e., mayflies) may provide dietary benefits which offset potential deleterious effects of the toxin. Our results provide evidence that threats posed by algal toxins are influenced by food web dependence on autochthonous and allochthonous sources of organic matter, and that these threats extend beyond the aquatic environments in which blooms occur.

HIGH-RESOLUTION TEMPORAL POPULATION GENETIC STRUCTURE OF *GAMBIERDISCUS CARIBAEUS* COMPARED TO SPATIAL DIFFERENTIATION ACROSS THE GREATER CARIBBEAN REGION

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Members of the benthic dinoflagellate genus *Gambierdiscus* are the causative agents of ciguatera fish poisoning worldwide. Incidence and range of ciguatera appear to be increasing, similar to many other HAB phenomena, with new outbreaks being reported from unprecedented regions. We investigated the genetic population structure of *Gambierdiscus caribaeus*, a common species at our main study sites in St. Thomas, US Virgin Islands (USVI), and the Florida Keys, to gain insights into population dynamics, dispersal routes and connectivity across the Greater Caribbean Region (GCR). A temporal population genetic analysis with strains repeatedly sampled over a period of eleven years at USVI, including a three-year period with monthly sampling, indicates high temporal variability in local population structure. This genetic differentiation is likely impacted by natural selection due to current speed as well as biotic factors such as benthic coverage of the habitat by corals and macroalgae. We also observed strong spatial genetic differentiation between our USVI and Florida Keys sites, which varies among years. This spatial differentiation can be an order of magnitude higher than temporal divergence within USVI, which suggests limited connectivity and dispersal across the GCR. However, local temporal differentiation sometimes exceeds spatial variability between USVI and the Florida Keys. By directly comparing genetic divergence on large, spatial scales with continuous temporal differentiation, this study illuminates short-term and long-term trends in intraspecific genetic diversity and population structure of the dinoflagellate genus *Gambierdiscus.*

CIGUATOXICITY AND MAITOTOXICITY OF REPRESENTATIVE *GAMBIERDISCUS* AND *FUKUYOA* SPECIES

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Dinoflagellate species belonging to the genera *Gambierdiscus* and *Fukuyoa* produce ciguatoxins (CTXs), potent neurotoxins that concentrate in fish causing ciguatera fish poisoning (CFP). They also produce maitotoxins (MTXs) that may also contribute to CFP when gut or liver tissues are consumed. Currently, few data exist on the intra- and interspecific variations in toxicity among these *Gambierdiscus* and *Fukuyoa* species. Quantifying these variations is critical for understanding how CFP events develop. An initial study analyzed the ciguatoxicity of 34 strains representing seven *Gambierdiscus* and one *Fukuyoa* species using the cell based Neuro-2a cytotoxicity assay. The average toxicity was inversely proportional to growth rate, suggesting an evolutionary trade-off between investments in growth versus production of defensive compounds. *Gambierdiscus belizeanus*, *G. caribaeus*, *G. carolinianus*, *G. carpenteri*, *Gambierdiscus* ribotype 2, *G. silvae* and *F. ruetzleri* exhibited low toxicities (0 to 24 fg CTX3C equivalents cell^{-1}), compared to *G. excentricus*, (469 -1426 fg CTX3C eq. cell^{-1}). These findings indicate *G. excentricus* may be a primary source of ciguatoxins in the Atlantic. In a related study, the maitotoxicity of 13 strains representing G. *australes*, *G. balechii*, *G. caribaeus*, *G. carpenteri*, *G. excentricus*, *G. pacificus, G. scabrosus* and an undescribed species from Vietnam, was measured using a human erythrocyte lysis assay. *G. excentricus* and the Vietnamese species exhibited average toxicities of >70 pg MTX equivalents cell^{-1} whereas the remaining species produced <23 pg MTX equivalents cell^{-1}. *G. excentricus* produced maitotoxin-4, a novel MTX analog. Production of maitotoxin-4 was well correlated with the high toxicity of *G. excentricus*.

Gambierdiscus has neighbors: an examination of the epiphytic community cohabiting macrophyte hosts with _Gambierdiscus._

Gambierdiscus is a benthic (epiphytic) genus of dinoflagellate that contains some species capable of producing ecologically-relevant amounts of ciguatoxin-precursor molecules (gambiertoxins) that can lead to ciguatera fish poisoning (CFP). CFP is caused by the consumption of (sub)tropical fish (primarily demersal reef fish) that accumulate significant amounts of ciguatoxins through trophic processes. People then are exposed to these potent neurotoxins by consuming the toxin-laden fish. Much research has been invested in the autecology of _Gambierdiscus_, in an effort to better learn the circumstances that lead to high concentrations of cells in the benthos (i.e., a benthic "bloom"). Largely ignored, however, is synecology of the epiphytic flora within which _Gambierdiscus_ cells reside. In this study, we examined 93 samples collected in the Florida Keys to determine how the epiphyte community structure varied by site, substrate (host macrophyte), season, and physico-chemical properties of the overlying water. We found that the epiphytic community varied by site and season, but that seasonal differences were more attenuated in Atlantic Ocean reef sites versus sites in Florida Bay to the north. Additionally, wave height, temperature, ammonium concentrations, and salinity provided the best synergistic relationship between environmental variables and epiphyte variation across sites, seasons, and substrate. There was no statistical difference in epiphytic community composition among the different host macrophytes, suggesting that the hosts served as neutral substrates in regards to the overall epiphyte community. _Gambierdiscus_ abundances were only correlated with 14% of the epiphytic taxa (11 out of 77) and examined and 17% of the environmental variables (3 out of 18). Additionally, the absolute and relative abundances of _Gambierdiscus_ cells were correlated, indicating that _Gambierdiscus_ will occupy a larger proportion of the epiphytic community as its abundance increases. These results suggest that _Gambierdiscus_ may not be strongly influenced by cohabitating epiphytes or changing environmental conditions.

Regional Growth Projections for Ciguatera-Associated Dinoflagellates in the Caribbean.

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Abstract

In a critical step toward forecasting capacity for ciguatera fish poisoning in the Caribbean, a dinoflagellate growth-distribution model was used to define locations in the greater Caribbean having the greatest potential for *Gambierdiscus* and *Fukuyoa* blooms. Satellite-derived sea surface temperature data from the Caribbean Sea, Gulf of Mexico and the southeast US coast were used to project bottom temperatures and growth rates of four *Gambierdiscus* and one *Fukuyoa* species. Bottom temperatures were validated using *in situ* temperature profiles from seven representative locations and dinoflagellate growth was estimated from experimental temperature vs. growth equations. Model output indicated the highest growth potential for each species in the shelf waters of the Caribbean Sea, more moderate growth in the Bahamas, southern Florida and Gulf of Mexico, and low growth potential along the northern Gulf and U.S. south Atlantic coasts. The highest projected growth rates generally coincided with the highest CFP incidences in the northeastern Caribbean. There were some exceptions in the southwestern Caribbean, likely due to terrestrial runoff, improper bottom type or various socio-economic factors. Despite this, statistical analysis showed a positive association between ciguatera incidence and projected dinoflagellate growth rates for the 29 regional nations, with the strongest correlation during the months with peak seasonal temperatures (July - November). The model results indicate spatial differences in *Gambierdiscus* and *Fukuyoa* growth may play a prominent role in governing the occurrence of ciguatera fish poisoning and that projected temperature may be a good first order predictor of CFP risk in the greater Caribbean.

THE IMPACT OF DOMOIC ACID ON MICROBIAL COMMUNITIES IN THE COASTAL OCEAN

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Domoic acid (DA) is an exudate produced by the diatom *Pseudo-nitzschia*, which is well studied for its negative physiological effects on humans, marine mammals and birds. What is largely unknown is its effect on natural microbial communities, and if it significantly influences their composition and community structure. Here we looked at the effect of domoic acid (DA) on bacterial diversity from a two-year weekly time-series data that comprised of several natural *Pseudo-nitzschia* bloom and non-bloom events in Santa Cruz Municipal Wharf, Monterey Bay, California. We measured physical and environmental parameters along with sequencing the 16S rRNA of size-fractionated water samples using Illumina Miseq sequencing platform. Statistical analysis showed that DA is negatively correlated with bacterial diversity from bacterial samples both taken from free-living (0.2 um) and particle-attached (3.0 micron) size fractions, while phytoplankton biomass, the proposed main driver of bacterial productivity, only showed weak correlation with bacterial diversity. Growth assay performed on several bacterial strains exposed to varying concentrations of DA exhibited distinct responses upon exposure and confirmed our field observations; some bacterial strains were growth-inhibited by DA while other bacterial strains were not affected. Our field and laboratory data provide strong support to the idea that an algal metabolite like DA not only impacts the physiology of mammals and seabirds but it also affects bacterial physiology and can play a strong role in structuring bacterial communities when present in the coastal ocean.

TROPHIC TRANSFER AND PERSISTENCE OF BREVETOXINS AND NEUROTOXIC SHELLFISH POISONING (NSP) IN CARNIVOROUS MOLLUSKS.

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Neurotoxins (brevetoxins) produced by the dinoflagellate, *Karenia brevis*, accumulate in filter-feeding mollusks as they consume *K. brevis* cells and toxins from the water. Human consumption of contaminated shellfish has resulted in cases of neurotoxic shellfish poisoning (NSP) from consumption of various filter-feeding as well as carnivorous mollusks. NSP in carnivorous mollusks is thought to result from trophic transfer of toxins from contaminated shellfish prey. This study was undertaken to investigate the accumulation, transformation and persistence of brevetoxins, metabolites and NSP toxicity in filter-feeding mollusks, with subsequent trophic transfer, accumulation and persistence in carnivorous mollusks following *K. brevis* blooms in Sarasota Bay and Gasparilla Bay, FL. The hard clam (*Mercenaria mercenaria*) and Eastern oyster (*Crassostrea virginica*) retained brevetoxin metabolites for several months after a *K. brevis* bloom, but *K. brevis*-derived toxins (PbTx-1, -2, -3, and PbTx-2-carboxylic acid) were not retained in detectable concentrations (LOD < 14 ng/L, <10 ng/L, <4.5 ng/L, and <9 ng/L, respectively) and did not retain NSP toxicity for more than two or three weeks. However, carnivorous mollusks, lightning whelk (*Busycon. contrarium*) and tulip snail (*Fasciolaria tulipa*), accumulated and retained elevated concentrations of PbTx-3 and metabolites in the viscera, for more than 12 months, and whelks retained NSP toxicity for at least five months following exposure to natural *K. brevis* blooms (NSP testing ended at 5 months post bloom).

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Pinfish ciguatoxicity in relation to varying *Gambierdiscus* densities in the Florida Keys

Ciguatera fish poisoning (CFP) is a phycotoxin- borne seafood poisoning. Originating in many tropical and subtropical reef regions around the world, ciguatoxin is gaining attention worldwide, including South Florida and the Florida Keys, which have the highest incidence of CFP in the continental US. The source of this toxin is the benthic dinoflagellate, *Gambierdiscus*, which commonly associates with macroalgae. CFP is a highly underreported problem for many reasons, the main being that the only indication of CFP is people getting sick from seafood ingestion. Other reasons include difficulty identifying toxin sources, which herbivores are ingesting said sources, and the amounts of ciguatoxin causing toxicity in predators. Because of these unknown factors, the purpose of this study was to determine the toxicity in pinfish (*Lagodon rhomboides*) in order to determine if and how pinfish toxicity is related to *Gambierdiscus* densities in the benthos. Toxicity was determined using the N2a cytotoxicity assay with ouabain/veratridine treatments to target sodium channel-specific activity. These data will provide insight into low to mid trophic level transfer of ciguatoxin into the food web.

NODULARIN FROM BENTHIC FRESHWATER PERIPHYTON AND IMPLICATIONS FOR TROPHIC TRANSFER

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Abstract: In 2013 and 2015, the Pennsylvania Department of Environmental Protection conducted a survey of lotic habitats within the Susquehanna, Delaware, and Ohio River basins in Pennsylvania, USA, to screen for microcystins/nodularins (MCs/NODs) in algae communities and smallmouth bass (*Micropterus dolomieu)*. Periphyton (68 from 41 sites), juvenile whole fish (153 from 19 sites) and adult fish liver (115 from 16 sites) samples were collected and screened using an Adda enzyme-linked immunosorbent assay (ELISA). Samples that were positive for MCs/NODs were further analyzed using LC-MS/MS, including 14 variants of microcystin and NOD-R and the MMPB technique. The ELISA was positive for 47% of the periphyton collections, with NOD-R confirmed (0.7-82.2 ng g^{-1} d.w.) in 20 samples. NOD-R was confirmed in 10 of 15 positive juvenile whole fish samples (0.8 – 16.7 ng g^{-1} w.w.) and in 2 of 8 liver samples (1.7 & 2.8 ng g^{-1} w.w.). The MMPB method resulted in total MCs/NODs measured in periphyton (2.2-1269 ng g^{-1} d.w.), juvenile whole fish (5.0 – 210 ng g^{-1} d.w.) and adult livers (8.5 – 29.5 ng g^{-1} d.w.). This work illustrates that NOD-R is present in freshwater benthic algae in the USA, which has broader implications for monitoring and trophic transfer.

CYANOTOXINS IN FISH: A GLOBAL ANALYSIS

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Cyanobacteria, the primary HAB-forming group in fresh water, elicit a spectrum of issues in lentic systems. The most immediate concern for people and animals are the toxins produced by cyanobacteria, which have been detected at variable concentrations in water and fish around the world. Cyanotoxins transfer through food webs, potentially increasing the risk of exposure to people who eat fish from affected waters. Compared to marine algal toxins, less is known about how cyanotoxins affect freshwater fisheries. More research is needed to understand different exposure pathways, including the accumulation of cyanotoxins in fish tissues. Existing studies on cyanotoxins in fish and fresh water from lakes around the world were compiled into a global dataset to test the hypothesis that cyanotoxin concentrations in fish increase with water toxin concentrations. Cyanotoxins are expected to be found in increasingly higher concentrations from carnivorous, to omnivorous, and planktivorous fish. Preliminary analyses show that planktivorous fish may accumulate higher toxin concentrations, however concentrations are highly variable and accumulation may be species and context dependent. Our data synthesis highlights limited knowledge on cyanotoxins in wild freshwater fish due to continually evolving detection methods, high associated costs, and a restricted number of analytical standards. Further, hundreds of cyanobacterial secondary metabolites have been discovered, but research on the environmental occurrence of cyanobacterial toxins is weighted toward the few toxins for which reference standards exist (e.g., microcystins). With HABs on the rise, our interaction with these compounds is only expected to increase.

METHOD DEVELOPMENT FOR ASSESSING TROPHIC TRANSFER OF DOMOIC ACID FROM *PSEUDO-NITZSCHIA* SPP. TO *ACARTIA TONSA*

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Domoic acid (DA) is a known neurotoxin responsible for amnesic shellfish poisoning. Trophic transfer of DA through the food web may pose a threat to human health. It is known that copepods (e.g. *Acartia tonsa*), shellfish, and fish act as vectors for DA. A short food chain (e.g. copepod consumption on diatoms) promotes an efficient trophic transfer of DA due to its water-soluble nature. However, it is unknown if copepods accumulate DA proportionally to the amount ingested from food or dissolved in water. The form in which copepods are exposed to DA, a pulsed high dose from particulate DA (PDA), or a long exposure to a low dose as dissolved DA (DDA), may affect total assimilation of DA in tissue. Grazing experiments are needed to assess relative grazing of *Pseudo-nitzschia* spp. (includes toxic and non-toxic species) and assimilation of DA by *A. tonsa* in relation to cell abundance and toxicity. A liquid chromatography-tandem mass spectrometry method was developed for the determination of PDA and DDA. The samples for DA determination are cleaned and concentrated using a C18 solid-phase extraction column. The LC conditions were optimized to separate DA from interfering matrices by reversed-phase chromatography. Three transitions (*m/z* 312→266, *m/z* 312→220, and *m/z* 312→193) were selected for selected reaction monitoring with transition *m/z* 312→266 for quantitation. The method is highly selective and sensitive, LOQ 12 pg/ml for DDA and a recovery of ~85%. The LC-MS/MS methodology is adequate for the confirmatory determination of DA in seawater, phytoplankton, and copepod tissue.

UNDERSTANDING THE PREVALENCE AND DISTRIBUTION OF CARIBBEAN CIGUATOXINS (C-CTXs) IN GRAYSBY GROUPER, *CEPHALOPHOLIS CRUENTATA*, FROM THE U.S. VIRGIN ISLANDS

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Ciguatoxins (CTX) are potent neurotoxins that bind to voltage gated sodium and potassium channels in mammalian neurons, causing severe cellular dysfunction, and are linked to the onset of ciguatera fish poisoning (CFP) in humans. The CTXs are produced by marine benthic dinoflagellates of the genus *Gambierdiscus* that occur globally in tropical and subtropical waters. These toxigenic algal metabolites are biotransformed through reef food webs increasing toxicity and risk to human populations relying on these fisheries resources. The graysby grouper (*Cephalopholis cruentata)*, is a small carnivorous fish of the Greater Caribbean and based on its size is thought to have a reduced CFP risk. However, there have been no reports on the risk posed by this species in any region. With high site fidelity, understanding the geospatial CTX prevalence in *C. cruentata* may allow higher resolution in subsequent risk assessments potentially expanding the known safe harvest zones. In this study, we evaluated the prevalence of Caribbean CTXs (C-CTXs) in graysbys collected from the USVIs compared to age, size, diet, and trophic level (via stable isotope analysis). Graysbys (n=56) were collected on scuba from four field sites south of St. Thomas in the USVIs. Sub-samples of fish were chemically extracted to isolate and test C-CTXs using standardized methods. Sodium channel dependent ciguatoxicity was assessed using the ouabain-veratridine dependent mouse neuroblastoma (N2A) assay with confirmation by LC-MS/MS. Relationships between C-CTX, site, age, size, and trophic level will be discussed along with the implications of novel CTX metabolites present in related reef species.

CIGUATOXIN ACCUMULATION IN A HYBRID FISH BETWEEN SPECIES OF LOW AND MEDIUM RISK FOR CIGUATERA FISH POISONING

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Ciguatera fish poisoning (CFP) is a circumtropical seafood-toxin illness, resulting from reef fish contaminated with neurotoxins, classed as ciguatoxins (CTXs). These toxins are metabolites of CTX precursors produced by benthic dinoflagellates in the genus *Gambierdiscus*. Ciguatoxic fish are difficult to identify. Therefore, to avoid CFP, consumers rely on local knowledge regarding species-specific CFP incidents. However, little is known about the CFP risks from hybrids between species with different historical CTX accumulation potential. To understand the importance of species-specific differences for CTX accumulation, we tested a suspected hybrid fish that was the resulting offspring between historically low and medium risk species for CTXs. The hybrid was identified by comparing two gene sequences (mitochondrial CO1 and nuclear S7) and it was determined that the low risk *Ocyurus chrysurus* and medium risk *Lutjanus apodus* were the paternal and maternal cross source, respectively. CTX content analysis relied on a two-tiered protocol, consisting of an *in vitro* mouse neuroblastoma cell assay (for composite toxicity) followed by LC-MS/MS for confirmation of the regional CTX biomarker, C-CTX-1. The hybrid contained 0.03 ppb C-CTX-1 eq. in the muscle tissue, a level that is below the FDA's guidance level (0.1 ppb C-CTX-1 eq.); yet, the presence of C-CTX-1 was confirmed. The maximum CTX level measured in *O. Chrysurus* (n=17) from the same region was 1.5X lower than the hybrid. Therefore, species-specific life histories likely play an important role in determining CTX accumulation in a hybrid, and these levels may deviate from parental species with a historically low CFP risk.

DEVELOPMENT OF AN ULTRA-HIGH-THROUGHPUT EXTRACTION METHOD FOR THE ANALYSIS OF CARIBBEAN CIGUATOXIN-1 (C-CTX-1) BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Globally, ciguatera fish poisoning (CFP) is among the most common causes of fish-related foodborne illnesses. In the U.S., the majority of CFP cases are attributed to the consumption of fish contaminated with Caribbean ciguatoxins (C-CTXs). Analyses of C-CTXs in fish are challenged by the lack of certified standards, along with labor and time intensive extraction procedures. In addition, traditional CTXs extraction methodologies often require large sample sizes and solvent volumes. These are concerns for the implementation of surveillance programs, targeted regulatory analyses, and CFP outbreak response by the U.S. FDA and other public health authorities. Therefore, there is a critical need for a high throuput C-CTXs extraction methodology that is effective, efficient, and more affordable. Here, we present an ultra-high-throughput (UHT) C-CTX extraction method for quantification and confirmation of the regional biomarker for C-CTXs, C-CTX-1, by LC-MS/MS. We compared our method to a traditional CTX extraction method using previously analyzed and reported CFP outbreak samples provided to the FDA. Preliminary data suggest that this UHT-CTX extraction method can be used to extract twenty samples per day in contrast to five to seven per day by other methods. Furthermore, spiked samples demonstrate the capability for this method to yield 50-70% recovery. Compared to traditional C-CTX-1 methods, the UHT method greatly reduces time, labor, and solvent volumes, and provides comparable toxin yields, making the method suitable for routine CTXs analysis.

TOXIC BLOOM OF *Ostreopsis* **cf.** *rhodesae* **IN A SUBTROPICAL BRAZILIAN COASTAL ISLAND.**

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Few studies considering the diversity and potential harmful effects of marine benthic dinoflagellates have been conducted along the Brazilian coast, especially in the subtropical region. As part of an ongoing nationwide research project, we identified an *Ostreopsis* cf. *rhodesae* bloom in the Currais Archipelago, a set of three small costal islands located in southern Brazil (25°44'S, 48°22'W). In February/2017, samples of seaweeds and artificial substrates (nylon mesh collectors left for 24 hours) were collected at four different depths (1.5, 3.0, 4.5 and 6.0 m) using SCUBA diving. Seaweeds and artificial substrates were shaken with local sea water, and sample aliquots were preserved in 1% lugol for cell counting. Maximum cell abundances – 3.2×10^5 cell.g^{-1} of seaweed (fresh weight) or 1.1×10^5 cell.cm^{-2} in artificial substrates – were detected near the surface, at 1.5 m depth. During the peak of the bloom, the sea floor was covered by *Ostreopsis* biofilm, and the formation of "*Ostreopsis* flowers" (cell aggregation in mucus) was noticeable in the water column. Ovatoxins (mostly ovatoxin-a) were found by LC-MS/MS in a cell pellet from the bloom, which was lethal to mice (~3×10^5 cells.mouse^{-1}). Further analysis will be undertaken to fully characterize the toxin profile of bloom samples and *Ostreopsis* cultures, and to assess the toxin accumulation in animals collected during the bloom. Additionally, molecular analysis will be conducted to confirm the species identification, currently determined by electron and fluorescence microscopy. If confirmed, this will be the first bloom reported for *O. rhodesae*, and the first occurrence of this species in the Atlantic Ocean.

AN EVALUATION OF THE GENUS *AMPHIDINIUM* (DINOPHYCEAE) COMBINING EVIDENCE FROM MOPHOLOGY, PHYLOGENETICS, AND TOXIN PRODUCTION WITH THE INTRODUCTION OF SIX NOVEL SPECIES

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The genus *Amphidinium* is an important group of athecated dinoflagellates because of its high abundance in marine habitats, its member's ability to live in a variety of environmental conditions and ability to produce toxins. Furthermore, the genus is of particular interest in the biotechnology field for its potential in the pharmaceutical arena. Taxonomically the there is a history of complication and confusion over the proper identities and placements of *Amphidinium* species due to high genetic variability coupled with high morphological conservation. Thirteen years has passed since the most recent review of the group, and while many issues were resolved, some remain. The present study used microscopy, phylogenetics of the 28S region of rDNA, secondary structure of the ITS2 region of rDNA, compensatory base change data, and cytotoxicity data from *Amphidinium* strains collected world-wide to elucidate remaining confusion. This holistic approach using multiple lines of evidence resulted in a more comprehensive understanding of the morphological, ecological, and genetic characteristics that are attributed to organisms belonging to *Amphidinium*, including six novel species.

IMPACTS OF ENVIRONMENTAL STRESSORS INCLUDING TWO HARMFUL ALGAL SPECIES ON OYSTERS CULTURED IN LOWER CHESAPEAKE BAY

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Understanding the impacts of environmental stressors in the context of harmful algal blooms (HABs) is crucial for managing economically and ecologically important species like the eastern oyster, *Crassostrea virginica*, in Chesapeake Bay (CB). Two toxigenic dinoflagellates, *Cochlodinium polykrikoides* and *Alexandrium monilatum* develop sequential summer blooms almost annually in the lower bay. Following local reemergence in 2007, *A. monilatum* blooms have increased in spatial distribution, density, and duration. Oyster aquaculture stakeholders have expressed concern about the impacts of both species on oysters and their human consumers. To assess potential HAB impacts on cultured shellfish, juvenile oysters have been deployed at two sites in lower CB where both blooms commonly occur, and at a reference site seldom impacted. Oyster growth, mortality, and phycotoxin tissue concentrations are being monitored through the 2017 bloom season. Histopathology and condition indices are being used to assess oyster health. Relevant water quality parameters and depth are being measured continuously at all sites, and composition and concentrations of HAB species will be monitored weekly during both blooms. Previous research indicates that oysters can close and cease feeding to avoid consumption of certain HAB species, including *A. monilatum*; however, intertidal oysters may be forced to feed while submerged because of increased respiratory and nutritional demands following aerial exposure. Intertidal oysters, especially juveniles, may therefore be particularly susceptible to bloom effects. Thus, oysters are being grown both inter- and sub-tidally at all sites to assess the influence of aerial exposure and related stressors on HAB impacts.

FIRST REPORT OF JUBILEE INVERTEBRATE/FISH KILLS IN A HEMPSTEAD HARBOR COVE
JOINS TOKYO BAY AND MOBILE BAY AS ONLY 3rd LOCATION IN WORLD

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In response to citizen complaints of fish kills in Nassau County waters, investigations were always performed between 1970 thru the early 1990's. Fish kills were periodically documented in Hempstead Harbor, an embayment of southwestern Long Island Sound, but one summer descriptions of the happening were not typical. Flounder flung themselves upon the beach and marine invertebrates actively climbed a rock jetty. Many floated just barely alive in inches of water. This became the first documented Jubilee in US waters other than Alabama. Infrequent Jubilees followed, but only in that eastern shore cove. It became possible to predict the day before an event, though it has not been possible to mitigate or prevent this natural occurrence. Jubilees have particular environmental pre-conditions including warmest weather, near-by deep sink, cloudy sky, gentle east wind, fairly calm surface water, stratified waters. During a flood tide usually early in the morning, mostly bottom dwellers seeking the more oxygenated surface waters, become trapped between a sloping shoreline and an advancing water mass low in dissolved oxygen. In the Hempstead Harbor episodes the settling decomposing load of identified recent dense diatom and then dinoflagellate bloom "crashes" contributed to oxygen-deficient waters. The 100+ year old name derives from masses of perfect fish, crabs, shrimp just dying on the shore or moribund in the shallows for easy taking by those who came from miles around. It became a happy festive event, a Jubilee.

ALGAL TOXINS IN MUSSELS AS INDICATORS OF PHYCOTOXIN OCCURRENCE IN SAN FRANCISCO BAY

S. Morgaine McKibben[1], Zephyr Sylvester[1], Raphael M. Kudela[2], Melissa B. Peacock[3], James E. Cloern[4], David B. Senn[1]

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Nutrient pollution is a contributing factor to the increased frequency of harmful algal blooms (HABs) observed worldwide. At the regional scale, however, the presence of eutrophic waters is not an indicator that HABs will proliferate. General bloom condition in San Francisco Bay (SFB), for example, is limited relative its high nutrient loading. Harmful algae and their toxins have been observed in SFB, yet the potential for toxigenic HAB initiation in these eutrophic waters and the range and frequency of phycotoxins routinely entering the SFB food web are poorly constrained. To investigate these questions, the first mussel monitoring program for SFB began in September 2015. Preliminary results from year one (through Sept. 2016) show domoic acid (DA), microcystins (MCY), and saxitoxins (STX) are present 64% (n=192), 68% (n=203), and 32% (n=127) of the time, respectively. Maximum DA levels were 2 orders of magnitude lower than the regulatory threshold for human consumption of shellfish (20 ppm). In contrast, two MCY samples surpassed a recommended safety threshold (10 ppb). Most mussels with elevated (>1 ppb) MCY, considered a freshwater class of toxins, were collected in relatively saline (~26–33 PSU) areas during warmer seasons when evaporation was greatest and rainfall the lowest. Maximum STX reached roughly half the regulatory threshold of 80 µg 100g^{-1} and this event coincided with elevated STX in coastal mussels outside of SFB. Mussel data are compared to other HAB monitoring proxies (dissolved, particulate toxins and cellular abundance) and coincident physical parameters to investigate patterns of occurrence and potential sources.

CLIMATE-MEDIATED IMPACTS ON TROPHIC TRANSFER BETWEEN *KARLODINIUM VENEFICUM* AND *ACARTIA TONSA*

Laura E. Smith[1], Nayani Vidyarathna[1], Mark Warner[1], and Jonathan Cohen[1]

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Global climate change will impact aquatic organisms directly by altering their abiotic conditions and indirectly through trophic transfer. Less efficient trophic transfer at the base of the food web could be particularly detrimental to the ecosystem by reducing the overall energy available to support higher trophic levels. Estuarine ecosystems are subject to blooms of harmful algae and are also characterized by low species diversity. This results in a system where changes in phytoplankton and their consumers can dramatically impact the health of the local community. Increased temperature and pCO_2 levels are predicted to change nutritional adequacy and/or toxicity of some HAB species and their copepod consumers. Interactions between *Karlodinium veneficum*, a HAB species present in the Delaware Inland Bays, and its consumer *Acartia tonsa*, a locally-dominant copepod, will be used to assess direct changes to physiology and/or indirect changes to trophic transfer. *A. tonsa* and *K. veneficum* are being grown in laboratory cultures at both ambient conditions (25 °C/400 ppm of CO_2) and those predicted for year 2100 (29 °C/ 1000 ppm of CO_2). Preliminary grazing studies using field-collected *A. tonsa* and *K. veneficum* have shown a decrease in copepod grazing on both toxic and control algae in the presence of the toxic species. Ongoing work will compare grazing efficiency under ambient and year 2100 conditions. Physiological changes will be assessed using respirometry and growth rates for copepods. *K.veneficum* will be analyzed for toxic production, fatty acid content, and carbon content to assess its nutritional adequacy.

Phycotoxins Standards and Certified Reference Materials: Needs and Sources

Monday Presentations

VARIABILITY OF MICROCYSTIN-LR STANDARDS AVAILABLE FROM COMMERCIAL VENDORS I: CHEMICAL ANALYSIS

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Microcystin standards are not generally produced synthetically but are extracted directly from cyanobacteria and the source water. Studies using accurate quantities of toxins purchased from vendors are necessary to characterize the toxic effects. Our laboratory has hypothesized that difficulties in replicating toxicology studies may in part be due to possible differences between stated and supplied toxin quantities obtained from vendors. This study was designed to investigate the extent of the inter-vendor variability of microcystin-LR (MCLR), a common environmental cyanotoxin. A comparison of the quantity and purity of MCLR purchased was determined by measuring concentrations with three independent methods in two laboratories using UV, LC-MS/MS, and LC-MS-TOF. Significant inter-vendor differences between stated and actual toxin masses were found with all three methods and in both laboratories. The amounts of toxin actually present in the purchased samples ranged from 33% to 129% of the amount stated by the vendors. Two of the seven MCLR samples purchased had amounts that differed by more than 30% from the stated quantities. Types and amounts of impurities also varied between vendor-supplied chemicals and the possibility exists that these impurities are themselves hepatotoxic. Amounts of MCLR present were correlated with the degree of toxicity observed in studies also reported at this Meeting. The potential for inaccuracy of the mass of MCLR reported by vendors indicates that users of these toxins should independently verify concentrations before studies are initiated.

MONITORING SOURCE AND DRINKING WATERS WITH A RAPID AND ROBUST ONLINE LC/MS/MS METHOD.

Dr. Judy Westrick[1] and Johnna Birbeck[1]

1 Lumigen Instrument Center, Wayne State University, Detroit, MI

In 2015 the USEPA announced an age-dependent drinking water Health Advisory (dwHA) for the natural freshwater toxins, microcystins (MCs). For pre-school age children and adults, the MC dwHA values are 0.3 □g/L and 1.6 □g/L, respectively. In parallel, EPA Method 544, a solid phase extraction/liquid chromatography tandem mass spectrometry (LC/MS/MS) method was released. Our goal was to create an online concentration LC/MS/MS method with 12 MCs that meets the EPA's quality assurance/quality control (QA/QC) criteria. MC concentrations were measured in samples from freshwater lakes and drinking water. Samples were prepared by three freeze/thaw cycles and centrifuging. Our LC/MS/MS platform included a Thermo Scientific EQuan MAX, UltiMate 3000 system and a TSQ Quantiva (MS/MS). This method included 12 MCs with calibration curves from 0.5 – 500 ppt with R^2 values greater than 0.996. The MCs eluted between 2.2 – 5.2 minutes with MC detection limits between 0.5 ng/L MC-RR and 4.26 ng/L MC-WR. Our run time was 12 minutes as compared to an 8-hour day for solid phase extraction plus a 26-minute run for EPA Method 544. Over 50 source and drinking water samples were analyzed and over 30% of the samples have MCs other than the six MCs in the EPA method. Our data suggests that 1) by not including 12 MCs in Method 544, the true risk potential of exposure to MCs in drinking and recreational waters will be underestimated greatly, and 2) an untargeted microcystin occurrence study needs to be performed in the USA.

WORKING WITHOUT A SAFETY NET: WHAT DO YOU DO WHEN STANDARDS ARE NOT AVAILABLE BUT YOU STILL NEED TO DO THE ANALYSIS?

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Analytical chemists are faced with a constant need for high quality analytical standards. However our ability to identify new toxins has rapidly outpaced our ability to produce the analytical standards needed for their measurement. Analyzing for toxins where standards are not readily available require that the analyst be creative and use all the information at their disposal. Using the cyanobacteria toxins as an example, two approaches to the analysis of toxins without the needed standards or access to a high resolution mass spectrometer will be discussed. Microcystins are a class of more than 200 related cyclic peptides which vary in their biological activity. However the class is defined by the presence of an ADDA amino acid residue with a distinct UV signature. By coupling chromatography, photodiode array analysis and mass spectroscopy, one can extend the number of toxins measured 2 or 3-fold, allowing for the examination of geographical differences in toxin distribution. Similarly, anatoxin-a is part of a family of 6-10 closely related neurotoxins. Only anatoxin-a is currently commercially available. By coupling chemical synthesis with knowledge of the fragmentation pattern of the anatoxins, it is possible to extend that analysis to include six members of the anatoxin-a family. This approach may not be suitable for use in highly regulated or legal situations, but may allow the individual researcher to answer specific environmental questions.

DEVELOPMENT AND CERTIFICATION OF REFERENCE MATERIALS FOR ALGAL BIOTOXINS: A PERSPECTIVE FROM THE NATIONAL RESEARCH COUNCIL CANADA (NRCC)

Pearse McCarron

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Toxins produced by harmful algae have negative impacts on human health, commercially associated industries, and on recreational activities around the world. The wide range of toxic algal species and the complex toxin analog profiles produced biosynthetically by the algae themselves, and as a result of metabolism in seafood, means that measurement and regulation is a significant challenge.

Sound measurements rely on good analytical methods, while good analytical methods require reliable standards and reference materials. The NRCC has put significant effort into this field of research and development for nearly three decades. Work has covered a broad range of disciplines including algal identification and culturing, natural product chemistry, development of analytical techniques, production of reference materials, and dissemination of those materials internationally. Reference materials are now available for domoic acid, paralytic shellfish toxins, lipophilic shellfish toxins and a selection of freshwater toxins. Much of the progress made at NRCC has been the result of collaborations with national and international partners, and this collaborative approach is crucial for addressing ongoing needs associated with toxin classes such as brevetoxins, ciguatoxins, and the broader range of freshwater toxins.

This presentation will give an overview of the approach taken to develop and certify algal toxin reference materials at the NRCC. Perspectives will be provided on their use and on the importance of sound measurements for toxin research in addition to screening and regulatory monitoring applications.

CERTIFIED REFERENCE MATERIALS CASE STUDY: REQUIREMENTS FOR AN INTERSTATE SHELLFISH SANITATION CONFERENCE VALIDATION OF A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR DIARRHETIC SHELLFISH POISONING TOXINS IN SHELLFISH

Whitney L. Stutts and Jonathan R. Deeds

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Since 2008, Diarrhetic Shellfish Poisoning (DSP) toxins exceeding the regulatory guidance level of 16 µg OA eq./100 g shellfish have required shellfish harvesting closures on the Texas Gulf Coast, in the Puget Sound region, and in the New England region of the US. To protect public health and minimize economic hardship to harvesters, approved methods are urgently needed for the control of DSP toxins in domestic molluscan shellfish. Domestic shellfish safety is managed through the National Shellfish Sanitation Program (NSSP), and methods for the control of natural toxin, and other, hazards must be formally validated following guidelines set by the Interstate Shellfish Sanitation Conference (ISSC) prior to official regulatory use. This presentation will describe the certified reference material (CRM) requirements for a current proposal entitled "Liquid Chromatography Tandem Mass Spectrometry (LCMS/MS) Method for the Determination of Diarrhetic Shellfish Poisoning (DSP) Toxins in Shellfish", which is under review by the ISSC for use in the NSSP. Additional CRM needs for similar regulatory method development projects will also be discussed.

THE PRODUCTION OF ISOTOPICALLY LABELED CYANOTOXIN STANDARDS: ^{15}N-LABELED MICROCYSTINS

Jeffrey L. C. Wright, Allison K. Stewart, Wendy K. Strangman, Eve Wright, Elizabeth Hutchins

University of North Carolina Wilmington

As the incidence of cyanobacterial blooms continue to rise worldwide, there is a global need for accurate and sensitive methods with which to monitor various cyanotoxins. Among these, the hepatotoxic microcystins are perhaps the most notorious, and as part of the Safe Water Drinking Act the EPA has announced six microcystins of concern. Both matrix effects and losses due to sample cleanup steps can be compensated for by using an internal standard for increased accuracy in quantitation. The optimum standards are isotopically labeled compounds and we have begun a program to address this need by first producing a series of ^{15}N-labeled microcystins for use in stable isotope dilution analysis (SIDA) using liquid chromatography tandem mass spectrometry. This talk will describe the evolution of the program, the approaches used and the challenge of producing milligram amounts of these toxins. To date ten isotopically enriched microcystins have been produced and characterized. The availability of these labeled compounds permitted a comparative MSMS study of the fragmentation of natural abundance and ^{15}N congeners for the confirmation of fragment ion annotations reported in the literature. In some cases the published fragments have been correctly annotated, whereas in others they are reported incorrectly. Due to the scale of this endeavor, it is worth noting that many other unrelated labeled cyanopeptides which are co-produced were isolated, purified, and characterized such as the cyanopeptolins, aeruginosins, and cyanobactins, some of which are also toxic in other biological screens. Using these methods, the preparation of other isotopically labeled nitrogen-containing toxins is now under development.

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Monitoring, Mitigation and Management of HABs Part 1

Tuesday Presentations

PROGRESS IN HAB MITIGATION FOR FRESH AND MARINE SYSTEMS?

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Harmful algal and cyanobacterial blooms are well known and projected to expand in the coming decades as anthropogenic loads follow human population increases and food production as well as a changing climate creating lager habitats and niches in the tropics, mid-latitudes, and sub-polar regions. Preventing nutrient enrichment of our waterways and oceans is more daunting each year as fiscal limitations, little political will, and minimal public awareness or interest constrain most efforts to minimize nutrient loads from existing land use. In lakes and ponds, *proactive* measures such as pre-bloom hydraulic/mixing controls, expansion of wetlands, or deployment of barley straw or occasionally ultrasound generators can limit bloom development. Most often, however, *reactive* intervention measures dominate mitigation activity such as dispersal of algicides, chemical oxidants, P-binding compounds, or flocculants. In flowing waters with hydraulic controls, manipulating current velocities and depths may limit benthic cyanobacteria blooms. Bloom mitigation in coastal ocean and marine systems is more problematic due to multi-seasonal blooms of various taxa over large geographic areas. When detected, local winds, tides, currents, and open boundaries reduce intervention effectiveness. In some mariculture-rich areas, locally applied chemical oxidants or flocculants, aerators, or fish pen screens have had some success while HA impacts to product and consumers are avoided through early harvest or fish pen relocation. Detection capabilities are increasingly sophisticated, allowing earlier detection of specific HA groups and taxa but even with early warning improvements, intervention to minimize impact thereafter remains limited.

USE OF HYDROGEN PEROXIDE TO MITIGATE TOXIC CYANOBACTERIAL BLOOMS: REFINING APPROACHES AND DIFFERENTIAL EFFECTS ON PROKARYOTES

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Blooms of toxic cyanobacteria are a serious threat to aquatic ecosystems, human health, and animals across the US. Though watershed management is the most effective long-term solution for preventing cyanobacterial blooms, mitigation strategies that are rapid, safe, and effective are required when serious health risks emerge. This talk will present the use of hydrogen peroxide (H_2O_2) to mitigate toxic cyanobacterial blooms. H_2O_2 has several distinct advantages over traditional cyanobacterial mitigation strategies including a higher sensitivity of cyanobacteria, but not other forms of aquatic life, to modest concentrations of H_2O_2, the destruction of toxins produced by cyanobacteria, the degradation of H_2O_2 to oxygen and water within hours of application, and its relatively low cost. While H_2O_2 has been used to mitigate cyanobacterial blooms abroad, its use in the US has been exceedingly limited. Multiple mesocosm experiments were performed in 2016 and 2017 in multiple NY lakes applying $2 - 6$ mg H_2O_2 L^{-1}. In all cases, H_2O_2 significantly reduced the biomass and relative abundance of cyanobacteria while significantly increasing the abundance of diatoms and green algae. There was, however, a gradient in the vulnerability of the cyanobacteria to the H_2O_2 with populations dominated by *Microcystis* being more resistant than blooms of other genera. In addition to pure, liquid H_2O_2 both solid forms of peroxide and commercial products containing H_2O_2 proved to be equally effective in mitigating cyanobacteria blooms. Beyond cyanobacteria, H_2O_2 proved effective at reducing the densities of some, but not all, bacteria indicative of pathogens.

EFFECTS OF POTENTIAL ALGICIDAL COMPOUNDS PRODUCED BY *SHEWANELLA* SP. IRI-160 ON HARMFUL DINOFLAGELLATES

Yanfei Wang[1], Eva Ternon[2] and Kathryn J. Coyne[1]

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Previous research identified a bacterium *Shewanella* sp. IRI-160, which can produce a water-soluble algicidal compound, IRI-160AA, targeting harmful dinoflagellates, while having no significant impacts on other phytoplankton species. Ammonium and three other amines, putrescine, diethylamine, and n-butylamine, were identified from the algicide by UHPLC-HRMS (Orbitrap) analysis. In this study, ammonium, putrescine, and its two major metabolites, spermidine and spermine were applied to harmful dinoflagellates, *Karlodinium veneficum*, *Prorocentrum minimum*, and *Gyrodinium instriatum*, as well as non-harmful cryptophyte alga, *Rhodomonas* spp. Addition of polyamines to laboratory cultures indicated dose-dependent and species-specific effects. Putrescine had negative impacts on the growth of all four species, spermine inhibited the growth of *P. minimum*, *G. instriatum*, and *Rhodomonas* spp., while spermidine only had negative effects on *Rhodomonas* spp. Synergistic effects were also investigated and interestingly, putrescine and ammonium in combination were found to affect the growth of *K. veneficum* at concentrations where no effect was observed by either compound alone. This synergistic effect was not observed on other species. The results showed that the effects of amines tested here differed from those of IRI-160AA, however, indicating that other compounds likely play a role in the algicidal effects of IRI-160AA. Effects of diethylamine, n-butylamine, as well as different combinations of amines found in IRI-160AA will also be tested, and cell morphology will be evaluated after treatment with these compounds. The results of this study will provide support for application of this algicide to control natural dinoflagellate blooms.

USE OF A MULTI-PLEX QPCR ASSAY AS A CYANOTOXIN SCREENING TOOL FOR OHIO PUBLIC WATER SYSTEMS AND INLAND LAKES

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In 2016, Ohio EPA began utilizing a multi-plex qPCR assay that identifies and quantifies total cyanobacteria (16s), microcystins production (mcyE), saxitoxins production (sxtA), and cylindrospermopsin production (cyrA). Source water samples were collected biweekly at 118 public water systems (PWSs) and analyzed with the assay and ELISA (total microcystins). ELISA analysis for saxitoxins and cylindrospermopsin was conducted if sxtA or cyrA were detected. Sampling frequency increased if cyanotoxins were detected. In addition, at 24 inland lakes, at least three samples were collected and analyzed for microcystins, saxitoxins, cylindrospermopsin, phytoplankton enumeration, and molecular analysis with the assay. mcyE was detected in source waters for 57 PWSs and six inland lakes and microcystins were detected at 45 PWSs and five inland lakes. 2% of PWS samples and 15% of inland lake samples had microcystins detections without corresponding mcyE detections. At several sites, detection of mcyE preceded microcystins detections by 1-4 weeks. At one inland lake site, high 16s may have inhibited mcyE detection. sxtA was detected at 33 PWSs and 14 inland lakes, and saxitoxins were detected at 15 PWSs and 10 inland lakes. Less than 1% of PWS samples and 1.9% of inland lakes samples had saxitoxins detections without corresponding stxA detections. Samples with cyanotoxin detections that lacked corresponding gene detections often occurred following bloom senescence and cyanotoxin concentrations in those samples were low. At one PWS, mcyE, sxtA, and cyrA were all detected, demonstrating multi-plex assay functionality. qPCR results out-performed cyanobacteria cell counts as a predictor for inland lake cyanotoxin production.

COMMUNITY-BASED MITIGATION OF HARMFUL ALGAL BLOOMS AND SHELLFISH POISONINGS IN SOUTHEAST ALASKA

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Paralytic Shellfish Toxins have been known in Alaskan shellfish for thousands of years and remain a present threat to all recreational and subsistence shellfish harvesters in the state. Limited State resources, dispersed and remote populations, and long coastlines have precluded the development of a statewide monitoring program or testing facilities for wild shellfish. As climate change has extended the HAB season in Alaska, traditional risk mitigation strategies such as harvesting only during winter months have become increasingly unreliable, causing many would-be harvesters to forgo shellfish entirely. To reduce the threats of PSP and harmful algal blooms in Southeast Alaska, the Sitka Tribe of Alaska has taken the lead in developing a community-based harmful algal bloom monitoring and toxin testing program. The Sitka Tribe's partners, fourteen tribal governments that comprise the Southeast Alaska Tribal Ocean Research group, each monitor one or more local shellfish sites. Samplers collect and analyze weekly phytoplankton samples at each site and ship bi-weekly shellfish samples to the Sitka Tribe's Environmental Lab for analysis using Receptor Binding Assay. Results are available within one to two business days and are immediately communicated to each local community. The Sitka Tribe and SEATOR group are building a regional database of shellfish toxins and phytoplankton abundances to make shellfish available as a safe, reliable wild food source. The SEATOR group's success has implications for all monitoring programs that rely heavily on community participation as well as programs that struggle to effectively serve remote sites.

MONITORING MULTIPLE HAB TOXINS AT THE LAND-SEA INTERFACE IN COASTAL CALIFORNIA

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The conventional focus of HAB monitoring has been to analyze toxins according to the waterbody type sampled, either as marine toxins or freshwater toxins, but not both. However, recent studies have shown that cyanotoxins have far reaching-effects downstream of their freshwater origin in brackish and marine waterbodies. Cyanotoxins produced in and transported by multiple inland waterbodies in California (lakes, reservoirs, streams, rivers, wetlands and lagoons) provide many unanticipated sources of toxins to downstream receiving waters. This presentation will focus on several aspects of HAB monitoring at the land-sea interface, including recent monitoring results, challenges encountered (and solutions), and the development of an integrated, multi-toxin HAB strategy.

Monitoring, assessment and intensive targeted studies were conducted to evaluate the transfer of cyanotoxins from fresh waterbodies to estuarine waterbodies, to determine the prevalence of cyanotoxins, and to identify predominant toxin producing cyanobacteria species. Multiple cyanotoxins were detected simultaneously in some systems indicating multiple physiological stressors in those ecosystems. The persistent detection of cyanotoxins temporally (months and seasons) and spatially (multiple systems and regions) indicates a low-level, chronic presence of these toxins. Our findings indicate a high risk for bioaccumulation of multiple HAB toxins into marine food webs and that the influence of toxic cyanobacterial blooms pose a much more complex mix of stressors than presently recognized. These studies underscore the importance of inland waters as potential conduits for transfer of freshwater toxins to the marine environment, and highlight the importance of a multi-toxin approach to monitoring at the land-sea interface.

MULTIPLE FRESHWATER AND MARINE ALGAL TOXINS AT THE LAND-SEA INTERFACE: IS BROADER MONITORING OF ALGAL TOXINS NECESSARY TO PROTECT NON-COMMERCIAL SHELLFISH HARVESTERS?

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The California Current spans nearly 3,000 km, from Southern British Columbia to Baja, California. Over the last few years, there have been multiple, dramatic algal toxin events within the California Current, including the 2015 Amnesic Shellfish Poisoning event, the emergence of Diarrhetic Shelfish Poisoning in the Pacific Northwest, and freshwater microcystin poisoning of California sea otters. These events have led to broad public, management, and scientific interest in algal toxins at the land-sea interface. Current management practices separate freshwater and marine harmful algae, and sample organisms or toxins that "should" be present, or have been historically problematic in water bodies. Here we look at San Francisco Bay, CA, USA, a region that has been monitored for harmful algae for more than 20 years, but historically has been assumed not to have harmful algal bloom problems. From 2011–2016 we used particulate, dissolved, and shellfish sampling to identify that multiple toxins are present nearly all the time, and that at least four toxins are present simultaneously in 37% of our sampled shellfish, including domoic acid, microcystins, Paralytic and *Dinophysis* Shellfish Toxins. Here we discuss what chronic or acute toxin exposure means, and ask the question: is broader monitoring necessary to protect subsistence, ceremonial, and recreational shellfish harvesters? These findings explore improved monitoring and management efforts to deal with the simultaneous presence of multiple, chronic toxins.

A DECADE AND A HALF OF *PSEUDO-NITZSCHIA* SPP. AND DOMOIC ACID ALONG THE COAST OF SOUTHERN CALIFORNIA

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Pseudo-nitzschia spp. has been documented along the coast of southern California since the 1930s, however, there has only recently been documentation of domoic acid in the region. We present a synthesis of coastal monitoring and major field efforts occurring from 2003 to 2017 that documented the phenology and magnitude of *Pseudo-nitzschia* abundances and domoic acid in the region. Domoic acid outbreaks have shown significant year-to-year variability, and toxic events were comparable in magnitude to some of the highest particulate toxin concentrations recorded in the literature. Observations have linked domoic acid in the diets of marine mammals and seabirds to mass mortality events in several years. Domoic acid has been detected in shellfish tissue in virtually all years throughout this period, although the magnitude and geographic extent of the toxin has varied considerably by year and county coastline. Our work has shown that toxic blooms exhibit strong seasonality, with toxin appearing primarily in the spring. Toxic events appear to have a strong relationship with upwelling and generally tend to coincide with cooler waters. *Pseudo-nitzschia* blooms have been associated with the subsurface chlorophyll maximum, and the upwelling of subsurface populations has been observed to give rise to 'instant' bloom and toxic conditions. The species composition of bloom events is an important factor controlling the magnitude of toxic events. While the general oceanographic factors that give rise to bloom events in the region are known, the subtle factors controlling spatial and interannual variability in bloom magnitude and toxicity remain elusive.

FORECASTING 'EVERY BEACH, EVERY' DAY TO IMPROVE PUBLIC HEALTH INFORMATION DURING KARENIA BREVIS BLOOMS

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Improving the forecasts of respiratory risk from *Karenia brevis* aerosols involves the integration of several types of data sets. Any one data set is insufficient to locate blooms at the required spatial and temporal resolution to achieve "every beach, every day". Our project uses three data sets to improve the respiratory forecasts from the county level to the individual beach level.

First, we use ocean color satellite data to detect blooms that are likely to be *Karenia brevis*. The analysis includes a standard ensemble of algorithms to process data from multiple sensors (MODIS, VIIRS, OLCI). Using multiple satellites reduces the impact of clouds—improving temporal resolution. This information allows us to identify areas that warrant beach monitoring.

Second, we use observations. Field monitoring programs typically sample once a week to determine *Karenia* concentration through taxonomic microscopy. We have developed HABscope, a rapid detection system that combines a microscope with smartphone acquisition, then subsequent real-time automated image recognition software. HABscope is intended for deployment by volunteers. The backbone of the Gulf Coastal Ocean Observing System, combines these concentrations with state sampling and respiratory impacts reported from the Beach Conditions Reporting System.

Third, we use models. Respiratory irritation is forecast from the cell concentration and the onshore wind component. Replacing the daily marine forecasts with the National Digital Forecast Database (NDFD), will provide hourly, 2.5 km resolution forecasts. NDFD allows forecasting at a resolution not previously achieved, leading to respiratory forecasts every day at every beach with samples.

INNOVATION THROUGH CITIZEN SCIENCE AND OUTREACH

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Neurotoxins from *Karenia brevis* blooms threaten human health. When toxic aerosols are present, respiratory irritation or illness can ensue, resulting in negative beach experiences. Informing the public of beach conditions is an effective strategy to protect beachgoers by encouraging visits to beaches not experiencing effects. Citizen reports can provide a cost effective strategy for this purpose. Three modern approaches to information dissemination/data g System (BCRS) is a website/ smartphone application displaying twice daily beach conditions and qualitative bloom effects from 33 Gulf Coast beaches. Due to the robust dataset, statistical analyses of BCRS respiratory irritation and sample toxin data were performed to validate reports; in response to a strong correlation, a map and model of collected sample, meteorological, and BCRS data were used to determine parameters most correlated with respiratory irritation. Results were considered in development of a BCRS complement called Citizen Science Information Collaboration (CSIC), a smartphone citizen reporting application, released June 2017, to empower the public, provide geo-located real-time effect data, improve outreach efforts, and spark public interest in environmental science. The third outreach tool presents a technological advancement in citizen science and gathers real-time quantitative data on *K. brevis* presence and concentration provided by trained citizens and a smartphone microscope, through a NASA ROSES funded project involving NOAA, GCOOS, FDOH and Mote Marine Laboratory. Our overall citizen science effort is a culmination of science, technology, community, and education to produce effective tools, protecting human health from the effects of neurotoxic aerosols.

USING NEW INSTRUMENTATION AND PIGMENT RATIOS TO MONITOR CYANOBACTERIA

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In vivo monitoring of cyanobacterial-specific marker pigments such as phycocyanin (PC) has been used as a general method for determining relative abundance of cyanobacteria. However it does not provide a definitive answer to the question, "Is a bloom on the way?", and that is the key question of interest. To answer this we must turn to pigment ratios, namely PC to Chlorophyll (CHL), which provides a more accurate way to: 1) estimate cyanobacterial abundances within total phytoplankton populations and 2) predict the onset of cyanobacterial blooms (HABs). Turner Designs recently introduced two instruments utilizing this ratio method to help researchers quickly and easily track cyanobacterial production to facilitate predicting cyanobacterial blooms. Early prediction is critical so proper protocols can be implemented to prevent or reduce the harmful bloom's effects. CyanoFluor is a Handheld HAB Indicator that quickly (<15 seconds) indicates through trend monitoring whether environmental conditions are changing, favoring cyanobacterial production, which may result in a HAB. PhytoFind is a self-contained *in situ* monitor that tracks real-time abundance of 3 algal groups using pigment ratios: Cyanobacteria (PC-containing algae), Mixed (PE-containing algae), and Green-Brown (all other algae). Using instruments designed for pigment ratio analyses will help monitoring efforts to quickly, easily, and accurately track HABs.

Exploring climate impacts on inland and coastal waters

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Questions surrounding water sustainability, climate change, and extreme events are often framed around water quantity – whether too much or too little. The massive impacts of water quality impairments are equally compelling, however, and recent years have provided a host of compelling examples of unprecedented harmful algal blooms and hypoxic dead zones. Linkages between climate change and water quality impacts are not well understood, however. Several factors explain this lack of understanding, including the relative complexity of underlying processes, the spatial and temporal scale mismatch between hydrologists and climatologists, and observational uncertainty leading to ambiguities in the historical record. Here, we draw on a number of recent studies that aim to quantitatively link meteorological variability and water quality impacts. Focusing on eutrophication, harmful algal blooms, and hypoxic dead zones, this talk will frame challenges and opportunities related to characterizing water quality, bridging from local to global scales, identifying key drivers, and understanding the role of climate. In each case, the availability of new tools makes it possible to develop and test novel hypotheses about the role of climate and what the future may hold.

THE USE OF TECHNOLOGY TO ASSIST CITIZEN SCIENTISTS MONITORING FRESHWATER HABS AND CHANGES IN ENVIRONMENTAL CONDITIONS

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The negative environmental public health impacts from harmful algal blooms (HABs) have been noted recently in association with pet deaths, fish kills, and disease outbreaks in marine birds and mammals. Mounting evidence indicates global climate changes support increased frequency and geographic extent of HABs. The Phytoplankton Monitoring Network (PMN) is a NOAA research-based program utilizing volunteers to monitor phytoplankton species composition and environmental conditions.   Volunteers are trained to identify general classes of phytoplantkon, including 14 known toxin producing species.   Since 2001, the network area includes coastal Atlantic, Gulf and Pacific waters. In 2014, NOAA and EPA collaborated to expand this citizen science program into freshwater habitats. Volunteers participate in hands-on research by collecting water samples, identifying species of interest, and entering data on a weekly or bi-weekly basis. Volunteers monitoring these sites represent public and private schools, colleges and universities, Native American tribes, state and national parks, aquariums, 4-H centers, civic groups, and other non-governmental organizations. The PMN was able to grow into a national monitoring program by use of various web-based tools such as an interactive web site and a geographic information system tool for data visualization and searchable database. NOAA scientists using internet teleconference capabilities and next generation digital microscopes give volunteer training sessions and workshops. This presentation will outline the use of these technologies and highlight the use of volunteer data.

CITIZEN SCIENCE INFORMATION COLLABORATION: THE SHIFTING TIDES OF CROWD SOURCED RESEARCH

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Due to the widespread and varying distribution of harmful algal blooms, such as the Florida red tide organism *Karenia brevis*; real-time, geo-located reports are a valuable resource to protect public health and ensure positive beach experiences. The Citizen Science Information Collaboration (CSIC) smartphone application, developed by Mote Marine Laboratory and Function on Phones, was created with a goal to enhance harmful algal bloom data collection through community engagement. CSIC, released June 2017, uses descriptive pictures to guide users through reporting environmental parameters as they relate to red-tide effects including: respiratory irritation, dead fish, and marine water discoloration. This Fall, 2017, parameters will be expanded to include beach and inland environmental conditions such as: beach conditions (such as crowds, debris, surf, etc.), freshwater algae blooms and fish kills, bird and mammal fatalities, invasive species sightings, odors, and observed ecosystem changes. This smartphone application is free and available for download through the Apple and Google Play stores, and the data will eventually be displayed on a layer added to Mote's established Beach Conditions Reporting System. The app hopes to incorporate an educational aspect so the user can learn about HABs and other environmental conditions as well as report and view reports. CSIC allows scientists to engage a larger portion of the community while facilitating a cost effective flow of information between citizens and researchers. Ultimately, CSIC hopes to empower community members, by allowing their voices to be heard, while engaging in scientific research and assisting scientists in data collection.

UTILIZING CITIZEN SCIENCE TO ESTABLISH A MONITORING PROGRAM FOR PUBLIC HEALTH PROTECTION

Devin Burris[5], Tracy Fanara[5], Aly Busse[5], Fitz Otis[5], Richard Stumpf[1], Barbara Kirkpatrick[2], R. Wayne Litaker[3], Michelle Tomlinson[1], Robert D. Currier[2], Rance Hardison[3], William C. Holland[3,], Andrew Reich[4]

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The patchiness and mobility (through wind and currents) of *Karenia brevis*, "red tide" blooms, creates variability of respiratory irritation among beaches. As a result, beaches that are a few miles apart may experience drastically different degrees of respiratory effects and these effects can change each day as currents move bloom patches along the coast. Providing the public with real-time red tide data is key for implementing an effective strategy to protect beachgoers. The HABscope design (described in the paper by Holland et al.) provides an opportunity for the creation of a citizen science network that can be deployed at multiple beaches and provide daily monitoring. It utilizes a novel smartphone application and algorithm, interfaced with a microscope to detect and rapidly calculate concentrations of *Karenia brevis* based on distinctive cell characteristics. Implementing a citizen science network for this approach allows for timely data collection across a much larger geographical range. Volunteers receive instruction on microscopy, basic cell identification of *Karenia brevis,* and the use of the HABscope app as well as online, laboratory, and hands-on field training to insure data quality. In this pilot year, trained volunteers from Mote Marine Laboratory are reporting from five different beaches. Training methods, field protocols, and technology has been improved as a result, developing the framework for the citizen science network that can be expanded for local application and well as in other regions.

HARMFUL ALGAL BLOOMS AND CULTURAL TIES

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Lummi Bay, located in Bellingham, Washington that surrounds the Lummi people and its Nation. Many Coast Salish tribes, including Lummi Nation, have fished and harvested from the Salish Sea for thousands of years. Today fishing and shellfish harvesting is still relevant for food, potlatch and ceremony purposes. Native people have always known about red tide (harmful algae), knowledge of red tide has been passed down with each generation and some of the teachings include what part of the shellfish to harvest when there is a bloom to minimize exposure to toxins. With excess nutrients entering the sea, such as pet waste, sewage, and fertilizers, along with heavy rainfall and warmer temperatures, harmful algal blooms are expected to increase in size, duration, and consequences. Many tribal and state agencies, in stewardship for their land, monitor for harmful algal blooms and toxins. Native Americans that live and work along the Salish Sea and harvest shellfish for subsistence are feeling the effects of harmful algae, where more harvesting closures means no work or food on the table. The Salish Sea Research Center at Northwest Indian College is supporting tribal monitoring, while working with Lummi Natural Resources, by including passive toxin tracking. This may provide early evidence of toxin contamination in the water column before it reaches shellfish, and decrease closure times due to toxigenic harmful algae by providing more data points and faster turn around time. Here we present our long-term monitoring plan and preliminary data while focusing on the cultural ties that the Lummi people have with harmful algae and shellfish harvesting.

EFFECTS OF THE BACTERIAL ALGICIDE IRI-160AA ON MICROBIAL COMMUNITY COMPOSITION OF THE DELAWARE INLAND BAYS

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Global increases in harmful algal blooms (HABs) have spurred interest in measures to control these blooms. Prevention, control, and mitigation of HABs are of human interest, and there are naturally-occurring compounds that may directly influence these processes. Use of these compounds appears promising, but effects to non-target organisms are also of concern. The Delaware Inland Bay (DIB) system is host to the algicidal marine bacterium *Shewanella* sp. IRI-160, which secretes a compound(s) having allelopathic effects to dinoflagellate algae. Here, the effects of the algicide, designated IRI-160AA, on microbial communities in the DIB will be evaluated. IRI-160AA will be added to natural DIB dinoflagellate bloom communities at 50% lethal concentrations (EC50) to determine the potential impact of the algicide on microzooplankton grazing activity. A second treatment will include addition of ammonium, which is present in high concentrations in the algicide and is toxic to some algae, in order to distinguish between effects of ammonium alone and the effects of the algicide. Changes in microbial community composition in response to these compounds will be assessed via molecular fingerprinting methods. A second set of natural community experiments will be conducted to assess proactive application efficiency of the algicide to communities as a preventative measure. Here, repeated dosing will be conducted during non-bloom conditions at EC5, EC50, and EC95 concentrations of the algicide, and changes in the microbial community will be evaluated as above. This study will provide valuable insight into the potential use of IRI-160AA as a reactive and proactive HAB control measure.

HYDROGEN PEROXIDE USED AS A REMEDIATION METHODS: A TARGETED APROACH TO TACKLE CYANOBACTERIAL BLOOMS?

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Algal blooms are increasing on a worldwide scale. This includes cyanobacteria blooms that produce toxins that impact recreation and human and ecosystem health. Hydrogen peroxide (H_2O_2) has been proposed as a control method to limit the growth of these cyanobacteria blooms. Here, the sensitivity of different species of cyanobacteria and chlorophytes to H_2O_2 was examined. Cyanobacteria in the genera *Dolichospermum*, *Anabaena*, *Trichormus*, and *Microcystis*, and chlorophytes in the genera *Chlorella*, *Chlamydomonas*, and *Auxenochlorella* were grown in Z8 using a 12:12 light:dark cycle at 22ºC with the addition of various concentrations of H_2O_2. *In vivo* fluorescence, catalase activity, and toxin production were measured. There was no significant difference ($p>0.05$) between the average LC_{50} of cyanobacteria (1.25 µM) and chlorophytes (0.075 µM). Also, different species, even within the same genus, varying survivability with the addition of H_2O_2, i.e. the LC_{50} of *Microcystis aeruginosa*, *M. botrys*, and *M. wesenbergii* were 0.213, 0.158, and 0.0002 µM. Furthermore, addition of H_2O_2 did not affect the toxicity of the samples (i.e. the negative control of *M. aeruginosa* had a toxicity level of 790 µg/L for microcystins, whereas the 3.0 mM addition of H_2O_2 had a toxicity of 768 µg/L). This suggests the use of H_2O_2 to control harmful cyanobacterial blooms must be carefully considered depending on which genera and species are present.

Emerging HABS and Toxins

Tuesday Presentations

First records of the genus *Azadinium* (Dinophyceae) from Puget Sound, Washington State

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Lipophilic toxins, in particular those associated with diarrhetic shellfish poisoning (DSP), are an emerging threat to shellfish harvesting in Washington State. While Washington was the first state to implement the routine testing of shellfish for DSP toxins in 2012 after the occurrence DSP in Sequim Bay, WA in 2011, there continue to be occasional reports of DSP-like illnesses likely tied to the consumption of shellfish from Puget Sound. To address these illness of unknown etiology, we began a Monitoring and Event Response to Harmful Algal Blooms project in 2015 to identify whether species of the genus *Azadinium* were present in Puget Sound. This small dinoflagellate, in particular *A. poporum*, *A. spinosum, and A. dexteroporum*, have been shown in other parts of the world to produce azaspiracids, lipophilic toxins which can produce DSP-like symptoms. The presence of the genus *Azadinium* was confirmed in whole water samples collected from several SoundToxins sites in Puget Sound based on the use of molecular probes. Here we report the establishment of *Azadinium* cultures from sediment samples from Puget Sound including *A. obesum*, *A. cuneatum*, *A. poporum*, *A. dalianense*. The production of a new azaspiracid, named AZA-59, was confirmed by liquid chromatography mass spectroscopy in several isolates of *A. poporum*. This first confirmation of the presence of the genus *Azadinium* in Puget Sound and the first report of azaspiracid on west coast of the U.S., underlining the potential risk of azaspiracid shellfish poisoning in this region.

TEMPORAL AND SPATIAL DISTRIBUTION OF *AZADINIUM* SPP. IN WASHINGTON STATE WATERS

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Azaspiracids, produced by species of dinoflagellate genera *Azadinium* and *Amphidoma*, can cause a syndrome in humans called azaspiracid shellfish poisoning. In 1995, mussels from the Irish west coast contaminated with azaspiracids were first linked to this human illness that causes symptoms of nausea, vomiting, severe diarrhea, and stomach cramps. Similar symptoms reported by consumers of Puget Sound shellfish with no detectable diarrhetic shellfish toxins or *Vibrio* contamination motivated our study of the distribution of *Azadinium* species in Washington State. During the summer months of 2014-2017, quantitative polymerase chain reaction (qPCR) analysis using probes specific to species of *Azadinium* from the North Sea detected the presence of *Azadinium poporum, A. spinosum*, and *A. obesum* in Puget Sound and on the outer coast of Washington State. In 2016 and 2017, standard curves developed using *Azadinium* isolates from Puget Sound (*A. poporum*) and the North Sea (*A. spinosum* and *A. obesum*) were used to quantify concentrations of up to 410, 250 and 150 cells/L of *A. poporum*, *A. obesum*, and *A. spinosum,* respectively. In some samples where these three species were not detected, an Amphidomataceae specific qPCR assay indicated that other species of *Azadinium* or *Amphidoma* were present. Further work is needed to isolate these species of *Azadinium* and/or *Amphidoma* and develop qPCR probes for their detection and quantification. The identification of *Azadinium* species in Washington State demonstrates the need to assess their toxicity and to incorporate their routine detection in monitoring programs.

LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY ANALYSIS OF A NEW AZASPIRACID PRODUCED BY *AZADINIUM POPORUM* IN WASHINGTON STATE

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Through the MERHAB funded project "Clear and Present Danger: Monitoring and Management of Lipophilic Shellfish Toxins in Washington State", a new azaspiracid (AZA-59) was detected in Puget Sound, WA. A liquid-chromatography tandem mass-spectrometry method, developed for monitoring AZA-1, -2, and -3, was extended to monitor AZA-59 in Solid-Phase Adsorption Toxin Tracking (SPATT) samples and shellfish extracts. Structurally informative transitions for each AZA were selected for multiple reaction monitoring. The first and second most abundant ions were utilized for quantitation and confirmation, respectively. Certified reference materials (CRMs) for AZA-1, -2, and -3, were used for external calibration; however, in the absence of a CRM for AZA-59, concentrations were determined by external calibration with AZA-1. Chromatographic separation was achieved using a reversed-phase UPLC column and acidic chromatographic conditions. To determine the retention time for AZA-59 and verify that the instrument parameters were sufficient for detection, an extracted cell pellet from a toxin-producing, Puget Sound *A. poporum* culture was analyzed. This LC-MS/MS method was then applied for the analysis of over eighty SPATT resin extracts, collected over two years at multiple sampling stations in Puget Sound and along the WA coast. Resin extracts from eight different sampling stations were found to contain concentrations of AZA-59 ranging from trace to 2.7 ng/g resin. These results were compared to quantitative data for diarrhetic shellfish poisoning (DSP) toxins obtained from the same sample extracts; however, no correlation in DSP and AZA toxin concentrations were observed.

CLADISTIC ASSESSMENT OF CYANOBACTERIAL BIOACTIVE METABOLITE ACCUMULATION IN MARINE, FRESHWATER, AND TERRESTRIAL CYANOBACTERIAL ISOLATES

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There has been no systematic assessment of toxin occurrence within the Cyanoprokaryota, even though cyanobacterial secondary metabolites are known to cause significant human health and environmental concerns. This study identified the accumulation of the known 48 classes of secondary metabolites (e.g. microcystins, anabaenopeptins, saxxitoxins, and anatoxin-a) in terrestrial (73 strains), freshwater (60) and marine (16) cyanobacteria from 58 genera in 8/9 orders using UPLC/TOF-MS. In a preliminary study of 149 strains, an average of 11.63 cyanobacterial toxins per cyanobacterium were identified. Protease inhibitors were the most commonly identified cyanotoxin group, followed by cytotoxins. Co-production of multiple bioactive compounds was determined in over 90% of the isolates. A second TOF analysis of these cyanobacterial cultures sampled at early and late exponential phases for toxins and cell numbers should provide clear patterns of the dynamics of metabolite production during the growth period and represents the first systematic assessment of cyanobacterial toxins and the diversity of toxin occurrence in different habitats.

SELF PROTECTION FOR THE DSP PRODUCER PROROCENTRUM LIMA: SULFATED DIESTERS AND A SPECIALIZED ESTERASE.

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Many species of the dinoflagellate *Prorocentrum* biosynthesize Diarrhetic Shellfish Poison (DSPs) okadaic acid and DTX-1. These toxins are potent inhibitors of the eukaryotic phosphatases PP1 and PP2A. Because these phosphatases are key enzymes in all eukaryotic organisms, it is possible to propose these compounds are effective deterrents of predators. Conversely, it begs the question as to how the *Prorocentrum lima* protects itself from self-destruction, especially since it has been shown the dinoflagellate possesses these phosphatases.

This challenge is not unusual in Nature, for example how do bacteria protect themselves from the very antibacterials they produce? Various strategies have been observed over the years. They include alteration of the target, compartmentalization of the toxin, or the biosynthesis of a protected form of the compound only to be released later in the active form, or efflux pumps that excrete the toxin or an adduct. In the case of the DSP toxins we have established that the toxins themselves are not the initial biosynthetic products but rather a series of inactive sulfated diesters of both okadaic acid and DTX-1 are the actual products.

We have also shown that when *P. lima* cells are disrupted they release an esterase that specifically and rapidly hydrolyses the ester links of the diesters to produce both the DSP toxins as well as the diol esters which are as potent as the DSP toxins *in vivo*.

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Paralytic Shellfish Toxin Monitoring in New York State Freshwater

Most research in the United States on Harmful Algal Blooms has focused on the occurrence of microcystins. However, the rich diversity in cyanobacteria flora may suppose a presence of other cyanobacterial toxins. Paralytic Shellfish Toxins (PSTs) are known to be produced by *Anabaena*, *Aphanizomenon*, *Lyngbya*, and *Planktothrix*, genera found in many New York Lakes. In the Northeast United States, reports of PST production by cyanobacteria have appeared in the literature, but little is known about 'typical' levels of toxin present in surface water. Approximately 6000 freshwater samples collected between 2015 and 2017 from >175 lakes across New York were analyzed for PSTs by HPLC with post-column oxidation and fluorescent detection. PST presence was confirmed using using Abraxis PST ELISA assays and/or HILIC chromatography with mass spectrometry detection. A description of the monitoring program, analytical techniques, and assessment of surface water toxicity will be presented.

    1. Monitoring, Mitigation, and Management of HABs

    2. Emerging HABs and Toxins

Disinfection by-products arising from *Microcystis Aeruginosa* algal DOM

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Toxic algal blooms become more widespread and specifically the alga *Microcystis aeruginosa* already caused several drinking water advisories in recent years. To treat toxic algal bloom related dissolved organic matter (DOM), enhanced chlorination in conjunction with activated carbon treatment were implemented in drinking water treatment plants, but a systematic evaluation of metabolites and formed disinfection by-products (DBPs) is still lacking. In this study, we performed chlorination of a *Microcystis aeruginosa algal DOM* extracted from a culture. Additionally, we purified the algal toxin microcystin-LR and also performed chlorination experiments to specifically characterize DBPs directly associated with this toxin. Non-targeted screening for unknown DBPs was undertaken using ultrahigh resolution 12 Tesla Fourier transform ion cyclotron resonance mass spectrometry. Results showed a complex suite of DBPs that were produced after chlorination of the algal DOM and indicated the efficient breakdown of the microcystin-LR toxin, but also the formation of specific DBPs directly related to this toxin. Most determined DBPs in this study have not been previously described.

UNTARGETED METABOLOMICS OF SPIROIMINE TOXIN PRODUCING DINOFLAGELLATES AND DISCOVERY OF THE POTENT CYTOTOXIN PORTIMINE B

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Spiroimine toxins are an emerging class of dinoflagellate metabolites of great importance to human health. They can accumulate in bivalve mollusks and have been shown to cause rapid, often fatal intoxication in animal models. Interestingly, at concentrations far below their oral toxicity levels, many of these spiroimines also possess intriguing, potentially therapeutic activities against diseases targets in cancer and Alzheimer's. Members of the spiroimine toxin class have been described from cultures of three different genera of dinoflagellates. These compounds show distinctive mass fragmentation pathways and thus lend themselves as ideal candidates for detailed metabolomics analysis. UPLC-QTOFMS untargeted metabolomics allows for rapid and detailed comparative analysis between species and genera.

Examination of spiroimine-producing dinoflagellate cultures from 3 genera (*Karenia selliformis*, *Alexandrium ostenfeldii*, and *Vulcanodium ruggosum*) revealed distinct chemical signatures between the species which could be partially attributed to their spiroimine toxin content. Detailed high resolution MS fragmentation analysis of ions highlighted through this metabolomic analysis further revealed potentially new analogs of these toxins. Rigorous chemical investigation of a large scale culture of *V. ruggosum* resulted in the purification and structure elucidation of portimine B, the first new congener of portimine. Interrogation of this new compound and purified portimine against a panel of cancer cell lines revealed insight into the structure-activity relationships between these two potent cytotoxins.

LATE SUMMER HARMFUL ALGAL BLOOMS IN LOWER CHESAPEAKE BAY: EMERGING PATTERNS AND IMPACTS

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Late summer blooms of *Alexandrium monilatum* and *Cochlodinium polykrikoides* have been particularly intense and widespread throughout lower Chesapeake Bay in recent years. A York River bloom in 2007 marked the re-emergence of *A. monilatum* in the mid-Atlantic since intermittent reports of blooms during the 1960's. *Cochlodinium polykrikoides* blooms have occurred almost annually in the York River for decades and throughout the southern bay since the early 1990's. Until 2011, *A. monilatum* blooms were localized in and near the lower York River. During blooms in 2012-2016, *A. monilatum* cells were found at increasing concentrations into the bay main stem, in many lower bay estuaries, as well as to the south along the North Carolina Outer Banks. A predictable population progression occurs with peak cell concentrations of *C. polykrikoides* followed a few weeks later by peak concentrations of *A. monilatum*. Reports of juvenile oyster mortalities from local aquaculturists during and immediately following these blooms have prompted investigation. Laboratory bioassays have demonstrated acute dose dependent toxicity of York River *A. monilatum* to both larval and juvenile oysters, and of *C. polykrikoides* to larvae. Field studies are being conducted to examine the impacts of these blooms and other stressors, including temperature and low dissolved oxygen, on various oyster life stages. The toxin goniodomin A, produced by *A. monilatum*, was isolated from York River water samples collected during blooms and was found to cause rapid mortality in larval shellfish and finfish. Field studies also will help determine if goniodomin A accumulates in oyster tissues.

EMERGENCE OF *ALEXANDRIUM MONILATUM* IN SOUTHWESTERN CHESAPEAKE BAY: SEDIMENT CYST DISTRIBUTION AND HEALTH IMPACTS ON ADULT OYSTERS

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The toxin-producing harmful algal bloom (HAB) species *Alexandrium monilatum* has historically been associated with finfish and shellfish mortalities in the Gulf of Mexico. Since its re-emergence in Chesapeake Bay during 2007, blooms of this dinoflagellate have expanded almost annually in range, duration, and cell density, raising concerns among the shellfish aquaculture community. Bottom sediment samples were collected in southwestern Chesapeake Bay from 2014-2016 to map and quantify overwintering resting cysts of *A. monilatum*. Quantitative PCR indicated that *A. monilatum* cysts were widespread in the region, with densities as high as 90,000 cysts/cc sediment. Laboratory bioassays were conducted to investigate toxicity of *A. monilatum* to adult eastern oysters, *Crassostrea virginica*. Valve gape and mortality were monitored, and histology was used to assess tissue damage. Oysters did not exhibit mortality or tissue damage in a 48-hour toxicity bioassay. In a 96-hour bioassay, however, exposure to *A. monilatum* densities of 1,000-7,600 cells/mL led to gill and mantle epithelial erosion in 75-100% of oysters and 50-80% mortality. Adult oysters deployed in the field during the 2015 *A. monilatum* bloom season exhibited no mortality, but minor epithelial erosion of the mantle was observed during the peak of the bloom. *Alexandrium monilatum* is widely established in southwestern Chesapeake Bay and, at least under laboratory conditions, can adversely affect adult oyster health.

ANABAENA AS THE LIKELY SOURCE OF MICROCYSTIN RESPONSIBLE FOR THE RAPID DEATH OF 31 STEERS DRINKING FROM A S.E. OREGON RESERVOIR

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During 19-21 June, 2017, thirty-one 14-15-month-old steers died rapidly after contact with a HAB on a 175-acre ranch reservoir near Lakeview, OR. Persistent northerly winds had accumulated dense scum at the southern end of the reservoir where the cattle were drinking. Samples of the reservoir water and rumen contents of a rapidly deceased steer were submitted to the California Animal Health and Food Safety Laboratory for testing. Extremely high levels of microcystin-LR were detected in the reservoir water/scum sample (3000 ppb) and in the rumen contents (7100 ppb). Liver and other organs from two mortalities analyzed at the Oregon Veterinary Diagnostic Laboratory revealed diffuse hepatic necrosis in one animal and post necrotic collapse and severe hepatic vacuolation in the other; these findings are consistent with microcystin exposure. Tissue damage was also observed in heart, spleen and lung. HAB samples obtained for analysis were either frozen and thawed (most cells lysed) or shipped over a weekend (substantial cellular degradation), making morphological assessment of cyanobacteria uncertain. However, high levels of an irregularly coiled *Anabaena* with abundant heterocysts and akinetes were present, with small amounts of presumptive *Aphanizomenon flos-aquae*. The progress of genetic analysis of the putative microcystin-producing *Anabaena* will be reported, and compared with mcy+ *Anabaena* HABs from two other Oregon lakes with elevated microcystin levels in the absence of *Microcystis*. *Anabaena/Dolichospermum* is not usually considered the source of high levels of microcystin in the U.S., but recent experience in Oregon indicates that it should be considered as a potentially potent source of microcystin.

PHYLOGENETICS, GROWTH AND TOXICITY ASSESSMENT OF COOLIA MONOTIS MEUNIER (DINOPHYCEAE) FROM NOVA SCOTIA, CANADA

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Benthic dinoflagellates of the genus *Coolia* are globally distributed with the majority of species found in tropical waters. The type species, *C. monotis,* has been reported from the Mediterranean Sea, the Northeast coast of the Atlantic and from Rhode Island in the Northwest Atlantic. Cells isolated from algal drift samples collected at several sites in Nova Scotia, Canada were identified with light and scanning electron microscopy and confirmed with genetic sequencing to be *C. monotis*. This is a first record of this species in Nova Scotia. Single cell isolates were established and incubated at 18 ºC. In culture, cells tolerated temperatures from 5- 25 ºC with optimum growth between 15- 25 ºC. Reports of toxin production in this genus have been inconsistent and appear to be limited to just two species, *C. malayensis* and *C. tropicalis*. LC-MS analysis of the Nova Scotian *C. monotis* did not detect Cooliatoxin. Subsequently the potential toxicity of the Nova Scotia *C. monotis* isolate was assessed using larval zebrafish (*Danio rerio*) assays. When compared with a yessotoxin producing strain of *Protoceratium reticulatum*, the Nova Scotia isolate of *C. monotis* gave no appreciable toxic response.

Abstract

Unusual "Cold Weather" Fish Kills in Maryland caused by Karlotoxin.

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The Maryland Department of the Environment (MDE) investigated a large fish kill in the upper tidal Gunpowder and Bird rivers near Joppatowne (Harford/Baltimore Counties) on December 19, 2016. Approximately 20,553 fish died involving ten species: gizzard shad, common carp, pumpkinseed sunfish, bluegill sunfish, yellow perch, channel catfish, spottail shiner, largemouth bass, black crappie, and chain pickerel. All affected fish were freshwater species. Many of the affected gizzard shad displayed lesions typical of chronic cold stress, which is common for this species. All other fish appeared virtually pristine with no signs of disease or chronic stress, other than their gills were bright red and bleeding. Water quality parameters were within normal ranges except salinity, which was unusually high for this typically fresh region of Chesapeake Bay at 7ppt (parts per thousand). Results for dissolved metals, nitrate/nitrite, chlorine, and cyanide were unremarkable. Algal samples revealed that the ichthyotoxic algae *Karlodinium veneficum* was present at cell counts as high as 4,300 cells/ml. Liquid Chromatograph/Mass Spectrophotometry identified a karlotoxin congener as the probable cause of the fish kill. The congener was present at levels as high as 168 ng/ml and was demonstrated to be hemolytic to fish red blood cells. Fish histology indicated that the fish died as a result of respiratory distress and failure from marked changes in the gill architecture and hemorrhage following exposure to an irritant and adverse water quality conditions. While normally a summer/fall blooming species, *K. veneficum* appeared to be actively feeding on cryptophytes and to be following a salt wedge into the freshwater reaches of the bay well into mid December when water temperature in the kill zone had fallen to below 1.0°C. As a consequence, the freshwater fish community was trapped between a gill damaging algal species and the salt wedge. These fish were subjected to a lethal one-two punch to gill function induced by a combination of karlotoxin and salinity stress. A similar event occurred a year earlier during November 2015 in the upper Middle River involving 201,789 fish. The cause was also concluded to be gill damage and respiratory failure caused by a combination of karlotoxin and osmotic stress associated with rising salinity.

GENOME SEQUENCES OF PLANKTONIC BLOOM-FORMING ANABAENA/DOLICHOSPERMUM/APHANIZOMENON BELONG TO A DISTINCT GENUS-LEVEL CLADE AND REVEAL PATTERNS OF GENOME EVOLUTION

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The Nostocales include potentially toxigenic cyanobacteria that form HABs around the world. Here, we present nine novel *Anabaena/Dolichospermum* and *Aphanizomenon* draft genome sequences and analyze a group of 15 recently completed genome sequences from planktonic HABs on four continents. Five of the novel genomes were extracted directly from environmental shotgun metagenome assemblies, while the remaining four were cultured prior to sequencing. Phylogenomic clustering revealed a distinct genus-level ADA clade (planktonic *Anabaena/Dolichospermum/Aphanizomenon*) that is distinct from other Nostocales, in particular *Nostoc* and several *Anabaena*. Genome-wide nucleotide identities suggest the presence of four species within the ADA clade, two of which contain both *Anabaena* and *Aphanizomenon flos-aquae* morphotypes. Genes for the production of toxins (anatoxin-a, microcystin, saxitoxin) and other secondary metabolites are variably present. Gene differences in light harvesting pigments, utilization of organic sulfur sources, urea utilization, and other characteristics often do not follow a phylogenetic pattern, indicating active recombination and gene gain and loss. Genomic sequences are important in distinguishing physiological characteristics that could provide competitive advantages in different environments and in facilitating strain- or clade-specific molecular monitoring. They are also valuable in guiding taxonomic reforms to best classify Nostocales members that are morphologically similar but distantly related (some *Anabaena/Dolichospermum* and *Nostoc*) and those that are closely related but morphologically distinct (*Anabaena/Dolichospermum* and *Aphanizomenon flos-aquae*).

TOXIN PROFILES IN SPECIES OF *ALEXANDRIUM* NATIVE TO NOVA SCOTIA, CANADA

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One of the major harmful algal bloom (HAB) genera, *Alexandrium*, is globally well known with respect to the biodiversity, scale and consequences of toxic events. Two species in this genus, *Alexandrium ostenfeldii* and *A. tamarense* are routinely found in coastal waters of Nova Scotia, Canada. Species of *A. ostenfeldii* from this area are known to produce spirolides whereas *A.tamarense* typically produce paralytic shellfish toxins (PSTs). Clonal cultures obtained from net tow samples collected from Ship Harbour and Halifax Harbour in Nova Scotia in 2016, along with existing in-house cultures, were screened for toxin profiles by target and non-target liquid chromatography mass spectrometry methods. Known PSTs were detected in all strains. De-sulfated and ring-open analogues (M-toxins) previously only considered as shellfish metabolites were also detected in the algae. Most strains of *Alexandrium ostenfeldii* produced low or trace level concentrations of PSTs. A variety of spirolide and gymnodimine-like compounds were detected in all *A. ostenfeldii* cultures, in addition to trace levels detected in 6 new isolates of *A. tamarense*. These results provide valuable new insight on the diverse toxin profiles of *Alexandrium* strains from Nova Scotia and demonstrate the importance of screening *Alexandrium* for a broad range of PSTs, SPXs and GYMs. This is of importance from a shellfish toxin monitoring point of view, but also in terms of algal cultures that should be maintained for the production of biotoxin reference materials at the National Research Council.

A COMBINATORIAL APPROACH TO MAP TOXIC CYANOBACTERIAL BLOOMS IN SWEDISH SURFACE WATER USED FOR DRINKING WATER WITH LC-MS/MS AND 16S rRNA AMPLICON SEQUENCING

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Blooms of toxin producing cyanobacteria are of increasing concern for countries relying on surface water for the production of drinking water. Global increasing temperatures and nutrient discharges make the blooms more severe and often occurring. In Sweden 65 % of the drinking water is produced from surface waters of which some are regularly subjected to cyanobacterial blooms. The current project aims to map the incidence of cyanobacterial blooms in Swedish surface waters by analyzing the toxin content with LC-MS/MS and the species composition with both microscopy and 16S rRNA amplicon sequencing. In 2016 cyanobacterial blooms were sampled from 20 different lakes. These blooms comprised a vast diversity of cyanobacterial species (570 Operational Taxonomic Units), of which *Microcystis aeruginosa* was the most abundant taxon. The LC-MS/MS analysis revealed that microcystin RR and its variants were most abundant in cyanobacterial blooms, as were microcystins LR and YR. The potent toxin anatoxin-a was detected both in blooms from fresh and brackish water. We observed a positive correlation between the relative *M. aeruginosa* abundance and the microcystin toxin levels. For the purpose of risk assessment, many drinking water producers solely rely on microscopic identification of toxin producing cyanobacterial genera. However, our study revealed taxonomic discrepancies between the microscopy observations and 16S amplicon sequencing analyses of cyanobacterial blooms. Induced by these findings, we have trained drinking water producers to employ 'easy to use' immunochemical assays to monitor cyanotoxin levels, in addition to microscopic analysis of cyanobacterial blooms.

DETECTION OF PHOTOTRANSFORMATION PRODUCT OF CYANOBACTERIAL HEPATOTOXIN MICROCYSTIN-LR BY NMR AND LC/MS

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Toxic cyanobacterial blooms are a growing environmental and human health concern. The most commonly occurring toxins produced by cyanobacteria are microcystins (MCs). This toxin affects the liver by forming a permanent covalent linkage with the enzyme protein phosphatase. Among the microcystins, microcystin-LR (MC-LR) is found to be one of the most abundant and the most toxic. It is thus necessary to develop effective treatment methods to remove microcystin-LR from contaminated water. Photodegradation is a very important way to remove contaminants in natural water system. MC-LR has a maximum absorbance at 239 nm, it may be removed under UV irradiation. We used 800MHz ^{1}H NMR to identify the structure of MC-LR and its photoproducts after UV irradiation. Microcystin-LR after exposure to UV, formed two new compounds which were identified as [4(E), 6(Z)-Adda]- and [4(Z), 6(E)-Adda] microcystin-LR. They were produced in equal amount. The two photoproducts and MC-LR have the same molecular weight and the existing method cannot separate them. To investigate their formation, we developed a new HPLC method to separate MC-LR and its photoproducts.

NODULARIN FROM BENTHIC FRESHWATER PERIPHYTON AND IMPLICATIONS FOR TROPHIC TRANSFER

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Abstract: In 2013 and 2015, the Pennsylvania Department of Environmental Protection conducted a survey of lotic habitats within the Susquehanna, Delaware, and Ohio River basins in Pennsylvania, USA, to screen for microcystins/nodularins (MCs/NODs) in algae communities and smallmouth bass (*Micropterus dolomieu*). Periphyton (68 from 41 sites), juvenile whole fish (153 from 19 sites) and adult fish liver (115 from 16 sites) samples were collected and screened using an Adda enzyme-linked immunosorbent assay (ELISA). Samples that were positive for MCs/NODs were further analyzed using LC-MS/MS, including 14 variants of microcystin and NOD-R and the MMPB technique. The ELISA was positive for 47% of the periphyton collections, with NOD-R confirmed (0.7-82.2 ng g^{-1} d.w.) in 20 samples. NOD-R was confirmed in 10 of 15 positive juvenile whole fish samples (0.8 – 16.7 ng g^{-1} w.w.) and in 2 of 8 liver samples (1.7 & 2.8 ng g^{-1} w.w.). The MMPB method resulted in total MCs/NODs measured in periphyton (2.2-1269 ng g^{-1} d.w.), juvenile whole fish (5.0 – 210 ng g^{-1} d.w.) and adult livers (8.5 – 29.5 ng g^{-1} d.w.). This work illustrates that NOD-R is present in freshwater benthic algae in the USA, which has broader implications for monitoring and trophic transfer.

A DECADE AND A HALF OF *PSEUDO-NITZSCHIA* SPP. AND DOMOIC ACID ALONG THE COAST OF SOUTHERN CALIFORNIA

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Pseudo-nitzschia spp. has been documented along the coast of southern California since the 1930s, however, there has only recently been documentation of domoic acid in the region. We present a synthesis of coastal monitoring and major field efforts occurring from 2003 to 2017 that documented the phenology and magnitude of *Pseudo-nitzschia* abundances and domoic acid in the region. Domoic acid outbreaks have shown significant year-to-year variability, and toxic events were comparable in magnitude to some of the highest particulate toxin concentrations recorded in the literature. Observations have linked domoic acid in the diets of marine mammals and seabirds to mass mortality events in several years. Domoic acid has been detected in shellfish tissue in virtually all years throughout this period, although the magnitude and geographic extent of the toxin has varied considerably by year and county coastline. Our work has shown that toxic blooms exhibit strong seasonality, with toxin appearing primarily in the spring. Toxic events appear to have a strong relationship with upwelling and generally tend to coincide with cooler waters. *Pseudo-nitzschia* blooms have been associated with the subsurface chlorophyll maximum, and the upwelling of subsurface populations has been observed to give rise to 'instant' bloom and toxic conditions. The species composition of bloom events is an important factor controlling the magnitude of toxic events. While the general oceanographic factors that give rise to bloom events in the region are known, the subtle factors controlling spatial and interannual variability in bloom magnitude and toxicity remain elusive.

Human & Natural Drivers for Watershed & Coastal HABS

Wednesday Presentations

MITIGATING A GLOBAL EXPANSION OF TOXIC CYANOBACTERIAL BLOOMS: THE CONFOUNDING IMPACTS AND CHALLENGES POSED BY CLIMATE CHANGE

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Managing and mitigating the global expansion of toxic cyanobacterial harmful blooms (CyanoHABs) is a major challenge facing researchers and water resource managers. A variety of traditional (e.g., nutrient load reduction) and experimental (e.g., artificial mixing and flushing, omnivorous fish removal, algaecide applications) approaches have been used to reduce bloom occurrences. However, managers now face the additional effects of climate change on watershed hydrologic and nutrient loading dynamics, temperature and mixing regimes, internal nutrient dynamics, and other factors. Rising temperatures, increasing frequencies and magnitudes of extreme weather events, such as tropical cyclones, floods and droughts generally favor CyanoHABs over other phytoplankton and could influence the efficacy of control measures. These climatic changes are "raising the bar" by requiring setting new nutrient (including both nitrogen and phosphorus) input reduction targets and establishing nutrient-bloom thresholds for impacted waters. In addition, physical-forcing mitigation techniques, such as flushing and artificial mixing, will need to adjust and compensate for a world facing increasing human population pressure and climate change.

CULTURAL EUTROPHICATION AND HARMFUL ALGAL BLOOMS IN THE INDIAN RIVER LAGOON, EAST-CENTRAL FLORIDA

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The Indian River Lagoon (IRL) has experienced increasing urbanization and macroalgal blooms for many decades. Following a multi-year drought in 2011, a "super bloom" of phytoplankton (> 100 µg/L chl a) developed in the Banana River, which was followed by a brown tide (*Aureoumbra lagunensis*) in the Mosquito Lagoon and the northern IRL in 2012/2013. Widespread seagrass die-off and wildlife mortality, including dolphins, pelicans, and endangered manatees, followed these blooms. To better understand the nutrient source(s) supporting these harmful algal blooms (HABs), seawater samples and primary producers (macroalgae, phytoplankton, seagrasses) were collected at a network of 20 fixed stations throughout the IRL. High TDN concentrations (up to 152 µM) and TDN:TDP ratios (>100:1) in the poorly flushed northern IRL and Banana River segments reflected the accumulation and cycling of N-rich groundwater and stormwater inputs in Brevard County. The $\delta^{15}N$ values in macroalgae were enriched throughout the IRL (mean = +6.5 o/oo; maximum values of + 9.2 o/oo in Brevard County) and similar to values reported for sewage-polluted coastal waters. The overall mean $\delta^{15}N$ of phytoplankton (+4.2 o/oo) was also enriched, whereas the seagrasses had lower values (+2.2 o/oo), indicating an increased reliance on N-fixation in the roots/rhizomes. Although many scientists and managers assumed that fertilizers from agricultural and urban areas were the primary nutrient source supporting these HABs, these results indicate a critical need for improved wastewater collection and treatment (nutrient removal and/or reuse) to moderate stormwater-related HABs in the future.

UNRAVELING THE MULTIFACETED EFFECTS OF CHANGING FLOW REGIMES AND HYDROLOGIC MODIFICATIONS ON CYANOBACTERIAL BLOOM POTENTIALS ALONG THE CAPE FEAR RIVER, NC

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Since 2009, the nutrient laden and hydrologically modified Cape Fear River, NC has experienced summertime blooms of the toxigenic cyanobacteria, Microcystis. Nutrient limitation bioassays indicate that nutrients are replete and the intrinsic growth rate of phytoplankton is light limited. River flow trends indicate an increasing frequency of summertime low flow events during which thermal stratification can develop upstream of run-of-river dams. At the population level, low flow provides longer transit times for biomass to accumulate. At the cellular level, low flow enhances light availability due to increased water transparency and shallower depths. Reduced vertical mixing and development of thermal stratification can increase or decrease light availability depending on cell buoyancy. A 1-D Lagrangian competition model of positively buoyant Microcystis and a negatively buoyant diatom was developed and reproduced the timing of observed Microcystis blooms based solely on river flow, temperature, and incident irradiance. Simulations of lower water levels due to removal of run-of-river dams significantly decreased the magnitude of Microcystis blooms and increased the relative abundance of diatoms. These positive effects on water quality were due to both a decrease in transit time and enhancement of vertical mixing in a shallower, faster-moving river.

INTERACTIVE EFFECTS OF SIMULATED HYPOXIA AND OCEAN ACIDIFICATION ON GROWTH OF THE HARMFUL DINOFLAGELLATE *AMPHIDINIUM CARTERAE*

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Ocean acidification and hypoxia frequently co-occur in coastal marine ecosystems. These combined stressors will likely become more intense and persistent with anthropogenic climate change. Although the separate effects of low seawater pH and low dissolved oxygen concentrations have been previously described for some marine phytoplankton, the interactive effects of ocean acidification and hypoxia on HABs are largely unknown. Here, factorial incubation experiments were performed using the harmful dinoflagellate *Amphidinium carterae* to examine the effects of ocean acidification and hypoxia both individually and in combination. Under both low pH and low dissolved oxygen concentrations, synergistically negative effects were observed for *A. carterae* growth and carbon metabolism. These findings suggest that *in vitro* RuBisCO specificity may not predict *in vivo* organismal responses to dissolved CO_2 and O_2 concentrations. In addition, the combined effects of ocean acidification and hypoxia may strongly impact the growth of harmful dinoflagellates in coastal marine ecosystems.

EARLY ONSET OF A MICROCYSTIN-PRODUCING CYANOBACTERIAL BLOOM IN THE MAUMEE RIVER (OH, USA)

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In late May 2016, a cyanobacterial harmful algal bloom (cHAB) was detected in the Maumee River, the largest tributary to Lake Erie. Testing on 31 May identified *Planktothrix agardhii* as the dominant cyanobacterium with cell abundance exceeding 1.7×10^9 cells/L and total microcystins (MC) reaching 19 μg/L MC-LR equivalents, a level over 10-fold higher than the 2015 revised U.S. Environmental Protection Agency national health advisory levels for drinking water exposure to adults. Low river discharge coincident with negligible precipitation through the latter half of May coincided with an 80% decline in river turbidity that likely favored bloom formation. Likewise, also contributing to the cHAB was a doubling of the river temperature from 13 °C to 26 °C over this same period. The bloom persisted through 5 June with microcystins exceeding 22 μg/L MC-LR equivalents at the bloom peak. By 6 June, the river had returned to its muddy character following a rain event and sampling on 7 June detected only low levels of toxin (<0.6 μg/L) at public water systems located near the bloom origin. The elevated toxin production associated with this early onset bloom was without precedent for the Maumee River. Whereas *Planktothrix* spp. is common in lotic environments, and has been previously detected in the Maumee, blooms are not commonly reported. This early onset, microcystin-producing cHAB provided a rare opportunity to glean insights into environmental factors that promote bloom development and dominance by toxin-producing genotypes of *Planktothrix* in lotic environments.

PSEUDO-NITZSCHIA SPECIES DETECTED IN BARNEGAT BAY-LITTLE EGG HARBOR, NEW JERSEY DURING 2012-2014

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Phytoplankton community was investigated in Barnegat Bay-Little Egg Harbor, New Jersey from 2012 to 2014, coincident with the New Jersey DEP's water quality monitoring. During the investigation, monthly and biweekly water samples were analyzed for phytoplankton species composition and cell density. *Pseudo-nitzschia* species were detected most often during the 3-year study in areas near the Barnegat Inlet and in the Little-Egg Harbor. The occurrence, abundance and species of *Pseudo-nitzschia*, however, varied in different years. In 2012, *Pseudo-nitzschia* spp. were found in about 20% of total samples with cell density magnitude mainly between 10^5 to 10^6 per liter. Much lower density (10^3 to 10^4 per liter) of *Pseudo-nitzschia* was observed in 2013 following Hurricane Sandy, likely related to the abrupt disturbance caused by the storm. *Pseudo-nitzschia* was detected in only a few 2014 samples with low density (10^3 per liter), except for one sample collected from south of the Barnegat Inlet which contained an abundance of *Pseudo-nitzschia* spp. of 10^6 cells per liter. Based on the light microscopic identification, we distinguished at least five different species of *Pseudo-nitzschia*, and the co-existence of two or three species was observed. These preliminary results indicate a diverse group of *Pseudo-nitzschia* in New Jersey coastal waters. Further examination of the taxonomy of *Pseudo-nitzschia* species and multivariate analyses of their relationships to environmental variables are underway.

HAB AT COAST OF BAJA CALIFORNIA: THE ROLE OF IRON IN POTENTIAL ALGAE-BACTERIAL MUTUALISM

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Increasing evidence has shown that iron is an important and often growth limiting nutrient for phytoplankton. Despite its necessity, iron is not only at low concentrations but also mostly biochemically unavailable under the oceanic alkaline aerobic conditions. Thus how phytoplankton including HAB species effectively acquire iron from such difficult environment is an interesting and yet unanswered question. Unlike bacteria, phytoplankton have not been proven to produce, nor directly utilize, iron chelating molecules such as siderophores for their iron acquisition which is a common practice for the former. A variety of potential iron uptake mechanisms for phytoplankton have been proposed which include a) iron reductive route via a cell surface reductase or b) direct iron uptake via cell surface enhanced processes. A third possibility is the indirect utilization of iron from unique marine siderophores such as vibrioferrin, which can readily reduce insoluble ferric iron quickly to more soluble ferrous iron due to its high photo-reactivity and weak iron biding affinity. In theory, the reduced iron made available by vibrioferrin producing bacteria could be utilized by phytoplankton. Our hypothesis is that certain phytoplankton are commensally associated with vibrioferrin producing bacteria for a benefit of their iron acquisition. To support our hypothesis, we present *in vivo* assay of Baja California coast samples collected during a bloom of spring 2017 to correlate abundance of iron, phytoplankton, and siderophore producing bacteria. Additionally, we present *in vitro* iron uptake assay of *Lingulodinium polyedrum*, one of the common bloom species in Southern California coast.

INVESTIGATING VERTICAL AND TEMPORAL HETEROGENIETIES OF CYANOBACTERIA BLOOMS USING A LONG TERM IN-SITU MONITORING STATION

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Cyanobacteria blooms are a global nuisance to freshwater bodies as they are capable of producing toxins which are harmful to wildlife and humans. Risk of contamination is compounded by the spatial and temporal unpredictability of cyanobacteria blooms. Because of this transient nature, long term and high resolution monitoring is necessary to capture the temporal and spatial dynamics of cyanobacteria blooms, in order to, develop relationships to understand what is driving them and predict when and where they will occur. To do this an *in situ* research station monitoring full depth water quality (hourly) and meteorological conditions (5 minutes) was deployed in a Minnesota lake during the summer of 2016. This research station observed temporal and vertical heterogeneities of cyanobacteria on a full depth and seasonal scale. A bloom of cyanobacteria, recorded from mid-July to the end of September, was shown to be driven by prolonged strong stratification within the water column, high surface water temperatures and high phosphate concentrations. High correlations among cyanobacteria biovolume, BV, stratification, surface water temperature, and stratification stability were used to develop a predictive relationship to determine how the thermal structure in the water column drives BV heterogeneities. The presence and location of maximum BV accumulation was determined to be driven by diurnal relationships. Finally, BV peaks at deeper levels of the lake coincided at the depth of PAR=10 $\mu E/m^2s$. These relationships explaining and predicting temporal and spatial heterogeneities are important to mitigate risks of contamination from drinking water, as well as recreation.

DISTRIBUTION OF CYANOBACTERIA IN LAKES AND RIVERS ON THE WEST COAST OF SOUTH AMERICA

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Blooms of toxic cyanobacteria are a problem worldwide. In Chile, these blooms have been reported since 1980 and are increasing in the central zone of the country, directly affecting water quality. In this study, phytoplankton samples of 23 water bodies (lotic and lentic) monitored by the General Water Directorate and by the EULA Center were examined. We found 10 water bodies with blooms (Maule River, Estero Llico, Vichuquén Lake, Torca Lagoon, Lencan Lagoon, Grande de San Pedro Lagoon, Lanalhue Lake, Lo Galindo, Lake Lloncao, Lake Villarrica), which are used for both recreational activities and drinking water. The most common genus was *Microcystis* (3 toxigenic species), followed by *Dolichospermum* (at least 4 toxigenic species). The -LA and -RR congeners of microcystin were the most common toxin reported. Cyanobacterial blooms occurred mainly in summer, although there were blooms at low temperatures in winter. Predictions of climate change in the center-south of the country indicate that there will be an increase in distribution and frequency of blooms. Chile does not have specific regulations for cyanobacteria or cyanotoxins in either recreational or drinking waters. Our results indicate that blooms of toxic cyanobacteria in Chilean waters are an emerging threat and a potential risk to the health of animals, including humans. These data are essential to begin a dialogue on managing the problem at the national level.

CONSIDERATION OF HARMFUL ALGAL BLOOMS IN THE U.S. ENVIRONMENTAL PROTECTION AGENCY'S REVIEW OF THE NATIONAL AMBIENT AIR QUALITY STANDARD FOR OXIDES OF NITROGEN-ECOLOGICAL CRITERIA

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The United States Environmental Protection Agency (EPA) is mandated under the Clean Air Act to periodically review the adequacy of the National Ambient Air Quality Standards (NAAQS) for human health and welfare, including effects on water and wildlife. The scientific foundation for review of the NAAQS is the Integrated Science Assessment (ISA), containing a synthesis and evaluation of the most policy-relevant science developed from a systematic literature review. Atmospheric deposition of oxides of nitrogen (NOx), one of six pollutants regulated under the NAAQS, combined with other nitrogen sources contributing to total nutrient loading in freshwater and coastal environments, can lead to harmful algal blooms (HABs). Atmospheric deposition typically represents <50% of total nitrogen contributions in estuaries, although it can be higher in some locations, representing an opportunity for mitigation. Over the last few decades in the U.S., atmospheric deposition of reduced nitrogen has increased relative to oxidized nitrogen in parts of the country, including the East and Midwest. Since the 2008 ISA for Oxides of Nitrogen and Sulfur - Ecological Effects, the role of nitrogen in stimulating algal blooms has been further characterized. Evidence from several recent studies indicates that availability and form of nitrogen influence freshwater algal bloom composition and inputs of inorganic nitrogen selectively favor some HAB species. The coastal systems evidence is stronger for atmospheric nitrogen deposition and HAB formation. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.

ENHANCED SEDIMENT TOXICITY ON GROWTH OF THE HARMFUL DINOFLAGELLATE *COCHLODINIUM POLYKRIKOIDES* UNDER SIMULATED OCEAN ACIDIFICATION

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Coastal waters of the Persian Gulf are strongly influenced by suspended sediments and may be particularly sensitive to ocean acidification conditions. However, the combined effects of suspended sediment loads and decreased seawater pH on the growth of harmful marine phytoplankton are not well understood. Here, incubation experiments were performed using *Cochlodinium polykrikoides*, a harmful dinoflagellate observed in the Persian Gulf and Sea of Oman in 2008-2009. Under low pH conditions, dissolved nickel concentrations from sediments increased over time and corresponded to significant decreases in *C. polykrikoides* growth rates. In addition, the response of *C. polykrikoides* to nickel was dose-dependent. These findings suggest that elevated dissolved nickel supplied from some sediments under ocean acidification conditions may significantly impact the growth of harmful dinoflagellates in the Persian Gulf, as well as in other coastal marine ecosystems.

DETECTION OF *VIBRIO PARAHAEMOLYTICUS* AND HARMFUL ALGAL SPECIES IN *CRASSOSTREA VIRGINIA* IN THE DELAWARE INLAND BAYS

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The Delaware Inland Bays (DIBS) are a collection of salt marshes, saltwater creeks, and shallow open waters. Over the years, the ecological health of certain areas in the DIBs has deteriorated because of poor water quality. Historically, oyster reefs were evenly distributed in the DIBS, but oyster disease caused the population to collapse in the 1950s. In 2013, the Delaware State Legislature instructed the Delaware Department of Natural Resources and Environmental Control to create an oyster aquaculture industry. Recent studies have suggested an association between the abundance of phytoplankton and the pathogenic bacterium *Vibrio parahaemolyticus* in the DIBs. The presence of *V. parahaemolyticus* and several harmful algal bloom (HAB) species causes concerns for the proposed aquaculture sites due to environmental and human health risks associated with these organisms. We are examining the areas near proposed aquaculture sites to determine the impacts of water quality and proliferation of pathogenic bacteria on oyster aquaculture. Using a combination of microscopy and PCR-based screening methodologies, we are comparing the HAB and bacteria communities residing inside the oyster, *Crassostrea virginica,* to environmental water samples. A number of HAB species, such as *Karlodinium veneficum, Dinophysis spp.*, *Heterosigma akashiwo* and *Chattonella subsalsa,* were found in the DIBs during year one of the study. *V. parahaemolyticus* was detected both in the water samples and *C. virginica* gut content samples. We will present data on HAB and pathogenic bacteria species from both water and oyster samples that analyzes potential human health risks at the proposed DIB oyster aquaculture sites.

COMMUNITY-BASED APPROACH TO MITIGATION OF PSP RISK IN SOUTHEAST ALASKA

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The receptor binding assay for detecting saxitoxin-activity in shellfish is an Interstate Shellfish Sanitation Conference (ISSC) accepted method for the analysis of mussels, clams and scallops. The Southeast Alaska Tribal Ocean Research (SEATOR) partnership has adopted a community-based approach to biotoxin management by utilizing a network of tribes that monitor for the presence of harmful algal species combined with saxitoxin analysis of shellfish species targeted for subsistence harvests. This network and its approach is based on a model proposed by Trainer et al. (2014). Identifying the presence of HAB species and their toxins in shellfish complements the regulatory testing of commercial harvests for paralytic shellfish toxins conducted by the Alaska Department of Health and Social Services. The Sitka Tribe of Alaska Environmental Research Laboratory uses the saxitoxin receptor binding assay to provide information on biotoxin risk to the SEATOR partnership. While many sites and shellfish species have been found to be below regulatory limits for saxitoxin contamination, potentially lethal levels ($>2500\mu gSTX/100g$ shellfish) of saxitoxin-like activity were measured in shellfish from two Southeast Alaska sites in May and June. Blue mussels and butter clams from these sites were found to contain up to 46 times the regulatory limit for saxitoxins. Although this information is not used to generate harvest closures, the results from saxitoxin testing are shared with community members providing potentially lifesaving information about the risks from algal toxins associated with consumption of shellfish collected during subsistence harvests.

HARMFUL ALGAL BLOOMS AND CULTURAL TIES

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Lummi Bay, located in Bellingham, Washington that surrounds the Lummi people and its Nation. Many Coast Salish tribes, including Lummi Nation, have fished and harvested from the Salish Sea for thousands of years. Today fishing and shellfish harvesting is still relevant for food, potlatch and ceremony purposes. Native people have always known about red tide (harmful algae), knowledge of red tide has been passed down with each generation and some of the teachings include what part of the shellfish to harvest when there is a bloom to minimize exposure to toxins. With excess nutrients entering the sea, such as pet waste, sewage, and fertilizers, along with heavy rainfall and warmer temperatures, harmful algal blooms are expected to increase in size, duration, and consequences. Many tribal and state agencies, in stewardship for their land, monitor for harmful algal blooms and toxins. Native Americans that live and work along the Salish Sea and harvest shellfish for subsistence are feeling the effects of harmful algae, where more harvesting closures means no work or food on the table. The Salish Sea Research Center at Northwest Indian College is supporting tribal monitoring, while working with Lummi Natural Resources, by including passive toxin tracking. This may provide early evidence of toxin contamination in the water column before it reaches shellfish, and decrease closure times due to toxigenic harmful algae by providing more data points and faster turn around time. Here we present our long-term monitoring plan and preliminary data while focusing on the cultural ties that the Lummi people have with harmful algae and shellfish harvesting.

HARMFUL ALGAL BLOOM AND CYANOTOXIN RISK MANAGEMENT IN HAMILTON HARBOUR AND ASSOCIATED BEACHES

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Harmful algal blooms present a risk to the safety of drinking and recreational waters including beachfronts through the production of toxins. Blooms are difficult to predict and show significant spatial heterogeneity in appearance and toxicity. The demand for reliable, cost-effective and rapid methods to detect toxins and reduce risk of public exposure cannot be met by most analytical lab turnaround times. Commercial cyanotoxin test kits are now available, but few have been rigorously field-tested or incorporated into monitoring programmes. Working with a local health agency, two kits with different operative ranges of detection for the hepatotoxins microcystins were evaluated using samples covering a wide range of water quality conditions, sample matrices, and bloom composition. The performance was compared against lab analyses using Enzyme-Linked Immunosorbent and Protein Phosphatase Inhibition assays. Both kits could resolve samples with high (<10 µg/L microcystin equivalents) and low/no toxins, but failed to reliably detect levels between 1 and 5 µg/L, at which threshold there were few false negatives (8%) but approximately one third of samples (32%) yielded false positives. Therefore, these kits are useful for screening and informed risk management decisions e.g. beach closures, but should be followed up with more rigorous tests where needed. The kits have been successfully incorporated into the routine municipal beach monitoring and advisory programme by the Hamilton Public Health Services (Ontario). Test kits for neurotoxins also exist and the recent report of anatoxin at the beaches provides an opportunity to similarly evaluate the efficacy of this type of test kit.

Assessing Microcystins in Lake Champlain Fish: A Potential Risk to Consumers?

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Microcystins (MCs), a class of cyclic heptapeptide toxins produced by cyanobacteria, present a variety of risk factors to consumers. While the risk of exposure to MCs through drinking water is well characterized, less is known about fish consumption as an exposure pathway. Seasonal safe consumption guidelines of fish have been suggested as 300 ug kg^{-1} and 40 ug kg^{-1} of MCs in fish tissue for adults and children respectively (Ibelings and Chorus, 2007, Environmental Pollution). Previous studies have indicated that MC concentrations vary greatly both within and between species. This variation, which may be due to behavioral and physiological differences in fish and temporal variation in blooms, makes risk assessment difficult. Lake Champlain has a documented history of cyanobacteria blooms, especially in eutrophic Missisquoi Bay; it is unknown if fish in the lake would exceed consumption guidelines. Paired liver and muscle samples were collected from 191 fish caught during Lake Champlain fishing derbies in 2016. Samples were extracted, purified with solid phase extraction, and analyzed with tandem mass spectroscopy (LC-MS/MS) for three MC congeners common within lake (LR, RR, and mRR). Internal standard recoveries ranged between 47% and 123% with an average of 76%. All samples were negative for MCs suggesting there was a low risk to consumers during the 2016 Lake Champlain bloom season.

Monitoring, Mitigation and Management of HABS Part 2

Wednesday Presentations

EXPANDING THE NETWORK OF IMAGING FLOW CYTOBOTS FOR EARLY WARNING OF HABS

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The time series at TOAST (Texas Observatory for Algal Succession Time series) has completed ten years at Port Aransas and a second site at Surfside Beach (205 km northeast) was added in May 2017. Preliminary observations during the first month of operation of the two locations have allowed comparisons of community composition between sites. Differences in the overall community composition and in the timing of individual species occurrences at the two locations suggest limited connectivity between these two sites. Together with a Texas-Louisiana shelf model of coastal circulation (pong.tamu.edu), the impact of forcing mechanisms such as wind and river discharge on bloom initiation will be compared to identify commonalities and differences in bloom initiation by simulating drifters using the model output. The Imaging FlowCytobot in Port Aransas has provided successful early warning for eight HAB events in the central Texas region; however, the locations and extent of HABs on the TX coast can vary among years. Thus, having a second site will facilitate improved early warning of HABs for the Texas coast.

DECADAL TRENDS IN OCEAN WARMING WITHIN TEMPERATE COASTAL OCEANS CONTRIBUTES TO INCREASED GROWTH, DISTRIBUTION, AND INTENSITY OF COCHLODINIUM POLYRKIKOIDES BLOOMS

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Identified in 1895, *Cochlodinium* spp. have been reported along North American and East Asian coastlines for many decades. Blooms, where present, have deleterious effects on marine life, resulting in severe economic losses to fisheries and aquaculture industries. Since the early 1990's, blooms have become more frequent, intense, and widespread across the Northern Hemisphere. The environmental mechanisms responsible for these trends, however, are not fully understood. Here we present modeled trends in growth rates and bloom season using high-resolution sea surface temperature records to identify effects of ocean warming on the persistence of *C. polykrikoides* blooms within temperate coastal oceans. Along the Northeast US coastline, significant expansions of the bloom season were observed with bloom favorable conditions becoming established earlier and persisting longer. In addition, warmer sea surface temperatures had significant positive effects on the growth rates of *C. polykrikoides* in areas ranging from Cape Cod to the southern extent of Chesapeake Bay, areas where annual blooms have become established this century. Similar trends were observed for regions within the Sea of Japan whereby mean potential growth and bloom season displayed significant positive correlation with rising sea surface temperatures since 1982 and documented bloom events have become more intense. Results suggest that warmer sea surface temperatures have expanded the fundamental niche of *C. polykrikoides,* blooms allowing them to initiate earlier and persist longer in North America and East Asia. As a result, the adverse economic and ecological effects of these blooms may become more pronounced as climate changes continue.

A 15-YEAR SUMMARY OF SOUTH CAROLINA HARMFUL ALGAL BLOOMS AND FISH KILLS: MONITORING, RESPONSE, AND OUTREACH

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South Carolina (SC) has one of the most rapid coastal population growth and urbanization rates in the nation. As a consequence, elevated stormwater runoff has increased the transport and volume of non-point source nutrients into receiving waters. To mitigate runoff, stormwater ponds are constructed as a best management practice (BMP) to reduce flooding and offset impervious surface cover. There are >21,000 coastal stormwater ponds in coastal SC alone, and development is ongoing. These systems are particularly susceptible to fish kills, and in SC, coastal fish kills are widespread, particularly in residential/recreational ponds. Harmful algal blooms (HABs) have been associated with one in four (27%) of SC coastal fish kills since 2001, with responses exceeding >1,100 separate events (excluding follow-up sampling), and dense, toxic, and pervasive HABs spanning numerous phytoplankton taxa commonly recur in stormwater ponds. Responses include site assessments, environmental sampling, and addressing public concerns about water quality. In this presentation, we present a 15-year (2001-2016) synthesis of SC fish kill and HAB responses. Specifically, we describe our coastal monitoring program, our evaluations of fish kills associated with HABs (quantified using microscopy, pigment, and molecular techniques), hypoxia, and other causes, HAB toxins, nutrients (particularly nitrogen and phosphorus), and other environmental parameters. We also highlight public outreach to engage managers, landowners, and other stakeholders in how to recognize, report, and prevent future fish kills and algal blooms.

Preferred format: Poster or Speed Talk/Poster

OVERVIEW OF U.S. EPA OFFICE OF RESEARCH AND DEVELOPMENT'S RESEARCH ON ANALYSIS AND MONITORING OF FRESH WATER AND COASTAL/ESTUARINE ENVIRONMENTS

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Several factors contribute to the increased frequency and magnitude of harmful algal blooms (HABs). Elevated temperatures, changes in precipitation, population demographics, agricultural land use linked to nitrogen loading increases, and an aging water treatment infrastructure all combine to increase the probability of toxins being present in consumers' tap water. Several notable HAB events have impacted a number of states. In 2014, a HAB event on Lake Erie contaminated the city of Toledo's drinking water supply with elevated levels of microcystins. In 2015 an algal bloom occurred along the Ohio River spanning over 680 miles. In 2016 Lake Okeechobee experienced an algal bloom that impacted the Caloosahatchee and St. Lucie River systems. As a result of the human, ecological, and economic impacts of HABs, the U.S. EPA has established a research program to address issues related to the detection, quantification, and monitoring of algal blooms. This research has several objectives: develop new or refine existing chemical, instrumental, and biological methods for detection of HABs and their toxins; test such methods in field studies; determine method(s) best used as early warning systems for detection of HABs and their toxins. This presentation focuses on the non-targeted analysis of algal products by mass spectrometry, but will also summarize current research on molecular detection methods of HAB species and genes responsible for toxin production, in vitro methods for detection of toxicity, multiple approaches for phyto/zooplankton identification, phone apps for HABs early warning and cyanobacterial and algal identification, and advanced instrumental and hyperspectral image analysis approaches.

SPATIOTEMPORAL SURVEY OF THE 2016 LAKE OKEECHOBEE AND ST. LUCIE RIVER CYANOBACTERIAL BLOOM

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In late June, 2016, a cyanobacterial harmful algal bloom (cyanoHAB) was transported from Lake Okeechobee (LO) through the St. Lucie River (SLR) into the coastal Atlantic Ocean causing environmental, recreational and economic issues. While cyanoHABs have been a common occurrence in LO and the connecting waterways since the early 1980s, this event attracted national interest and initiated a state of emergency for two counties. In response to this, spatial surveys were conducted from LO to the SLR in early July and late September. Samples were collected for general physiochemical parameters, algal pigments, phytoplankton community composition, molecular analysis (qPCR and metagenomics) and nutrient amendment experiments. Results indicated that *Microcystis* was the dominant cyanobacterium but other potentially-toxic cyanobacteria were also present (e.g. *Cylindrospermopsis, Cuspidothrix* and *Dolichospermum*). Molecular analysis of the July samples indicated that the bloom was comprised of both potential-microcystins and potential-saxitoxins producers with the saxitoxins producers being two orders of magnitude less than the microcystins producers. Metagenomic analysis suggested that *Microcystis* was the likely microcystin-producer whereas *Dolichospermum* was the likely saxitoxin-producer. ELISA toxin analyses revealed that microcystins were detected at up to 34 µg L^{-1} in July but levels were low in September and saxitoxins were below detection in all samples. Inorganic N:P ratios suggested nitrogen limitation across the SLR, a finding confirmed by nutrient amendment experiments that revealed the cyanobacterial community was promoted by nitrogen, but not phosphorus. Collectively, this study demonstrates that the 2016 cyanoHAB in LO and the SLR was toxic, dominated by *Microcystis* and nitrogen limited.

SPATIAL AND TEMPORAL VARIATION IN MICROCYSTIN OCCURRENCE IN WADEABLE STREAMS IN THE SOUTHEASTERN UNITED STATES

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Wadeable streams have historically been overlooked as a source of cyanobacterial harmful algal blooms (cyanoHABs) and their associated toxins. However, historical evaluation of stream periphyton in the Southeastern United States revealed potential cyanotoxin producers present in 74% of headwater streams sampled by USGS between 1993 and 2011 in Alabama, Georgia, South Carolina, and North Carolina. Benthic, filamentous, potential cyanotoxin-producers dominated the cyanobacteria assemblages and included species of *Leptolyngbya, Phormidium, Pseudanabaena,* and *Anabaena, which* are known to produce microcystins as well as other cyanotoxins in a few cases. A follow up survey of 75 wadeable streams in the same Piedmont region of the Southeastern U.S. was conducted in June of 2014 where microcystins were detected in 39% of sampled waters with a maximum observed concentration of 3.2 µg/L. Microcystin concentrations were significantly ($\alpha=0.05$) correlated with stream flow, total nitrogen to total phosphorus ratios, and water temperature, however each factor independently explained 38% of the variability or less. Six sites were sampled monthly through October 2014 revealed a persistent occurrence of microcystins. These fluvial microcystin results have implications for understanding the algal loading hypothesis, the impact downstream toxin transport may have at the land-sea interface, and an identified need to better understand ecological exposure to microcystins through food web dynamics for chronic, low-level exposure.

TOXIC *PLANKTOTHRIX* BLOOMS IN THE LAKE ERIE WATERSHED

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Whereas much attention has been devoted to studying the *Microcystis* harmful algal bloom (HAB) events in the lower Laurentian Great Lakes, tributary sites are often affected by persistent blooms of *Planktothrix*, a filamentous microcystin-producing cyanobacterium. Our labs are examining the factors that allow these organisms to occupy different ecological niches. Regarding *Planktothrix*, HABs are typically of longer duration (May-October), routinely yielding microcystin levels in excess of 20 ppb. n agreement with prior studies on the *Planktothrix* bloom in Grand Lake St. Marys (OH), Sandusky Bay midsummer dissolved inorganic N concentrations often fall below detection, and bottle assays indicate that enhancement of summertime bloom conditions is often dependent on N additions, rather than P. Losses of N in Sandusky Bay are attributed in part to high rates of denitrification in the sediments. We hypothesize that N availability is a major driver of *Planktothrix* dominance in the Bay, distinct from open water *Microcystis* HABs in which P is traditionally viewed as the key factor for biomass production. Indeed, work in other labs has shown that *Microcystis* is an effective scavenger for P. The success of *Planktothrix* in an N-limited system is notable given that this genus is not an N fixer, but metatranscriptomic analyses reveal a minor cyanobacterial community of N fixers contributing new N into the system. Analysis of Sandusky Bay blooms by qPCR and 16S iTags also reveals that *Planktothrix* presence and abundance is correlated with N speciation.

Lake Harsha: Three Years of HABs Monitoring

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USEPA's Office of Research and Development has partnered with the Clermont County, OH Water Resources Department in an on-going study to assess HAB trends and develop monitoring tools and approaches. Lake Harsha, a multi-use reservoir and primary drinking water source in southwest OH, has experienced an increase in cyanoHAB frequency and intensity over the past several decades. The goal of this work is to develop relationships between HAB indicator measures and cyanotoxin occurrence which can provide time-relevant information regarding source water quality for DWTP operators and other public health stakeholders. In order to characterize the cyanobacterial population and both intracellular and extracellular cyanotoxin production, an intensive sampling regime was implemented beginning in 2015. High frequency (HF) monitoring (in-vivo fluorescence and physico-chemical parameters) was used to determine the timing and rate of discrete sampling of cyanotoxins (LC-MSMS analysis of select MC congeners and a total MC surrogate, cylindrospermopsin, and anatoxin-a, MC ELISA), molecular indicators, taxonomic enumeration, and nutrients, varying from biweekly, to daily collection during the observed bloom peak. Resulting data provide a time-series of the cyanobacterial population dynamic and greatest periods of cyanotoxin production. HF data coupled with cyanotoxin analyses demonstrate the utility of HF data for tracking the cyanoHAB status of the reservoir. It is also apparent that cyanotoxin concentrations may potentially be underestimated if cyanotoxin sampling is not coupled with bloom status. Data and observations from 2015 to the present will be presented.

USING HISTORICAL DATA FROM 20 MIDWESTERN USA RESERVOIRS TO IDENTIFY TRENDS IN HARMFUL CYANOBACTERIA BLOOMS AND FACTORS AFFECTING THEIR SEVERITY

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Water quality and cyanobacteria densities from 1988-2015 were compiled for 20 Midwestern USA reservoirs. Maximum summer cyanobacteria densities were dominated by taxa capable of producing toxins and increased over the last 7-15 years of the record, with greatest increases typically observed in reservoirs with low watershed forest cover. In 2015, 17 reservoirs experienced densities posing high probabilities of human health risks (>100 million cells/liter). In watersheds dominated by agricultural land cover, longer water residence times were associated with greater increases in cyanobacteria densities over time. Summer months had little increase in surface water temperature over the 27-year record, but May temperatures increased with deeper reservoirs warming the most (1–2 degrees Celsius/decade). In one reservoir (analysis ongoing for others), mean dissolved oxygen in May increased in the epilimnion (8.3 to 10.8 mg/L) and decreased in the hypolimnion (5.4 to 2.9 mg/L) since 1989, indicating earlier increases in productivity and onset of deep water anoxia. Weaker stratification occurred in summers with greater precipitation and was associated with warmer hypolimnetic water temperatures and higher cyanobacteria densities. Earlier seasonal warming of surface waters, the amount of precipitation, and physical characteristics of reservoirs likely contribute to differences in stratification regimes among reservoirs and interact with how watershed land cover and nutrients affect the severity of cyanobacteria blooms and subsequent human health risks. In addition to describing trends in harmful algal blooms, these results and ongoing work can be used to inform predictive models and future management decisions.

CYANOBACTERIAL HARMFUL ALGAL BLOOMS AND U.S. GEOLOGICAL SURVEY SCIENCE CAPABILITIES

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Cyanobacterial harmful algal blooms (CyanoHABs) are increasingly a global concern because CyanoHABs pose a threat to human and aquatic ecosystem health and cause economic damages. Despite advances in scientific understanding of cyanobacteria and associated compounds, many unanswered questions remain about occurrence, environmental triggers for toxicity, and the ability to predict timing, duration, and toxicity of CyanoHABs. Scientific data and mechanistic understanding of environmental factors and adverse health effects related to cyanotoxin exposure are required to develop reliable early warning systems and predictive tools that guide management decisions. Advanced warning at time scales relevant to CyanoHAB management, allow proactive, rather than reactive, responses to CyanoHAB events. U.S. Geological Survey (USGS) scientists are leading a diverse range of studies to address CyanoHAB issues in water bodies throughout the Nation, including: developing field and laboratory methods to identify and quantify cyanobacteria and associated compounds; understanding causal factors, environmental fate and transport, ecological processes, and effects of environmental exposure; and developing early warning systems for potentially harmful blooms. Study approaches use a combination of traditional methods and emerging technologies, including advanced analytical techniques, stable isotopes, molecular techniques, sensor technology, and satellite imagery. Studies range in scale and include laboratory experiments, individual water body research, and studies that are regional or national in scope, and are completed in collaboration with local, State, Federal, Tribal, university, and industry partners. By providing practical applications of cutting edge CyanoHAB research, USGS studies have advanced scientific understanding, enabling the development of approaches to help protect ecological and human health.

OCCURRENCE OF MICROCYSTIN CONTAMINATION IN BLUE-GREEN ALGAL DIETARY SUPPLEMENTS IN THE UNITED STATES AND THE EVALUATION OF A COMMERCIALLY AVAILABLE PROTEIN PHOSPHATASE INHIBITION ASSAY (PPIA) SCREENING KIT.

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The cyanobacterium *Aphanizomenon flos-aquae* (AFA), from Upper Klamath Lake, Oregon, are used in blue-green algal (BGA) dietary supplements. The periodic co-occurrence of hepatotoxin-producing contaminant species prompted the Oregon Health Division to establish a limit of 1 μg/g microcystin (MC) for products sold in Oregon. Despite this, international surveys have reported MC in excess of this limit in AFA-BGA products. The objective of this study was to identify a screening method and assess the occurrence of MC contamination in AFA-BGA products in the U.S. A commercially available protein phosphatase inhibition assay (PPIA) was evaluated and used to screen AFA-BGA products for MC contamination. The PPIA kit had acceptable cross-reactivity and accuracy for MC-LR, RR, LA, YR, LY, and nodularin, but overestimated MC-LW and underestimated MC-LF by 2-fold at the 1 μg/g limit. Using the PPIA kit, 51% (26 of 51) distinct AFA-BGA products had MC contamination above the detection limit of the kit (0.25 μg/g), 10 products contained MC between 0.5-1.0 μg/g, and 4 products were in excess of the Oregon limit (1.1-2.8 μg/g). LC-MS/MS confirmed PPIA results $\geq$0.5 μg/g and determined that MC-LR and MC-LA were the main congeners present. PPIA is a reliable screening method for the detection of MC contamination in dried BGA dietary supplements. While the majority of AFA-BGA products did contain > 0.25 μg/g MC, most were close to or just below the Oregon limit, but variability in recommended serving sizes prevented further analysis of consumer exposure based on the levels of MC contamination found.

HOW TO BETTER TARGET HAB RESEARCH BY UNDERSTANDING THE ROLE OF THE INTERSTATE SHELLFISH SANITATION CONFERENCE

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Over the years, many funding proposals submitted to the NOAA ECOHAB, MERHAB, and PCMHAB programs claim to be developing methods for HAB cell and toxin detection that will be useful to resource and public health managers responsible for ensuring seafood safety. Often the connection to managers is tenuous and the resulting methods are not fit for purpose. In part this is because HAB researchers often do not understand how shellfish safety is managed and regulated.

Established guidance levels and approved methods for HAB toxins in shellfish are provided in the National Shellfish Sanitation Program (NSSP) Guide for the Control of Molluscan Shellfish, which is updated every two years by the Interstate Shellfish Sanitation Conference (ISSC). The ISSC fosters and promotes shellfish sanitation through cooperation of FDA, NOAA, EPA, states, industry, and academia. New methods to be incorporated into the NSSP are evaluated by a rigorous process that starts with pre-proposals being submitted to the ISSC Laboratory Committee, following a specified format. This talk will provide a description of the kinds of methods that are used for marine biotoxin management and the process by which new methods are incorporated in the NSSP.

DINOFLAGELLATE DIVERSITY ALONG A SUBTROPICAL PERMANENTLY OPEN ESTUARY: EMPHASIS ON POTENTIAL HARMFUL SPECIES.

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Dinoflagellates are one of the most economically and ecologically important phytoplankton groups in aquatic systems as they are primary producers, heterotrophs and causative agents of harmful algae blooms. It is therefore imperative that in aquatic systems the potential harmful species are known and their driving factors understood. Dinoflagellate abundance, species composition and diversity was investigated in a subtropical permanently open estuary (Mlalazi, South Africa) to understand the variation of species with emphasis on harmful species. Microscopy was used to enumerate and identify cells alongside relevant literature. 42 dinoflagellate species, 10 of which are harmful, were recorded for a year. Species composition and abundance differed between the three sites [p=0.001, F=2.98] along estuary length and four seasons [p=0.001, F=2.55]. Total abundance ranged from 4.00×10^1-6.03×10^5 Cells/L with highest and lowest values in the middle and upper reaches respectively. The harmful *Prorocentrum cordatum* was most dominant and present in all sites and seasons. Amongst the sites, species richness and diversity ranged from 26-35 and 0.39-0.48 respectively. Richness was highest at the lower reach while diversity was highest in the middle reach. Some other dominant species recorded were *P. micans*, *P. triestinum*, *Scrippsiella spinifera*, *Protoperidinium steinii*, *Gyrodinium estuariale*, *Diplopsalis lenticulata*, *Gonyaulax spinifera* and *Peridinium quinquecorne*. Dinoflagellate composition and abundance in this subtropical permanently open estuary was influenced by temperature, turbidity and dissolved inorganic phosphorus and nitrogen. This study reveals the presence of harmful dinoflagellate species which have the potential to bloom with increase in dissolved inorganic nutrients, a common occurrence in our degrading estuaries.

DISTRIBUTION AND ABUNDANCE OF *ALEXANDRIUM CATENELLA* IN KACHEMAK BAY AND LOWER COOK INLET, ALASKA

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Despite the long history of paralytic shellfish poisoning (PSP) events in Alaska, little is known about *Alexandrium* distribution and abundance or environmental factors that control *Alexandrium* bloom development. To address this issue, we undertook a four-year (2012 – 2016) study in Kachemak Bay and Lower Cook Inlet, Alaska to determine how the distribution and abundance of *A. catenella*, the dominant *Alexandrium* species, was influenced by prevailing temperature, salinity, and nutrient conditions. *A. catenella* cell concentrations from 572 surface water samples were estimated using quantitative PCR. High frequency sampling revealed a cyclic pattern of *A. catenella* cell growth that was positively correlated with water temperature. Average water temperatures increased approximately 2°C over the course of the study and were accompanied by an increase in *Alexandrium* cell abundances and saxitoxin concentrations in shellfish. Prevailing salinity conditions did not significantly influence abundance, and residual nutrient concentrations indicated no evidence of nutrient limitation. Most of the samples (92%) contained low cell concentrations (≤ 10 cells L^{-1}), consistent with the historically low occurrence of PSP in this region of Alaska. Bloom concentrations were detected in 35 samples (8%) from Kachemak Bay (100 – 3,050 cells L^{-1}). In contrast, the highest cell concentration detected in Lower Cook Inlet was only 67 cells L^{-1}. These data indicate *A. catenella* blooms originate in Kachemak Bay and are not transported from Cook Inlet. This study demonstrated quantitative PCR assays are an effective monitoring tool for the *A. catenella* blooms that pose a substantial risk of paralytic shellfish poisoning in the region.

MORPHOLOGICAL, MOLECULAR, AND TOXIN ANALYSIS OF MID-ATLANTIC *DINOPHYSIS*

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Over the past decade shellfish harvesting area closures have occurred in the Gulf of Mexico, Puget Sound, and New England regions due to the detection of elevated concentrations of okadaic acid (OA) and dinophysistoxins (DTXs) produced by various species of *Dinophysis*. *Dinophysis* is an emerging harmful algal bloom (HAB) species in Mid-Atlantic coastal and intracoastal waterways in that these species and toxins are present, but have not resulted in illnesses or harvesting area closures to date. The morphological variability of several *Dinophysis* species exhibiting varying degrees of toxicity creates complications for HAB management programs responsible for monitoring *Dinophysis* and its toxins. Here we present a morphological, molecular, and toxin analysis of *Dinophysis* found within Mid-Atlantic waters from New Jersey to Virginia. The morphological variability of cells in the Mid-Atlantic *D. acuminata* and *D. norvegica* populations is illustrated with light and scanning electron microscopy and is compared to *D. acuminata*, *D. norvegica*, *D. ovum* and *D. sacculus* from other regions. Data from the analyses of ~2.0kb ribosomal DNA sequences indicates that Mid-Atlantic *D. norvegica* can be differentiated from *D. acuminata* using molecular analysis, but *D. acuminata* cannot be molecularly distinguished from the Mediterranean species *D. sacculus*. In addition, the toxin profile of the Mid-Atlantic *D. acuminata* population differs from other regions of the US. This work highlights the considerable intraspecific morphological variability of the *D. acuminata*-complex and the need to use morphological analysis, coupled with molecular and toxin analyses, to provide a comprehensive response to blooms of Mid-Atlantic *Dinophysis* populations.

EXPANDING ALGAL BLOOM MONITORING IN VIRGINIA: COLLABORATIVE RESPONSES TO FRESHWATER AND COASTAL HABS IN RECREATIONAL AND SHELLFISH WATERS

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The Virginia Harmful Algal Bloom Taskforce is a collaboration of state regulatory agencies and academic institutions which has addressed the growing concern of HABs throughout the commonwealth for 20 years, largely through long-term phytoplankton and water quality monitoring. These efforts have documented the presence of potentially toxic taxa in Chesapeake Bay including *Dinophysis* and *Pseudo-nitzschia* species along with increases in annual seasonal blooms of *Cochlodinium polykrikoides*, *Alexandrium monilatum* and other dinoflagellates. While comparatively less studied, recent surveys of Virginia freshwater recreational lakes, ponds and reservoirs have also identified potentially toxic cyanobacteria in a majority of the waters examined, including *Anabaena, Aphanizomenon, Cylindrospermopsis, Microcystis* and *Planktothrix* species. Building on the phytoplankton composition data, taskforce members have increasingly utilized analytical toxin monitoring to inform management decisions, including the use of both laboratory and field measurements by ELISA and other techniques. Most recently, solid phase adsorption toxin tracking in both estuarine and freshwater systems has been conducted to monitor toxin concentrations in dynamic systems that might otherwise be mischaracterized by traditional grab sampling. Ongoing efforts relating phytoplankton identification and enumeration data to toxin presence and concentrations work to inform risk assessments by management agencies to better protect public health.

MULTIPLE FRESHWATER AND MARINE ALGAL TOXINS AT THE LAND-SEA INTERFACE: IS BROADER MONITORING OF ALGAL TOXINS NECESSARY TO PROTECT NON-COMMERCIAL SHELLFISH HARVESTERS?

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The California Current spans nearly 3,000 km, from Southern British Columbia to Baja, California. Over the last few years, there have been multiple, dramatic algal toxin events within the California Current, including the 2015 Amnesic Shellfish Poisoning event, the emergence of Diarrhetic Shelfish Poisoning in the Pacific Northwest, and freshwater microcystin poisoning of California sea otters. These events have led to broad public, management, and scientific interest in algal toxins at the land-sea interface. Current management practices separate freshwater and marine harmful algae, and sample organisms or toxins that "should" be present, or have been historically problematic in water bodies. Here we look at San Francisco Bay, CA, USA, a region that has been monitored for harmful algae for more than 20 years, but historically has been assumed not to have harmful algal bloom problems. From 2011–2016 we used particulate, dissolved, and shellfish sampling to identify that multiple toxins are present nearly all the time, and that at least four toxins are present simultaneously in 37% of our sampled shellfish, including domoic acid, microcystins, Paralytic and *Dinophysis* Shellfish Toxins. Here we discuss what chronic or acute toxin exposure means, and ask the question: is broader monitoring necessary to protect subsistence, ceremonial, and recreational shellfish harvesters? These findings explore improved monitoring and management efforts to deal with the simultaneous presence of multiple, chronic toxins.

HARMFUL ALGAL BLOOMS: ROLE OF THE NATIONAL PARK SERVICE IN PROTECTING RESOURCES

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Harmful Algal Blooms (HAB) can result in negative impacts on fresh and salt water ecosystems by depleting oxygen in aquatic environments and releasing toxins, which have the ability to physically injure fish, wildlife, pets, or humans, and compromise environmental quality. HABs impose challenges to our ability to safeguard the health of our nation's coastal and freshwater natural resources, including those inside or surrounding national parks. The mission of the National Park Service is to "preserve unimpaired the natural and cultural resources and values of the National Park System for the enjoyment, education, and inspiration of this and future generations." HABs present a new challenge for up to protect the resources inside national parks. Recently, there has been a marked increase in the number, frequency, and duration of HAB events in U.S. waters, including inside national parks. Effective management of HABs is critical to minimizing negative impacts to park system resources, values, and visitor health and safety. The NPS has recently introduced a new community of practice for NPS parks that deal with HAB and hypoxic issues. A new website was created by the Ocean and Coastal Resources Branch that will help parks respond to HAB events and provide resources and information for park managers. We are currently soliciting feedback from parks, regions, and partners on ways to help parks deal with HAB events. This presentation will also explain the role of the NPS in managing HABs within our parks.

CYANOBACTERIAL HARMFUL ALGAL BLOOMS AND U.S. GEOLOGICAL SURVEY SCIENCE CAPABILITIES

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Cyanobacterial harmful algal blooms (CyanoHABs) are increasingly a global concern because CyanoHABs pose a threat to human and aquatic ecosystem health and cause economic damages. Despite advances in scientific understanding of cyanobacteria and associated compounds, many unanswered questions remain about occurrence, environmental triggers for toxicity, and the ability to predict timing, duration, and toxicity of CyanoHABs. Scientific data and mechanistic understanding of environmental factors and adverse health effects related to cyanotoxin exposure are required to develop reliable early warning systems and predictive tools that guide management decisions. Advanced warning at time scales relevant to CyanoHAB management, allow proactive, rather than reactive, responses to CyanoHAB events. U.S. Geological Survey (USGS) scientists are leading a diverse range of studies to address CyanoHAB issues in water bodies throughout the Nation, including: developing field and laboratory methods to identify and quantify cyanobacteria and associated compounds; understanding causal factors, environmental fate and transport, ecological processes, and effects of environmental exposure; and developing early warning systems for potentially harmful blooms. Study approaches use a combination of traditional methods and emerging technologies, including advanced analytical techniques, stable isotopes, molecular techniques, sensor technology, and satellite imagery. Studies range in scale and include laboratory experiments, individual water body research, and studies that are regional or national in scope, and are completed in collaboration with local, State, Federal, Tribal, university, and industry partners. By providing practical applications of cutting edge CyanoHAB research, USGS studies have advanced scientific understanding, enabling the development of approaches to help protect ecological and human health.

CAN TASTE AND ODOR COMPOUNDS PREDICT HARMFUL ALGAL BLOOMS IN SURFACE DRINKING WATER SOURCES?

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Cyanobacteria threaten diverse aquatic ecosystems around the world, including drinking water sources, through the production of toxic secondary metabolites. Water resource managers need reliable tools to predict harmful algal blooms. Considering some cyanobacteria are also capable of producing taste and odor compounds, such as geosmin and 2-methylisoborneol (MIB), that cause an earthy or musty flavor in water, we used data from an ongoing large-scale survey of drinking water sources throughout Alabama to determine if geosmin and MIB concentrations could be used to predict microcystin concentrations. During the first four months of the project, microcystin has been generally low (<0.02 ug/L) to undetectable across ~70 surface water intakes in Alabama. Interestingly, geosmin has been persistent, albeit at low concentrations, during the entire project while MIB has only recently increased in concentrations across the study sites. If these pattern hold for the remainder of the project, taste and odor compounds are not expected to be valuable indicators of cyanobacterial toxicity. Such a finding would not be surprising given that other organisms, including actinobacteria, produce taste and odor compounds and that there appears to be an ecophysiological trade-off in the production of taste and odor compounds and toxic secondary metabolites within an organism.

COMPARISON BETWEEN QUANTITATIVE REAL TIME PCR (QPCR) AND SANDWICH HYBRIDIZATION ASSAY (SHA) FOR DETECTION AND QUANTIFICATION OF HETEROSIGMA AKASHIWO IN FIELD SAMPLES

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Quantitative real time PCR (qPCR) and Sandwich Hybridization Assay (SHA) have been independently developed for quantification of the harmful alga, *Heterosigma akashiwo*. These methods provide a powerful alternative to microscopy but differ in the how they determine cell abundance. qPCR is based on exponential amplification of extracted DNA, while SHA measures the target species' rRNA directly within a cellular homogenate. Past research using laboratory cultures showed significant correlation between qPCR and SHA, while highlighting shortcomings that may impact research or monitoring efforts: qPCR had a larger range of detection and greater sensitivity to detection at low cell abundance, while SHA showed a greater degree of precision when measuring replicate samples. Growth stage also affected results of SHA analysis, while nitrogen status affected both SHA and qPCR. Here, compare qPCR and SHA for quantification of *H. akashiwo* in field samples. As expected, both molecular methods were able to detect *H. akashiwo* at abundances below the level of detection for microscopy. qPCR and SHA results were significantly correlated at cell abundances above bloom level, but not at lower levels. Interestingly, qPCR and SHA results for samples collected at high (vs. low) chlorophyll *a* concentration, or at low (vs. high) water temperature, were more highly correlated even at low cell abundance, suggesting that environmental factors may affect the ability of each method to accurately determine concentrations of this species. Results of this investigation will aid management by providing critical information on the applicability of molecular tools for monitoring harmful algae in coastal estuaries.

INTEGRATING TOOLS FOR IN SITU DETECTION OF THE RED TIDE ALGA *KARENIA BREVIS* INTO A STATE MONITORING PROGRAM

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The Florida Fish and Wildlife Conservation Commission conducts statewide monitoring for the harmful dinoflagellate *Karenia brevis* and analyzes ~100 samples weekly by light microscopy. Working with partners, we integrated two affordable, in situ tools for rapid quantification of *K. brevis* – a handheld genetic sensor that uses real time nucleic acid sequence-based amplification (RT-NASBA) and a portable microscope that interfaces with an iPod touch (HABscope) to capture video of swimming phytoplankton cells – into weekly monitoring in the greater Tampa Bay region. Automated data processing to determine cellular abundance for each tool is accomplished through products developed for end-users by the Gulf of Mexico Coastal Ocean Observing System. Sampling with NASBA, initiated in late 2015, quantifies transcripts of the *K. brevis rbcL* gene as a proxy for cellular abundance and has accurately quantified cell concentrations spanning six orders of magnitude. Ongoing lab validations examining intracellular *rbcL* transcript copies in *K. brevis* strains grown under ecologically relevant conditions expand existing datasets that inform the conversion of field NASBA signal to cells L^{-1}. Initiated only in spring 2017, HABscope sampling recognizes the unique swimming pattern of *K. brevis*. Light microscopy data indicates that field efforts thus far have captured bloom/non-bloom conditions, a diversity of flagellate species, at least six *Karenia* spp., and other harmful taxa. This integration within state sampling provides a rich spatiotemporal and taxonomic dataset to inform management and bloom prediction, and an opportunity to conduct additional validations that inform the implementation of these in situ detection tools within citizen monitoring networks.

Hot Topic Session Recent East and West Coast ASP Events

Thursday Presentations

SYNERGISTIC CHARACTERIZATION OF *PSEUDO-NITZSCHIA* COMMUNITIES DURING UNPRECEDENTED DOMOIC ACID EVENTS

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The diatom genus *Pseudo-nitzschia* now includes ~50 species indistinguishable by light microscopy and yet unique with respect to morphology, biogeography, genomic content, and toxicity. We integrated genetic tools for screening species diversity into state monitoring and event response in New England and the Gulf of Mexico, where domoic acid (DA) from toxic *Pseudo-nitzschia* blooms has only recently warranted shellfish harvest closures. Multiyear routine sampling with Automated Ribosomal Intergenic Spacer Analysis (ARISA) detected >20 *Pseudo-nitzschia* taxa in each region, with assemblages consisting of toxic species observed prior to and/or in synchrony with toxicity. These monitoring efforts informed a streamlined species identification approach combining ARISA, sequencing from nested PCR products, and electron microscopy. Implemented for event response, this approach provided resolution of diversity within the *P. pseudodelicatissima/P. cuspidata* species complex during Florida's first shellfish harvest closures (2013, 2014, 2017). It also proved invaluable in documenting *P. australis,* never before observed in New England waters, during the first DA closures in Maine (2016) and Rhode Island (2017). We have taken steps to couple sensitive *Pseudo-nitzschia* spp. detection with other HAB monitoring tools, including qPCR, high throughput sequencing, and the Imaging Flow Cytobot. These synergistic observations provide sufficient spatiotemporal and taxonomic resolution for both managing *Pseudo-nitzschia* blooms and their impacts, and evolving our understanding of the mechanisms underlying species coexistence and toxic bloom formation in the marine environment.

WHAT'S FOR DINNER? PERSISTENT AND EXTENSIVE DOMOIC ACID CONTAMINATION IN THE FOOD WEB BEFORE, DURING, AND AFTER THE 2015 MEGA-BLOOM IN CALIFORNIA

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Pseudo-nitzschia blooms along the U.S. West Coast have been documented since 1991, with good evidence for trophic transfer of domoic acid to birds at least since the 1950s. Until recently the management response for both humans and wildlife has focused on acute toxicity associated with episodic bloom events, with short-term closures of fish and shellfish, under the assumption that domoic acid, a water-soluble toxin, will depurate rapidly from contaminated organisms. However, the 2015 "mega-bloom" that extended from Southern California to coastal Alaska demonstrated the potential for widespread accumulation of domoic acid in multiple trophic levels. Re-examination of data from before, during, and after that event suggests that domoic acid is ubiquitous in the food web both during these massive bloom events and during more "normal" periods. Here we document the presence and persistence of domoic acid in multiple organisms including shellfish, fish, squid, and marine mammals, as well as particulate and dissolved domoic acid in estuarine water, seawater, desalinated water, the benthos, and shallow benthic systems. With increasing evidence for chronic exposure leading to impairment in both humans and marine animal populations, we suggest that the existing paradigms need to be reexamined, and that chronic exposure to domoic acid is a common feature of estuarine and marine foodwebs.

PSEUDO-NITZSCHIA EARLY WARNING BULLETIN INCREASES CLAMMING OPPORTUNITIES ON PACIFIC NORTHWEST OUTER COAST BEACHES

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The 2015 coastwide bloom of toxic *Pseudo-nitzschia* resulted in over $125 million losses to shellfish harvesters and contributed to the deaths of marine mammals. This event illustrated the need for shellfish managers to receive complete and timely information to allow them to plan for seasonal closures and provide safe and timely harvest opportunities. Toward this end, a Pacific Northwest Harmful Algal Bloom (PNW HAB) Bulletin provides forecasts intended to help these resource managers from Neah Bay to Newport to target their beachside monitoring of toxicity levels in shellfish and fine-tune decisions regarding closures of beaches to shellfish harvest. This forecast system will include sampling by the Makah Tribe in a documented offshore HAB hotspot at two-week intervals. These offshore HAB data will be combined with a wealth of other information sources: beachside monitoring by Olympic Region HAB (ORHAB) partners and Oregon Department of Fish and Wildlife, the LiveOcean forecast model, and near real-time data from an offshore biological sensor (the Environmental Sample Processor). The first PNW HAB Bulletin in May 2017, gave managers the confidence in their highly unusual decision to increase the daily limit from 15 to 25 razor clams per day. This significant increase in bag limit resulted in a record number of one-day digger trips and over $5.3 million injected into the local economy. Future PNW HAB Bulletins will help coastal managers make rapid, informed decisions about seafood safety and is planned to transition to operations starting in 2019.

ECONOMIC AND SOCIOCULTURAL IMPACTS OF THE 2015 WEST COAST DOMOIC ACID EVENT: IDENTIFYING COMMUNITY NEEDS TO HELP FISHING COMMUNITIES COPE

Stephanie K. Moore[1], Edward H. Allison[2], Kathryn Blair[2], Amy Brodbeck[2], Sara Brostrom[2], Michael Cline[2], Jerilyn Coberly[2], Stacia Dreyer[2], Julia Ekstrom[3], Sunny Jardine[2], Terrie Klinger[2], Junwei Mao[4], Scott McGrew[2], Karma Norman[5] and Vera L. Trainer[5]

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The 2015 U.S. west coast domoic acid event resulted in fisheries harvest closures that generated an economic shock for coastal fishing communities, causing them considerable disruption. Here we report on the economic and sociocultural impacts of the Dungeness crab and razor clam fisheries harvest closures that began in 2015 and lasted into 2016. We conducted semi-structured interviews with 36 community members involved in the fisheries, hospitality, and retail industries, local government officials, recreational harvesters, and others from two communities impacted by the event – Long Beach, WA and Crescent City, CA. Common themes that emerged from the interview responses indicate that economic hardships extended far beyond fishing-related operations, and permeated through other sectors, particularly the hospitality industry. Some of these impacts persisted long after the bloom subsided. Long-held traditions surrounding crab and shellfish were disrupted, threatening the cultural identities of the affected communities. To minimize these impacts, community members expressed a desire for clearer, more thorough and more rapidly disseminated information regarding fisheries closures and the health risks associated with harmful algal blooms (HABs). Many also hoped for financial and employment assistance following HAB-related fisheries harvest closures that is tailored to the unique circumstances of fishing communities. Interview responses informed a comprehensive survey targeting 18 fishing communities on the west coast, and an associated economic impacts assessment aimed at exploring the impact of consumer responses to media coverage of the 2015 event on seafood prices. This survey is ongoing, as of July 2017. Preliminary results from these analyses will be presented.

NOVEL COGNITIVE PROFILES ASSOCIATED WITH FETAL AND GERIATRIC EXPOSURE TO DOMOIC ACID IN NATIVE AMERICANS

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In humans, high levels of dietary exposure to domoic acid (DA) causes Amnesic Shellfish Poisoning with lower level, repeated exposures associated with mild memory decrements and difficulties with "everyday memory." The impact of low level exposure to DA on vulnerable groups, i.e., fetal and geriatric, however, remains unknown. To address this knowledge gap we conducted preliminary studies of fetal and geriatric exposure to DA in coastal dwelling Native Americans of the Pacific NW. Findings indicated that fetal exposure via maternal razor clam consumption (compared to non-exposed) resulted in delayed development of gross motor and visual spatial skills in infants/toddlers. These deficits reversed themselves by ages 7-12 years, at which time, difficulties with executive functions emerged. Findings of the geriatric group, (M=72.53 years) indicated that the low/non consumers (M=23.92, sd=3.62) outperformed the high consumers (M=19.25, sd=3.10) on a general cognitive screen (p=.035). Impairments were found in domains beyond memory, specifically in visual/spatial executive abilities. Fetal and geriatric groups appear to have unique DA related cognitive profiles, possibly reflecting the neuroplasticity and protracted development of frontal lobe functions in the infants and children and the effects of lifetime DA exposure combined with reduced brain reserve capacity in the geriatric group.

THE GULF OF MAINE DOMOIC ACID EVENT OF 2016: AN EMERGING PUBLIC HEALTH CONCERN

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In the fall of 2016, a bloom of *Pseudo-nitzschia spp.* caused the Maine Department of Marine Resources to initiate its first Amnesic Shellfish Poisoning (ASP) closure. Blue mussels (*Mytilus edulis*) and soft shelled clams (*Mya arenaria*) became toxic more rapidly and to a higher extent than had previously been recorded off the Maine coastline. While shellfish toxicity levels reached record levels, phytoplankton cell counts were not as high as observed in prior years. Due to this combination of observations, Bigelow Laboratory for Ocean Sciences and Woods Hole Oceanographic Institute launched research cruises to identify the extent of the harmful algal bloom near-shore and off-shore as well as to quantify domoic acid levels within the phytoplankton. In conjunction, scanning electron microscopy and DNA sequencing was used to identify the *Pseudo-nitzschia* species composition in coastal waters from Massachusetts to Maine. A novel species, *Pseudo-nitzschia australis,* known to produce domoic acid in other parts of the world was observed within some of the samples. High abundance of *P. australis* correlated with high shellfish and phytoplankton toxicity levels.

WASHINGTON DEPARTMENT OF FISH AND WILDLIFE: MONITORING AND MANAGEMENT ACTIONS IN RESPONSE TO RECENT DOMOIC ACID EVENTS ALONG WASHINGTON'S COAST

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Harmful algal blooms (HABs) pose a significant threat to human health and fish and wildlife including threatened and endangered marine mammals and birds. Washington State's coastal economies also depend on revenue generated by recreational and commercial fisheries which can be disrupted by HAB events. Washington Department of Fish and Wildlife co-manages coast shellfish resources with four coastal Tribes. Together with Washington Department of Health (WDOH) we currently collect and test shellfish samples on a regular basis. If elevated levels of marine toxins are found, seasons are closed or postponed. To better understand and mitigate for the impact of HABs managers and researchers from local state, federal, academic and tribal nations partnered to form Olympic Region Harmful Algal Bloom Monitoring (ORHAB) collaboration in 1999. ORHAB was designed to provide resource managers an early warning to shellfish toxicity. The program relies on partners consistently collecting and sharing data which is ultimately disseminated to mangers to aid in safely and efficiently executing shellfish fisheries.

Over the past two decades domoic acid (DA) events in Washington State have been somewhat episodic, with intermittent fishery closures occurring between the years of, 1998-2003 and 2015 to present. The most recent DA events, including the unprecedented west coast-wide *Pseudo-nitzschia* bloom in 2015, have caused abrupt, widespread and often long lasting disruptions to commercial and recreational fisheries along the coast. Some of the most significant actions coastal shellfish mangers have taken since 2015 are: closing commercial Dungeness Crab fishing coast-wide, closing commercial harvest on the Willapa spits for the last three seasons, and implementing countless closures, delays and openings of the recreational razor clam fishery. We will present some of our monitoring data and management actions in response to these recent DA events, highlighting some of the most significant closures, and the impacts to Washington State. We will also discuss some measures we have used to attempt to mitigate the economic impacts of these HAB events on commercial and recreational fisheries and the communities that depend on them.

COMPARISONS OF *PSEUDO-NITZSCHIA* BLOOM INITIATION AND TOXICITY IN RELATION TO SPRING TRANSITION ENVIRONMENTS OFF THE OREGON COAST DURING 2015-17

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Twice monthly observations of hydrography, nutrients and phytoplankton species composition in the coastal waters off Newport, Oregon have been conducted annually since 2001 allowing us to track the harmful algal blooms by the diatom *Pseudo-nitzschia* (PN) and oceanographic conditions. In spring 2015, a toxic PN bloom was initiated following the onset of coastal upwelling in mid-April which led to closures of the harvest of razor clams and Dungeness crab. Macronutrient conditions appear to play a critical role in triggering the bloom and its toxicity, particularly stress from silicic acid limitation. In 2016, the physical spring transition to coastal upwelling conditions started early, in late-March; however, PN did not bloom as expected until June although low numbers of different cell-sized PN species had been present from January to May. Domoic acid tests in razor clam tissues did not show an elevation signal. Instead, a toxic PN bloom occurred later in September-October. The spring transition in 2017 started in late-April, and similar to 2015, PN abundance increased immediately to moderate bloom levels and those cells were toxic. However, unlike in 2015, the bloom decayed quickly 2-3 weeks later. The relatively sufficient silicate acid but constantly low nitrate concentrations before spring transition and pre- PN bloom in 2016 seemed to make the differences in the PN bloom formation and potential toxicity from the 2015 event. Our extended PN observing data continue to confirm the primary role of nutrients associated with local upwelling environments in controlling the occurrence of PN bloom off Newport.

REPEATED LOW LEVEL DIETARY EXPOSURE TO DOMOIC ACID CAUSES PROBLEMS IN EVERYDAY MEMORY

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Domoic Acid (DA) is a marine based toxin produced by the diatom, *Pseudo-nitzchia.* High levels of dietary exposure has been associated with Amnesic Shellfish Poisoning in humans. In two recent studies, a milder memory decrement has been reported in people with repeated exposure to lower levels via consuming Pacific Razor Clams, a known vector for DA. In both studies, the effect size of the memory decrement was small, with overall memory scores remaining within normal limits. While statistically significant and of academic interest, questions remain about the actual *meaningfulness* of the mild, memory decrement. To answer this question a cross sectional study of DA exposure and "everyday memory" was conducted. Participants included 60 Native Americans (NA) from a coastal reservation in the Pacific NW. DA exposure over the prior 7-9 days (target week) and past year was obtained based upon dietary consumption of razor clams (RC) with a well validated measure. The Everyday Memory Questionnaire-Revised was administered and demographic data was obtained. Based upon a logistic regression model (controlling for age, gender, and education), findings indicated that high consumers of razor clams are five to six times more likely to have problems with everyday memory than non or low consumers for the target week or past year (OR = 5.75, $p < .005$;   OR=5.55, $p < .005$, respectively). Higher levels of DA exposure appear to be associated with everyday memory complaints. Everyday memory may represent a meaningful approach to assessing dietary exposure to DA than traditional laboratory based neuropsychological measures.

PSEUDO-NITZSCHIA SPECIES DETECTED IN BARNEGAT BAY-LITTLE EGG HARBOR, NEW JERSEY DURING 2012-2014

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Phytoplankton community was investigated in Barnegat Bay-Little Egg Harbor, New Jersey from 2012 to 2014, coincident with the New Jersey DEP's water quality monitoring. During the investigation, monthly and biweekly water samples were analyzed for phytoplankton species composition and cell density. *Pseudo-nitzschia* species were detected most often during the 3-year study in areas near the Barnegat Inlet and in the Little-Egg Harbor. The occurrence, abundance and species of *Pseudo-nitzschia*, however, varied in different years. In 2012, *Pseudo-nitzschia* spp. were found in about 20% of total samples with cell density magnitude mainly between 10^5 to 10^6 per liter. Much lower density (10^3 to 10^4 per liter) of *Pseudo-nitzschia* was observed in 2013 following Hurricane Sandy, likely related to the abrupt disturbance caused by the storm. *Pseudo-nitzschia* was detected in only a few 2014 samples with low density (10^3 per liter), except for one sample collected from south of the Barnegat Inlet which contained an abundance of *Pseudo-nitzschia* spp. of 10^6 cells per liter. Based on the light microscopic identification, we distinguished at least five different species of *Pseudo-nitzschia*, and the co-existence of two or three species was observed. These preliminary results indicate a diverse group of *Pseudo-nitzschia* in New Jersey coastal waters. Further examination of the taxonomy of *Pseudo-nitzschia* species and multivariate analyses of their relationships to environmental variables are underway.

VARIABILITY OF *PSEUDO-NITZSCHIA* AND DOMOIC ACID ALONG THE U.S. WEST COAST

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A massive, destructive harmful algal bloom (HAB) along the U.S. West Coast in 2015 illustrated the threat posed to fisheries, marine mammal survival, and public health by toxigenic diatom *Pseudo-nitzschia.* Certain species of the genus *Pseudo-nitzschia* produce the neurotoxin domoic acid (DA), which can cause amnesic shellfish poisoning (ASP) in humans who consume shellfish contaminated with DA. The specific environmental conditions producing toxic algal blooms remain largely unclear. The continued assessment of the abundance and toxicity of *Pseudo-nitzschia* is used to identify emerging hotspots, investigate the occurrence of *Pseudo-nitzschia* and DA relative to environmental phenomena such as El Niño and the Blob, and make more robust the data that are incorporated into the HAB early-warning system. Seawater samples collected on NOAA research vessel *Bell M. Shimada* in May 2017 were analyzed in the laboratory using the following methods: 1. indirect cBASI Enzyme-linked Immunosorbent Assays (ELISAs) to determine DA concentration; 2. light microscopy to determine abundance of *Pseudo-nitzschia*; and 3. scanning electron microscopy to determine *Pseudo-nitzschia* species composition at select locations. Results indicated that freshwater plumes can affect the location and abundance of *Pseudo-nitzschia* at HAB hotspots near Heceta Bank. Future analysis will examine the potential connection between the abundance of toxic species of *Pseudo-nitzschia* with nutrient and chlorophyll data. This research adds to the time series data needed to determine the relative influence of various drivers of *Pseudo-nitzschia* blooms, including local factors such as macronutrient concentrations, and climatic influences such as El Niño, La Niña, and the Pacific Decadal Oscillation.

THE COMPLEX SEASCAPE OF HARMFUL ALGAL BLOOMS IN CALIFORNIA BEFORE, DURING, AND AFTER THE 'BLOB'-TO-EL NINO TRANSITION

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The 2016 El Niño was flanked by massive toxic *Pseudo-nitzschia* blooms off the coast of California that coincided with the Pacific Warm Anomaly (a.k.a. "blob") in 2015 and with ENSO-neutral conditions in 2017. This contribution examines blooms in three major hot spots: the Humboldt coast, Monterey Bay, and Santa Barbara Channel (SBC) over the span of three physically dynamic years in the California Current in an attempt to understand local and regional drivers. Variability in domoic acid concentration, the neurotoxin produced by *Pseudo-nitzschia*, is associated with various fishery closures and unusual mammal mortality events over the time period. While the warm temperature anomalies seen during the 'blob' were akin to those that resurged during the El Niño, HAB activity in 2016 did not approach anything like that seen from the Santa Barbara Channel to Alaska in 2015. Unexpectedly, HAB activity in 2017 was dramatic and mostly restricted to the Southern California Bight, with major ecosystem disruption occurring in the SBC region. This southern California bloom followed an unusually wet year along the coast that was decoupled from the ENSO event. Here we examine the similarities and differences in environmental drivers across years, to tease apart basin-scale factors from environmental stochasticity.

NOVEL TOXIGENIC *PSEUDO-NITZSCHIA* BLOOMS IN NARRAGANSETT BAY, RHODE ISLAND

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An unprecedented bloom of toxigenic *Pseudo-nitzschia* spp. occurred in Rhode Island waters during October 2016 leading to a 21-day shellfish harvest closure in lower Narragansett Bay, RI. This was the first widespread shellfish closure caused by HABs in Rhode Island, and was part of a regional (Maine, Massachusetts, Rhode Island) bloom. A series of sampling cruises tracked the *Pseudo-nitzschia* bloom in RI waters. *Pseudo-nitzschia* spp. were at elevated levels of > 20,000 cells L^{-1} for approximately one month (26 September 2016 to 24 October 2016), and abundance was frequently >100,000 cells L^{-1} with a maximum of 2 x 10^6 *Pseudo-nitzschia* spp. cells L^{-1} (20 October 2016) in lower Narragansett Bay. Cell counts and domoic acid levels in plankton and shellfish were greatest in the lower Bay, indicating that this was a coastal bloom. The greatest concentration of *Pseudo-nitzschia* spp. cells were consistently found in the coastal areas near the mouth of Narragansett Bay, with *Pseudo-nitzschia* spp. either absent or present at reduced abundance in the landward portions of upper Narragansett Bay and coastal salt ponds. Morphological evidence indicated that several *Pseudo-nitzschia* species were present during the bloom, but species identification awaits further analyses. A second toxigenic *Pseudo-nitzschia* bloom and shellfish closure occurred during March 2017. While *Pseudo-nitzschia* spp. have long been present in RI waters, toxin production has not previously been recorded. Recent observation of the repeated presence of toxigenic *Pseudo-nitzschia* spp. has led to modification of HAB monitoring and contingency plans in Rhode Island.

Bloom Prediction, Forecasting and Modeling

Thursday Presentations

EXAMINING AND FORECASTING CHARACTERISTICS OF THE ANNUAL CYANOBACTERIAL BLOOM IN LAKE ERIE

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In this century, western Lake Erie has experienced a recurrence of substantial cyanobacterial blooms—dominated by *Microcystis aeruginosa*. Since 2012, NOAA and partners have issued a severity forecast that predicts the annual cyanobacteria bloom in western Lake Erie. Of the nine largest blooms, eight have occurred in the last ten years, which raises questions about whether the system has changed with a changing climate—or climate cyclicity—to promote bloom development. An additional complicating factor is that core models for evaluating the 16 years of bloom data are based on statistical climate models. These models use the spring load (phosphorus primarily, but also discharge) as an input to the forecast. They are useful, but inherently simplistic, which limits their effectiveness in specifically targeting changes in the system due to changes in management. We have developed a semi-deterministic model to help with this effort. This model can explain some characteristics of the existing statistical model and allows closer examination of factors that cannot be explained under the current models, including non-linearity in the model, and the relative importance of loads in different months. The results may allow for retrospective analysis of blooms, and improve our understanding of likely future scenarios of management and climate.

Using predictive lake modeling to assess the development of Cyanobacteria blooms: lake ecosystem responses to nutrient stressor gradients

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The U.S Geological Survey (USGS), in partnership with the Minnesota Department of Natural Resources (MDNR), the St. Croix Watershed Research Station, and the National Park Service, has developed a number of mechanistic, bio-physical lake models that simulate trophic dynamics and track changes in algal populations, including dominance by Cyanobacteria. For three deep, cold-water Sentinel lakes, calibrated models captured the trajectories of phytoplankton seasonal succession over time at multiple depths. Model simulations successfully tracked the seasonal dominance of cyanophytes as well as the development of lake-specific algal biomass distributions. Calibrated models were used to evaluate changes in biomass of major algal groups under changing nutrient loading and meteorological stressor gradients. In Lake St. Croix, a multi-basin, riverine glacial scour lake, a calibrated model was able to simulate the spatial and temporal development of a Cyanobacteria bloom that is known to produce algal toxins. Sensitivity analysis of the Lake St. Croix model revealed how specific parameters were driving algal bloom dynamics. Model simulations provided an understanding of how cyanobacterial production and biomass accumulation in Lake St. Croix results from complex interactions between algal physiology, lake stratification, and hypoxia in the deep pools of the lake. In all of the lakes modeled to date, dissolved oxygen distributions reflect the interactions between lake stratification, primary production in the upper mixed layer of the lake, and water column oxygen demand below the photic zone. Dynamic, mechanistic models provide the necessary tool to simultaneously evaluate the influence of multiple stressors on whole-lake metabolism.

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INFLUENCE OF WIND FORCING ON DURATION AND SAMPLING OF FLORIDA RED TIDE, *KARENIA BREVIS*

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The toxic dinoflagellate, *Karenia brevis* blooms along the west coast of Florida repetitively cause shellfish poisoning and respiration irritation in humans. Although the blooms have been sampled since the 1950s, the routine sampling did not start until after 1995. In this study, combining in-situ wind and data-assimilative meteorological model products, along with bloom severity metrics defined by our recent work (see also Stumpf et al., this symposium), we analyzed the inter-connection between surface wind forcing and *K. brevis* blooms on seasonal to interannual time scales. Preliminary analysis shows that in many cases, the under-sampling of *K. brevis* in the 1980s was associated with relatively low frequencies of wind blowing onshore. This suggests the lack of respiratory irritation (or dead fish) might have resulted in lack of sampling. After 1995 when bloom sampling became routine, the duration of the bloom was highly correlated with the northerly wind, suggesting that the winds may alter the bloom duration. Our finding highlights the role of wind on the historical sampling effort, and its impact on modulating the spatial and temporal extent of the bloom. These analyses lend us confidence that we may be able to predict on the first order the potential duration of *K. brevis* blooms based on the wind forecast.

DEVELOPMENT OF A MECHANISTIC ROMS-RCA-HAB MODEL FOR PREDICTING *PROROCENTRUM MINIMUM* AND *KARLODINIUM VENEFICUM* BLOOMS IN CHESAPEAKE BAY

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The over-enrichment of Chesapeake Bay by nutrients has been well recognized and documented. Among the effects of this over-enrichment is an increasing proliferation of HABs, ranging from toxic dinoflagellates (e.g. *Karlodinium veneficum*), to ecosystem disruptive high-biomass dinoflagellates (e.g., *Prorocentrum minimum*) as well as toxic and high-biomass cyanobacterial blooms. HABs in Chesapeake Bay are now more frequent, and of significantly higher densities, than several decades ago. Through a new NOAA-ECOHAB project, a spatially explicit, mechanistic HAB model that integrates physics, nutrient cycling, food web interactions, physical factors and nutrient physiology will be developed to improve predictions of *P. minimum* and *K. veneficum,* seasonally and under various scenarios of change. The model will be built based on a coupled hydrodynamic-biogeochemical model (ROMS-RCA) and a new HAB model for *P. minimum* and *K. veneficum*. The RCA biogeochemical model includes 3 phytoplankton functional groups (cyanobacteria, diatoms and flagellates), separate cycling of N, P, Si and C, and is coupled to a sediment diagenesis model. The HAB model will consider nutrient preferences, life history including diel regulation, internal cellular nutrient storage and mixotrophy. The models will be applied to 1) spatially explicit seasonal forecasts, i.e. forecast summer HAB based on winter-spring nutrient loading and spring bloom development, and 2) longer-term scenario analysis to examine how HABs respond to nutrient management strategies (higher or lower loading) and climate change (warming, salinity change due to sea-level rise or changes in stream flows etc.) as projected by global and regional climate models.

SEASONAL TEMPERATURE CONDITIONING MEDIATES DORMANCY IN TAMPA BAY POPULATIONS OF *PYRODINIUM BAHAMENSE*

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The toxic dinoflagellate *Pyrodinium bahamense* has been documented in Florida's Tampa Bay since the 1960's, but only since 2000 have high biomass blooms -- occurring in fourteen of the last seventeen summers -- become a persistent issue for ecosystem health. *Pyrodinium bahamense* produces saxitoxins, neurotoxins that can accumulate in filter-feeding shellfish and cause paralytic shellfish poisoning in humans. Like many dinoflagellates, *P. bahamense* forms resting cysts as part of its life cycle; cysts deposited on the seafloor create a seed bank that can later initiate blooms. *Pyrodinium bahamense* cyst abundance is commonly high (>1000 cysts g^{-1} of wet sediment) in surficial sediments of northern Tampa Bay, an area where dense blooms commonly occur. We hypothesized that near-synchronous germination in *P. bahamense* would be an important driver of recurring blooms. However, we found that nondormant cysts can germinate across the range of temperature and light regimes encountered in Tampa Bay, suggesting that a cyclical period of dormancy could be more important than environmentally triggered germination. We examined changes in the dormancy status of cysts collected throughout one year from sediment cores in Tampa Bay and applied lessons from terrestrial plant literature to explore controls of dormancy. Collectively, our findings suggest temperature-mediated dormancy cycling occurs in *P. bahamense*, and we demonstrate, for the first time in dinoflagellates, the phenomenon of environmentally induced secondary dormancy. Ongoing research examines the roles of benthic resuspension and *P. bahamense* physiology to further understand bloom dynamics in Tampa Bay and other Florida systems.

ACROSS THE RESEARCH 'VALLEY OF DEATH' TO OPERATIONS: NOAA'S LAKE ERIE HARMFUL ALGAL BLOOM FORECAST SYSTEM

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The valley of death is a commonly-used term that characterizes the challenging progression of research from its initial inception to an operational application that reliably serves its users sustainably with dedicated resources and the necessary technical support. A key priority of NOAA's Harmful Algal Bloom Operational Forecast System (HAB-OFS) is systematically transitioning scientifically mature HAB forecasts to operations to ensure forecasts that are vital for public health are incorporated into a system where they are produced on a routine basis through a repeatable, rigorous methodology. NOAA prioritizes transitioning forecasts in specific regions of the country with clearly identifiable user requirements that can be met by forecasts supported by an operational framework. Creating a completely custom infrastructure for each forecast region would not be sustainable. Yet, developing a standardized approach is difficult because of inherent regional differences in local ecology and stakeholder needs. Based on lessons learned from the recent transition of the Lake Erie HAB forecasts to operations, NOAA has built a bridge across the valley of death through the development of a framework that will streamline the process for future transitions.

SPACE-TIME GEOSTATISTICAL MODELING OF HARMFUL ALGAL BLOOMS DYNAMICS IN WESTERN LAKE ERIE

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Harmful algal blooms (HABs) have occurred more frequently in western Lake Erie over the last two decades, and substantial efforts have been made to track these blooms using in-situ sampling and remote sensing. The resulting bloom measurements are critical to HAB management, but do not fully capture their spatial and temporal variability. Our study is focused on improving HAB tracking through development of a spatio-temporal geostatistical model. To synthesize available chlorophyll a data from four independent in-situ sampling programs in western Lake Erie, we treat each observation as a realization from a Gaussian random process subject to a Matérn space-time covariance function parameterized through restricted maximum likelihood estimation. Model performance is characterized through cross-validation exercise, and uncertainty is quantified. The results illustrate bloom dynamics, with blooms typically forming near the Maumee River outfall in early August and persisting for different durations in different years. Through conditional simulation, we develop probabilistic estimates of bloom extent, duration, and severity for each year (2008-2015). The results generally compare well with satellite imagery, though some exceptions are noted. We discuss future plans to incorporate remote sensing information within the geostatistical modeling framework, along with potential challenges related to the vertical movement of the bloom within the water column.

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Modeling spatio-temporal variability of algal bloom using MODIS imagery of inland waters

Water colour satellite data are increasingly used to manage and monitor water quality for ocean and coastal waters, particularly for monitoring harmful algal blooms (HAB) which have increased in frequency and intensity over the last decades. HAB monitoring using satellite sensors is possible due to the bio-optical activity of their principal pigment, the Chlorophyll-a (Chl-a), but these algorithms could be less effective when it has not been specifically designed to work in inland waters - like lakes, rivers and estuaries - where water contains a lot more optically active components like chlorophyll-a (chl-a), total suspended solids (TSS) and coloured dissolved organic matter (CDOM).

The first objective of this study is to develop an automated algorithm generating historical database of HAB episodes designed to work well in inland waters. A chl-a concentration estimator designed to perform in optically complex inland waters (El-Alem *et al.*, 2014) and a 250m cloud masking algorithm for MODIS water colour has been developed and applied to MODIS images. The second objective is to establish a regional portrait of the HAB occurrence on Southern Quebec using a geospatial database including the phenology features of HAB (e.g. beginning, duration, intensity). Finally, the third objective is to develop a statistical model which will estimate the predisposition of lakes to develop HAB according to their physiographic and climatic characteristics. This model will describe the relations between phenology features (e.g. beginning, duration and intensity) and climatic, physiographic and anthropological characteristics in connection with HAB's development (e.g. temperature, precipitation, snow coverage, lake depth, watershed area, agricultural land uses).

Preliminary results focus on the first and second objectives of the study. The automated algorithm applied on MODIS images can estimate and detect chl-a concentration well at 250m of resolution. The actual cloud masking product available for MODIS images is recorded at 1 km spatial resolution, making it unsuitable for monitoring algal blooms in small to medium-sized inland waters. Also, the product does not work well for turbid coastal waters due to the use of a NIR threshold algorithm compared to the new algorithm based on a probabilistic method (linear discriminant analysis). A regional portrait of HAB occurrence in Southern Quebec is then described and presented using five phenology features of HAB defined as being the intensity, the duration, the beginning, the frequency, and the scale (amplitude).

Reference: El-Alem A, Chokmani K, Laurion I & El-Adlouni SE (2014) An adaptive model to monitor chlorophyll-a in inland waters in southern Quebec using downscaled MODIS imagery. *Remote Sensing* 6(7):6446-6471.

FORECASTING HARMFUL ALGAL BLOOMS USING ARCGIS TOOLS

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The NOAA Harmful Algal Bloom Operational Forecast System (HAB-OFS) uses geographic information system (GIS) techniques to efficiently assimilate, visualize and publish spatial data. NOAA has an ESRI ArcGIS-based infrastructure to more efficiently and effectively produce operational forecast products. ArcGIS software provides the flexibility to integrate different types and sources of data, from emerging HAB monitoring technologies to region-specific partner observations.

ArcGIS readily georeferences dissimilar data types using physical location as the common thread. Georeferenced data can be easily visualized as layers, allowing for simultaneous assessment of bloom conditions while reducing the potential for error in forecasting. NOAA has developed custom, Python-based ArcGIS toolboxes to incorporate the data layers necessary for analysis such as satellite imagery, water samples, real-time beach observations, wind forecasts, and current data--all of which go into the creation of operational products. These tools are flexible enough that they can be modified to incorporate future input, like real-time, *in situ* sample data.

NOAA's ArcGIS-based infrastructure is used to create georeferenced PDFs, providing users with additional metadata such as sample concentration and location to help mitigate the effects of HABs. Improved mapping techniques enhance visualization of forecasts and analyses, creating an easily consumable product for public health officials, water resource managers, and researchers. The development of this flexible framework will enable a streamlined transition of future research products into operational forecasting systems.

WHAT THREE WATER QUALITY PARAMETERS MAY TELL US ABOUT HABS AND BLOOM FREQUENCY IN CHESAPEAKE BAY

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In an era of shrinking budgets and scaled-down phytoplankton monitoring programs, inferences about phytoplankton can be made using a relatively simple water quality binning method. Secchi depth, dissolved inorganic nitrogen, and ortho-phosphate are classified with empirically determined thresholds and grouped into distinctly different habitat categories. Categories range from nutrient-enriched, excessively turbid habitats ("Degraded") to habitats with bloom-limiting nutrient concentrations and water clarity adequate for unstressed phytoplankton photosynthesis ("Reference"). Chesapeake Bay phytoplankton show observable differences in total biomass, taxonomic composition, HAB frequency, bloom frequency, and physiological stress across this disturbance gradient. Recovery trajectories are evident that indicate how Bay phytoplankton communities will respond to ongoing, TMDL-driven nutrient and sediment reductions. The results can be used to check model-based projections of chlorophyll *a* and HAB concentrations.

PREDICTING THE BLOOM: CAN TIME SERIES DATA AND MACHINE LEARNING PRODUCE INFORMATIVE FORECASTS OF HARMFUL ALGAL BLOOM ABUNDANCE?

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Predicting the occurrence of harmful algal blooms (HABs) has been a longstanding goal of HAB researchers and managers. The increase in availability of large, long-term datasets from marine environments coupled with Imaging FlowCytobot instruments capable of providing high resolution counts of phytoplankton abundance has the potential to make the goal of predicting HABs more attainable. In this work, time series data of environmental factors (e.g. coastal currents, temperature, salinity, winds) from several coastal regions of Texas and Louisiana were combined with the time series of phytoplankton abundance from the TOAST station in Port Aransas, TX. Using machine learning algorithms, a forecast was generated for one, three, and seven time steps into the future (time steps varied from 3 - 24h) for various target taxa (both HAB forming and non-HAB forming). Preliminary results showed a small improvement compared to a naïve model (i.e. abundance tomorrow will be the same as today) for a forecast one time step into the future but the results were taxon dependent. The resulting forecasts for three and seven time steps into the future showed no improvement over a naïve model. Gaps in the various time series, often occurring at different points, represent a major difficulty encountered in the analysis. As time series become longer and additional data streams become available online (e.g. GCOOS data portal; http://data.gcoos.org/), the ability of machine learning algorithms to extract patterns from the data and provide better forecasts is expected to improve.

FORECASTING ABOVE REPLACEMENT: A SABERMETRIC APPROACH TO ENSURING HARMFUL ALGAL BLOOM FORECASTS MEET USER NEEDS

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NOAA's Harmful Algal Bloom Operational Forecast System (HAB-OFS) issues weekly forecasts for the Gulf of Mexico and Lake Erie that assist with bloom response efforts. Forecast performance is evaluated regularly based on observational data; this assessment guides research to advance the forecasts. But how good is good enough? Scientific advancement can continue indefinitely so establishing user defined acceptance criteria for forecast performance is essential to ensure that the forecasts meet user needs. NOAA is currently developing a more systematic approach for setting forecast acceptance criteria.

This balance between forecast performance and user requirements can be understood by comparing the performance of new features against existing methods, and how the performance of new components compare to user requirements. This operates in much the same way as a baseball statistic known as Wins Above Replacement (WAR) which estimates a team's expected improvement of adding a new player by measuring him against the performance of an average player for that position. Using this concept, the HAB team is developing a value index for understanding how our existing forecasts and proposed changes meet user requirements and improve efficacy.

USING HIGH FREQUENCY MONITORING OF ENVIRONMENTAL FACTORS TO PREDICT THE MICROCYSTIN CONCENTRATIONS IN A MULTI-USE, INLAND RESERVOIR

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Cyanobacteria, known as blue-green algae, are photosynthetic bacteria found naturally in marine, freshwater, and estuarine ecosystems. An increase in nutrient input and changes in the climate have contributed to the proliferation of cyanobacteria, forming harmful algal blooms, or HABs. Beyond the ecosystem effects, loss of recreational use and economic impact, a serious issue associated with these blooms is the production and release of cyanotoxins. While some factors that influence the proliferation of HABs have been identified, how these factors affect blooms and the resulting production of toxins are still unknown. In order to investigate the environmental factors and nutrient concentrations that results in an increase of total microcystin, high frequency water quality data and discrete grab sampling were compared for 2015 and 2016 sampling at William H. Harsha Lake, Ohio in order to determine significant correlations between chlorophyll-a, phycocyanin, temperature, rainfall, photosynthetic active radiation, nitrogen, phosphorus, and resulting total microcystin concentrations at four sites, on Harsha Lake. A time series approach was then used to first determine significant correlations and investigate useful lag-times for management decisions such as drinking water treatment options, beach closures, and do not drink or consume warnings, to be enacted.

COMPARISON OF LOCAL AND REGIONAL (C-HARM SYSTEM) PREDICTIVE BLOOM MODELS FOR TOXIGENIC *PSEUDO-NITZSCHIA* IN MONTEREY BAY, CALIFORNIA

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Pseudo-nitzschia blooms in California have been a recognized human and wildlife health concern since 1991 and are the primary harmful algal bloom (HAB) issue in Monterey Bay, CA. Over the past 25 years, domoic acid (DA), the toxin produced by *Pseudo-nitzschia*, has caused several marine bird and mammal stranding events and extended the closures of shellfish harvests (both commercial and recreational) in the bay. In 2009, a predictive model for toxigenic *Pseudo-nitzschia* blooms in Monterey Bay was developed utilizing in-situ water data over an 8 year period. Due to the nature of the inputs, the model requires on-site sampling and further lab analysis, generally causing a delay in the prediction output. However, the burden of HABs on CA's coastal ecosystems and a need for forecasting such events real-time, led to the development of a regional predictive model, California Harmful Algae Risk Mapping (C-HARM) system. The C-HARM system uses numerical and ecological forecast models in addition to satellite imagery to predict *Pseudo-nitzschia* blooms and the presence of domoic acid for the CA coast. Both models perform well at the Santa Cruz Municipal Wharf (SCMW), a weekly monitoring site in the northern part of the bay at the time of comparison, but have not been directly compared to each other. This study updates the local model with recent SCMW data and data from the Monterey Municipal Wharf II and evaluates the performance of both the local model and C-HARM system in Monterey Bay, CA.

HIGH RESOLUTION MODELING AND FORECASTING OF *KARENIA BREVIS* RESPIRATORY IRRITATION USING HABSCOPE AND HIGH RESOLUTION WEATHER FORECAST (NATIONAL DIGITAL FORECAST DATABASE)

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Along the Gulf of Mexico, aerosolized brevetoxins produced by *Karenia brevis* can cause respiratory irritation at nearby beaches in the presence of onshore winds. A model was constructed to capture these conditions by ingesting observed HABscope cell counts, forecast winds obtained from the high resolution weather forecast (National Digital Forecast Database) winds, and shoreline orientation. NDFD provides hourly forecasts with high spatial resolution of < 2.5 km which can resolve sea breeze, and thus can be evaluated against the meteorological measurements at Venice pier. The respiratory impact is a function of cell counts and wind speed (Kirkpatrick et al., Science of the Total Environment, 2008, pp. 1-8). To validate the model, data from past bloom events and verified buoy winds were ingested into the model and compared against observed respiratory irritation from the Beach Conditions Reporting System at Venice, Sarasota and St. Petersburg, Florida. Preliminary results suggest that as long as the wind blows onshore, the magnitude of absolute wind speed, rather than the onshore wind speed component , may be more important in governing respiratory irritation. Even weak onshore wind componentconditions with high total wind speeds produce respiratory impacts. At least part of this finding can be explained by the formulation of wind stress- onshore component of wind stress increases when overall wind speed increases, not just the onshore wind speed. The model is expected to be used for experimental forecasting in Fall 2017.

Emerging Use of 'Omics Approaches to Understand Freshwater and Marine HABS

Thursday Presentations

GENOME SEQUENCES OF PLANKTONIC BLOOM-FORMING ANABAENA/DOLICHOSPERMUM/APHANIZOMENON BELONG TO A DISTINCT GENUS-LEVEL CLADE AND REVEAL PATTERNS OF GENOME EVOLUTION

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The Nostocales include potentially toxigenic cyanobacteria that form HABs around the world. Here, we present nine novel *Anabaena/Dolichospermum* and *Aphanizomenon* draft genome sequences and analyze a group of 15 recently completed genome sequences from planktonic HABs on four continents. Five of the novel genomes were extracted directly from environmental shotgun metagenome assemblies, while the remaining four were cultured prior to sequencing. Phylogenomic clustering revealed a distinct genus-level ADA clade (planktonic *Anabaena/Dolichospermum/Aphanizomenon*) that is distinct from other Nostocales, in particular *Nostoc* and several *Anabaena*. Genome-wide nucleotide identities suggest the presence of four species within the ADA clade, two of which contain both *Anabaena* and *Aphanizomenon flos-aquae* morphotypes. Genes for the production of toxins (anatoxin-a, microcystin, saxitoxin) and other secondary metabolites are variably present. Gene differences in light harvesting pigments, utilization of organic sulfur sources, urea utilization, and other characteristics often do not follow a phylogenetic pattern, indicating active recombination and gene gain and loss. Genomic sequences are important in distinguishing physiological characteristics that could provide competitive advantages in different environments and in facilitating strain- or clade-specific molecular monitoring. They are also valuable in guiding taxonomic reforms to best classify Nostocales members that are morphologically similar but distantly related (some *Anabaena/Dolichospermum* and *Nostoc*) and those that are closely related but morphologically distinct (*Anabaena/Dolichospermum* and *Aphanizomenon flos-aquae*).

ABUNDANCE AND EXPRESSION OF NITROGEN UPTAKE AND METABOLIC GENES IN A LAKE ERIE *MICROCYSTIS* BLOOM DEMONSTRATE A DIVERSE AND ACTIVE NITROGEN METABOLISM IN THE *MICROCYSTIS* COMMUNITY

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Multiple lines of evidence suggest that nitrogen plays a key role in the structure and toxicity of *Microcystis* cyanobacterial harmful algal blooms in freshwater and estuarine systems. Observations from a 2014 *Microcystis* bloom in western Lake Erie show a succession between toxin and non-toxic genotypes that coincide with shifts in nitrogen form and availability. We used comparative genomics to identify the diversity of nitrogen uptake pathways in cultured isolates of *Microcystis* from the lower Great Lakes. Data were then incorporated into a newly developed graph database with thirty-four metagenomes and seven metatranscriptomes from the 2014 western Lake Erie *Microcystis* bloom. This approach allowed for the analysis of relative abundance and expression patterns of nitrogen uptake and metabolic pathways in addition to gene pathways associated with toxin production. We found that while *Microcystis* has a diverse array of nitrogen transport and metabolic genes, expression of these genes corresponded to a shift from high to low Nitrate as the bloom progressed. –Omics data were then analyzed for shifts in microcystin synthesis operons and expression to investigate the link between nitrogen pools and bloom toxicity. Abundance and expression of microcystin genes decreased as the bloom progressed, suggesting that *Microcystis*-community functional capability and physiological state change in relation to nitrogen form and availability. Combined, these data improve the understanding of drivers behind community composition, physiological function, and toxicity in *Microcystis* blooms and provide a new tool to further explore links between environmental factors and genetic responses.

GENOME-RESOLVED METAGENOMICS REVEALS THE MICROBIAL DIVERSITY AND METABOLIC POTENTIAL OF TOXIC BENTHIC FRESHWATER PHORMIDIUM MATS

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In many rivers and streams cyanotoxins are produced by benthic mats, not pelagic blooms. Cyanobacterial mats are complex structured habitats hosting a diverse assemblage of microbial taxa. The strong geochemical gradients and high density of organisms in cyanobacterial mats results in complex interactions among microbes to produce and process molecular compounds. In spite of their important ecological and public health impacts, few benthic freshwater toxin producing cyanobacterial genomes have been sequenced, and the microbial assemblages associated with toxin producing cyanobacterial mats have rarely been investigated. Using genome-resolved metagenomics we sampled 22 *Phormidium* spp. mats in summer 2015 in the Eel River, CA to 1) understand the diversity of toxin producing *Phormidium* spp. and the associated microbial assemblage in the mat and 2) identify the different metabolic and toxigenic potential in the mat. We recovered 28 near-complete *Phormidium* genomes from four potentially novel *Phormidium* genotypes, and an additional 93 non-cyanobacterial genomes were also recovered. The microbial assemblage of *Phormidium* mats are primarily comprised of Burkholderiales (Proteobacteria) and Cytophagales (Bacteroidetes). Genes for nitrogen metabolism and phosphorus acquisition were abundant in the mats. The anatoxin-a operon was recovered in seven *Phormidium* genomes and found to have a novel gene arrangement. These results are the first culture independent genomes for toxigenic freshwater benthic cyanobacteria, and improve our understanding of the genes controlling how energy flows, nutrients cycle, and toxins are produced in these mats, which can help predict what factors drive the proliferation of toxic benthic cyanobacterial mats in rivers.

METABOLOMICS OF CYANOBACTERIAL HARMFUL ALGAL BLOOMS: RAPID ACCESS TO METABOLITE PROFILES AND IDENTIFICAITON OF NOVEL CHEMISTRY

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Cyanobacterial harmful algal blooms (CyanoHABs) are on the rise worldwide, resulting in increased attention to monitoring and regulation of the hepatotoxic microcystins often produced by these events. A number of other cyanobacterial peptides are often co-produced by these bloom forming species, many of which display potent activity in various enzyme systems and in some cases display toxicity comparable to microcystins. The human health impact of these non-microcystin cyanopeptides has yet to be determined. Parallel screening of bloom events for complete metabolite profiles in addition to targeted quantitation of cyanotoxins would allow the CyanoHAB community to observe trends and identify commonly encountered peptide classes produced by these bloom events.

In July 2016, a massive CyanoHAB consumed much of Florida's coastline. Our laboratory received samples from Martin County, FL spanning five sites, four of which contained bloom material and one control site with no visible bloom. Targeted analysis of bloom samples resulted in the rapid identification of toxic microcystin congeners present in bloom samples. Additional untargeted UPLC-QTOFMS metabolomics analysis of bloom samples revealed that markers of the bloom included two structurally novel aeruginosin congeners in addition to toxic microcystins and the acylated glycoglycerolipid DGMG. This presentation will discuss the application of UPLC-QTOFMS for rapid access to metabolite profiles of cyanoHABs as well as in depth structure elucidation and preliminary biological assessment of two novel secondary metabolites belonging to the aeruginosin class of linear peptides.

EUKARYOTIC PHYTOPLANKTON COMMUNITY SPATIOTEMPORAL DYNAMICS AS IDENTIFIED THROUGH GENE EXPRESSION WITHIN A EUTROPHIC ESTUARY

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Estuaries are highly dynamic and productive environments. A clear understanding of how phytoplankton, supporting the base of the food web, respond to spatiotemporal dynamics is necessary to ensuring the health and sustainability of these ecosystems. Over the span of a year, we investigated the interactions between biotic and abiotic factors within the eutrophic Neuse River Estuary (NRE). Through metatranscriptomic sequencing in combination with water quality measurements, we show that there are different metabolic strategies deployed along the NRE, and nitrogen availability is the main driving factor for such divergence. In the upper estuary, phytoplankton express more transcripts of genes for synthesis of cellular components and carbon metabolism whereas in the lower estuary, transcripts allocated to nutrient metabolism and transport were more highly expressed. Metabolisms for polysaccharide synthesis and transportation were elevated in lower estuary and could be reflective of unbalanced growth and/or interactions with their surrounding microbial consortia. We advocate for the use of molecular sequencing approaches to complement coastal water quality monitoring programs as a way to examine microbial community dynamics in response to changing environmental conditions.

EFFECTS OF NUTRIENT ENRICHMENT AND ELEVATED TEMPERATURE ON CYANOBACTERIA BLOOM DIVERSITY AND TOXICITY IN TEMPERATE FRESHWATER LAKES

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Toxic freshwater CyanoHABs have intensified globally, being linked to accelerated anthropogenic eutrophication and climatic warming. However, there remains a limited understanding of how entire communities respond to multiple environmental drivers in an ecosystem setting. This study aimed to assess the shifts in the cyanobacteria and microcystin-producing communities' diversity in response to elevated temperature, nitrogen, phosphorus, and their interactions during spatial/temporal monitoring and incubation experiments in Lake Erie (USA) and Mill Pond (Long Island, NY). Bar-coded metagenomic analyses of cyanobacterial-specific ribosomal RNA (16S) and the microcystin production (mcyE) genes was conducted using next generation sequencing. Cyanobacteria biomass significantly increased in response to elevated nutrients and temperature while the abundance of green algae and diatoms decreased. 16S rRNA sequencing revealed high cyanobacteria genera diversity (37-49 OTUs) with N, P and temperature all significant sources of variation in abundance. N was a significant source of variation for non-diazotrophic genera (*Microcystis/Planktothrix*), which dominated under high N and P, and lower temperature, while diazotrophic genera (*Anabaena*) were most abundant under low nutrient, high temperature conditions and promoted by elevated P. Sequencing analyses also revealed cryptic cyanobacteria diversity, detecting elevated abundances of uncultured cyanobacteria associated with *Microcystis*-dominated communities, and two distinct clades of *Synechococcus* in Lake Erie. *mcyE* sequencing detected low diversity in the potential microcystin-producing community, with *Microcystis* being the sole toxigenic genus in Mill Pond and toxigenic *Planktothrix* and *Microcystis* present in Lake Erie. Microcystin concentrations coincided with the 16S relative abundance of these genera and were significantly increased by N enrichment.

STRUCTURE AND FUNCTION OF THE MICROBIOME OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE*

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Like all organisms, algae appear to harbor specific microbiomes that can have both positive and negative effects on the algal partner. Our previous work with the toxic dinoflagellate *Alexandrium tamarense* has shown that the presence of bacteria can be associated with faster growth, larger cell size, higher photosynthetic efficiency, and greater cellular chlorophyll and toxin content. To characterize interactions between *A. tamarense* and its microbiome, we used two sub-cultures of a single genetic strain of this species, one bacterized (xenic) and one axenic, which show significant differences in morphology and physiology. Bacteria from the xenic strain were re-inoculated into the axenic strain, producing replicate "rexenic" strains. At 9 and 15 months, we characterized algal physiology and the community composition of both the algal-attached bacteria and those that were free in the medium. Parental microbiomes were stable over a long time scale (6 years). Despite differences in physiology between the two rexenic strains, their free bacterial communities were similar to each other, and distinct from the xenic parent. The attached bacterial communities of the rexenic strains were similar to the parent at 9 months, but had diverged by 15 months. Microbiome differences were driven primarily by variation in the abundance of the dominant species, which were shared between strains. These results suggest that the algal microbiome composition is not resilient to severe disturbance. Nonetheless, structurally dissimilar communities can be functionally equivalent in terms of impact on the algal partner.

BOTH MODULAR AND SINGLE-DOMAIN TYPE I POLYKETIDE SYNTHASES ARE EXPRESSED IN THE BREVETOXIN-PRODUCING DINOFLAGELLATE, *KARENIA BREVIS*

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Dinoflagellates are prolific producers of polyketide compounds, many of which are potent toxins with adverse impacts on human and marine animal health. To identify polyketide synthase (PKS) genes in the brevetoxin-producing dinoflagellate, *Karenia brevis*, we assembled a transcriptome from 595 million Illumina reads, sampled under different growth conditions. The assembly included 125,687 transcripts greater than 300 nt in length, with over half having >100x coverage. We found 121 transcripts encoding Type I ketosynthase (KS) domains, of which 99 encoded single KS domains, while 22 contained multiple KS domains arranged in 1-3 protein modules. Phylogenetic analysis placed all single domain and a majority of multidomain KSs within a monophyletic clade of protist PKSs. In contrast to the highly amplified single-domain KSs, only 8 single-domain ketoreductase (KR) transcripts were found in the assembly, suggesting they are more evolutionarily conserved. The multidomain PKSs were dominated by *trans*-acyltransferase (AT) architectures, which were recently shown to be prevalent in other eukaryotic microalgae. *K. brevis* also expressed several hybrid nonribosomal peptide synthetase (NRPS)/PKS sequences, including a *burA*-like sequence previously reported in a wide variety of dinoflagellates. The longest PKS found in this study consisted of three modules, insufficient by itself to synthesize any of polyethers known to be produced by *K. brevis*. This suggests that multiple multidomain PKSs likely work in tandem in the biosynthetic process. How, and if, the single domain KS sequences interact with multidomain PKSs is currently unknown, and is a question of key interest for understanding HAB toxin biosynthesis.

USE OF HIGH THROUGHPUT SEQUENCING TO ASSESS DIFFERENTIAL GRAZING ON HARMFUL ALGAL BLOOMS BY ZOOPLANKTON

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Harmful algal blooms (HABs) have increased globally within both marine and freshwater habitats. While cyanobacteria, which can be extremely toxic, dominate HABs within freshwater systems and are linked to reduced zooplankton grazing pressure, the relative grazing pressure on differing genera of cyanobacteria is poorly understood. The use of 16S barcoding and high throughput sequence analysis offers a tool to assess the genera of cyanobacteria grazed upon by zooplankton during HABs. To assess its utility, a set of grazer addition experiments with *Daphnia pulex* and *Daphnia magna*, as well as zooplankton dilution experiments were performed at the periphery and closer to the epicenter of the cyanobacterial HABs in Lake Erie at the Maumee River and Sandusky Bay. Pigment-based phytoplankton community analysis showed that there was active grazing cyanobacteria at all sites but more rapid grazing rates on diatoms when they were abundant. 16S high throughput sequencing revealed multiple genera including *Prochlorococcus, Synechococcus, Spirulina, and Anabaena* were actively grazed by both indigenous zooplankton and *Daphnia* spp. In contrast, other genera including *Microcystis* and *Planktothrix* were not grazed or became more abundant in the presence of enhanced grazing pressure. This talk will also focus on the differential grazing on eukaryotic algae and the general utility of using high throughput sequencing to quantify grazing on HABs and other phytoplankton.

SCALING RIBOSOMAL RNA DIFFERENCES WITHIN DINOFLAGELLATES FOR SPECIES IDENTIFICATION

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Defining species within dinoflagellates based on ribosomal DNA (rDNA) or other molecular markers underpins many environmental and HAB detection methods. Ideally a single molecular marker could be used to predict or detect toxic events. The rDNA is also often used in environmental sequencing to describe biodiversity with more than 25,000 unique molecular taxa assigned to dinoflagellates. However, the rate and type of sequence changes vary across different dinoflagellate genera. There are 'slow' genera, such as *Dinophysis* or *Scrippsiella* where only a handful of differences are seen between species within the genus, while similar comparisons in the 'fast' genera *Alexandrium* or *Blastodinium* reveal many more differences. The small subunit ribosomal gene tree can be compared to the well-resolved dinoflagellate phylogeny and tested for lineage specific differences in rate. Similarly for genera such as *Karlodinium* and *Symbiodinium*, the internal transcribed spacers (ITS) are sufficiently sampled to make valid comparisons. Nucleotide bias, covarying sites, the functional constraints of different rDNA regions, and lineage-specific rates of change all need to be accounted for to practically and accurately match sequences to species. Similarly, the molecular taxonomic units of environmental sequence surveys may not match one to one with distinct species, especially when lineage specific effects are not taken into account.

ACETYL-COA CARBOXYLASES IN DINOFLAGELLATES: FUELING THE POLYKETIDE SYNTHASE PATHWAYS

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Dinoflagellates are phytoplankton that make a diverse array of fatty acids and polyketide toxins which are responsible for harmful algal blooms worldwide. A necessary precursor, and rate limiting step, for their synthesis is malonyl-CoA formed by carboxylating acetyl CoA using the enzyme acetyl-CoA carboxylase (ACC). To date, information on dinoflagellate ACCs is lacking. Through transcriptome analysis in *Amphidinium carterae* (CCMP 1314) we found two full-length homomeric type ACC sequences; no heteromeric type ACCs were found. We assigned the putative cellular location for these two ACCs based on transit peptide predictions. Using mass spectrometry-based proteomics on isolated gel bands, we validated the presence of ACC proteins. Transcript abundance for these ACCs follow the established global pattern of expression for dinoflagellate mRNA messages over a diel cycle. A census of core dinoflagellates reveals that there are 3-4 versions of homomeric type ACCs that exist. Streptavidin-HRP western blots corroborate these findings with biotinylated bands present around 250 kDa, which is close to the estimated protein size in *Amphidinium carterae, Karlodinium veneficum,* and *Akashiwo sanguinea.* This is one of the few descriptions at the transcriptomic and protein level of ACCs in dinoflagellates. This work provides insight into the enzymes required to produce CoA precursors for fatty acid and toxin synthesis in dinoflagellates. Future work will establish ACC protein expression across the diel cycle, visualize cellular location using confocal microscopy, and identify all acyl CoA pools present in the cell.

PHOSPHOPANTETHEINYL TRANSFERASES IN DINOFLAGELLATES: FAT VERSUS TOXIN SYNTHESIS

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The phosphopantetheinyl transferase performs the first step in fatty acid and polyketide/non-ribosomal protein synthesis by covalently attaching the phosphopantetheinyl arm of CoA to a protein. This provides the substrate upon which acetate is sequentially added to a bound starter molecule and is essential for both lipid and toxin synthesis. Many dinoflagellates toxins exhibit the hallmarks of polyketide-based synthesis, but phylogenetic analyses of associated proteins have been unable to separate type I (toxins) and type II (fats) based enzymes using canonical classifications. In order to simplify this problem, a phylogeny of phosphopantetheinyl transferases was constructed to look for duplications and divergence that correlate with toxin production using transcriptomes from 42 dinoflagellate species. Transcriptomes were mined for PPTases using three putative sequences from *Amphidinium carterae*, a basal photosynthetic dinoflagellate that produces amphidinols. These sequences were also cloned into a bacterial expression vector and co-transformed with a BpsA reporter construct into *Escherichia coli*. Successful phosphopantetheination of BpsA results in the production of the blue dye indigoidine and indicates that a PPTase can activate a type I multi-domain synthesis pathway. All transcriptomes examined contained two to three PPTases with an absence of only PPTase 1 in both toxic and non-toxic species. All three PPTases from *A. carterae* were able to activate indigoidine synthesis with PPTase 3 being the most efficient. If PPTase 3 is responsible for polyketide-based synthesis and present in all dinoflagellates, this would indicate that synthesis of polyketide type molecules may be irrespective of a species' status as a toxin producer.

STABLE ISOTOPES AS TOOL TO LINK NITROGEN SOURCE TO BLOOM AND TOXICITY

C. Taylor Armstrong[1], Deana L. Erdner [2], James W. McClelland[2], Marta P. Sanderson[1], Donald M. Anderson[3], Christopher J. Gobler[4], and Juliette L. Smith[1]

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The impact of natural versus anthropogenic nutrient sources on species composition or toxicity of HABs remains unclear. Stable isotopes are used to identify and track nitrogen (N) sources to water bodies, and thus can be used to ascertain the N source(s) used by the phytoplankton in those systems. While literature is available describing N fractionation by diatoms and coccolithophores, data are lacking regarding dinoflagellates. Here we investigated the effects of N chemical form on isotope fractionation (Δ) and toxin content using isolates of the dinoflagellate, *Alexandrium catenella,* in single-N and mixed-N experiments. Growth of *A. catenella* exclusively on nitrate (NO_3^-), ammonium (NH_4^+), or urea, resulted in Δ of 2.7±1.4‰, 29±9.3‰, or 0.3±0.1‰, respectively, with the lowest cellular toxicity reported during urea utilization relative to NH_4^+. No matter the N chemical form to which *A. catenella* were preconditioned, the cells initially utilized NH_4^+ and urea when exposed to mixed-N medium, and only utilized NO_3^- after NH_4^+ decreased below 2-4 µM. This result suggests that there is no effect of preconditioning on chemical preference. Overall, the results suggest that in NO_3^- and urea-rich environments, the $\delta^{15}N$ of dinoflagellates would reliably look like the source(s) of N utilized, but caution should be taken in NH_4^+ rich environments where the large Δ value could lead to misinterpretation of the signal and more toxic blooms may result. We continue to build on this information by focusing in on an N-rich compound that is unique to *A. catenella*, i.e., saxitoxin, and ultimately, create a species-specific N-tracer that identifies the N source and chemical form(s) facilitating blooms and toxin production.

SPATIO-TEMPORAL DYNAMICS OF PLANKTONIC EUKARYOTES IN A HAB-PRONE MULTIPURPOSE RESERVOIR

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Harmful algal blooms (HABs) constitute an increasingly frequent and severe threat to human health and the environment worldwide. The US EPA's research into how to anticipate and mitigate HABs emphasizes the important role of abiotic factors such as nutrient pollution and temperature. Though informative, these research efforts may be insufficient by themselves. Accounting for the influences of biotic interactions such as predation, competition, and disease on HAB formation/dissipation may improve the power of prediction and mitigation strategies. We conducted 18S rRNA gene sequence metabarcoding analyses to profile changes in the aquatic community of a multipurpose reservoir in southeastern Ohio, USA, that has become prone to summertime blooms of toxic cyanobacteria. This metabarcoding approach revealed spatial and temporal patterns among a far greater set of biota than traditional morphology-based monitoring allows. A total of 1,314 unique observational taxonomic units (OTUs) were detected, comprising 15 distinct metazoan clades, 25 distinct protist clades, and 8 distinct fungal clades. Our preliminary data suggest that peak cyanobacterial abundances and peak microcystin concentrations during the growth season coincide with calanoid copepods and dinoflagellates dominating the planktonic eukaryote community and that declines in each coincide with spikes in the relative abundances of rotifers, green algae, and chytrid fungi. Ongoing analyses are intended to elucidate key community dynamics foretelling HAB formation/dissipation. In addition, controlled laboratory experiments will be used to test hypotheses regarding mechanisms driving these dynamics.

Some Assembly Required - Beyond the type I, II and III polyketide synthase paradigms in dinoflagellates

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We have uncovered a single transcript (>14Kb) with three consecutive KS modules, that are conserved across three species of dinoflagellates (*Karlodinium veneficum* and *Amphidinium carterae,* have known toxins , and *Akashiwo sanguinea*, which is non-toxic), as well as many single module KS subunits using RNA sequencing analysis. When comparing the three species, the acyl transferase (AT) subunit in the triple module KS was present only in the non-toxic species and missing in the toxin producing species. To test the functionality of PKS in these species, we added cerulenin, an inhibitor that covalently binds to the KS subunits (Funabashi et al, 1989), to an exponential phase culture of *A. carterae and A. sanguinea*. Using ^{14}C labeled acetate and liquid chromatography mass spectrometry (LC/MS), we found that cerulenin inhibition resulted in the cessation of both fatty acid and toxin production. This shows KS plays a role in both fat and toxin synthesis leading us to our hypothesis: The triple module KS acts as a scaffold for both toxin and fatty acid production where the final product is mediated by accessory *trans*-AT subunits, which exist in parallel with the KS. To substantiate that the triple KS transcript produces a protein, we have generated an antibody to an epitope in the TE domain. We clearly have evidence that a partial multi-module protein (~285,000 daltons) is expressed and is predicted to catalyze two carbon additions to a growing fatty acid/polyketide chain. However, an antibody to KS1 domain found a discrete protein containing the KS1 and KS2 domains but not the KS3 domain indicating post-translational processing. We are currently investigating the interacting partners with these two protein scaffolds.

IMPACT OF *SYNECHOCOCCUS SP.* ON NUTRIENT AND REACTIVE OXYGEN SPECIES DYNAMICS SURROUNDING BLOOMS OF *MICROCYSTIS* IN LAKE ERIE

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Analysis of 16S gene sequences from a 2014 *Microcystis* bloom in western Lake Erie found multiple *Synechococcus* operational taxonomic units (OTUs) that were abundant and occasionally dominant within the cyanobacteria community (Berry et al., 2017). While much focus in placed on the ecological role and toxicity of *Microcystis*, few studies have investigated the composition and physiology of co-occurring *Synechoccoccus*. Evidence suggests that microbial sinks of reactive oxygen species (ROS), which negatively impact cell integrity and physiology, in Lake Erie *Microcystis* blooms are driven by non-*Microcystis* members of the bloom community. *Synechococcus* may contribute to community-wide defenses against ROS, impacting bloom fitness, duration, and toxicity. We used comparative genomics to investigate the diversity and activity of nutrient uptake and metabolic pathways and ROS defense mechanisms within the *Synechococcus* community during the 2014 western Lake Erie *Microcystis* bloom. A cultured isolate and assembled genome of *Synechococcus* from Lake Erie were compared to thirty-four metagenomes and seven metatranscriptomes from western Lake Erie as well as publicly available *Synechoccocus* genomes using a newly developed graph database which included environmental metadata. This allowed for changes in the abundance and expression of these pathways in *Synechococcus* to be tracked over the course of a *Microcystis* bloom. This work builds a mechanistic understanding of the physiological state and potential ecological roles of co-occurring cyanobacteria found within a large *Microcystis* bloom and works towards a community-level understanding of drivers behind bloom and toxin dynamics.

Emerging Technologies and Advances in Remote Sensing

Friday Presentations

THE 3RD GENERATION ENVIRONMENTAL SAMPLE PROCESSOR (ESP) – TOWARDS MULTIPLEXED, WAVEGUIDE-BASED HAB SPECIES & TOXIN DETECTION ON A LONG-RANGE AUTONOMOUS UNDERWATER VEHICLE

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The push towards autonomous, near-real time detection of HAB species and toxins in marine and freshwater systems is driven by research and management applications. The former focuses on scientific and technological advances needed to assess ecosystem conditions governing bloom dynamics and toxicity, whereas the latter aims to capitalize on that knowledge and capability to provide early warning of potential HAB outbreaks and data to enhance HAB forecasts.

The 2nd generation (2G) ESP (McLane Research Laboratories) is being employed for such research and management initiatives. This instrument automates molecular-based detection of HAB taxa (e.g., *Pseudo-nitzschia*, *Alexandrium*, *Heterosigma*) and phycotoxins (e.g., domoic acid (DA), saxitoxins, microcystins) in deployments spanning marine and freshwater environments. A 3rd generation (3G) instrument advances ESP technology via integration with a long-range AUV (LRAUV) and coupling cartridge-based sample acquisition/processing with a standalone surface plasmon resonance (SPR) system for target molecule detection. Development of the 3G ESP/SPR system has focused primarily on DA detection, whereas design of a microcystin assay is underway.

The 3G ESP/SPR system shows considerable promise when the number of analytes is limited; however, detection of multiple HAB species and toxins in one sample poses many technical challenges. Therefore, we are exploring incorporation of a planar waveguide-based molecular detection technology (MBio Diagnostics) with the 3G ESP. The waveguide can be integrated with a sample cartridge and derivatized with protein and/or nucleic acid arrays for multiplexed detection. Results of proof-of-concept experiments demonstrate the feasibility of this approach and have informed design of a new 3G ESP/waveguide system.

DYNAMICS OF PSP TOXIN PRODUCTION BY AN INSHORE BLOOM OF *A. CATENELLA* OBSERVED THROUGH CO-DEPLOYMENT OF COMPLEMENTARY, AUTOMATED IN SITU PHYTOPLANKTON SENSORS

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Blooms of the dinoflagellate *Alexandrium catenella* are the most widespread cause of paralytic shellfish poisoning (PSP) worldwide. Recent application of autonomous in situ phytoplankton sensors has shown that the growth, swimming, and life cycle conversions of these blooms are far more robust than previously predicted. In this study, the toxin dynamics of a bloom within the Nauset Marsh estuary (Cape Cod, MA USA) were examined again using autonomous in situ sensors. These included two highly complementary robotic instruments, the Environmental Sample Processor (ESP), which is able to measure the PSP toxin content of particle concentrates, and the Imaging FlowCytobot (IFCB), a submersible imaging in-flow cytometer that produces rich image records of nano- and microphytoplankton species abundance. Through real-time analysis of IFCB images, ESP toxin assays were configured and triggered to assess changes in per cell toxin content over the bloom's full course, from its early development to its sexual induction and decline. Toxin measurements from the ESP were compared to results of liquid chromatography-mass spectrometry (LCMS) of >15 μm cell fractions collected weekly. Both LCMS and ESP analyses revealed much higher per cell toxin quotas than previously measured from cultures of Nauset isolates, but ESP measurements (from >5 μm fractions) were up to two orders of magnitude greater than LCMS during the bloom's sexual induction. The discrepancy suggests that a large fraction of toxin is transferred to smaller size fractions as a consequence of sexual processes and/or increased grazing pressure late in bloom development.

DEVELOPMENT OF A RAMAN SPECTROSCOPIC LIBRARY FOR RAPID DETECTION AND CLASSIFICATION OF HAB SPECIES AND THEIR TOXINS

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Harmful algal blooms (HABs) are a significant and growing threat to fisheries as well as human and ecosystem health, yet there remains no simple, precise, rapid, and low-cost technique to identify the diverse array of HAB cells and their toxins from unprocessed water samples. Here we show that Raman spectroscopy provides a strong and distinct signal or fingerprint of HAB cells and their toxins without the need for sample preparation. Raman spectra were obtained at wavelengths of 532 and 785 nm, on whole cultures using a laser beam of 500 μm diameter, and on single cells with a 2 μm beam diameter. Chemometrics used on the spectra included PCA to reduce dimensionality of the feature set followed by classification by Linear Discriminant Analyses and machine-learning techniques such as random forests and SVM. 532 nm provided a stronger and more characteristic signal in the finger print region of the spectra, while 785 nm produced lower autofluorescence, which was only an issue with the Cryptophyceae. Separation and identification was possible for 26 strains of algae, 19 of which were toxic marine dinoflagellates, one marine toxic diatom, and one freshwater toxic cyanobacterium representing nine Classes, 12 Orders, and 13 Families. The *Alexandrium catenella* complex was separable with 96% accuracy. Separation was mainly a function of the ratios of fatty acids (triglyceride), to β-carotene, chlorophyll a,b, and other photosynthetic pigments. The key to rapid detection of a variety of species is the development of a library of known spectra for comparison against samples.

Commented [DMA1]: I haven't seen the data – this sentence implies you separated the complex – which consists of 5 species now. I don't think that is what you mean. You should probably say Alexandrium catenella – if you looked at one species. We used to call this A. fundyense.

Commented [DMK2]: I think a few words in one of these sentences could be chopped.

HABSCOPE: AUTOMATED CLASSIFICATION OF *KARENIA BREVIS* USING A LOW-COST FIELD KIT, OPEN SOURCE COMPUTER VISION LIBRARIES AND CONVOLUTIONAL NEURAL NETWORKS

Robert D. Currier[1], Barbara Kirkpatrick[1], Richard Stumpf[2], Rance Hardison[3], William C. Holland[3,] R. Wayne Litaker[3], Andrew Reich[4], Tracy Fanara[5], Devin Burris[5], Aly Busse[5], Fitz Otis[5]

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Cell counts for *Karenia brevis* samples are typically completed manually by a technician using a laboratory microscope. The counts can take up to one week to complete and at the height of the bloom season are unlikely to be valid when published. HABscope was designed to be usable by a volunteer with minimal training and to provide real-time cell counts from the sampling location. The HABscope field kit consists of an Omax microscope, Apple iPod Touch, 3D printed adapter, StraightTalk hot spot, power supply and case. An app is loaded on the iPod Touch and provides sentinels with the ability to record a thirty second video and upload the video to a cloud server. When a video is uploaded to the server, it is first rotated for proper orientation and then run through the detection algorithm. The algorithm uses visual characteristics in the first pass to discriminate between particles of interest and detritus. Based on morphological characteristics, regions of interest (ROI) are identified. Each ROI is clipped from a frame and fed to a Google TensorFlow model. Using image recognition techniques each ROI is classified as '*Karenia*' or '*Not Karenia*.' *Karenia* cells are marked with a green target indicator. Other moving objects are marked with a red target indicator. The maximum number of visible cells is used to calculate cells/Liter. The scale used in the calculation is self-generated by the algorithm. Testing against known cell quantities has shown that HABscope consistently provides cell counts within 20% of manual counts.

RAPID AND LOW-COST FIELD DETECTION OF MARINE TOXINS

Avni A. Argun[1], Mourad Manoukian[1], Richard H. Pierce[2], Vincent J. Lovko[2], and Michael Henry[2]

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Brevetoxins (PbTx) are a group of marine algal toxic compounds that are produced by the bloom-forming dinoflagellate *Karenia brevis*. During harmful algal blooms (HAB), brevetoxins can reach concentrations high enough to cause human neurological and gastrointestinal illnesses through inhalation of the airborne toxins or consumption of contaminated shellfish. The timely recognition of this toxin in marine environment is of great importance for public safety, human health, human recreation, marine ecology, and the marine economy. We present the development of a low-cost and easy-to-use test for rapid and quantitative detection of brevetoxin species in seawater. Specifically, we utilize an electrochemical assay to enable measurement of PbTx-2 and PbTx-3 with high specificity while lowering the field detection limits to sub-parts-per-billion (ppb) levels via unique use of disposable electrode chips. Key sensor specifications include PbTx-2 detection limit of 0.25 ppb, a linear range of 0.25 - 50 ppb, and sample-to-result turnaround time of < 5 minutes. The assay also works interference-free; the measured electrochemical signal maintains 90% of its magnitude in seawater in the presence of other aquatic toxins.

This versatile field sensor will allow near real-time monitoring of brevetoxin levels in seawater throughout the progression of a red tide bloom. Our platform technology also has the potential to go beyond detection of brevetoxin as indicator of HABs and could find applications in areas where rapid, accurate, and selective detection of other marine and freshwater toxins are needed.

SIMULTANEOUS EXTRACTION AND SEPARATION OF CHLOROPHYLL AND PHYCOCYANINE FROM CYANOBACTERIA WITH OCTANOL-WATER

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Toxin-producing cyanobacteria are significant contributors to harmful algal blooms (HABs), an increasingly serious threat to the health and safety of fresh water lakes, ponds, and streams worldwide. Cyanobacteria are characterized by the presence of phycocyanine, a blue green, proteinaceous, water soluble pigment, in addition to chlorophyll a, a green, hydrophobic pigment. The amounts of phycocyanine and chlorophyll in water bodies are a commonly used and useful parameter for estimation of the amounts of cyanobacteria and total algae, respectively, in water bodies. However, it is customary to employ separate extraction procedures for these two pigments. Most chlorophyll extraction procedures employ organic solvents which may denature phycocyanine, and chlorophyll is not soluble in the aqueous-based extraction procedures for phycocyanine. We have found that the representative species of cyanobacteria grown in culture, *Microcystis aeruginosa* and *Limnothrix 144*, can be gently extracted with 1:1 mixtures of octanol:water, resulting in chlorophyll concentrated in an upper octanol phase, and phycocyanine concentrated in the lower aqueous phase. A representative green algae, Scenedesmus, was not extracted, thus suggesting the possibility that octanol-water will provide a means of selectively extracting phycocyanine and chlorophyll from cyanobacteria but not from non-bacterial alga. This possibility is being explored. The simultaneous extraction and separation of phycocyanine and chlorophyll also facilitates their respective measurements, as the overlapping characteristics of their absorbance spectra shows. Work in progress is focused on optimizing the extraction for environmental samples, and measuring the pigments by fluorescence. Funding is provided by East Georgia State College.

DEVELOPMENT OF A SANDWICH HYBRIDIZATION ASSAY FOR DETECTING THE HARMFUL CYANOBACTERIA *MICROCYSTIS*

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Cyanobacteria are responsible for the largest number of harmful algal blooms (HABs) worldwide. The genus *Microcystis* is a major contributor to these HABs, and several species produce the hepatotoxin microcystin that affect humans, pets, and wildlife. Since *Microcystis* blooms occur in lakes, rivers, and stormwater detention ponds associated with residential and recreational areas, they pose public health risks. Several molecular techniques enable rapid and accurate identification and quantification of HABs, facilitating 'early warnings' of blooms for management responses that safeguard public health. This presentation describes the adaptation of one such technique, sandwich hybridization assay (SHA), for *Microcystis*. SHA enables direct (e.g. no amplification of genetic material) cell identification and quantification using ribosomal RNA (rRNA) with two DNA probes, capture and signal, that bind to the targeted rRNA sequence creating a "sandwich" wherein results are measured by absorbance. Capture probes were designed using 16S rRNA sequences from GenBank[®], and the assay was validated using cultures of *M. aeruginosa, M. botrys,* and *M. wesenbergii* as well as environmental samples from a cyanobacteria bloom that included *Microcystis* spp. The bloom occurred in a South Carolina stormwater detention pond during the summer of 2016. *Anabaena, Microcystis,* and *Anabaenopsis* were observed in succession with microcystin detected on multiple occasions. *M. aeruginosa* cultures were also used to test the effect of different irradiances and temperatures on SHA response because these factors affect bloom dynamics. Ultimately, this technology will enhance *Microcystis* bloom tracking and prediction.

Oral Presentation
Session Choices:
1. Monitoring, Mitigation, and Management of HABs
2. Emerging Technologies, Instrumentation, and Methodologies

PRIMARY STRUCTURE ELUCIDATION OF HEMOLYTIC TOXINS FROM DINOFLAGELLATES PRESUMED RESPONSIBLE FOR FISH KILLS BY ACCURATE TANDEM MASS SPECTROMETRY

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Dinoflagellates are motile, unicellular protists found in many aquatic environments and capable of causing harmful blooms, sometimes referred to as "red tide." Karlotoxins and amphidinols are hemolytic polyol toxins produced by *Karlodinium* and *Amphidinium* dinoflagellate species, respectively, that have been associated with fish kills worldwide. The goal of this research was to develop a comprehensive mass spectrometric methodology to identify and define primary chemical structures of polyol toxins for support of applied attribution studies and basic dinoflagellate biology studies. Previously archived extracts and newly acquired water samples, from areas in which fish kills were observed, were analyzed by either a hybrid 3D ion trap-time of flight mass spectrometer (MS) or an ultra-high mass resolving power and mass accuracy 21 T FT-ICR MS. Dissociation schema were proposed for each toxin using several gas-phase dissociation techniques and compared to similar schema from a library of known toxins to make positive identifications. Accurate mass precursor ion measurements, isotopic distributions, and accurate tandem MS were all used to inform these decisions. Karlotoxins were observed in blinded experiments and quantified in samples from areas where fish kills occurred, without exception, supporting the hypothesis that blooms of *Karlodinium* species were responsible for the events. Gilthead seabream erythrocyte hemolytic assays were also consistent with the other analytical results. No samples from very recent fish kills associated with *Amphidinium* species were available for analysis; however, several purified amphidinols and amphidinols from archived fish kill water samples were characterized in the same way as the karlotoxins for future reference.

MONITORING HABS BY ISOTHERMAL RNA AMPLIFICATION USING HANDHELD GENETIC SENSORS (AKA "TRICORDERS")

John H. Paul[1], Katherine A. Hubbard[2], Dana M. Nieuwkerk[1], Alicia E. Hoeglund[2], Charles L. Tilney[2], Robert M. Ulrich[1]

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Accurate identification and quantification (AIQ) of bloom-forming harmful algae is critical for human health in the coastal zone, safe shellfish harvesting and aquaculture, surface water quality, and understanding fish, avian, and marine mammal mortality events. The HAB AIQ needs have traditionally been met by microscopy, although cytometry, image analyses, and pigment analyses have contributed significantly to this cause. More recently, real-time PCR (qPCR) has provided a powerful tool for HAB AIQ, yet in most instances cannot distinguish between functionally active or dead and detrital targets. Isothermal RNA amplification assays based upon Nucleic Acid Sequence-based Amplification (NASBA) can determine if the gene targets were ***transcriptionally active*** when sampled. We have developed NASBA assays for *Karenia brevis, K. mikimotoi,* and *Pseudo-nitzschia multiseries* as well as the freshwater *Microcystis aeruginosa, Anabaena,* and *Planktothrix.* Because NASBA analysis is isothermal, detection of positive targets does not require the associated energy requirements of thermal cycling. We have chosen the robust Douglass Scientific AmpliFire as our detection platform, a small battery operating system that can analyze 8 samples simultaneously. This point of use (POU) technology is not only ideal for portable analyses in the field but is also affordable for state, local, city, and neighborhood associations.

IMPROVING STANDARDS FOR MOLECULAR QUANTIFICATION OF THE FLORIDA RED TIDE DINOFLAGELLATE

Dana M. Nieuwkerk[1], Robert M. Ulrich[1], Alicia A. Hoeglund[2], Charles L. Tilney[2], Katherine A. Hubbard[2], John H. Paul[1]

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The dinoflagellate *Karenia brevis* commonly forms harmful algal blooms and produces brevetoxins, which can cause massive fish kills, marine mammal mortalities, and negative impacts on human health. The current standard method for quantifying *K. brevis* employs light microscopy. We have previously developed a genetic quantification assay that targets the *K. brevis rbcL* gene for Real Time Nucleic Acid Sequence Based Amplification (RT-NASBA), with a limit of detection of 10 cells/L. To adapt the bench-top RT-NASBA assay to a handheld method that can be used in situ, we first demonstrated a strong correlation between RT-NASBA and microscopy data (R-squared = 0.98). We selected the AmpliFire platform to conduct field-based NASBA, and tested an *rbcL* mRNA standard and an *rbcL* single-stranded DNA (ssDNA) standard against cellular standards. The *rbcL* mRNA standard (ng/mL) and the cell standard (cells/mL) corresponded well, and we are currently testing the more stable ssDNA standard and evaluating *rbcL* copy number per cell to better automate analysis of field data. Samples taken as part of an 18-month time series at Anna Maria Island, FL were analyzed using cellular and *rbcL* standard curves to further evaluate differences in quantification and reproducibility. These data will be used to inform the incorporation of handheld RT-NASBA sensors into state and citizen *K. brevis* monitoring networks throughout the Gulf of Mexico. Results from these sensors will be reported through regional ocean observation systems (e.g. Gulf of Mexico Ocean Observing System) to provide near real-time, sensitive, in situ monitoring of *K. brevis*.

PORTABLE SYSTEM FOR EARLY DETECTION OF HARMFUL ALGAL BLOOM TOXINS

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Harmful Algal Blooms in freshwater and marine environments are increasing in frequency and duration and constitute a growing public health threat while also carrying substantial economic, ecologic, and food supply implications. Recent development of guidelines for microcystin (MC) and cylindrospermopsin (CYN) in drinking water has increased the need for rapid onsite testing for these toxins. MBio Diagnostics is developing a transformative platform technology that will enable users in the field to perform laboratory-quality, cost-effective cyanotoxin testing that will help protect drinking water, monitor commercial food resources, and provide critical data for ecosystems management. MBio's patent-protected cartridge and reader system provide an unprecedented combination of speed, ease-of-use, quantitation and results multiplexing in a field-portable system. Here, we present results from a one-step, 10-minute duplex assay for microcystin and cylindrospermopsin. All reagents are dried onboard the cartridge allowing the user to simply add sample and insert the cartridge into the instrument. With this simple and rapid protocol, 50% inhibition concentrations for both assays are 1 ng/mL. To demonstrate variant coverage on the microcystin assay, eight common MC congeners were measured and had 50% inhibition concentrations ranging from 0.6-2.9 ng/mL. A one-step, 10-minute, field-portable mechanical cell lysis method will also be described. By adding the portable lysis module, a fully integrated, field-portable kit is enabled.

SPATIAL VARIABILITY OF HARMFUL ALGAL BLOOMS IN MILFORD LAKE, KANSAS

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Cyanobacterial harmful algal blooms (CyanoHABs) are spatially variable both vertically in the water column and horizontally across the lake surface because of in-lake and weather-driven processes, and can vary by orders of magnitude in concentration across relatively short distances (meters or less). Spatial variability in CyanoHABs poses unique challenges to collecting representative samples for scientific study and public health protection. During July and August, 2015 the U.S. Geological Survey (USGS) and the U.S. Army Corps of Engineers (USACE) collected open-water microcystin data and the Kansas Department of Health and Environment (KDHE) collected near-shore microcystin data throughout the lake. Microcystin concentrations ranged from <0.5 to 381 micrograms per liter (µg/L; Minimum detection limit was <0.5 µg/L for KDHE) in discrete samples, and decreased in the down-lake direction. Based on KDHE's guidance levels, the public health advisory status of designated lake zones (A, B, and C) was consistent regardless of where or how the samples were collected within each zone. An additional study was conducted in the upper-reaches of the lake during the summer (May–September) of 2016, that expanded data collection to include continuous water quality (including algal fluorescence) and time-lapse cameras. Preliminary results from USGS samples collected in 2016 indicate that microcystin concentrations ranged from <0.10 to 29,000 µg/L a (<0.10 µg/L was the USGS minimum detection limit) and decreased in the down-lake direction. Six time-lapse cameras operated around the upper-reaches of the lake and captured changes in bloom conditions that occurred over periods as short as five minutes.

~~Decoding the Spectral Signature of~~DETECTING HARMFUL ALGAL BLOOMS IN THE CHESAPEAKE BAY USING SENTINEL-2 EARTH OBSERVATIONS

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Harmful Algal Blooms (HABs) have an increasingly negative effect on water quality and ecosystems in Virginia's Chesapeake Bay. H~~ABs deprive the water of oxygen, produce harmful toxins, and disrupt water chemistry causing large-scale fish mortality events. Scientists from the Virginia Institute of Marine Science (VIMS) monitor HABs using fixed monitoring stations and data flow cruises; however~~owever, the Chesapeake and its estuaries are geographically too large for continuous monitoring of HABs and associated water conditions. Thus, it would be beneficial to develop a method to detect harmful algal blooms in the Chesapeake Bay watershed using remotely sensed data.

~~Daily Aqua Moderate Resolution Imaging Spectrometer) chlorophyll-a estimates are readily available for the Chesapeake Bay, yet the one kilometer resolution is too coarse for Virginia's rivers. While Landsat 8Operational Land Imager) has appropriate 30-meter resolution, there are no readily available chlorophyll products. Early attempts to overcome this challenge included cross-calibrating Landsat and MODIS data in order to visualize chlorophyll at 30-meter resolution. However, results varied.~~

~~On August 17, 2015, hyperspectral~~Hyperspectral data w~~ere~~ obtained for a large bloom of *Alexandrium monilatum* in the York River. A~~t the same time, a~~ data cruise obtained in-situ chlorophyll-a measurements from the bloom. ~~Landsat 8 OLI and MODIS~~ Satellite data were also available. ~~A spectral hourglass tool was used to select 13 spectral bands for analysis.~~ The bloom was analyzed using true color, false color, and ISODATA classification techniques. Spectral profiles were obtained for each class using ENVI's class statistics work flow. Chlorophyll-a estimates were obtained using band ratios in the red (665 nm) and near infrared (710 nm) wavelengths. Comparison with data cruise measurements showed the chlorophyll-a estimates were consistent with in-situ data.

Because Landsat 8 does not collect data in the 670 nm to 850 nm range, this chlorophyll-estimation ratio could not be applied to Landsat imagery. However, when chlorophyll ratios were applied to imagery of the Chesapeake Bay using Sentinel-2 MultiSpectral Instrument's (MSI) band 4 (665 nm) and band 5 (705 nm) ~~data~~, algal blooms were clearly visible. The Sentinel-2 satellite has a 5-day latency with 20-meter spatial resolution. The data is readily available through USGS's EarthExplorer. ~~provi~~Therefore, Sentinel-2 can provide a cost-effective and reliable means to track algal blooms in Virginia's Chesapeake Bay watershed.

CHARACTERIZING CYANOBACTERIA BLOOMS USING REMOTE SENSING AND NON-TRADITIONAL METHODS

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Harmful algal blooms of toxigenic cyanobacteria (cyanoHABs) are becoming more prevalent with the continued eutrophication of inland surface waters. CyanoHABs can be detrimental to lake ecosystems and threaten the health of organisms exposed to cyanotoxins. Monitoring all lakes affected by cyanoHABs via traditional sampling methods at a time scale necessary for early warning is expensive and unrealistic. Remote sensing could provide less expensive, fast, and reliable data for monitoring cyanoHABs. Current modeling strategies focus on algal concentrations, which do not easily translate into management decisions and implementation. Here we propose an alternate approach using classification and regression tree (CART) modeling to predict bloom presence and absence, which provides more readily useful binary output for management decisions. We used a nine-year water quality (i.e., chlorophyll-a and phycocyanin concentrations as proxies for algal and cyanobacterial abundances) dataset from a large southern reservoir paired with corresponding Landsat 7 ETM+ satellite images to build a CART model. Model output was compared to output from traditional linear and logistic models. Classification trees built using reflectance values of band (1-red, 2-blue, 3-green and, 4- NIR) ratios and season accurately predicted chlorophyll-a ($\geq$ or $<$ 50 µg/L) and phycocyanin ($\geq$ or $<$ 30 µg/L). Using the same thresholds, linear and logistic models performed poorly for chlorophyll-a compared to the classification tree while the phycocyanin linear and logistic models performed comparably and slightly worse, respectively, to the CART model. Our results show that CART models could be used as an effective monitoring tool for cyanoHABs in southern reservoirs.

EXAMINING TEMPORAL CHANGES IN CYANOBACTERIA HARMFUL ALGAE BLOOM SPATIAL EXTENT IN THE CONTINENTAL UNITED STATES USING SATELLITE REMOTE SENSING

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CyanoHABs are thought to be increasing globally over the past few decades, but relatively little quantitative information is available about the spatial extent of blooms at varying spatial scales. Satellite remote sensing provides a technology for identifying cyanoHABs in multiple water bodies and across geo-political boundaries. A previously developed method, employing MEdium Resolution Imaging Spectrometer (MERIS) imagery, was used to quantify cyanoHAB special extent, transferable to different spatial areas, in the continental United States for the test period of 2008 to 2012. Temporal assessment was used to examine changes in satellite resolvable inland waterbodies for each state. To further assess cyanoHAB risk within the states, the World Health Organization's (WHO) recreational guidance level thresholds were used to categorize surface area of cyanoHABs into three risk categories: low, moderate, and high-risk bloom area. This can assist in prioritizing the degree of potential exposure risk to cyanobacteria. This work builds upon numerous studies by using satellite remote sensing to extend trend identification for inland cyanoHABs across numerous water bodies and to an entire state scale. The temporal assessment method developed here will be relevant into the future as it is transferable to the Ocean Land Colour Instrument (OLCI) on Sentinel-3A/3B missions.

ESTIMATING ALGAL BLOOM SEVERITY IN EAST FLORIDA INLAND LAKES FROM 10 YEARS OF MERIS OBSERVATION

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Cyanobacterial Harmful algal blooms (CyanoHAB) are a serious environmental, water quality and public health issue worldwide because of their ability to form dense biomass, scum, and produce toxins. Models and algorithms have been developed to detect and quantify CyanoHAB presence and biomass using remotely sensed data. However, managers need information on which water bodies have significant blooms and whether those bloom intensities have changed. Consequently, there is a need to develop methods in order to estimate CyanoHAB annual severity in inland lakes. A particular need is quantitative information on spatio-temporal variability in lake level CyanoHAB severity would be useful for effective monitoring and decision-making for recreational and drinking water sources. In this study, we present a method to estimate CyanoHAB severity in inland lakes and reservoirs using satellite observations. Areal CyanoHAB biomass is quantified using a reflectance spectral shape based algorithm that uses data from the European Space Agency's Medium Resolution Imaging Spectrometer (MERIS). As a case study, we present the estimates of CyanoHAB severity in inland lakes and reservoirs in East Florida using 10 years based on available MERIS observations. Methods developed in this study can also be applied to satellite observations by Sentinel-3 Ocean and Land Colour Imagers (OLCI) for mapping CyanoHAB severity in inland lakes.

HYPERSPECTRAL IMAGING FOR THE LOW-LEVEL DETECTION OF CYANOBACTERIA

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To assist US Army Corps of Engineer resource managers in controlling cyanobacteria blooms we are developing a hyperspectral imaging technique to improve the low-level detection and identification of cyanobacteria. Hyperspectral imaging of laboratory monocultures of cyanobacteria revealed a difference in spectral signatures, based on unique qualities of the photosynthetic absorbance spectrum, that may be used for identification. To use this technique in the field we need to understand how these signatures change with stress. Therefore we induced varying degrees of stress (N, P, Mg and Fe limitation) in *Microcystis aeruginosa* and *Anabaena* sp. cultures to identify physiological ranges of each species and record their associated hyperspectral signatures using a Resonon hyperspectral camera (400-1000nm). Concurrently we evaluated these samples for cyanotoxins to align changes in cell health with toxin production. Laboratory experiments showed a change in spectral signatures under all nutrient stressors examined. This coincided largely with culture senescence, which also corresponded with higher cyanotoxin levels. We are working to create a database of hyperspectral signatures that includes both healthy and stressed conditions. Additionally, we are currently performing outdoor 500 gallon mesocosms using a cyanoHAB bloom sample obtained from the USACE Kentucky District along with monocultures from the laboratory. To scale-up, we are using a hyperspectral sensor mounted on a UAS (unmanned aerial system) for aerial imaging and comparing it to the laboratory hyperspectral camera images. This platform will enable routine whole-lake monitoring and rapid identification of cyanobacteria at low-levels for the prevention and/or early management of cyanoHAB events.

ASSESSMENT OF AN LC-MS METHOD FOR MONITORING NSP TOXINS IN *KARENIA BREVIS* EXPOSED SHELLFISH

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Neurotoxic shellfish poisoning (NSP) is caused by consumption of shellfish contaminated with brevetoxins, a suite of cyclic polyether compounds produced by the marine dinoflagellate *Karenia brevis*. To prevent NSP, shellfish harvest areas are closed when *K. brevis* density exceeds 5,000 cells/L and re-opened when the shellfish toxicity assessed by mouse bioassay (MBA) is < 20 MU/100g. The NSP MBA, the only NSSP approved method for regulatory NSP testing, was developed in the 1960s. This assay has several drawbacks. It is laborious, time-consuming, and delays regulatory decisions. There has long been a need for methodologies that are more sensitive and precise, do not require live test animals, and provide public health protections equal to the MBA. Brevetoxins are extensively metabolized in molluscan shellfish. Three metabolites, BTX-B1, BTX-B2, and desoxy BTX-B2, have been identified as biomarkers of brevetoxin exposure in Eastern oyster and hard clam. We developed an LC-MS method for the determination of the biomarkers and are currently assessing the application of these biomarkers for monitoring brevetoxins in shellfish. Shellfish sampling and testing is routinely conducted by state authorities in the Gulf of Mexico region where *K. brevis* blooms frequently occur. We are examining these samples (Eastern oyster, hard clam, and sunray venus clam) by LC-MS to evaluate the method for monitoring purposes. Preliminary results show correlation of biomarker levels by LC-MS with composite toxins by ELISA and toxicity assessment by MBA. The data indicate the LC-MS is a method of higher precision, sensitivity, and throughput for monitoring brevetoxins in shellfish.

HABscope: A Tool For Use By Citizen Scientists To Facilitate Near Real-Time Warning of Respiratory Irritation Caused By Toxic Blooms of *Karenia brevis*

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Millions of dollars in lost revenue occur along Florida beaches every year due to *Karenia brevis* algal blooms. These blooms produce toxic aerosols that cause mild to severe respiratory irritation in beachgoers. When respiratory impacts are severe, people avoid beaches all together to minimize potential exposure. In reality, the distribution of blooms is highly patchy with some beaches strongly impacted whereas others nearby are not. To provide the public with near real-time information on local beach conditions, we have begun organizing a citizen scientist network to monitor *K. brevis* cell density at local beaches daily. Critical to the success of the project was development of the HABscope for use by volunteers. This rapid detection system combines a OMAX microscope with 40X -2000X objectives along with smartphone acquisition via Apple iPOD touch® and a wireless hotspot (~$450 total). Volunteers take video of water samples and upload them to the GCOOS website, which estimates cell densities using real-time automated image recognition software. The system was calibrated with cultured *K. brevis* cells. A comparison of the cell counts from the HABscope system versus hand counts had an R^2= 0.994, and slope = 0.919 resulting in a near 1:1 relationship within standard error. These near real-time results are currently being integrated into an "Every Beach, Every Day" respiratory forecast that incorporates local wind speeds and direction as well as remote sensing. The system will be demonstrated in conjunction with the poster presentation.

DEVELOPMENT OF OPTICAL DETECTION TOOLS OF HARMFUL ALGAL BLOOMS ON LAKES

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The occurrence of harmful algae blooms (HAB) has become a growing concern for society, especially when they are dominated by toxic cyanobacteria. New detection technologies such as hyperspectral cameras will be used to spatially define HABs with an high spectral resolution and to distinguish those dominated by cyanobacteria. A spectral signatures bank will first be generated in the laboratory using algal cultures at different stages of their growth to determine the changes in the shape and / or intensity of the signature according to the physiological state of the cells. Spectral signatures of mixtures of algal species will also be analyzed and in addition of interfering factors (suspended matter, colored dissolved organic matter). A deconvolution of the spectrum will allow to estimate the biomass of the different groups of algae. Water samples (Chl-a, taxonomy, MOD, MES, inherent optical properties) will be collected to calibrate these estimates, in parallel with the deployment of two EXO fluorescence probes (YSI). The data collected by the mobile probe will interpolate those obtained by conventional time-consuming sampling. A stationary probe attached to a buoy will collect data at high temporal frequency. The validation of these tools will help to better target and evaluate the potential of hyperspectral satellite sensors in service and planned for the coming years, and to provide the scientific community with unprecedented data on accuracy and limitations of these tools.

APPLICATION OF OPTICAL REMOTE SENSING AND ECOLOGICAL ASSOCIATIONS FOR NEAR REAL-TIME MONITORING OF HARMFUL ALGAL BLOOMS IN CHESAPEAKE BAY

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High frequency monitoring through satellite remote sensing has been beneficial to protecting public and environmental health in several key US waterways. In most instances, the success of these tools relies on strong optical signatures of some high biomass harmful algal bloom species (HABs). The near monospecific nature of blooms, like *Karenia* in the Gulf of Mexico and *Microcystis* in Lake Erie, make them suitable for optical detection. In southern Chesapeake Bay, monospecific blooms of *Alexandrium monilatum* and *Cochlodinium polykrikoides* are also amenable to detection with satellite data. In contrast, a majority of blooms in northern Chesapeake Bay occur as mixed assemblages that are optically complex, making it difficult for direct detection of individual species. However, these mixed assemblages may produce a unique signature if properly validated. An approach using optical detection combined with ecological associations may aid in further classification of blooms (e.g., *Heterocapsa* blooms during the winter). This presentation examines how fluorescence and high biomass algorithms applied to the 300 m Ocean and Land Colour Imager (OLCI) on the Sentinel-3 satellite have improved our ability to detect and characterize algal blooms at higher resolution. Statistical models constructed with information regarding the ecological niche of individual species (time of year; bloom succession; salinity, temperature and nutrient regimes), combined with remote detection of blooms can potentially provide a real-time monitoring system for HAB events in Chesapeake Bay. This type of integrated system would be a valuable tool for resource managers responsible for safeguarding fisheries and public health within the region.

AN ASSESSMENT OF ANNUAL SEVERITY OF FLORIDA RED TIDE, *KARENIA BREVIS*

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Karenia brevis has produced "red tide" events in the Gulf of Mexico for centuries. In Florida, monitoring by cell counts of water samples began over 60 years ago, and routine sampling has continued since 1995. The availability of this dataset has provided researchers with tools to examine many characteristics of the blooms, including economic and health impacts. Observations of potential respiratory irritation have also been made for over a decade by the Beach Conditions Report System (BCRS). Starting with the cell count dataset, we can characterize the relative severity of blooms between different years and the association between these blooms and potential impacts.

Bloom severity can be defined in many ways. We examine severity as the potential for direct impact on the public, and also review that potential against the observed impact, as reported by BCRS and other metrics (such as animal mortalities). Differences resulting from changes in the sampling approach before 1995 (event response sampling) and after 1995 (routine monitoring) are also considered. The results provide a potential metric for severity that can be used to characterize annual blooms, and to evaluate inter-annual differences.

DESCRIMINATION OF CYANOBACTERIA FROM MIXED BLOOM ASSEMBLAGES IN GREEN BAY, WISCONSIN

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Green Bay, a sub-basin on the west coast of Lake Michigan, is one of the main hotspots for cyanobacteria blooms in the Laurentian Great Lakes. The Fox River drains the eutrophic Lake Winnebago, into Green Bay. Lake Winnebago and Green Bay experience large mixed assemblage phytoplankton blooms of which cyanobacteria can make up a significant percentage of the biomass. This study will use satellite-derived reflectance to make synoptic biomass maps of the cyanobacterial blooms. We will utilize a previously published algorithm that has been successfully used to detect monospecific blooms elsewhere in the Great Lakes, and parse out cyanobacteria from non-cyanobacteria (Wynne et al., 2010). The initial algorithm uses the relatively novel chlorophyll fluorescence signature as an indicator. This newly proposed algorithm will use the same Wynne et al. (2010) algorithm to quantify the bloom, but use an additional criteria based on phycocyanin absorption to eliminate pixels which are not likely to have an elevated concentration of this key accessory pigment unique to cyanobacteria. The presentation will conclude with basic probability maps of cyanobacteria biomass.

HUMAN HEALTH EFFECTS AND REMOTELY SENSED CYANOBACTERIA

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Cyanobacteria blooms (HAB) pose a potential health risk to beachgoers, including HAB-associated gastrointestinal, respiratory and dermal illness. We conducted a prospective study of beachgoers at a Great Lakes beach during July – September, 2003. We recorded each participant's health status and activity during their beach visit, and their health effects that occurred within 12 days after the beach visit. We evaluated the presence of blooms retrospectively using the default MODIS ocean chlorophyll-a (OC4) algorithm and MERIS cyanobacteria index (CI). During 16 weekend study days, we recruited 2,840 participants, of which 1305 (46%) reported water contact (bathers) and were included in analysis; 55% percent were female, and 26% were children. During a retrospective assessment of MODIS OC4 imagery, chlorophyll-a concentrations suggested a HAB during August 16 – 24, whereas the MERIS CI did not indicate a HAB was present. We detected no significant increase in reported gastrointestinal, respiratory or dermal health effects among bathers who recreated during August 16 – 24 compared to other study days. Although the MODIS OC4 is useful to detect chlorophyll-a and HABs in coastal environments, the MERIS CI appears to have provided a better assessment of bather health risk at this Great Lakes beach. Reliance upon the default MODIS OC4 algorithm alone would have led to erroneous bather HAB exposure assessment. This is the first time concurrent MODIS OC4 and MERIS CI results have been informed by temporally-associated health information. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

EFFORTS TO OPTICALLY SEPARATE HARMFUL BLOOMS SPECIES IN THE CHESAPEAKE BAY

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Numerous harmful algal bloom (HAB) species occur in the Chesapeake Bay. These HABs can impact fish, shellfish, animal and human health via the production of toxins and/or degradation of water quality. Resource managers are seeking ways to discriminate different HABs on large temporal and synoptic scales. Satellite-derived algorithms and techniques have been developed for monitoring high biomass blooms that are visible in surface waters. A red-band difference (RBD) fluorescence algorithm has been developed for detecting toxic Karenia brevis in the Gulf of Mexico. When applied to MODIS imagery for the Chesapeake Bay, the RBD has been successful in monitoring blooms of several species of concern at 1 km resolution. The addition of higher resolution (300 m) products from the Ocean and Land Color Imager (OLCI) on the Sentinel-3 satellite (launched by the European Space Agency in February 2016) provides detail that allows monitoring of the tributaries of the bay, where shellfish resources and recreation beaches are located. OLCI also has several additional spectral bands not found on MODIS that can provide additional information on the various blooms. Field measurements of water-leaving spectral radiance have been collected to validate and fine tune algorithms. The majority of blooms in the Bay are mixed making it challenging to separate bloom type by optics alone. Satellite-derived products will need to be combined with bloom ecology to further separate bloom type.

IDENTIFYING CYANOBACTERIA BLOOMS FROM SPACE WITH SENTINEL-3 **OLCI**

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The Cyanobacteria Assessment Network (CyAN) project is a EPA, NASA, NOAA, and USGS inter-agency effort to use satellite data to detect cyanobacteria blooms. Toxic cyanobacteria blooms threaten ecosystem function, public health, and water supplies and are an increasing concern. The cyanobacteria index (CI) algorithm was applied to the Ocean Land Colour Instrument (OLCI) data on Sentinel-3A for a number of lakes in the United States. Then, validation was completed using a USGS in-situ dataset to assess algorithm performance. A reliable OLCI cyanobacteria algorithm will be useful for the monitoring of and near real-time warnings of HAB events.

EFFECTIVE RETRIEVAL OF KARENIA BREVIS HARMFUL ALGAL BLOOMS IN THE WEST FLORIDA SHELF FROM THE VIIRS SATELLITE USING RECENTLY DEVELOPED NEURAL NETWORK ALGORITHMS HIGHLIGHTING THE IMPACT OF TEMPORAL VARIABILITIES

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Detecting, quantifying and tracking of *Karenia* brevis Harmful Algal blooms (KB HABS) that plague the coasts of the West Florida Shelf (WFS) is important because of their impact on health, tourism and the economy. Previously, satellite monitoring from MODIS-A has proved effective using the normalized fluorescence height (nFLH) technique. Unfortunately, VIIRS, the current successor satellite to MODIS-A, lacks the fluorescence band at 678 nm used in the nFLH technique. We report on a recently developed effective alternative approach. This uses neural network (NN) algorithms, and does not require observations at the fluorescence channel. The NN requires as inputs the remote sensing reflectance (Rrs) measurements at 486, 551 and 671 and 488, 555 and 667 nm channels, available from VIIRS and MODIS-A respectively. These channels are also less vulnerable to atmospheric correction inadequacies affecting observations at the shorter blue wavelengths which are used with other algorithms. NN VIIRS WFS retrievals were compared against all the in-situ measurements available over the 2012-2016 period for which near concurrent match ups could be obtained with VIIRS observations. The NN technique achieved significantly better accuracies than all other retrieval algorithms tested, including OCI/OC3, GIOP and QAA version 5. Results also highlighted the impact of temporal variabilities on retrieval accuracies. Thus retrievals within a 15 minute overlap time window showed very significantly improved accuracies over those attained with a 100 minute window. KN HABs temporal variabilities are also confirmed by results of in-situ field measurements as well as observations from consecutive overlapping VIIRS and MODIS-A images.