

10TH US HAB Symposium



10th

US HAB SYMPOSIUM 11/19

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BLOOM DYNAMICS

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MODELLING OF CYANOBACTERIAL BLOOMS DYNAMICS IN MESOCOSM EXPERIMENT

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Research on the cyanobacterial blooms continues to accelerate, yet we still know a little about *Microcystis* spp. bloom dynamics. To address this question, we developed imaging flow cytometry approach to differentiate five major *Microcystis* spp. morphoforms (*M. wesenbergii*, *M. novacekii*, *M. smithii*, *M. aeruginosa*, *M. ichthyoblabe*), and used it to carry out mesocosm experiments in LMWE (Lake Mesocosm Warming Experiment, Lemming, Denmark) during May-October 2018. Weekly analysis by FlowCam imaging flow cytometer (Fluid Technologies Inc., USA) allowed us to differentiate and quantitate the spatiotemporal distribution of colonial cyanobacteria, green algae, dinoflagellates, different groups of diatoms, cryptomonads and other phytoplankton groups. Also, water parameters, turbidity, pH, nutrient composition (TN, TP), oxygen, conductivity, and chlorophyll-a were recorded. In this work we show a seasonal succession of different *Microcystis* spp. during summer months blooms. Increase in temperature was responsible for early cyanobacterial bloom formation and colonies size growth, however, further temperature change suppressed colonial cyanobacteria and led to cryptomonad dominance not associated with a particular season. Data showing forming a “half-empty” phenotype colonies; i.e. sheets filled with occasional empty spaces or even without any cell inside (“empty” sheets) and their association with “dying-off” blooms will be presented in the context of blooms development. The potential scenario is that during *Microcystis* spp. bloom formation, *M. wesenbergii* colonies are sequentially growing in size, losing cells, sharply change density and increase buoyancy of colonies. It may facilitate a wind-forced migration of colonies in surface layer of water even against flow stream (rivers). Our results closely illustrate mechanisms of *Microcystis* spp. bloom dynamics formation and a role of temperature factor in bloom development. The data obtained provide a new framework for modelling cyanobacterial blooms in mesocosm experiments.

BIOAVAILABILITY OF ORGANIC PHOSPHORUS COMPOUNDS WITH RESPECT TO THE GROWTH KINETICS OF *MICROCYSTIS AERUGINOSA*

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Organic phosphorus (Po) compounds display differing levels of bioavailability for *Microcystis aeruginosa* (*M. aeruginosa*). The growth patterns of *M. aeruginosa* with orthophosphate, D(+)-glucose 6-phosphate, β -glycerophosphate, inositol hexaphosphate (phytic acid) and mixtures of the inorganic P and Po compounds were investigated. Results show that both D(+)-glucose 6-phosphate and β -glycerophosphate can serve as the sole P source for *M. aeruginosa* growth. A mixture of orthophosphate and Po compounds consistently yielded higher maximum cell densities than orthophosphate alone. *M. aeruginosa* showed no detectable growth with phytic acid as the sole P source. The concentrations of the Po compounds present in and during cultures were confirmed using ³¹P Nuclear Magnetic Resonance (NMR) spectroscopy. This study shows that various forms of organic phosphorus are bioavailable to *M. aeruginosa*, providing a deeper insight in the role of phosphorus loading and dynamics for the growth of cyanobacteria harmful algal blooms (a.k.a. CyanoHABs). These findings should offer improved predictions of *M. aeruginosa* growth with various forms of environmental phosphorus.

A YEAR IN THE LIFE OF A *LYNGBYA WOLLEI* BLOOM IN LAKE WATEREE, SC: TOXINS, TRENDS, AND FATES

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The occurrence and spatial distribution of the persistent benthic filamentous algae *Lyngbya wollei* was determined for Lake Wateree, SC over the period of July 2018 - August 2019. A 16S rRNA gene sequence was recovered from a sample of collected algae to verify microscopic determination of the presence of *Lyngbya wollei* as the dominant algal species in grab samples from the bloom. Grab samples presented as fibrous masses and the organic carbon and ash contents were also determined for all samples taken. Samples exhibited a surprisingly high inorganic content at a mean 35% ash by gravimetric determination. The mass of inorganic material trapped in the fibers was applied as a correcting factor for grab sample biomass. Samples were lyophilized, extracted, and extracts were subjected to analysis by liquid chromatography-mass spectrometry (LCMS) for toxin determination, focusing on the neurotoxins *Lyngbya wollei* toxins (LWTs) 1-6. The LWTs 1, 4, 5, and 6 were observed in all locations sampled over the measured time period. Standard reference LWTs were unavailable; therefore, LWT 5 and LWT 6 were quantified against their structural analogue saxitoxin and normalized against organic carbon content. Based on the same standard LWTs 1 and 4 were present at much lower concentrations and are reported as qualified but not quantified. Samples were analyzed for spatial or temporal trends over the 13 month period. Algal occurrence maps and mean toxin concentrations were used to estimate an approximate total *Lyngbya wollei* toxin inventory for Lake Wateree. The cation exchange capacity of several Lake Wateree sediments was determined to evaluate the possibility of sedimentary LWT sinks.

RETURN OF THE “AGE OF DINOFLAGELLATES”: DRIVERS OF UNUSUAL DINOFLAGELLATE DOMINANCE IN NORTHERN MONTEREY BAY EXAMINED USING AUTOMATED IMAGING FLOW CYTOMETRY

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Monterey Bay is the largest open embayment along the U.S. west coast and is subject to intense autumn dinoflagellate blooms, many of which are harmful. During 2004–2007 these blooms were so dominant that this period was called the “age of dinoflagellates”. From mid-2017 through 2019, after a decade of absence, a diversity of dinoflagellates returned to dominate the phytoplankton biomass. To quantify bloom development at high temporal resolution, an Imaging FlowCytobot was deployed within the upwelling shadow of the northern bay at Santa Cruz Municipal Wharf (SCW) and a machine learning image classifier was developed to identify the local phytoplankton assemblage. In 2018, dinoflagellates comprised 57% of the annual phytoplankton-specific carbon biomass, and 62% from January to May. This is unusual given that Monterey Bay is typically diatom-dominated during spring upwelling. 2018 was characterized by strong upwelling interspersed with frequent relaxation, which contributed to unusually retentive winds and currents. The resulting warm and stratified northern bay was frequently resupplied with upwelled nutrients and frontal aggregations of dinoflagellates. Analysis of a 15-year weekly SCW time-series suggests that both “age of dinoflagellate” periods were linked to a negative NPGO and its associated effects of increased sea surface temperatures and stratification. As the climate continues to warm, periods of increased dinoflagellate dominance in the California Current System may become more common.

DIVERSITY AND DYNAMICS OF HARMFUL ALGAL BLOOM SPECIES IN THE GULF OF MEXICO FOLLOWING HURRICANE HARVEY

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Phytoplankton are a vital component of the coastal-pelagic ecosystem and are directly affected by changes in the hydrodynamic conditions (e.g., temperature, salinity, light availability, increasing nutrient inputs, and modifications in flow regimes). Tropical cyclones, such as hurricanes, create extreme disturbances which can drive rapid changes in the phytoplankton community. The present study investigated the changes in the phytoplankton community after the 2017 Hurricane Harvey in the Gulf of Mexico (GoM), with special emphasis on the harmful algal bloom species

(HABs). In the aftermath of the hurricane, multiple cruises were conducted along the Texas coast starting from Galveston Island extending south to Port Aransas to examine the phytoplankton community response on both a temporal and a spatial scale. Phytoplankton diversity and abundance was assessed using an Imaging FlowCytobot (IFCB) and high throughput sequence (HTS) metabarcoding. Molecular assemblages of protists differed along the spatial scale in the GoM. Metabarcoding reported a total of ca. 40 putative HAB species in both the V4 dataset and in the V8-V9 dataset belonging to 18 genera. The HTS result indicated that dinoflagellates were the dominant groups among the phytoplankton, which agreed with the IFCB data. From the HTS data, it was evident that the dinoflagellate abundance increased with time. Among the HAB species, *Karenia mikimotoi/selliformis* revealed highest abundance in both V4 and V8-V9 datasets. IFCB showed that initially *Karenia* cells were present in low numbers but rapidly increased after a week especially at the northernmost stations close to Galveston Bay, which was the area most impacted by the freshwater outflow and were much lower in abundance towards the southwest stations towards Port Aransas. Rapidly changing climate and increasing number of tropical cyclones predicts the potential for an increase in HAB events.

LONG-TERM NUTRIENT TRENDS IN THE GULF OF MEXICO IN RELATION TO FLORIDA RED TIDE

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Blooms of the dinoflagellate *Karenia brevis* occur nearly annually along the coast of southwest Florida and initiate offshore. To better relate offshore and nearshore bloom dynamics and increase routine nutrient sampling, a collaborative offshore sampling program on the west-central Florida shelf (~ 26.5°N to 27.5°N, spanning the 15-40m isobaths) was initiated in 2015. Discrete vertical samples from three depths at 14 sites were analyzed to determine nutrient (dissolved silicates, inorganic N and P species, total dissolved N and P, urea, particulate C, N, and P, and Si) and dissolved organic matter (CDOM) concentrations; vertical profiling (conductivity, temperature, dissolved oxygen, chlorophyll fluorescence, and PAR) provided further context. Sampling coincided with three *K. brevis* blooms of varying duration and severity (9/2015-2/2016, 9/2016-4/2017, and 11/2017-2/2019). Similar weekly event response sampling was conducted offshore of Charlotte Harbor in summer/fall 2018. Significant interannual and/or cross-shelf trends were observed for certain nutrient species. Nitrate concentrations were elevated only at the beginning of the bloom season (August – October), in the deepest, (often) northern, and furthest offshore stations, and in 2015, 2016, and 2018. Ammonium concentrations exceeded nitrate concentrations in most samples, and preceding and throughout the first ~9 months of the 2017-2019 bloom. Particulate C was significantly correlated with particulate N, P, and to a lesser extent, Si, and was generally higher in October 2018 relative to other time points. Underlying mechanisms contributing to reduced cross-shelf variability in 2017 as well as the flux of nitrate onto the shelf during the latter phase of the 2017-2019 bloom were further considered by examining spatiotemporal differences in other nutrients, water column stratification, and *K. brevis* abundance. This examination of chemical patterns prior to, during, and after the formation of *K. brevis* blooms provides critical insight into predicting these dynamics at seasonal to interannual time scales.

A POTENTIAL RESERVOIR FOR THE BROWN TIDE ORGANISM, *AUREOUMBRA LAGUNENSIS*, IN A EUTROPHIC SOUTH TEXAS ESTUARY, BAFFIN BAY

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Blooms of the “brown tide” organism, *Aureoumbra lagunensis*, have occurred regularly since 1990 in Texas’ Laguna Madre and Baffin Bay, yet the source population for the blooms is unknown. Field sampling from 2013-2016 showed persistently high abundances in one tributary (Laguna Salada) of Baffin Bay, even when the bloom had dissipated in the rest of the bay. Here we present results from a study that aimed to characterize the environmental conditions, as well as phytoplankton dynamics, in Laguna Salada. Results show that chlorophyll in Laguna Salada is at times tenfold higher than Baffin Bay proper, averaging 136 µg-l vs. 15 µg-l and with *A. lagunensis* abundance reaching one million cells ml⁻¹. During the study, dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN) concentrations in Laguna Salada were high (200 µM and 30 µM, respectively) compared to Baffin Bay proper (62 µM and 4 µM,

respectively). DIN:DIP ratios were similar (10.9 and 14.3) for Laguna Salada and Baffin Bay, suggesting nitrogen limitation of algal growth. Additional findings will be presented to highlight the characteristics of Laguna Salada that are favorable to *A. lagunensis* persistence.

POTENTIAL 'NEW' NITROGEN AND PHOSPHORUS INPUTS TO THE 2018 *KARENIA BREVIS* BLOOM FROM LAKE OKEECHOBEE DISCHARGES AND THE DISPLACED *MICROCYSTIS AERUGINOSA* BLOOM

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Translocated freshwater blooms of *Microcystis aeruginosa* represented a potential nutrient source to coastal blooms of the toxic dinoflagellate *Karenia brevis* in southwest Florida in 2018 when Lake Okeechobee water was released out the Caloosahatchee River for regulatory purposes. The nitrogen (N) and phosphorus (P) requirements of adjacent coastal *Karenia brevis* bloom waters were calculated and compared with 1) the dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) Caloosahatchee River loadings and 2) potential regenerated N and P available from displaced *Microcystis* populations. River nutrient loadings increased in mid-May coincident with increased river flow, to a maximum of 3.75×10^{10} uM/d⁻¹ PO₄⁻², 3.71×10^{10} uM d⁻¹ NO₃⁺² and 4.85×10^{10} uM d⁻¹ NH₄⁺. The percentage of coastal bloom N and P needs met by River loading was dependent upon the *K. brevis* concentration: at >300,000 cells l⁻¹, <10% of bloom N and P needs were met by River inputs, while at 200,000 cells l⁻¹, up to 30% of bloom N and 70% of bloom P needs could be met. Populations of *M. aeruginosa* in the lower River could potentially supply a maximum of 8.44×10^{-2} uM l⁻¹ d⁻¹ N and 8.65×10^{-4} uM l⁻¹ d⁻¹ P after degradation and nutrient regeneration. On a comparative basis, regenerated *M. aeruginosa* biomass provided an order of magnitude less N to *K. brevis* blooms than benthic N flux and two or more orders of magnitude less than decaying fish, zooplankton excretion, mixotrophy and *Trichodesmium* N₂ fixation, N release and decay. Decaying *M. aeruginosa* biomass met more of the N needs of the *K. brevis* bloom than P needs, possibly reflecting P limitation of translocated *M. aeruginosa* biomass. Regenerated *Microcystis aeruginosa* blooms thus represent a 13th quantified nutrient source for adjacent estuarine and coastal *K. brevis* blooms.

SURVEY OF CYANOBACTERIAL PARAMETERS IN FLORIDA SURFACE SEDIMENTS

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Cyanobacteria toxins (cyanotoxins) are metabolites found in water bodies around the world that can have negative effects on aquatic ecosystems and human health. An increase in cyanobacteria and cyanotoxin occurrences suggest drivers that promote their production are becoming more common. Microcystin (MY) is a toxin of increasing concern and has been frequently documented in Florida, USA. Prior research has attempted to identify the drivers that promote cyanotoxin occurrence—including MY—but to a lack of scientific consensus. Therefore, the purpose of this study is to document cyanobacterial parameters on a multi-lake scale to identify possible drivers of cyanobacterial and cyanotoxin presence. In July 2018, a 47-lake water column and surface sediment survey was conducted throughout the state of Florida using paleolimnological methods; data collected includes (MY) concentrations, nutrient concentrations, photosynthetic pigments, and additional parameters. Results of this study will contribute to a better understanding of cyanobacterial and cyanotoxin behavior in the subtropics.

POLYPHASIC EVIDENCE FOR CYLINDROSPERMOPSIS PRODUCTION BY *CYLINDROSPERMOPSIS RACIBORSKII* AT MATTAMUSKEET NATIONAL WILDLIFE REFUGE, NORTH CAROLINA, USA

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Infrequent historical detections of cylindrospermopsin (CYN) have been attributed to a few cyanobacteria species in the USA. However, no strains of *Cylindrospermopsis* have been proven previously to produce CYN. Some have considered *Cylindrospermopsis raciborskii* to be invasive to surface waters of the United States and genomics has supported other cyanobacteria such as *Chrysothrix* (*Aphanizomenon*) and *Oscillatoria* as being responsible for cylindrospermopsin production when encountered. CYN was only detected in 4% of the 2007 National Lakes Assessment and *Cylindrospermopsis* was rarely detected and never associated with CYN production. However, in a study initiated in 2015 in Lake Mattamuskeet National Wildlife Refuge, cylindrospermopsin was detected with peak concentration slightly lagging after peak concentration of a cyanobacteria identified morphologically as *Cylindrospermopsis raciborskii*. qPCR results supported that a CYN producing operon was present in environmental samples, and genomics supported that the samples contained an organism binned to the *Cylindrospermopsis/Raphidiopsis* clade. Culturing of isolates was unsuccessful. To our knowledge this is the first case with strong converging lines of evidence supporting the production of CYN by *Cylindrospermopsis raciborskii* in the United States.

TAYLOR CREEK: NUTRIENT POLLUTION FEEDING CYANOHABS IN LAKE OKEECHOBEE FLORIDA

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Lake Okeechobee Florida is plagued with recurrent blooms of the non-nitrogen-fixing cyanobacterium (aka blue-green alga) *Microcystis aeruginosa*. It is therefore apparent that nitrogen pollution has increased in the lake since the 1990s when nitrogen-fixing cyanobacteria (*Anabaena*, *Aphanizomenon*) were abundant. The results presented here represent the first 8 months of a planned 3-5 year study on mainly so-called “non-point” nutrient pollution sources and its effects on phytoplankton abundance and types. One sampling of phytoplankton increase in relation to nutrient pollution showed that Taylor Creek just after a Stormwater Treatment Area (STA) had CHLa at 16.1 micro-g/L and after flowing through the Okeechobee City area it increased to 63 micro-g/L. Nubbin Slough, also starting after the STA at the 16.1 micro-g/L level, had decreased to 13.1 micro-g/L at its terminus. Nutrient sources along Taylor Creek include a fertilizer plant, cattle that enter and leave manure directly in the creek and a high number of side canals with septic tanks. The lower stretch of Taylor Creek has contrasting developments. That is, on the west side sewerage is mainly municipal sewers with only scattered septic tanks. On the East side is all septic tanks with drain fields only feet from the side canals. Sucralose analyses showed that the east side has four times the amount of sucralose as the west side. The Okeechobee Utility Authority has detailed plans for the conversion of the east side to municipal sewage but that would require > 24 million dollars. CHLa levels in the canals feeding lower Taylor Creek often surpass 90-100 mg/L and are thus far dominated by chlorophytes with lesser amounts of cyanobacteria, diatoms and cryptophytes. Pigment analyses indicate that the cyanobacteria are mainly of *Synechococcus* and not *M. aeruginosa*. We have found a potential highly polar *M. aeruginosa* biomarker carotenoid and are working on its structural identification.

SPATIOTEMPORAL TRENDS AND ENVIRONMENTAL DRIVERS OF CYANOBACTERIAL BLOOMS AND MICROCYSTIN PRODUCTION IN THE NORTHERN GULF OF MEXICO FOLLOWING UNPRECEDENTED FRESHWATER DISCHARGE INTO COASTAL ZONES FROM THE 2019 BONNET CARRÉ SPILLWAY RELEASE

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During the first half of 2019, the Bonnet Carré Spillway was opened in order to protect the city of New Orleans from Mississippi River flooding and remained open for a record 118+ days. This resulted in an 83% increase in freshwater discharge relative to typical spring conditions. Throughout this period, low salinities across coastal Mississippi persisted for several months and resulted in blooms of *Microcystis* spp. and *Dolichospermum* spp. along the Mississippi coastline. While these genera have been identified locally in freshwater systems, blooms of these cyanobacterial HABs have not been reported in the northern Gulf of Mexico coastal waters. The objective of this work was to examine how environmental conditions such as water temperature, salinity, dissolved oxygen, total suspended solids, and nutrients, affected phytoplankton community composition and in-situ toxin production through time. A west – east salinity gradient persisted throughout the duration of the study as well as many zones of hypoxia that extended from the surface to the water in some instances. Microcystins were detected by enzyme linked immunosorbent assay and liquid chromatography/mass spectrometry in surface integrated samples and persisted at concentrations >1 ppb total microcystin along the coastal Mississippi transect, even in the absence of visible blooms. As climate disturbances are predicted to increase over the next several decades, so too will prolonged openings of spillways to protect property and livelihoods of communities living at or below sea level. These data have allowed a deeper understanding of cyanobacterial bloom dynamics in freshwater dominated coastal systems and will directly inform on response efforts for future events.

INTERANNUAL VARIABILITY IN BLOOMS OF *MARGILEFIDINIUM POLYKRIKOIDES* IN THE SOUTHERN CHESAPEAKE BAY

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Blooms of *Margilefidinium polykrikoides* in the lower Chesapeake Bay have occurred in most of the last 20 years. Blooms occur in late summer and thus temperature is thought to play a primary role in bloom initiation. However, the absence of blooms in several years when water temperatures were thought to be conducive suggest that water temperature alone is not sufficient for bloom initiation. Here we examine factors thought to contribute to bloom initiation (e.g., nutrient concentrations, temperature, salinity) and relate these to the timing of bloom initiation or the absence of blooms between 2012 and 2018 in the Lafayette River, a sub-estuary of the lower Chesapeake Bay. We also provide simulation results for bloom initiation and development that address controls on bloom initiation.

COMMUNITY EXPRESSION IN A DIEL STUDY OF A *MICROCYSTIS AERUGINOSA* BLOOM IN LAKE ERIE 2014

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August of 2014 was a notable year for cHABs in the western basin of Lake Erie. The city of Toledo, OH issued a Do Not Drink warning for 3 days early in the month due to elevated toxin levels (microcystin) produced by the cyanobacteria *Microcystis aeruginosa*. A 48-h Lagrangian survey was conducted August 26-28, 2014 in order to study the physiology of the community of organisms through metatranscriptomics. RNA was extracted from the water samples and sequenced. The metatranscriptome was analyzed using RNASeq function of the CLC Genomics Workbench v. 10.2.0 aligning the reads to 10 reference genomes representing the general community, including a *Microcystis aeruginosa* genome endemic to Lake Erie. Gene expression of metabolic cycles such as nitrogen and phosphorus were studied, along with photosystem I and II, toxin production and stress responses. We are currently looking at genetic expression profiles of other cyanobacteria and bacteria to understand the broader interactions among the microbial community under bloom conditions.

SPATIAL AND TEMPORAL DISTRIBUTION OF PHYCOTOXINS THROUGHOUT LOWER CHESAPEAKE BAY

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Lower Chesapeake Bay harbors multiple harmful algal species, including toxigenic *Dinophysis* spp., *Pseudo-nitzschia* spp., *Karlodinium veneficum*, *Alexandrium monilatum* and cyanobacteria. Although the region has not experienced recurring harvest closures or bans due to seafood contaminated with phycotoxins, low concentrations of multiple phycotoxins may be present and even co-occur, having potentially detrimental effects on environmental health. To conduct a comprehensive study of existing and emerging phycotoxins, twelve stations were sampled across the lower Chesapeake Bay spanning the mouth, northern neck, and the bayside and seaside of the Eastern Shore for a year. Solid-Phase Adsorption Toxin Tracking (SPATT) was used in conjunction with traditional water samples for cell enumeration to explore the possibility of trace level concentrations present in the water and to explore the relationships between cell abundance and phycotoxin persistence in the system. A multi-toxin extraction method was developed for SPATT samples, and extracts were analyzed by a combination of ultra-performance liquid chromatography coupled to tandem mass spectrometry with at-column dilution for 13 phycotoxins and ELISA for domoic acid. The former technology allows for high-volume injections leading to low detection limits, and when combined with SPATT enables the investigation of trace levels in the environment. This study is the first to showcase the spatial and temporal changes in the distribution of multiple phycotoxins across the lower Chesapeake Bay.

PATTERNS AND IMPACTS OF SUMMER BLOOMS IN THE LOWER CHESAPEAKE BAY

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Since 2007 during most summer seasons, blooms of *Margalefidinium* (formerly *Cochlodinium*) *polykrikoides* and *Alexandrium monilatum* have occurred in the York River, VA. Blooms of both species were confined to the York River region for several years and subsequently expanded throughout lower Chesapeake Bay. Annual variations in cyst densities and environmental parameters including rainfall, temperature, wind, salinity and nutrient levels affect

the occurrence, timing, density and distribution of blooms of these species. The relative importance of the various drivers is unknown and requires more study. Annual sediment cyst surveys have been conducted since 2014. Beginning in 2016 weekly cruises have been done in the York River during the intense summer blooms using a DataFlow system for determination of water quality parameters. In addition, bi-monthly year-round cruises were started in 2018. Water samples were collected during cruises for microscopic and molecular phytoplankton counts, for measuring metabolic rates, nutrients, dissolved inorganic and organic carbon, and examining microbiome composition via next generation sequencing of 16S and 18S rRNA genes. These data will help inform design and validation of bloom development models. Reported economic and ecological impacts of *M. polykrikoides* and *A. monilatum* blooms in the lower Chesapeake Bay have varied with year and location. Reports of juvenile oyster mortalities from local shellfish aquaculturists during and immediately following these blooms prompted field studies examining lethal and sub-lethal impacts of these blooms and other stressors using different grow-out strategies at aquaculture sites with differing physical characteristics with an aim toward identifying mitigation strategies. Mortality was higher for oysters grown intertidally than for those grown subtidally and higher at the bloom-impacted low energy site compared to the reference site during a bloom year. In addition, oysters exposed to the highest bloom cell concentrations were smaller than those at the reference site during that same year.

UNDERSTANDING THE CONTRIBUTION OF BENTHIC FLUXES TO THE PROLIFERATION OF HABs FORMED BY MULTIPLE GENERA OF DINOFLAGELLATE

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Globally, the range, frequency, and intensity of harmful algal blooms (HABs) are increasing, partially due to increasing nutrient loading (nitrogen) into coastal systems. Some HABs produce toxins that can contaminate seafood and cause economic losses while others are harmful to marine life. On Long Island, NY, HABs are formed by *Alexandrium catenella*, a neurotoxin producing species, *Cochlodinium* (aka *Margalefidinium*) *polykrikoides*, an ichthyotoxic species, and *Dinophysis acuminata*, a toxin producing species, on an annual basis. While prior research has demonstrated that increased nitrogen loading, often in the form of ammonium, can increase the intensity and, in some cases, toxicity of these HABs, the ultimate source of nitrogen that intensifies these events is unknown. While wastewater is the largest watershed-based nitrogen source to estuaries on Long Island, the relative contribution of sediment benthic fluxes of nitrogen, which are often dominated by ammonium, are unknown. In this study, we determined the effects of sediment nutrient fluxes on *A. catenella*, *C. polykrikoides*, and *D. acuminata* using mesocosm experiments, in which bloom water was collected in 300 L mesocosms that were amended with either sand or mud sediments and were, in some cases, supplemented with inorganic nutrients. In all cases, organically enriched muddy sediments with elevated fluxes of ammonium yielded significantly higher levels of eukaryotic phytoplankton densities and chlorophyll a. In experiments with *C. polykrikoides*, cell densities of this HAB were 50% higher in the mud sediment treatment than the sand treatment with or without additional inorganic nutrient loading. These findings suggest that nutrient fluxes from organic matter mineralization can exceed those from nutrient loading, and be a major driver of HABs in some estuaries. Results on the responses of *D. acuminata* and *A. catenella* will also be presented.

PYRODINIUM BAHAMENSE GROWTH AND TOXICITY IN TWO GEOGRAPHICALLY DISTINCT POPULATIONS OF FLORIDA

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Pyrodinium bahamense blooms at abundances greater than 100,000 cells L⁻¹ occur nearly every summer in two of Florida's largest estuaries: Tampa Bay and the Indian River Lagoon. High biomass blooms of *P. bahamense* can reduce light penetration, degrade water quality, and deplete dissolved oxygen. In addition, this alga produces potent neurotoxins (saxitoxins) that can accumulate in filter-feeding shellfish and cause paralytic shellfish poisoning (PSP) in humans if contaminated shellfish are consumed. Variability in *P. bahamense* growth and cellular toxicity (within and between populations) can have implications for resource management. Analysis of bloom samples collected from

these areas have confirmed the presence of three PSP toxin congeners: decarbamoylsaxitoxin (dcSTX), gonyautoxin 5 (GTX 5), and saxitoxin (STX) and suggest that cells present in the Indian River Lagoon may demonstrate higher toxicity than *P. bahamense* in Tampa Bay. To investigate this variability, we characterized growth-irradiance curves and compared cellular toxin profiles and concentrations of two Florida *P. bahamense* isolates (from Tampa Bay and the Indian River Lagoon). Results suggest *P. bahamense* is a high-light adapted species, so additional experiments examining toxin production were subsequently conducted under the observed optimal irradiances for growth. We will present an analysis of laboratory growth and toxicity results and discuss them in relation to field observations.

MONITORING AND SURVEILLANCE OF THE 2019 ALGAL BLOOM IN LAKE PONTCHARTRAIN

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Unprecedented rainfall inundated the Mississippi River basin in 2019, necessitating the operation of the Bonnet Carré Spillway for the third time in four years, and for the first time in history, operation in consecutive years and two different opening events in 2019. The structure is opened to prevent velocity of the river from exceeding 1.25 million cubic feet per second, an engineering parameter intended to protect the New Orleans metro area. Operation is not intended to minimize the impact of freshwater inundation or nutrient infusion into the basin. As a result, eutrophication events can be problematic. Over the course of the summer, LPBF and LSU collected and analyzed 111 samples over the course of June through September from both recreational and beach locations, as well as targeted boat sample locations. Highest concentrations of measured microcystin in ambient water samples coincided with a June 25th sampling date at 7.5 g/L and salinity measures of 0.2 parts per thousand. *Microcystis* and *Anabaena* species were both found in collected samples throughout the monitoring period. Sentinel Satellite 3 imagery sourced from the National Oceanic and Atmospheric Administration indicated high concentrations of cyanobacteria species in areas along the northern shore of Lake Pontchartrain in June, and remained high through August. However, microcystin concentrations fell to less than 0.2 ug/L across all samples with the passage of Hurricane Barry (July 11th – 19th). Statistical assessments of physical in situ parameters and nutrient water quality data will be presented.

M. AERUGINOSA BLOOM-INDUCED PH EFFECTS ON FRESHWATER DIATOMS

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Harmful algal blooms (HABs) are defined as phytoplankton masses undergoing prolific and unregulated growth. These blooms have detrimental ecological and economic ramifications due to their production of endogenous toxins, and/or the sheer magnitude of biomass resulting in eutrophication and hypoxia. One HABs species, the cyanobacterium *Microcystis aeruginosa*, poses a global threat to freshwater systems such as Lake Erie and Lake Taihu. While *M. aeruginosa* dominates the water column during summer blooms events, diatoms such as *Fragilaria crotonensis* dominate the water column during the spring months as well as some summer bloom-free regions. The mechanisms driving this community shift from diatoms to *M. aeruginosa* are commonly overlooked, yet a crucial area of study which may shed insight into the numerous advantages *M. aeruginosa* has during ecological competition. One such factor responsible for this shift in community composition is postulated to be pH: *M. aeruginosa* blooms drive up the pH to an average of 9.2, and upward of 9.8 during peak bloom events. These elevated pH levels have the potential to not only alter carbon availability to phototrophs, but to also compromise the integrity of silica frustules crucial to freshwater diatom viability. Growth studies as well as in vivo studies utilizing a novel fluorescent dye that incorporates into newly formed diatom frustules have elucidated the effects of environmentally-relevant pHs on the freshwater diatom *F. crotonensis*, serving as a vital piece to the puzzle of *M. aeruginosa*'s annual proliferation.

INNOVATION AND ADVANCES IN HAB DETECTION

DETERMINATION OF DIFFERENT ALGAL GROUPS WITH SPECIAL EMPHASIS ON CYANOBACTERIA AND THEIR TOXINS IN NATURAL WATERS

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A multi-wavelength fluorometer has been developed to quantify different algal groups and detect unbound phycocyanin (“free PC”) as an indicator of cyanobacterial taste and odor (T&O) compounds and cyanotoxins. Free (or unbound) phycocyanin describes that part of the cyanobacterial pigment phycocyanin which is no longer bound to chlorophyll due to cellular stress or lysis. Cell lysis releases T&O compounds and cyanotoxins in addition to creating free PC. This instrument is designed to estimate the risk of T&O compounds and cyanotoxins from cyanobacteria in natural waters by detecting free PC. The device uses two highly sensitive photomultipliers to detect and differentiate algal pigments. Continuous monitoring and determination of the phytoplankton composition can be used to determine the behavior of the waterbody. This submersible instrument measures continuously, giving it a distinct advantage in comparison with discrete sampling or laboratory analysis with delayed results. The instrument is a good tool for water quality management and can be used for the detection of natural horizontal and vertical variability in phytoplankton communities and for early detection of cyanobacterial blooms. A specially developed model and fingerprint calculation optimizes the results and provides an early warning with regard to the WHO cyanobacteria guidelines. Results from Clear Lake (California) will be presented.

THE 3RD GENERATION ESP/LONG-RANGE AUV: FIRST TESTS OF AUTONOMOUS, UNDERWAY SAMPLING AND ANALYSIS OF MICROCYSTIN IN WESTERN LAKE ERIE

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Unmanned technologies are rapidly advancing our biological sampling and detection capabilities in marine and freshwater systems. Data streams generated by autonomous assets have the potential to re-shape monitoring and management strategies for HAB events by providing assessments of bloom intensity/distribution and toxin measurements, coupled with a persistent in-water presence. The cyanobacterial blooms that threaten drinking/recreational water usage and human/animal health throughout the U.S., including the Great Lakes, vary spatiotemporally in toxicity thereby requiring real-time toxin data assessments to inform bloom toxicity forecasts and support water managers’ timely decision-making. The 2nd and 3rd generation (2G, 3G) Environmental Sample Processors (ESPs) – “labs in a can,” are autonomous, electromechanical fluidic devices capable of acquiring, processing, and analyzing water samples to generate real-time measurements of HAB cell and toxin concentrations. The commercially-available 2G ESP is typically deployed on a fixed-position mooring, whereas the 3G ESP prototype is integrated with a long-range autonomous underwater vehicle (LRAUV), providing a mobile platform capable of underway sampling, analysis, and sample preservation. Both ESP classes have been co-deployed in Lake Erie with the aim of operationalizing use of these complementary assets to provide real-time monitoring and assessment of cyanobacterial bloom intensity, distribution, and toxicity. The first 3G ESP-LRAUV prototype field trial in a freshwater system was conducted in western Lake Erie during August 2018, carrying miniature, re-usable surface plasmon resonance (SPR) sensor chips that enabled “on-the-fly” microcystin analyses. This landmark deployment highlighted the following emergent capabilities: ability to operate in a shallow, freshwater environment; intelligent, directed sampling of cyanobacterial ‘hot spots’ and frontal areas; underway sample processing/SPR-based microcystin analyses; sample preservation for ‘omics analyses; post-deployment lysate recovery/confirmatory analyses.

Interpretation of results within the framework of contextual data from sensors on the LRAUV, various moored and aerial observing assets is underway, providing unique insights into future HAB assessment strategies.

DYNAMICS OF A CYANOBACTERIA COMMUNITY AND DETECTION OF MICROCYSTIN-LR IN SANTUIT POND, MASHPEE, MA MEASURED BY HABSTATS, AN IMAGING RAMAN FLOW CYTOMETER

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HABStats uses a white light source for imaging cells and colonies at 100x as they flow past an interrogation window followed by triggering a pulsed 1 W 532nm laser to collect a Raman spectrum on each target as it passes by. Cell images and Raman spectra are combined and passed to a deep learning convolutional neural network classifier that has been trained to identify some number of species and quantify toxin, if present. A prototype HABStats is located on a dock on Santuit Pond, Mashpee, MA and continuously samples pond water at a depth of 10 cm and rate of 9 mL min⁻¹ while logging and telemetering data on concentration of colonies, cells per colony, and physical integrity of *Microcystis* sp., *Anabaena* (*Dolichospermum*), *Aphanizomenon* sp., *Melosira* sp., multi-cellular grazers (rotifers and copepods), other algae *Staurastrum* sp., *Pediastrum* sp., *Ceratium* sp., and the concentration of microcystin-LR per cell or colony. Results are compared to cell counts conducted by the Mashpee Department of Natural Resources throughout the summer and Fall of 2019. A strong diel pattern in *Microcystis* sp. and *Dolichospermum* cell concentration increasing from a mean of 811 cells mL⁻¹ during dark hours to 1800 cells mL⁻¹ during daylight hours and 6,000 to >14,000 cells mL⁻¹ for *Dolichospermum* and *Microcystis* sp., respectively. This 12-hour cycle was superimposed on a higher frequency of cell concentration variation with a period of 1.5 to 2 h with cell concentration ranging by ~30% in both species. Raman peaks for chlorophyll a (987, 1239, 1325 cm⁻¹), fucoxanthin (1152, 1529 cm⁻¹), and phycocyanin (1000, 1230, 1389, 1639 cm⁻¹) were evident in the spectra. Phycocyanin dominated photosynthetic pigments and correlated with cyanobacteria concentrations. Microcystin-LR (1004, 1307, 1645 cm⁻¹) was detected in both *Microcystis* sp. and *Dolichospermum*. We are working on calibrating to absolute concentration per cell.

EARLY WARNING OF SHELLFISH TOXICITY ALONG THE EASTERN MAINE COAST USING ENVIRONMENTAL SAMPLE PROCESSORS (ESPs): EVALUATION OF THE “LEAKY GYRE” HYPOTHESIS

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An ECOHAB study in the northeastern Gulf of Maine (GOM) and the Bay of Fundy (BoF) region was initiated with the goal to understand bloom dynamics and improve management, modeling, and forecasting of *Alexandrium catenella* blooms along the eastern Maine coast (EGOM). The “leaky gyre” hypothesis asserts that interannual variability in EGOM PSP toxicity is regulated by the “leakiness” of the BoF gyre; a strong gyre retains bloom cells whereas a weak gyre allows cells to escape into the GOM coastal current, ultimately affecting shellfish resources downstream. A two-year field effort (2017 and 2019) involved the deployment of four ESPs each year to capture high frequency bloom events, as well as CTD/water collection surveys and drifters to characterize the hydrography, nutrient field, and distribution and transport of cells. 2019 results highlighted the value of autonomous sensors in documenting episodic phenomena underlying bloom dynamics in this hydrographically complex region. One ESP at the mouth of the BOF documented a large source population in the gyre, while another revealed cells leaking out of the BoF along the eastern exit pathway around Grand Manan Island in mid-June. At that time, the western exit pathway from the Bay was cell free, but in late June, a pulse of cells with a rapid rise to extremely high cell concentrations was documented by the western, nearshore ESP, followed by a rapid rise in shellfish toxicity at a nearby PSP site in eastern Maine. We are evaluating an alternative hypothesis with an offshore source of cells with growth fueled by upwelling, but the leaky gyre with two exit pathways appears to be a valid mechanism to explain some of the complex patterns of toxicity in eastern Maine. This study has clearly demonstrated the long-promised value of offshore in situ sensors in providing timely warnings to shellfish managers.

USE OF AN IMAGING FLOWCYTOBOT TO ASSESS DIFFERENTIAL GRAZING BY ZOOPLANKTON DURING THE HARMFUL *DINOPHYSIS ACUMINATA* BLOOMS ON LONG ISLAND, NEW YORK

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Blooms of *Dinophysis acuminata* have been recorded for decades in the bays of Long Island, NY, with bloom populations co-occurring with many other plankton species. *Dinophysis* is mixotrophic and a potential prey item for larger zooplankton grazers. The complexity of plankton interactions during *Dinophysis* bloom events requires a robust assessment of plankton community composition. The use of Imaging FlowCytobot (IFCB) to record live images of plankton during blooms and zooplankton grazing experiments offers such a holistic determination of plankton communities. For this project, the IFCB has been used to assess differential grazing by copepod (*Acartia tonsa*) adults and nauplii as well as by the protozooplankton community via dilution experiments using water from *Dinophysis* blooms. Dilution experiments revealed active grazing on populations of *Dinophysis* and *Myrionecta rubra* by the protozooplankton community. Regarding grazing by *A. tonsa*, differential abundance analyses revealed *Dinophysis* become significantly more abundant following nauplii enrichment whereas diatoms became significantly less abundant. This suggests *A. tonsa* nauplii may alter plankton community by preferentially consuming diatoms and facilitating an increase in the abundance of *Dinophysis*. It also suggests that there is active community grazing on *Dinophysis* and/or *M. rubra* during blooms. This talk will discuss the general utility of the IFCB as well as statistical approaches for detecting differential grazing during *Dinophysis* HABs, with data from multiple locations and blooms.

HYPERSPECTRAL IMAGING OF CYANOBACTERIA: TAKING IT FROM THE LABORATORY TO THE FIELD

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The US Army Corps of Engineers is developing methods for high resolution hyperspectral imaging to improve freshwater cyanobacteria identification and toxin prediction. First, in the laboratory, we determined that cyanobacterial monocultures have unique spectral characteristics that could be used for identification. We then assessed overall cyanobacterial spectral stability under nutrient stress in the laboratory using a Resonon Pika XC high-performance visible-near infrared (VNIR) hyperspectral imager. We paired these changes with toxin data and found differences in signatures under varying stress conditions that were linked to toxin status, where horizontal shifts were associated with nitrogen limitation and vertical shifts were associated with phosphorus limitation. The next phase of this work included cross-validating aerial (Headwall nano-hyperspectral sensor on SkyCrane unmanned aircraft system) and laboratory sensors. To do this, we conducted mesoscale (400-800gal) trials throughout a bloom, initiated from a natural bloom sample of *Microcystis* sp. from Milford Lake, KS. We then compared the spectra between the two different sensors as the bloom intensified and found similar spectral increases in the 500-671nm range along with the 680-740nm range. After cross validation, we conducted a demonstration at Port Mayaca Lock and Dam in Canal Point, FL. Currently, we are working through spectral extractions for known targets to map the extent of the bloom using this imagery. Future projects should consider database generation and development of a GUI interface for rapid cyanobacteria identification. Additionally, multi-spectral imagers should be explored with customized bands to optimize cyanobacterial classification without having to collect, store and process all wavelengths in the VNIR. As this technology advances, these types of platforms have the potential to enable routine monitoring and rapid identification of cyanobacteria bloom events, potentially coupled to relative cyanotoxin concentrations, the information from which can be used for early management of HAB events.

MULTI-METHOD COMPARISON FOR THE DETECTION FRESHWATER PARALYTIC SHELLFISH TOXINS IN NEW YORK STATE LAKES

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Paralytic shellfish toxins (PSTs) are of increasing concern in freshwater ecosystems. While there are a number of analytical methods developed for the analysis of PSTs in marine systems, the analysis of PSTs produced by freshwater cyanobacteria has primarily used STX-ELISA and/or LC-MS/MS. These methods were originally developed for the detection the marine congeners. They have been applied for the detection of freshwater PSTs, however freshwater cyanobacteria often have congener profiles that are different from their marine dinoflagellate counterparts. The ability of ELISA or LC-MS/MS methods to detect freshwater cyanobacteria toxins, such as the *Lyngbya wollei* toxins or other novel cyanobacterial PSTs, has not been adequately evaluated. Samples from 200 lakes in New York State were initially screened for PSTs by HPLC fluorescence with post column chemical oxidation (PCOX). A subset of samples with high levels of PSTs measured by PCOX were reanalyzed by three LC-MS/MS methods, STX-ELISA, and the STX receptor-binding assay. We applied a combination of these methods for the detection of PSTs from 2017 and 2018 blooms in New York including blooms from the New York Finger Lakes. Agreement between the methods was poor, with some samples negative by ELISA exhibiting a strong response in the PCOX HPLC method and some samples positive by ELISA showing little to no response in the PCOX and LC-MS/MS methods. LC-MS/MS methods run by different laboratories often gave conflicting results for the presence or absence of particular or novel PSTs. Analysis of freshwater PSTs clearly presents a significant challenge regarding the protection of human and environmental health. We recommend using a combination of methods for detecting freshwater PSTs as this reduced the likelihood of false negative results.

CELL & MOLECULAR ADVANCES

ALGIX BIOPLASTIC CONVERSION OF ALGAE BIOMASS INTO BLOOM FOAM

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ALGIX is a clean technology company creating biomaterials from algae biomass. We developed technology to harvest, dry, micronize, compound and mold algae bioplastics for applications in commercial products under the brand Bloom. Bloom collaborates with dozens of leading brands launching eco-friendly products containing Bloom resins to support the initiatives for eco-restoration and point-source transformation of pollution into products.

PROPOSAL FOR DEFINING DINOFLAGELLATE SPECIES BASED PRIMARILY ON MOLECULAR CRITERIA

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Dinoflagellates are ecologically important protists best known for the small fraction of species that produce toxins and form harmful algal blooms (HABs). Due to the adverse ecological, economic and health impacts of these species, along with growing evidence that closely related species can exhibit significantly different toxicities, there has been renewed interest in accurately delineating dinoflagellate species. The resultant taxonomic work has frequently included both detailed morphological and molecular examination of closely related species. An emerging consensus from this work is that morphological characters alone are insufficient to reliably define and identify closely related species. Instead, molecular sequences, primarily certain sections of ribosomal (rDNA) genes, which can be detected by various molecular methods have gradually become accepted as the more reliable character upon which to base species identifications. rDNA has been similarly employed in other protest groups to successfully define species. Despite this fact, concerns persists that using a single gene for defining species is less reliable than a multiple gene approach. Given this concern, the current study specifically tested to see whether the species level distinctions in the genus *Gambierdiscus* indicated by D1-D3 phylogenies were equivalent to those produced using multigene phylogenies. High-resolution transcriptomes for seven *Gambierdiscus* species were produced and two independent phylogenies based on 28 core eukaryotic genes or 17 of the common dinoflagellate gene identified by Janoušková et al. were constructed. The results of the multigene and rDNA phylogenies were consistent, indicating rDNA are sufficient to identify dinoflagellate species. However, this result does not negate the importance of morphology, understanding how much variation occurs among rDNA sequences within a species, and the use of multigene phylogenies when defining dinoflagellate species. Taking these factors into consideration, a scheme will be presented for defining dinoflagellate species, which emphasizes rDNA sequences as a defining characteristic to be used in the future.

OMICS-BASED TOOLKIT FOR MONITORING THE GOLDEN ALGA *PRYMNESUM PARVUM* (HAPTOPHYTA) AND ITS TOXIC METABOLITES, *PRYMNESINS*

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Prymnesium parvum forms seasonal blooms coupled with the presence of potent exotoxins. We present a suite of genomic, metabolomic, and transcriptomic analyses that can be used to rapidly monitor this microalga. Multiplex PCR assays were developed for quantifying *P. parvum* wherein sets of primers simultaneously amplify four species- and gene-specific products using genomic DNA or whole cells. PCR products resolved by gel electrophoresis generated a diagnostic banding pattern; molecular beacons were also designed for real-time quantitative PCR. Both methods were capable of detecting 1-2 cells in 50 cycles. Streamlined methods were developed for the enrichment of prymnesin-1

(prym1) and prymnesin-2 (prym2). Prymnesins were semi-quantified using a chemifluorescence assay and positive-mode ESI-MS metabolic fingerprinting. More than 10 ions were detected that agreed with predicted isotopic distributions for intact compounds and related fragments. The most abundant ion was observed at 919.88 m/z, representing the aglycone structure common to both molecules. We also report a de novo transcriptome assembly generating 47,289 transcripts. Thirty-two different polyketide synthase transcripts were identified, although none were differentially expressed under our experimental conditions. However, analyses revealed evidence of post-transcriptional regulation regarding the production of polyketide prymnesins and the synergistic effects of putative hemolysins and PUFAs toward ichthyotoxicity.

DEVELOPMENT OF UNIVERSAL PCR PRIMER SUITES FOR THE RAPID DETECTION OF ANATOXIN-A AND MICROCYSTIN-RELATED GENES IN FRESHWATER CYANOBACTERIAL COMMUNITIES

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Cyanobacterial blooms pose significant threats to freshwater systems around the world. The ubiquity of cyanobacteria and their ability to produce a variety of toxins make it difficult to predict and manage toxic bloom-forming genera. Moreover, there is a lack of reliable tools to test the potential for toxicity in diverse cyanobacterial communities. The overall aim of this study was to develop universal, gene-specific PCR primers to amplify the suite of genes related to microcystin and anatoxin-a biosynthesis, which should function regardless of which cyanobacterium contained the targeted genes. Sets of primers were designed for the detection of microcystin and anatoxin-a genes using available sequences in the NCBI database. Primer sets were tested with DNA isolated from diverse cyanobacterial genera in the UTEX Culture Collection of Algae to evaluate the specificity of the primers and to screen for potentially toxic strains to use in future research. The anatoxin-a primer sets were positive for anaA-anaJ using DNA isolated from *Lyngbya kuetzii* UTEX B 1547, and they were successfully tested against other Oscillatoriales and Nostocales. Published genera-specific primer sets were used to identify toxin-producing strains of *Microcystis* in the Collection; *Microcystis aeruginosa* UTEX LB 2385 and UTEX 3037 confirmed positive for mcyA-mcyE and mcyG. However, microcystin primer sets designed for this study (mcyA-mcyJ, mcyT) have been difficult to validate since the genetic diversity of toxin-producing genera required the design and use of highly-degenerate oligonucleotides, resulting in non-specific priming. These data, and the challenges associated with the development of universal gene-specific primers, will be presented along with future directions for the rapid in situ detection of these toxin biosynthesis genes.

BEYOND THE TRANSCRIPTOMES: BIOCHEMICAL AND PROTEOMIC VALIDATION OF THE PKS MACHINERY INVOLVED IN STEROLYSIN PRODUCTION

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We have uncovered a single large transcript (>14Kb) with three consecutive KS modules, that are conserved across three species of dinoflagellates (*Karlodinium veneficum* and *Amphidinium carterae*, have known sterolysin toxins, and *Akashiwo sanguinea*, which does not produce a sterolysin), as well as many single module KS subunits. 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 to an exponential phase culture of *A. carterae* and *A. sanguinea*. Using ¹⁴C labeled acetate and liquid chromatography mass spectrometry (LC/MS), we found that cerulenin inhibition resulted in the cessation of both fatty acid and sterolysin production. This shows KS plays a role in both fat and sterolysin synthesis leading us to our hypothesis: The triple module KS acts as a scaffold for both sterolysin and fatty acid production where the final product is mediated by accessory trans-AT subunits, which exist in parallel with the KS. Western analysis clearly finds evidence that a partial multi-module protein (~285,000 daltons, KR2 and TE domains) 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. In *A. carterae*, there are 11 single trans-AT domain proteins which could provide the missing AT activity. We are currently investigating the interacting partners with this triple KS scaffold to determine how fatty acid and sterolysin are selected as final products.

OBTAINING BIOMASS FROM DIFFICULT TO GROW, SENSITIVE MICROALGAE IN PHOTOBIOREACTORS: *KARENIA BREVIS* AS A CASE STUDY

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Photobioreactors provide an attractive and strategic tool to minimize the resources and time spent in microalgae mass culturing. These innovative platforms provide optimal growth conditions in a conveniently small space that can provide 8-10 times the biomass achieved in static system. Unfortunately, some microalgal groups (mainly dinoflagellates) exhibit difficulty in adapting to the growth conditions in these instruments. Although some success has been achieved in recent years around the development of photobioreactors customized for dinoflagellate growth requirements, the culturing of some sensitive species in these instruments represents an ongoing challenge. We have developed a new growth protocol suited to individual species that are difficult to grow under normal photobioreactor conditions. As a prime example, we chose the toxic dinoflagellate *Karenia brevis* as our model organism, as it represents one of the most challenging species to grow at large scale. Not only is this species responsible for massive algal blooms with serious health, social, and economical repercussions, it also produces bioactive compounds that are of keen interest to the pharmaceutical sector. We developed a special, adapted protocol that allowed us to successfully grow *K. brevis* in a photobioreactor using conditions that can also be translated to grow other microalgae with similar requirements. The described protocol highlights the benefit of utilizing bioreactors under modified conditions to produce the desired biomass of sensitive species containing bioactive compounds.

METHOD VALIDATION AND REFERENCE MATERIALS

RAPID, MULTIPLEXED DETECTION OF ALGAL TOXINS IN SHELLFISH AND SEAWATER

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MBio Diagnostics is developing a portable, rapid, inexpensive technology for more effective detection of harmful algal bloom (HAB) toxins in shellfish. The product will help producers and managers get more product to market while ensuring the safety of this commercially important food supply during HAB events. Shellfish are filter feeders and can accumulate HAB toxins during blooms. These toxins can cause serious health effects including temporary paralysis, intestinal or respiratory distress, or even death. Shellfish harvest closures due to HAB toxins are required to protect public health. The United States has regulatory limits for common toxins found in shellfish meat. The US requires testing of shellfish meat for saxitoxin (STX) which causes paralytic shellfish poisoning (PSP), domoic acid (DA) which causes amnesic shellfish poisoning (ASP), and okadaic acid (OA) which causes diarrhetic shellfish poisoning (DSP). Currently, tests for these three toxins can be time consuming and expensive, which hinders the distribution of harvested shellfish. MBio Diagnostics is developing a transformative platform technology that will enable users in the field to perform cost-effective, multiplexed, rapid, laboratory-quality HAB toxin testing. This technology will protect the safety of the nation's food supply while enabling expansion of aquaculture by reducing the time and cost necessary to bring shellfish to market. Here, we present an assay to simultaneously measure all three of these toxins in either shellfish or seawater in a portable, low-cost system.

INTEGRATION OF AN ALTERNATIVE METHOD OF BREVETOXIN ANALYSIS INTO NEUROTOXIC SHELLFISH POISONING MONITORING AND MANAGEMENT IN THE GULF OF MEXICO

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Blooms of the dinoflagellate *Karenia brevis* threaten the productive Gulf of Mexico shellfish industry. Brevetoxins produced by *K. brevis* are toxic to humans and can result in Neurotoxic Shellfish Poisoning (NSP) if contaminated shellfish are eaten. To prevent NSP, shellfish harvesting areas (SHAs) are closed during *K. brevis* blooms and are re-opened once *K. brevis* levels decrease and testing demonstrates that shellfish are no longer toxic. This biotoxin plan successfully prevents occurrences of NSP from lawfully harvested shellfish, but NSP closures come at a steep economic cost to the shellfish industry. The APHA mouse bioassay - the only approved method for regulatory NSP testing - has many drawbacks, and the delays caused by the time required to analyze samples and low sample throughput compound economic losses. To mitigate economic harm to the shellfish industry, a rapid brevetoxin enzyme-linked immunosorbent assay (ELISA) marketed by MARBIONC Development Group was validated according to Interstate Shellfish Sanitation Conference (ISSC) guidelines and was approved as a Limited Use Method for NSP testing in October 2017. Since then, with the support of the NOAA PCMHAB program, we have continued to move this effort forward. Here we present on the successes and challenges associated with the transition of an NSP management program from complete reliance on the mouse bioassay to a hybrid approach that incorporates rapid NSP testing by ELISA. We also report on our progress in transferring this technology to shellfish management entities in other Gulf states.

METHOD VALIDATION AND REFERENCE MATERIALS: ACTIVITIES AND NEEDS IN THE HAB COMMUNITY

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The expanding issue of harmful algal blooms (HABs) in US marine and freshwater systems highlights the need for continued development of fit-for-purpose analytical techniques for identification and quantitation of algal toxins. Reference materials play an essential role in method development, and are necessary for validation and ongoing quality control. Methods and reference materials are needed to address conventional analytical measurement challenges (e.g. regulatory testing for toxins), but also to further research on important questions around the environmental distribution and ecological impact of HABs. This session will cover recent activities in this area with the aim of highlighting and discussing current and future needs. There will be oral presentations on recent method development work, including reference material production and application, and the session will conclude with a series of 'user opinion' discussions to highlight the needs for a range of activities including event response, validation of biological detection methods, toxin survey work and broader ecological issues around HABs. There will also be a roundtable discussion later in the evening to focus on reference material priorities for the US HAB community with the goal of developing a recommendation for the US National HAB Committee.

APPLICATION OF RETENTION INDEX STANDARDS IN CIGUATOXIN RELATED FISH AND ALGAL SAMPLES

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The lack of authentic and characterized reference materials for many marine algal toxins poses a significant challenge to laboratories. Identification of new compounds or comparing datasets between laboratories relies heavily on retention times and MS spectra. Incorrect peak assignment can occur due to variations in retention times between different instruments, especially when several isomers are present. Retention indices (RIs) are instrument independent, dimensionless values to catalog toxins and allow reliable comparisons between laboratories. A retention index standard (NRC RM-RILC) has been developed composed of a homologous series of LC-MS compatible N-alkylpyrinium-3-sulfonates to span RI values from 100 to 2000, which correspond to increased alkyl chain lengths. Ciguatoxins are a complex class of ladder-shaped cyclic polyethers that accumulate in fish and are associated with ciguatera fish poisoning. Ciguatoxin (CTX) variants are characterized by region (Pacific, Indian and Caribbean) for which many congeners are only tentatively identified and authentic standards are not commercially available. Using an Agilent LC coupled to a Thermo Q-Exactive MS, the RIs of several Pacific and Caribbean CTX congeners have been determined. RIs have been applied to the analysis of *Gambierdiscus* and *Coolia* extracts where several potential maitotoxin-like compounds were detected. In the absence of individual toxin standards the adoption of an RI system in the analysis of marine algal toxins will improve between laboratory comparisons, toxin characterization, and data sharing and will prevent mis-identification of toxins.

EXPANDED ANALYSIS OF CYANOBACTERIAL TOXINS IN RECREATIONAL AND DRINKING WATER USING UPLC/MS/MS DETECTION...MORE TOXINS!

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Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) is a powerful tool for the analysis of various analytes in a wide variety of matrices. What is especially attractive about LC/MS/MS is its sensitivity and selectivity. Microcystins, in particular, represent an emerging class of algal toxins of concern to the drinking water industry. Other potential toxins such as anabaenopeptins, have also been discovered in bloom samples. Recognizing the potential

health risk, the World Health Organization, and other nations throughout the world have established guidelines for the amount of microcystins permissible in drinking water. In this paper we investigate the use of smaller column packing (sub 2µm particles) to both improve the selectivity, speed, sensitivity and resolution to screen for many of these toxins. Specifically microcystins, anatoxin-a, cylindrospermopsin as well as newer toxins, such as euglenophycin, anabaenopeptins and micropeptin using Ultra-Performance Liquid Chromatography (UPLC®) combined with tandem mass spectrometry. Specifically we will investigate the analysis of a wide variety of freshwater samples from throughout the United States, including California, Utah, Florida, Ohio, West Virginia, Kentucky and North Carolina. Examples showing the various parameter changes as well as additional toxins detected in freshwater samples will be presented including data from the recent animal deaths in North Carolina due to toxic algae.

INTERNATIONAL VALIDATION OF THE UHPLC-HILIC-MS/MS DETERMINATION OF PSP TOXINS AND TETRODOTOXINS IN BIVALVE MOLLUSC SHELLFISH

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A UHPLC-MS/MS method for simultaneous determination of paralytic shellfish toxins (PST) and tetrodotoxin (TTX) was developed in 2014 for the analysis of bivalve molluscs. The Boundy method, employing a rapid, single-step dispersive extraction with carbon solid-phase extraction clean-up was single-laboratory validated and shown to be fit-for-purpose for routine monitoring. In order for the method to be used for regulatory control, however, the method has to be “internationally accepted”, which required a full collaborative study. A pre-study was first conducted to enable the refinement of the method protocol and to provide potential participants the opportunity to test the method and demonstrate acceptable performance. For the main study, twenty-one laboratories situated in fourteen countries across five continents participated, including participants from around Europe, North and South America, Asia, Australasia and the Indian sub-continent. The study incorporated 15 shellfish species with a global HAB toxin profile representation. Mean values for method trueness showed excellent method performance against expected values. No significant difference was found in the trueness results determined using two different chromatographic column types. The within-laboratory repeatability based on the analysis of blind duplicates and the between laboratory reproducibility was acceptable, with >99% of valid HorRat values less than the 2.0 limit of acceptability. With excellent linearity and sensitivity fit-for-purpose over a range of mass spectrometer instruments, the method compared well against other internationally accepted PST-detection methods. The LC-MS/MS method includes additional PST analogues to those incorporated into FLD methods as well as TTX, which to date has not been incorporated into any other hydrophilic marine toxin official method of analysis. The results from this study therefore demonstrated that the UHPLC-MS/MS method is suitable for the analysis of PST analogues and TTX in shellfish tissues and is recommended as an official alternative method of analysis for regulatory control.

PREDICTIVE MODELS & FORECASTING

PREDICTING MICROCYSTIN OCCURRENCE IN FRESHWATER LAKES AND RESERVOIRS ON GLOBAL SCALE USING MACHINE LEARNING AND GENERALIZED ADDITIVE MODELING

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Determining the environmental conditions that influence the occurrence of microcystin (MC) is a necessary step to predict when the toxin will affect drinking water sources, recreational water bodies, aquaculture ponds, and other freshwater ecosystems. Although widely studied, little consensus exists regarding the environmental factors that influence MC on a global scale. The objective of this research was to identify the environmental parameters associated with MC concentrations using monitoring data from lakes and reservoirs around the world, while addressing large amounts of missing values within an aggregated dataset. We present a machine learning technique for imputing missing data within such a dataset. Further, we used gradient boosting (GB) to indicate predictor variables of importance, and constructed generalized additive models (GAM) to develop predictions about MC occurrence. A total 127 studies containing data from an estimated 2,041 lakes in 24 countries were used to construct the global dataset. From this dataset, 4 sub-datasets were made based on the percentage of missing data within each of the predictor variables; 95% (28 variables incorporated), 70% (16), 50% (13), and 30% (8). Missing values within each of the sub-datasets were then imputed using random forest machine learning. GB performed on each of the imputed sub-datasets revealed variables of relative importance, including turbidity, ammonium, total phosphorus, and pH, although variables of greatest relative importance did vary between sub-datasets. Finally, GAM also revealed variables of differing significance between datasets. Variables showing repeated, and most influential, significance between datasets in the GAM included latitude and longitude, total nitrogen, ammonia, ammonium, and total phosphorus. As expected, we observed that models outputs changed significantly between datasets of varying missingness. Although AIC values of the GAM estimates were significantly greater with the larger amount of missing values, we do not recommend the use of data with >50% imputed values for inference.

FORECASTING LAKE ERIE CYANOBACTERIAL BLOOM TOXICITY

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In recent years, a Lake Erie cyanobacterial bloom biomass forecast has been issued in early July based on the spring phosphorus load. Although bloom size and location can be reasonably well forecasted, questions remain about the controls and predictability of microcystins (MC) concentrations. One complexity in field studies has been the coexistence of toxic and non-toxic strains of *Microcystis*. In addition to competition between strains, environmental factors are also important in the occurrence and biomass of cyanobacteria and the toxicity within blooms. Phosphorus is important in overall bloom dynamics but nitrogen, interacting with light intensity and temperature, may play a major role in MC production. A Lake Erie cyanobacterial bloom toxicity forecast is being developed by linking process-based models with laboratory experiments. This presentation will present (1) historical (since 2014) MC and cyanobacterial biomass correlations from 4,758 samples collected between June and October in the western basin, (2) estimates of total MC mass in Lake Erie on weekly intervals, (3) numerical models of ecological and physical processes to develop hindcasting, nowcasting, and forecasting capabilities, and (4) laboratory experiments that quantified MC production and biodegradation rates under ambient and elevated nutrient concentrations. The factors influencing the dynamics of toxic and non-toxic strains within blooms, and the environmental variables that influence toxin production are only beginning to be understood, but they may be at the point where reasonable predictions of changes in MC concentrations could be possible.

USING MULTI-MEDIA MODELING AND MACHINE LEARNING TO ASSESS PARAMETERS ASSOCIATED WITH HARMFUL ALGAL BLOOMS

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Predicting water quality in lakes is important because healthy lakes provide diverse ecosystem services and environmental benefits that positively influence our quality of life and the strength of our economy. A combination of urban areas, industries, and agricultural activities have undoubtedly contributed to an increased loading of nutrient pollution into Lake Erie, particularly phosphorus and nitrogen. Today, harmful algal blooms (HABs) in Lake Erie are a frequently occurring seasonal issue. In this study, we use a suite of physical modeling systems with in-situ measurements of chlorophyll- α (chlor- α) in Lake Erie to serve as a proxy for HABs. Observations are provided by the Lake Erie Committee Forage Task Group (LEC FTG) and the Great Lakes National Program Office (GLNPO) for the period 2002-2012. Modeling systems involved are the: 1) Weather Research and Forecasting Model (WRF); 2) Variable Infiltration Capacity Model (VIC); 3) Community Multiscale Air Quality Model (CMAQ); and 4) Environmental Policy Integrated Climate Model (EPIC). Meteorological weather variables from WRF, hydrological variables from VIC, nitrogen deposition from CMAQ, and agricultural management practice variables from EPIC for the 11 year period are used to fit a random forest machine learning model to predict concentrations of chlor- α . Via random forest regression, the chlor- α predictive model is able to explain 57% of the variance in chlor- α and identifies eutrophic conditions 85.1% of the time. The importance of environmental variables is evaluated, and the contribution of each covariate in the model is analyzed with Accumulated Local Effect (ALE) plots to better understand the occurrence of HABs.

DEMONSTRATION OF AN INTEGRATED OBSERVATION AND FORECASTING NETWORK DURING THE 2017-2019 *KARENIA BREVIS* BLOOM

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The 2017-2019 *Karenia brevis* bloom endured for sixteen months – the fifth longest on record – and had widespread negative impacts on human, wildlife, ecosystem, and economic health. This event intensified in fall of 2018 when it expanded to Florida's northwest and east coasts, affecting 22 counties and >400 miles of coastline, and requiring innovative, adaptive, and interdisciplinary responses to evolving stakeholder needs. Microscopy results from >14,000 samples were provided online via status reports and a new GIS-based daily mapping tool, with >2 million website visits recorded. Cell abundance data were further synthesized through online remote sensing products and bloom trajectory and respiratory irritation forecasts, collectively guiding event response efforts that implemented in situ tools for bloom tracking including those specific to *K. brevis* (handheld genetic sensors, the HABScope, an Imaging Flow Cytobot [IFCB]), brevetoxins (Solid Phase Adsorption Toxin Tracking) and chlorophyll (ocean gliders, aerial surveys, and remote sensing). This toolbox was especially useful in characterizing conditions in southwest Florida during the

2018 intensification – which coincided with the passages of Tropical Storm Gordon and Hurricane Matthew – when enhanced forecasting tools were employed for tracking the bloom’s development in the Tampa Bay region. Offshore at that time, remote sensing and aerial surveys showed surface *Trichodesmium* sp. and *K. brevis* blooms co-occurring while glider and SPATT sampling respectively indicated higher subsurface chlorophyll fluorescence and toxin levels. Further south, where the bloom had persisted for ~10 months, nearshore cellular division was confirmed via the IFCB and event response surveys identified subsurface hypoxia offshore of Charlotte Harbor. This demonstration of an integrated network included >60 partners, and the spatiotemporal variability observed during and among *K. brevis* blooms underscores the need for sustained observations and enhanced forecasting to address remaining gaps in our understanding of how and when physical, chemical, and biological drivers contribute to bloom dynamics.

OBSERVED CORRELATION BETWEEN SUNSPOT NUMBERS AND INTENSE BLOOMS OF *KARENIA* SP. ON THE WEST FLORIDA SHELF

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Recent intense *Karenia* sp blooms on the west Florida continental shelf, that raised significant public concern, occurred during the years 1994-1996, 2005-2007 and 2016-2018. Noting that these events were separated by approximately 11 years led to the hypothesis that the solar cycle (11-year period) was somehow linked to intense bloom formation. The microscope cell count record extends back to 1954. Beyond that there are a variety of qualitative reports of toxic ‘red tide’ impacts dating back to 1844. The solar activity data records extend back to 1610 represented by sunspot numbers. Qualitative and quantitative comparisons of the two time-series (*Karenia* sp. cell counts and sunspot numbers) yielded strong correlations between large *Karenia* sp. blooms and low sunspot numbers. Every (approximately 56) intense bloom event was preceded, within 60 days, by a period of low to medium sunspot numbers. Sunspots are manifestations of solar activity. The sunspots themselves do not have any direct impact on earth, but instead their numbers indicate the levels of heliophysical events taking place. During times of low sunspot numbers the levels of ultraviolet radiation reaching the earth’s surface are increased and the heliomagnetic field is weaker allowing higher energy cosmic rays to reach the earth. During periods of high sunspot numbers geomagnetic storms are more intense on earth. Hypotheses were formed that could help explain how the slow-growing *Karenia* sp. out compete their phytoplankton community competitors allowing intense blooms to form. It is important to note that solar activity is thought to influence bloom formation but not control it. There are many environmental factors that come together to ‘cause’ *Karenia* sp. blooms

MODELING MICROCYSTIN CONCENTRATION IN GREEN BAY, LAKE MICHIGAN USING HIGH FREQUENCY BUOY DATA AND HYDRODYNAMIC MODEL OUTPUTS

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Green Bay is the largest freshwater estuary in the world and an important resource for tourism, recreational activities and drinking water. The city of Green Bay is developing the largest municipal swimming beach on Green Bay. Lower Green Bay (LGB) is a 303d listed Area of Concern due, in part, to toxic cyanobacterial blooms, mainly, microcystin producing *Microcystis*. In 2018 we studied trends in microcystin across LGB to better understand relationships between toxin concentration and high frequency buoy data at two sites on the West and East side of the LGB. Toxins were measured by liquid chromatography tandem mass spectrometry, enzyme-linked immunosorbent assay, and protein phosphatase inhibition assay. Buoy data included water temperature, dissolved oxygen, chlorophyll and phycocyanin fluorescence, windspeed/direction, pH, conductivity, turbidity, and total dissolved solids. A hydrodynamic model of LGB provided wave power, height and direction. Microcystin concentrations were over the United States Environmental Protection Agency’s recreational threshold of 8 µg/L in over 20% of samples. Microcystin was detected in all samples with a maximum concentration of 35 µg/L. Buoy sensor data and wave data were subjected to a Boxcox power transformation and used in multiple parameter regression modeling to explore

relationships between these predictor variables and microcystin concentration. Dissolved oxygen, wave power, water temperature, and phycocyanin were the best predictor variables and a model using these variables provided the best model fit ($R=0.8$, $p<0.01$) and lowest complexity of thousands of models considered. We then determined the range of values of these variables during periods when microcystin exceeded the recreational threshold. This information was used in 2019 to determine if these conditions were predictive of exceedances in microcystin concentrations above the recreational threshold. The results suggest that while location specific, this modeling approach may be useful for managing human health risks from microcystins in this recreational environment.

REAL-TIME FORECASTS FOR BREVETOXIN RESPIRATORY IRRITATION

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Brevetoxins produced by *Karenia brevis* can be aerosolized and can lead to severe respiratory irritation. In people with asthma, brevetoxins pose a substantial health risk, with sustained health impacts after a short (1 hour) exposure, and local emergency room visits increasing during blooms. Economic impacts occur as even healthy people avoid beach businesses when a “red tide” is reported to be in the region. The distribution of brevetoxin aerosol impact varies greatly, depending on patchiness of blooms and wind direction. Forecasts of location and timing of impacts may reduce all of these impacts.

In September, 2018, a severe *Karenia brevis* red tide reached Pinellas County, Florida. The county began sampling of water 3-7 days each week at 12-15 public beaches for cell count determination by microscopy. We combined that data with 3-hourly forecasts of wind speed and direction to indicate the potential risk of respiratory irritation for the next 24-36 hours. The results were updated every three hours with either current cell counts or newer forecasts and posted on a web-page. The forecasts varied through the day with changes in winds. Forecasts could be validated only during the morning to mid-day sampling. The results indicate good accuracy with low false negatives (which would otherwise present the greatest health risk). Both sampling and forecasts will continue during the next red tide event. We plan on expanding the forecast to other areas that have samples at least two days per week. We are also looking to include monitoring with the “HABscope”, which allows citizen science engagement.

MICROBIAL INTERACTIONS

A SURVEY OF THE GLOBAL *MICROCYSTIS* MICROBIOME

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Although the subject of ample study in recent years, little is known about the biogeographical diversity of bacteria associated with blooms of harmful cyanobacteria (cyanoHABs). CyanoHABs are known to shape bacterial community composition and to rely on functions provided by associated bacteria (microbiome), leading to the hypothesis that cyanoHABs and their microbiome together form an interactome, a coevolved community of synergistic and interacting taxa – each necessary for the communities' success. As an initial test of this hypothesis, we surveyed the microbiome associated with *Microcystis aeruginosa* blooms in nine lakes on four continents. We used 16S taxonomic data and community metagenomic data to test this hypothesis. Our survey spanned a 280° longitudinal and 90° latitudinal gradient and the majority of *Microcystis* OTUs were classified as *M. aeruginosa*, indicating that *M. aeruginosa* is a cosmopolitan bloom former. The microbiome communities were represented by a wide range of OTUs and relative abundances with most of the bacteria belonging to the Alphaproteobacteria, Bacteroidia, and Gammaproteobacteria classes. Highly abundant taxa were more related and shared across most sites and did not vary with geographic distance. This shows neither *Microcystis* nor the microbiome are dispersal limited. The microbiome communities had high phylogenetic relatedness, both within and across lakes, indicating similar functional potential was associated with all blooms. As expected among prokaryotes, the *Microcystis* and microbiome bacteria shared many genes, but the whole-community metagenomic analysis also revealed a suite of biochemical pathways found only in the microbiome that could be considered complementary to *Microcystis*. These included pathways involved in vitamin B12 synthesis, carbon cycling (e.g., d-galacturonate, galactose and glycogen degradation, as well as proline, maltose, and galactose transport), and nitrogen cycling (e.g., N₂-fixation and denitrification). Our results demonstrate a high degree of microbiome similarity across global *Microcystis* blooms thereby providing initial support for the hypothesized *Microcystis* interactome.

SHIFTS IN MICROBIAL COMMUNITY COMPOSITION AND MICROBIAL MEDIATED PROCESSES WITH CYANOBACTERIAL ALGAL BLOOM FORMATION AND CYANOTOXIN OCCURRENCE

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The occurrence of cyanobacterial harmful algal blooms (HABs) in eutrophic freshwater systems remains a human and ecological health problem in the Great Lakes region. The U.S. Geological Survey (USGS) continues to work in the Western Lake Erie Basin to characterize the spatio-temporal life cycle of cyanobacteria HABs, associated cyanotoxins and the genes responsible for cyanotoxin production, and HAB-associated microbial communities in relation to algal bloom formation and cyanotoxin production. High-throughput amplicon sequencing was used to assess the microbial

community of lake sediment prior to bloom formation and the microbial community succession in the water column prior to, during, and after algal blooms. Analysis of sediment and water column samples collected from 2014 to 2018 suggests that *Microcystis* sp. may overwinter in sediment and act as a source population for summer HAB formation. Additionally, a seasonal shift in the water column cyanobacterial community from *Synechococcus* sp. to a *Microcystis* sp.-dominated community (as observed in our 16S rRNA amplicon sequencing data set) correlates with algal bloom formation and cyanotoxin production. This research was expanded to incorporate shotgun metagenomics sequencing approach, allowing us to evaluate the functional capabilities of microbial communities with algal bloom formation and cyanotoxin production. Data were analyzed to determine whether there is a correlation between shifts in microbial-mediated processes and biotic and abiotic factors associated with bloom formation and cyanotoxin production. Understanding how microbial populations change over time with respect to concurrently-collected, water-quality data will help infer which parameters influence changes in blooms and identify potential triggers for cyanotoxin production.

CHANGES IN SUPPORTIVE BACTERIAL ASSEMBLAGES IN RESPONSE TO NUTRIENT FORM AND CONCENTRATION IN CYANOBACTERIAL COMMUNITIES

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The attention given to toxigenic cyanobacterial blooms over the past few decades has led to an abundance of new information on these phenomena. This influx of new information has brought forth the potential importance of supportive bacteria that reside within cyanobacterial blooms. Recent metagenomics studies have begun to support the importance of the supportive bacterial taxa and the interactions that may be occurring within these communities. This experiment (planned to end ~August 19th 2019) looks at the entire cyanobacterial community as well as the supportive, heterotrophic, bacteria using metagenomics techniques. In this mesocosm experiment, we subjected cyanobacterial bloom communities dominated by *Microcystis aeruginosa* to three different forms of nitrogen (NO₃, NH₃, urea) at double and quadruple the ambient total nitrogen concentrations. Expected results will either indicate (1) a shift in the cyanobacterial community to greater density of supportive bacteria in response to an increased amount of dissolved nitrogen in an energetically expensive form (NO₃) and a low density in response to an increased amount of nitrogen in an energetically efficient form (urea), (2) the opposite shift or (3) no shift in the cyanobacterial community indicating the stability of such communities to disturbances in the form of sudden increases in nitrogen and may point to the strength of the relationships that exist between the cyanobacteria and the supportive bacteria.

MICROCYSTIS COLONIES HARBOR MICROBIOMES LESS DIVERSE AND SIGNIFICANTLY DIFFERENT FROM FREE-LIVING AND PARTICLE ATTACHED BACTERIAL COMMUNITIES IN LAKE ERIE AND LAKE AGAWAM, NY

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The toxic cyanobacterium *Microcystis* is one of the most pervasive cyanoHAB genera and naturally occurs in colonies held together by a mucilage known to harbor diverse heterotrophic bacteria assemblages that may influence *Microcystis* proliferation and toxicity. Still, there remains a limited understanding of the structural and functional potential of the colony-associated microbiomes and how they may be shaped by nutrient and temperature conditions. To gain further insight into these interactions, we used next-generation amplicon sequencing of the 16S rRNA gene to characterize the community dynamics of the bacterial (16S) assemblages during *Microcystis* blooms in two temperate US lakes, Lake Erie (LE) and Lake Agawam (LA; Long Island, NY). We examined the *Microcystis*-attached (MCA), free-living (FL), and whole water (W) microbiomes through a spatial/temporal monitoring study in 2017 to identify differences between these fractions and along naturally occurring temperature-nutrient gradients. Across lakes, the MCA microbiomes were significantly less diverse, with diversity inversely related to bloom intensity, and significantly different than the FL and W fractions. The Gemmatimonadetes, Burkholderiaceae, Rhizobiales, and Cytophagales were significantly enriched in the MCA fraction, with their functions being related to macromolecule degradation nutrient cycling, and nitrogen fixation, which may play a role in the ability of non-diazotrophic *Microcystis* to persistence under N deplete conditions. Colonies were depleted of Actinobacteria, Sphingobacteriales, Chitinophagales, Verrucomicrobia and Chloroflexi, groups associated with cell lysis and microcystin degradation. PICRUSt predicted metagenome analysis identified conserved functional potential despite taxonomic differences in

the MCA fractions increased abundance of N/P cycling and microcystin-degrading pathways. Microbiomes showed strong temporal variation, associated with seasonal changes in temperature and N availability, with the MCA microbiomes being the least variable over time. Collectively, these findings indicate that *Microcystis* colonies exert a selective pressure on associated microbiome, selecting for bacteria which may facilitate bloom persistence, even under environmentally unfavorable conditions.

ENVIRONMENTAL EFFECTS ON PARASITIC INFECTIONS BY CHYTRIDS ON *PLANKTOTHRIX AGARDHII*

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Planktothrix agardhii is a nuisance cyanobacterial harmful algal bloom (cHAB) former in many water bodies across the world. This filamentous cyanobacterial genus has a known obligate parasite; chytrid fungal species *Rhizophyidium* sp. Chy-Kol 2008 (originally isolated from Lake Kolbotvatnet, Norway). The purpose of our work has been to establish how these parasitic interactions affect the host population, specifically during a bloom. To this end, chytrids and *P. agardhii* hosts were isolated from Sandusky Bay, Lake Erie during the 2018 bloom year. Utilizing dilution and single filament isolation techniques, 8 chytrid isolates have been obtained for characterization and assessments of host specificity and modes of pathogenesis. Environmental factors analyzed for effects on infection rates include conductivity, temperature, nitrogen limitation, and surf agitation. Additional work takes an 'omics approach at understanding the spatial and temporal prevalence of chytrid infection through qPCR of historic samples. Future work will include genomic sequencing of chytrid isolates and metatranscriptomic analysis of active infections.

MICROBIOME AND CHEMICAL DYNAMICS OF A TOXIC DINOFLAGELLATE BLOOM

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Harmful Algal Blooms (HABs) exert considerable ecological and economic damage and are becoming increasingly frequent worldwide. However, the biological factors underlying HABs remain uncertain. Relationships between algae and bacteria may contribute to bloom formation, strength, and duration. We investigated the microbial communities and metabolomes associated with a HAB of the toxic dinoflagellate *Karenia brevis* off the west coast of Florida in June 2018. Microbial communities and metabolite pools differed based on both bacterial lifestyle and bloom level, suggesting a complex role for blooms in reshaping microbial processes. Network analyses show *K. brevis* is an ecological hub in the planktonic ecosystem, with more and stronger connections to co-occurring microbial taxa in the free-living size fraction compared to the particle-associated fraction. We further identify eight microbial taxa that are associated with blooming *K. brevis*, including four flavobacteria and one SV unidentified past the domain level, suggesting uncharacterized diversity exists in phytoplankton-associated microbial communities. Additionally, metabolomic analyses associated high *K. brevis* levels with higher levels of aromatic compounds and lipids. These findings reveal water column microbial and chemical characteristics with potentially important implications for understanding HAB onset and duration.

THE “NEGLECTED VIRUSES” OF TAIHU: ABUNDANT TRANSCRIPTS FOR VIRUSES INFECTING EUKARYOTES AND THEIR POTENTIAL ROLE IN PHYTOPLANKTON SUCCESSION

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Viruses provide a checks and balance system in microbial communities, modulating population sizes and altering community diversity. The role of viruses in harmful algal bloom dynamics are not fully understood, but they have been implicated as a potential control mechanism. Lake Tai, China experiences yearly algal blooms that are commonly

dominated by toxic cyanobacteria, predominately *Microcystis* spp. and cause significant economic and environmental damage. To date virus research in bloom systems has been restricted to bacteriophage that infect *Microcystis* spp. A broad diversity of viral groups has thus been neglected, limiting our knowledge of interactions within these systems. Here, we present the seasonal, non-phage virus diversity during the 2014 Lake Tai bloom. Hallmark virus genes were examined in a co-assembled metatranscriptomic library to identify active nucleocytoplasmic large DNA viruses (NCLDVs), RNA viruses, ssDNA viruses, bacteriophage, and viroplasm. Phylogenetic analyses revealed a broad diversity of viruses that show seasonal and spatial variability. We observed disproportionately high levels of expression associated with NCLDVs and ssRNA viruses that are consistent with viruses that infect photosynthetic eukaryotes as opposed to those that typically infect heterotrophic bacteria. Under a modified kill-the-winner scheme, we hypothesize active eukaryotic viruses have the potential to help suppress the photosynthetic eukaryotic community, which might allow for the proliferation of cyanobacteria, such as *Microcystis*.

ECOPHYSIOLOGY

(Sessions I-II, alphabetical by author)

TEMPERATURE AFFECTS THE BIOLOGICAL CONTROL OF DINOFLAGELLATE BLOOMS BY A GENERALIST PARASITE

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The increase in emerging harmful algal blooms in the last decades has led to extensive concern in understanding the mechanisms behind these events. In this sense, generalist parasites are of special interest as they have the potential to control invasive species. However, the outcomes of such newly established host-parasite interactions depend on the biotic and abiotic context in which both partners are inserted. Here, we assessed the growth of three blooming dinoflagellates (*Alexandrium minimum*, *Scrippsiella trochoidea*, and *Heterocapsa triquetra*) and their susceptibility to infection by the generalist parasite *Parvilucifera* sp. under a temperature gradient (from 13°C to 22°C). The three dinoflagellates showed different responses to temperature that varied from a positive relationship in *A. minimum* (maximal growth at 20-22°C), to unimodal in *S. trochoidea* (maximal growth at 18°C), and sigmoidal inverse in *H. triquetra* (maximal growth at 13-18°C). Interestingly, the effect of temperature on the parasite infectivity also changed depending on which dinoflagellate was infected, with a positive response observed in both *A. minimum* and *H. triquetra* (maximal infections at 22°C) and unimodal in *S. trochoidea* (maximal infections at 18°C). Although low temperatures (13-15°C) negatively affected the parasite infections in the three hosts, this effect was more dramatic in *H. triquetra* (resulting in the failure of *Parvilucifera* sp. to control this dinoflagellate under low temperatures). These results are particularly relevant under the current scenario of climate change, with shifts in temperature conditions potentially affecting not only the competition between microalgal species but also their control by parasites.

NUTRIENT OVER-ENRICHMENT AND BROWN TIDE RESULT IN LIGHT LIMITATION OF SEAGRASS COMMUNITIES IN THE INDIAN RIVER LAGOON

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Historically, extensive seagrass meadows were common throughout the Indian River Lagoon (IRL) in east-central Florida, USA. Between 2011 and 2017, widespread catastrophic seagrass losses (~ 95%) occurred in the IRL following unprecedented harmful algal blooms (HABs), including persistent brown tides (*Aureoumbra lagunensis*). Little is known about how dissolved nutrients and chlorophyll a are related to light limitation or how biochemical factors, such as the elemental composition (C:N:P) and stable isotope signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), of seagrasses within the IRL relate to coverage. Accordingly, we conducted a survey from 2013 - 2015 at 20 sites to better understand these relationships. Results showed a negative correlation between DIN and salinity, indicating freshwater inputs as a DIN source. Seawater N:P ratios and chlorophyll a concentrations were higher in the urbanized, poorly-flushed northern IRL segments. Kd values were higher in the wet season and exceeded seagrass light requirements (0.8 m^{-1}) for restoration, demonstrating light limitation. Species distribution varied by location. *Halodule wrightii* was ubiquitous, whereas *Syringodium filiforme* was not found in the northernmost segments. *Thalassia testudinum* was only present in the two southernmost segments that had the lowest TDN and highest light availability (Kd). Blade %N and %P also frequently exceeded critical values of 1.8% and 0.2%, respectively, especially in the northern segments. Further, $\delta^{15}\text{N}$ was positively correlated with ammonium, suggesting wastewater as a major N source. The $\delta^{13}\text{C}$ values indicated a trend of increasing light limitation from south to north, which helps explain the recent catastrophic loss of seagrasses in the northern IRL. Overall, elemental composition reflected high N-availability and seagrass species distributions were relatable to spatial trends in N and light limitation. For effective restoration, resource managers must reduce N-loading to the IRL to diminish HABs and increase light availability. Regular biochemical monitoring of seagrass tissue should also be implemented during restoration efforts.

DISRUPTING THE CYCLE: DOES MIXOTROPHY DESYNCHRONIZE CELL DIVISION IN *KARLODINIUM VENEFICUM*?

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Blooms of the toxic dinoflagellate *Karlodinium veneficum* are responsible for fish kills in estuaries worldwide. Populations in the Chesapeake Bay regularly co-occur with cryptophyte microalgae, which are readily preyed upon by mixotrophic strains of *K. veneficum*. Previous research has demonstrated that toxin production aids in prey capture, and that during mixotrophic growth cryptophytes provide significant contributions to the carbon, nitrogen, and phosphorous needs of *K. veneficum*. Specific growth rates of mixotrophic cultures have been reported to reach 0.94 day⁻¹, a rate exceeding one doubling per day (1.4 divisions day⁻¹). Additionally, the occurrence of cryptophyte cells and *K. veneficum* blooms is correlated in the Chesapeake Bay, with evidence strongly suggesting that an abundance of cryptophyte cells can trigger *K. veneficum* blooms. It has recently been found that toxin production in *K. veneficum* is light dependent and toxicity is inversely related to growth rate, suggesting a role for the cell cycle arrest in toxicity. The cell cycle of autotrophically grown *K. veneficum* is strongly synchronized to the diel light cycle, with cell division occurring only during dark hours, which theoretically limits growth rates to a doubling per day or less. The higher growth rates observed under mixotrophic conditions suggests that a decoupling of the cell cycle and diel light cycle occurs during mixotrophic growth. In order to test this hypothesis, mixotrophic versus autotrophic cultures were analyzed for cell cycle synchrony and progression by image and flow cytometry on an hourly basis for 27 hours during mid-log growth and again every eight hours in stationary phase for 96 hours. Hourly cellular toxin content was also measured to correlate toxicity with cell cycle phase. Results from these experiments will be influential in better modeling of *K. veneficum* bloom development and predicting bloom toxicity.

TRACKING PATTERNS OF PHYSIOLOGICAL ECOLOGY IN *AUREOCOCCUS* AND ITS COMPETITORS OVER THE COURSE OF A BLOOM

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Aureococcus forms dense ecosystem-destructive blooms in the systems where it occurs, but understanding the drivers of these bloom dynamics is still a challenge. Working in embayments in coastal New York we used a novel dual metatranscriptome sequencing approach to profile the eco-transcriptomic responses of *A. anophagefferens* and co-occurring phytoplankton. Samples were taken over the course of a brown tide bloom and paired with regular incubation experiments to evaluate the factors driving bloom dynamics. In *A. anophagefferens*, expression of known markers of N and P stress suggests this bloom was P-stressed during the early stages, and transitioned to N-stressed when inorganic N levels were low and *A. anophagefferens* growth rates were significantly increased by the addition of ammonium. Analysis of the *Synechococcus* genes expressed in the same time series revealed that P and N stress genes had opposite patterns of expression from each other. These apparent stress responses were similar to *A. anophagefferens* during the initial part of the time series, but differed later in the bloom. If these initial observations are borne out with further work, these differences may indicate shifts in competitive outcomes. Taken together, our analysis of *A. anophagefferens* transcripts over the course of the brown tide has provided molecular insight into the extent to which both N and P can control the initiation and intensification of brown tides.

THE EFFECTS OF ENVIRONMENTAL STRESSORS ON CELL DEATH RESPONSES OF TOXIC DINOFLAGELLATE *KARENIA BREVIS* AND POTENTIAL BLOOM DECLINE PROCESSES

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Historically, marine phytoplankton were presumed to replicate indefinitely by binary fission. However, drastic environmental changes have been shown to induce death of algal cells, and may therefore affect development of harmful algal blooms (HABs). The Gulf of Mexico (GoM) is subject to frequent toxic blooms of the dinoflagellate *Karenia brevis*, but the mechanisms of bloom termination are not well known. Here we used established cell death markers and photosynthetic parameters to define short-term cellular responses to different levels of acute osmotic stress (from salinity 35 to 30, 25 and 20, respectively) and irradiance stress (from 50 to 500, 750 and 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$, respectively). Intensity-dependent apoptosis cell death processes were clearly observed with increasing osmotic stress. Under acute light increase, autocatalytic cell death was only found under 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$, potentially revealing strong photoacclimation ability of *Karenia* in the field. However it appears that irradiance stress responses and photosynthetic processes are closely linked under different levels of light stress. The observed increase of reactive oxygen species (ROS) prevalence and photosynthesis reduction may correspond to the over-reduction of electron transport chains. Stronger light stress induced higher Non-Photochemical Quenching (NPQ) which may further decrease photosynthetic capacities and mitigate the production of ROS. Our research revealed detailed cellular responses on *K. brevis* to environmental stressors and evaluated potential bloom-declined indicators. Meanwhile, the presence of cell death markers in untreated populations may also highlight their house-keeping functions in cell division and aging process.

DIFFERENT PHYSIOLOGICAL RESPONSES AND TOXICITY RELATED TO N-SPECIATION ARE REVEALED BY TRACING LABELED N THROUGH THE METABOLOME OF *MICROCYSTIS AERUGINOSA*

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Increased nitrogen (N) loads have corresponded to the increase of cyanobacterial harmful algal blooms (cyanoHABs) in freshwater ecosystems and the production of N-rich cyanotoxins. Most often, the dominant species in freshwater blooms are *Microcystis* spp., a non-N₂ fixing cyanobacterium that can produce the cyanotoxin microcystin. *Microcystis* uses inorganic forms of N (i.e. ammonium and nitrate), and organic forms of N (i.e. urea), and N speciation has been proposed to play a role in *Microcystis* bloom dynamics and influence microcystin production. On a physiological level, the effects of N speciation remain unclear. This is especially true for urea, which has been replacing nitrate-based fertilizers for the past several decades and can help satisfy both C and N demands of *Microcystis*. We aimed to determine the impact of N speciation on the metabolism and production of microcystins using stable labeled isotopes and a metabolomics approach. ¹⁵N-ammonium, ¹⁵N-nitrate and ¹⁵N-urea were traced using axenic cultures of the model *M. aeruginosa* NIES843, and distinct global metabolic profiles were observed on each form of N. Specifically, a greater flux of N was observed into the production of amino acids and pyrimidines for cells grown with urea. Glutamate and glutamine dynamics suggested N uptake was linked to photosynthesis, suggesting N assimilation is regulated by C assimilation. All N species were used to produce microcystin and did not impact microcystin cell quotas. However, N speciation influenced the enrichment of different microcystin congeners. Studies using ¹³C-urea confirmed that the C released from the hydrolysis of urea was also assimilated into cell metabolites, including microcystin, and may explain physiological differences in cells grown on urea. These studies indicate that changes in N speciation cause physiological changes that culminate in global metabolic shifts that can impact the persistence and toxin dynamics of cyanoHABs in the environment.

TOP-DOWN REGULATION OF FILAMENTOUS CYANOBACTERIA CONTRASTS AMONG RAPTORIAL VS. ACTIVE FILTER FEEDING COPEPODS

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Top-down regulation of producers depends on the traits of grazers and their prey. Copepods may facilitate cyanobacteria by grazing selectively on their 'edible' competitors. However, the role of copepod feeding modes on the top-down regulation of phytoplankton is largely unexplored. We ran laboratory, microcosm experiments in order to test the grazing effect of two copepods with different feeding modes – the calanoid *Notodiaptomus iheringi* (active, current feeder) and the cyclopoid *Thermocyclops decipiens* (passive, ambush feeder) – on the abundance and dominance of the filamentous cyanobacteria *Cylindrospermopsis raciborskii* co-cultured with the nutritious 'edible' eukaryotic phytoplankton *Cryptomonas obovata*, which had an initial 9-fold dominance. Unlike short-term grazing assays, our experiment ran over one week accounting for multiple prey generations, revealing shifts in prey selection. During the first half of the experiment, both copepods avoided ingestion of *C. raciborskii*, which increased the dominance of this cyanobacterium. In the second half of the experiment, 'edible' prey became scarce, and the calanoid switched grazing to *C. raciborskii*, reducing its growth to negative values. The cyclopoid also increased grazing on the cyanobacterium with time, but grazing did not reduce cyanobacterial growth compared to controls. Both copepods decreased the length of cyanobacteria filaments by ~70%. Results suggest that regulation of filamentous cyanobacteria growth by copepods may differ among ambush vs. current feeder modes, varying with prey relative abundance. In conclusion, top-down control of toxic phytoplankton likely depends on feeding traits of dominant zooplankton and availability of alternative prey. We suggest future work to clarify the interactions of zooplankton feeding traits, especially understudied cyclopoids, with the morpho-physiological traits of cyanobacteria.

REDUCED LIGHT INTENSITY COUNTERACTS THE COOL-TEMPERATURE-INDUCED INCREASE IN MICROCYSTIN QUOTA OF *MICROCYSTIS AERUGINOSA*

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Despite decades of research, a definitive intracellular function of microcystin has yet to be demonstrated, but a favored explanation is that microcystin protects photosynthetic proteins during periods of oxidative stress. In previous work, a strong phenotype was demonstrated in *Microcystis aeruginosa* NIES-843 whereby microcystin quota increased >10-fold when cultures were grown at environmentally relevant cool temperatures (~18°C, relative to ~26°C) and constant light intensity, but the mechanism of this phenomenon is not yet clear. We are using this phenotype to test two mechanistic hypotheses: 1) constant light with sharp reduction in temperature increases excitation pressure in PSII; 2) photodamage repair is suppressed at cooler temperatures. Each explanation is predicted to lead to oxidative stress and increased cellular microcystin quota. Using a factorial design, we grew *M. aeruginosa* NIES-843 and PCC7806 in batch culture at 18 and 26°C and 10, 50, and 75 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. PCC7806 displayed the cool-temperature phenotype, with quotas ~3-fold higher at 18°C and constant light, confirming this phenomenon in disparate strains. As predicted, in NIES-843, growth under 10 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at 18 °C completely offset the ~2-fold increase in quota observed under either 50 or 75 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. In PCC7806, growth under 10 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at 18°C moderated the increase in quota, reducing the increase to ~1.5-fold from ~3-fold observed under 50 or 75 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. These results lend support to a leading explanation of the function of microcystin and provide new insight into factors that need to be considered in predictive models of toxin production.

UNDERSTANDING THE EFFECT OF SALINITY TOLERANCE ON CYANOBACTERIA ASSOCIATED WITH A HARMFUL ALGAL BLOOM IN LAKE OKEECHOBEE, FLORIDA

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In an effort to simulate the survival of cyanobacteria as they are transported from Lake Okeechobee to the estuarine habitats that receive waters from the lake, a bioassay encompassing a range of salinities was performed. An overall decline in cyanobacteria health in salinity treatments greater than 18 practical salinity units (psu) was indicated by loss of cell membrane integrity based on SYTOX® Green staining, but this loss varied by the kind of cyanobacteria present. *Microcystis aeruginosa* was tolerant of salinities up to 18 psu; however, higher salinities caused leaking of microcystin from the cells. *Dolichospermum circinale*, another common bloom former in this system, did not tolerate salinities greater than 7.5 psu. Stimulation of mucilage production was observed and is likely a mechanism used by both species to protect organism viability. At 7.5 psu, microcystin increased relative to chlorophyll-a, providing some evidence of biosynthesis when *M. aeruginosa* is exposed to this salinity. This study indicates that as freshwater cyanobacteria are transported to brackish and marine waters, there will be a loss of membrane integrity which will lead to the release of cellular microcystin into the surrounding waterbody. Additional research would be needed to determine the exact effect of salinity on this relationship.

ALLELOPATHIC INHIBITION OF THE HARMFUL DINOFLAGELLATE *ALEXANDRIUM CATENELLA* BY MULTIPLE SPECIES OF CULTIVABLE MACROALGAE

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Alexandrium catenella, a harmful algal bloom (HAB) forming dinoflagellate, produces paralytic shellfish poisoning (PSP) toxins, leading to shellfishery closures that can persist for weeks and cause economic losses of millions of USD. There is, therefore, broad interest in identifying approaches for the mitigation of *A. catenella* blooms. Several genera of macroalgae have broad inhibitory allelopathic effects on phytoplankton, including HAB dinoflagellates; however, the effects of cultivable macroalgae on *A. catenella* have yet to be investigated. In this study we determined the effect of four cultivable macroalgae – *Chondrus crispus*, *Saccharina latissima*, *Porphyra* spp., and *Ulva rigida* – on *A. catenella* in various settings, using both cultured *A. catenella* and field populations taken from blooms in Long Island, NY estuaries. Growth assays of lab-cultured *A. catenella* together with various macroalgae concentrations showed that macroalgae of all species had significant dose-dependent algicidal effects on *A. catenella*, typically within 3 days. Low aquaculture concentrations of both *U. rigida* and *S. latissima* (0.5 g L⁻¹) reduced dense *A. catenella* populations (initial: ~6.5x10⁵ cells L⁻¹) relative to the control by ~42% (*U. rigida*) and 22% (*S. latissima*) over 3 days. Higher densities of macroalgae, and increased time, showed greater effects; over 9 days, high aquaculture concentrations of *U. rigida* and *S. latissima* (3 g L⁻¹) reduced *A. catenella* population by 77% and 80%, respectively. The addition of aquacultured *S. latissima* to mesocosms filled with a field population of *A. catenella* (initial density: 3.2x10⁴ cells L⁻¹) reduced the population by 46% over 2 days, and by 71% over 5 days. In a similar experiment, *U. rigida* caused a 73% reduction in *A. catenella* over 6 days. These results suggest that the integration of macroalgae into shellfish aquaculture should be considered as a non-invasive, environmentally friendly, and profit-generating measure to mitigate *A. catenella*-caused damage to the shellfish industry.

CELL CYCLE REGULATION OF THE MIXOTROPHIC DINOFLAGELLATE *DINOPHYSIS ACUMINATA*: GROWTH, PHOTOSYNTHETIC EFFICIENCY AND TOXIN PRODUCTION

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The mixotrophic dinoflagellate *Dinophysis acuminata* is a widely known diarrhetic shellfish poisoning (DSP) producer. Toxin variability of *Dinophysis* spp. have been well studied, but little is known of the manner in which toxin production is regulated throughout the cell cycle in these species, in part due to their mixotrophic characteristics. Therefore, an experiment was conducted to investigate cell cycle regulation of growth, photosynthetic efficiency, and toxin production in *D. acuminata*. First, a three-step synchronization approach, termed “starvation-feeding-dark”, was used to achieve a high degree of synchrony of *Dinophysis* cells by starving the cells for 2 weeks, feeding them once, and then placing them in darkness for 58 hours. The synchronized cells started DNA synthesis (S phase) 10 hours after being released into the light, initiated G2 growth stage eight hours later, and completed mitosis (M phase) 2 h before lights were turned on. The toxin content of three dominant toxins, okadaic acid (OA), dinophysistoxin-1 (DTX1) and pectenotoxin-2 (PTX2), followed a common pattern of increasing in G1 phase, decreasing on entry into the S phase, then increasing again in S phase and decreasing in M phase during the diel cell cycle. Specific toxin production rates were positive throughout G1 and S phases, but negative during the transition from G1 to S phase, and late in M phase, the latter reflecting cell division. All toxins were initially induced by the light and positively correlated with the percentage of cells in S phase, indicating that biosynthesis of *Dinophysis* toxins might be under circadian regulation and be most active during DNA synthesis.

BIOLOGICAL STOICHIOMETRY REGULATES TOXIN PRODUCTION IN *MICROCYSTIS AERUGINOSA*

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Harmful algal blooms (HABs) are increasing in frequency and magnitude globally. Even though a limited number of phytoplankton species can be toxic, they are becoming one of the greatest water quality threats to public health due to their intrinsic toxicity to humans and the numerous interacting factors that undermine HAB forecasting. Here, we show that the carbon:nitrogen:phosphorus (C:N:P) stoichiometry of a common toxic phytoplankton species, *Microcystis*, regulates toxin production during blooms through a tradeoff between primary and secondary metabolism. Blooms with optimal C:N and C:P cellular stoichiometry consistently produced more toxins than blooms exhibiting stoichiometric plasticity. Phosphorus availability in water exerted a strong control on bloom biomass and C:P stoichiometry, but N availability exerted a stronger control on toxin production by regulating bloom biomass and C:N:P stoichiometry. Microcystin-LR, like many phytoplankton toxins, is a N-rich secondary metabolite with a C:N stoichiometry that is similar to the optimal growth stoichiometry of *Microcystis*. Thus, N availability relative to P and light provides a dual regulatory mechanism that controls both biomass production and cellular toxin synthesis. Overall, our results provide a quantitative framework for improving forecasting of toxin production during HABs and compelling support for water quality regulations that limit both N and P inputs from anthropogenic sources.

CLIMATE

(Sessions I-II, alphabetical by author)

EVIDENCE FOR MASSIVE AND RECURRENT TOXIC BLOOMS OF *ALEXANDRIUM CATENELLA* IN THE ALASKAN ARCTIC

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The Pacific sector of the Arctic Ocean is experiencing rapid and dramatic changes in response to climate-driven warming. Many organisms may spread into and flourish in Arctic waters as a result of rising temperatures and loss of sea ice, but few present such significant threats to human and ecosystem health as harmful algal bloom (HAB) species. Here we present results from cruises in 2018-2019 that collected sediment and plankton samples from the Bering Strait to the Chukchi and Beaufort Seas, a large region with little history of toxic HABs. A massive, well-defined *Alexandrium catenella* cyst seedbed was documented in the northeastern Chukchi Sea in 2018. This feature extends at least 200 km offshore and up to 600 km alongshore in a region of reduced flow, and has cyst concentrations (up to 11,000 cysts cm⁻³) that are among the highest ever reported for this species globally. In both survey years, large-scale blooms of *A. catenella* cells at densities sufficient to cause shellfish toxicity elsewhere in the world were observed over much of the cyst seedbed area. High cell concentrations were also observed near the Bering Strait in 2019, suggestive of transport of established populations from southern waters. Notably, high cell concentrations were observed east of Barrow Canyon in the Beaufort Sea Shelfbreak Current. Bottom and surface water temperatures measured both years were within the ranges that support rapid cyst germination and cell growth. These data suggest that Arctic blooms may be derived from both advected as well as in situ populations, with strong potential for recurrent blooms in the future. *A. catenella* populations thus represent a new and significant threat to Alaskan Arctic communities who are justifiably concerned about their health and the health of the ecosystems on which they depend for food.

MODEL OF HEATING- AND CHILLING-BASED DORMANCY CONTROLS IN *A. CATENELLA* CYSTS

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Dormancy cycling is an endogenous mechanism that controls when dinoflagellate cysts can germinate and initiate new blooms. During their lifetime, cysts may alternate between dormant and quiescent (germinable) states many times. Prior work has shown that cysts of the PSP species *Alexandrium catenella* will cycle between these states with an approximately annual rhythm when stored in constant cold. More recent experiments have revealed two opposing temperature relationships that underlie these cycles. The first is chilling mediated passage through dormancy. Like many terrestrial seeds and bulbs, cysts must meet a chilling requirement to reach quiescence and will do so faster when exposed to colder temperatures. On exit from dormancy, cysts' passage through quiescence is controlled by heat accumulation. Cysts stored at higher temperatures return to dormancy faster than colder cysts. These relationships are revealed through a series of experiments that monitored the dormancy state of *A. catenella* collected from a deep cyst bed in the Gulf of Maine. Subsamples were stored in a range of constant and alternating temperature conditions. Both chilling and heating relationships fit simple heuristics that are commonly used to predict phenology in diverse agricultural applications. These heuristics are combined in a simple model of dormancy cycling that is used to explore potential consequences of climate warming on the behavior of *A. catenella*. The model reveals the importance of both mean temperature and temperature seasonality for the timing and intensity of germling fluxes from cyst beds and also provides a basis for statistical comparison of chilling and heating responses in different populations. Tools like this one will be critical for determining the capacity of *A. catenella* and other cyst forming dinoflagellates to adapt to changing ocean conditions.

WILL *ALEXANDRIUM* THRIVE IN A GREENHOUSE WORLD? IT'S COMPLICATED

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Our ability to forecast the fate of HAB under climate change is constrained by insufficient information on the response of species to projected warming and acidification. Because of their low CO₂ affinity, it is hypothesized that dinoflagellates should thrive under elevated CO₂ conditions. Yet few studies have considered the interaction of temperature and CO₂ on both cell growth and toxin content. Using a common garden design, we first measured cell growth rate (CGR) and cell toxin content (CTC) of six strains of the dinoflagellate *Alexandrium catenella* in response to temperature (8 to 28°C) and CO₂ (280 to 4000 ppm), followed by 2X2 factorial experiments (15.5 and 19.5°C and 400 and 2000 ppm CO₂). Strains were originally collected from the NE Atlantic (Long Island Sound to the Bay of Fundy) and varied in constitutive CTC by five-fold. There was considerable, but not random, variation in strain performance in response to warming and acidification. CTC was either independent or increased linearly with CO₂, whereas the norm for CGR was (surprisingly) a linear decrease. By contrast, CTC was independent or decreased linearly with temperature, and CGR showed the characteristic domed pattern for thermal performance curves. Results from the factorial experiments confirm the considerable among-strain variation in the response to temperature and CO₂, and more importantly in the interaction effects. Thus, prediction of even ecological responses of *Alexandrium catenella* to climate change remains challenging, but the extant genetic variation among strains suggests scope for evolution that may favor strains from the Bay of Fundy.

THE ROLE OF SURFACE WATER WARMING IN THE TIMING OF THE *MICROCYSTIS*-DOMINATED CYANOBACTERIAL BLOOMS IN WESTERN LAKE ERIE

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In Lake Erie, the smallest and shallowest of the Laurentian Great Lakes, cyanobacterial harmful algal blooms (cyanoHABs) in the western basin (WLE), dominated by potentially toxigenic *Microcystis* spp., have intensified over the past 15 years. It is well known that spring nutrient loads, primarily from the Maumee River watershed, plays a key role in determining the magnitude of the annual cyanoHAB. Furthermore, shifts in regional climatology, specifically warming and more extreme hydrologic events, leading to increased nutrient loading, is predicted to exacerbate the WLE cyanoHAB. However, one factor that is often overlooked is the potential shift in the initiation and duration of the bloom that may accompany earlier increases in spring water temperatures and warmer waters later into the fall, respectively. Currently the WLE cyanoHAB initiates around mid to late-July, peaks in August/September and dissipates by mid-October. However, if climate shifts cause warmer springs, the timing of bloom initiation could be shifted earlier in the summer. Furthermore, warmer fall temperatures may allow the blooms to persist longer than they currently do. We used high-resolution lake surface temperature retrievals from 2002-2017 and temperature-dependent growth rates of multiple *Microcystis* strains to evaluate recent changes in the phenology and growth rates. Our findings suggest that if the biogeochemical conditions for bloom formation in WLE persist, rising temperatures will indeed lead to an earlier onset and longer growing season for the *Microcystis*-dominated blooms with potential negative impacts for water managers.

USING SEDIMENTARY DIATOM AND CHIRONOMID ASSEMBLAGES TO DETERMINE THE ENVIRONMENTAL TRIGGERS FOR RECENT CYANOBACTERIAL BLOOMS IN CALLANDER BAY, LAKE NIPISSING, ONTARIO

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Callander Bay in Lake Nipissing (Ontario, Canada) has reported increased occurrences of cyanobacterial blooms since 2000. However, monitoring data and previously published diatom-inferred total phosphorus reconstructions indicate that nutrients have not changed in Callander Bay over the past four decades. To examine potential environmental triggers for recent blooms, we use sedimentary diatoms, chironomids, and chlorophyll-a from a Pb²¹⁰-dated sediment core to track long-term trends in water quality as well as to make comparisons to regional climate data. Prior to ~1900, biological proxies undergo pronounced changes characterized by a shift in dominance in diatom assemblages from *Aulacoseira* spp. to *Fragilaria mesolepta*, and in chironomid assemblages from *Procladius* spp. to *Chironomus* spp. Since ~1990, increases in the relative abundances of small-celled, planktonic diatoms (*Lindavia comensis*, *Discostella stelligera*, *Cyclostephanos tholiformis*), together with increases in colonial chrysophyte scales, likely indicate enhanced thermal stability in the bay. Pre-~1900 shifts in chironomid assemblages to *Chironomus* spp. dominance and the appearance of *Endochironomus* spp. around 1960 might indicate lower hypolimnetic oxygen in recent decades. We hypothesize that longer and stronger periods of thermal stratification, together with the development of anoxia in bottom waters, could release phosphorus and ferrous iron from the sediments. Collectively, these changes would be advantageous to cyanobacteria and fuel algal growth. Increasing primary production (inferred from sedimentary chlorophyll-a) since ~1860 is temporally consistent with the onset of cyanobacterial blooms. Our weight-of-evidence approach suggests that recent algal blooms are associated with climate warming, as they occur during some of the warmest air temperatures on record.

HOW TEMPERATURE AND NUTRIENTS INTERACT TO REGULATE THRESHOLDS OF CHANGE BETWEEN CYANOBACTERIA AND DIATOMS IN STREAM ECOSYSTEMS

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Climate warming and nutrient pollution are important global drivers of ecological change in streams, including shifts in the structure and function of algal assemblages. Warm conditions can promote the growth of noxious and toxin producing cyanobacteria. The addition of a limiting nutrient, like nitrogen (N) or phosphorus (P) can also promote the growth of nuisance or harmful algae, or result in species shifts from nutritious, palatable diatoms to toxic or less palatable cyanobacteria. Interactions between climate warming and nutrient additions further complicate our ability to predict, and thus manage, how a warm, nutrient-rich world might influence biofilm assemblages of current concern. We manipulated temperature (8°C–25°C) as well as N and P availability (individually and together), in a streamside channel experiment in Iceland to assess nutrients and temperature effects on biofilm species composition and abundance, particularly with respect cyanobacteria and diatoms. Nitrogen-fixing cyanobacteria (e.g. *Anabaena*, *Nostoc*) dominated N-poor channels, but diatoms with cyanobacteria endosymbionts like *Rhopalodia* were only abundant in warmer temperatures. Biomass accrual depended on temperature. As N availability increased, green algae and diatoms (e.g. *Melosira*, *Diatoma*) increased, but nitrogen-fixing taxa were generally only abundant in warmer temperatures. With the addition P, biomass accrual depended on temperature, but was also influenced by P level. Algal assemblages shifted to general dominance by *Nostoc*, or *Rhopalodiaceae* (diatom)-*Nostoc* associations. At the warmest temperatures, *Anabaena* dominated along with *Nostoc* and diatoms in the *Rhopalodiaceae*, with a consistent presence of green algae. With manipulation of N:P ratios, N-addition shifted biofilms to dominance by diatoms, but intersections between temperature and N:P ratios shaped diatom species assemblage structure. Understanding how temperature and nutrients interact to regulate thresholds of change between diatoms and cyanobacteria will strengthen

predictive modeling of how climate warming and eutrophication will influence the development of nuisance or harmful algal taxa.

CLIMATE- INDUCED INTERANNUAL VARIABILITY AND LONG-TERM CHANGE IN SEVERAL COMMON HABS OF CHESAPEAKE BAY

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Retrospective analysis of water quality monitoring data reveals strong interannual shifts in the spatial distribution of two harmful algal species (HABs), *Prorocentrum minimum* and *Karlodinium veneficum*, common to the mid and upper reaches of Chesapeake Bay. A habitat model, based on the temperature and salinity tolerance of the two species, as well as their nutrient preferences, provides good fidelity with both their observed seasonal progressions and spatial distributions. The habitat model also correctly hindcasts interannual differences in wet and dry years. Climate downscaling simulations using the habitat model suggest upstream shifts of the two species in the estuary, slightly longer blooming seasons (earlier spring events and later fall events) but slight reduction in their bloom frequency by the mid-21st century. A companion mechanistic model of these species which incorporates their mixotrophic growth is also being developed.

HARMFUL ALGAL BLOOMS: A CLIMATE CHANGE CO-STRESSOR IN MARINE AND FRESHWATER ECOSYSTEMS

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Marine and freshwater ecosystems are warming, acidifying, and deoxygenating as a consequence of climate change. In parallel, the impacts of harmful algal blooms (HABs) on these ecosystems are increasing. Many shallow, eutrophic habitats that host recurring HABs already experience thermal extremes, low dissolved oxygen, and low pH, making these locations potential sentinel sites with conditions that will become more common in larger-scale systems as climate change accelerates. While studies of the effects of HABs or individual climate change stressors on aquatic life have been relatively common, studies assessing their combined impacts have been rare. Those doing so have often reported strong species- and strain-specific interactions between HABs and climate change co-stressors, yielding outcomes for aquatic organisms that could not have been predicted based on investigations of these factors individually. This talk will provide an ecological and physiological framework for considering HABs as a climate change co-stressor and will consider the consequences of their combined occurrence for coastal animals. As an example, a recent international workshop identified mechanisms by which HABs promote the occurrence of hypoxia in some ecosystems, a co-occurrence that represents an ecosystem threat that will become more likely and/or intense under climate change. Very little is known with regard to the physiological consequences of such a dual threat and policies to protect fisheries and/or aquaculture from against such a threat do not exist. Hence, this talk will also highlight gaps in understanding of HABs as a climate change co-stressor that must be addressed to develop management plans that adequately protect fisheries, aquaculture, aquatic ecosystems, and human health. Ultimately, incorporating HAB species into experiments and monitoring programs where the effects of multiple climate change stressors are considered will provide a more ecologically relevant perspective regarding the structure and function of marine ecosystems in future, climate-altered systems.

THE EFFECT OF CLIMATE CHANGE AND EUTROPHICATION ON *DOLICHOSPERMUM* A HARMFUL, DIAZOTROPHIC CYANOBACTERIAL GENUS

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Freshwater ecosystems experiencing rising global temperatures and eutrophication have been subjected to toxic cyanobacterial blooms of an expanding size and duration that threaten human and animal health. Excessive nitrogen loading is often associated with blooms dominated by toxin-producing, non-diazotrophic (N₂-fixing) cyanobacteria, though intense blooms of diazotrophs such as *Dolichospermum* have also been reported in eutrophic systems. Moreover, the potential for elevated carbon dioxide (CO₂) levels to affect toxin production and N₂-fixation in freshwater cyanobacterial communities and diazotrophs in tandem with nutrient availability remains largely unknown. Thus, we have investigated the individual and interactive effects of nutrients and CO₂ on toxic strains and natural populations of *Dolichospermum*. Results indicate that when grown under elevated CO₂ levels the microcystin-producing *Dolichospermum* strain sp. 90 exhibited significantly higher biomass; *Dolichospermum* also exhibited N-limitation under CO₂ enrichment. N₂-fixation also significantly increased when cultures of strain sp. 90 were subjected to higher CO₂ levels, particularly in those cultures amended with nitrate (NO₃⁻). This strongly suggests that *Dolichospermum* relies more heavily on nitrogenase activity in tandem with external N assimilation in order to meet the demands of enhanced growth under elevated CO₂ availability. Furthermore, microcystin content exhibited N-limitation when strain sp. 90 was grown under elevated CO₂ levels, suggesting that eutrophication will play a greater role in the toxin production of *Dolichospermum* blooms in the near future. We also report that natural populations of *Dolichospermum* experienced significantly enhanced growth in response to CO₂-enrichment, particularly when amended with nutrients, indicating that the proportion of cyanobacterial diazotrophs in freshwater algal populations may benefit from future increases in atmospheric CO₂. The effects of CO₂ and NO₃⁻ availability on an anatoxin-producing strain of *Dolichospermum* (sp 54) will also be presented.

RESOLVING THE APPARENT PARADOX OF TEMPERATURE AND *PSEUDO-NITZSCHIA* EVENTS

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Highly toxic blooms of *Pseudo-nitzschia* regularly recur along the US west coast in the California Current System. The massive 2015 event was linked to the Pacific Warm Anomaly (warm blob), and historical analysis of blooms shows a correlation between increased temperature and toxic blooms in the northern CCS. However, in the southern CCS (roughly San Francisco Bay southward) increasing temperature does not appear to increase bloom frequency or toxicity. During 2015, the Gulf of the Farallones was one of several “hotspots” but despite the close proximity, San Francisco Bay has few reports of harmful algal blooms and low concentrations of domoic acid. Here we evaluate the potential growth and toxicity of the dominant toxigenic species in California coastal waters, *P. australis* and *P. multiseriata*, to directly test the hypothesis that San Francisco Bay waters confer resistance to blooms. We specifically evaluate the effect of varying temperature, salinity, and to a lesser extent, nutrients on growth and toxin production. This analysis is combined with a much larger dataset of toxicity from 2002-2019 for the CCS to identify the relationship, if any, between temperature and blooms. Based on both the physiological experiments with cultures and the much larger field dataset, we suggest that *Pseudo-nitzschia* exhibit a temperature envelope, with maximum toxicity occurring in a reasonably narrow range, which can rectify the apparent paradox of bigger and more toxic blooms (i.e. during the warm blob) in some, but not all, parts of the CCS. This has obvious implications for the future California Current, suggesting that blooms may shift temporally and/or geographically with increasing coastal temperatures.

THE BIGGEST ALGAE BLOOM ON EARTH: DEVELOPMENT OF THE GREAT ATLANTIC SARGASSUM BELT

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Since 2011, unprecedented coastal strandings of the brown pelagic seaweed *Sargassum* have occurred over broad areas of the North Atlantic basin and Caribbean Sea. These strandings, characterized by excessive biomass, are harmful because of their growing impacts on both the environment (fish kills, dead zones, toxic H₂S) and the tourist-based economies of affected areas. We used long-term *Sargassum* tissue C:N:P data, satellite imagery, numerical models, and field measurements to assess changes in the ecology and oceanography of these blooms. Comparison of *Sargassum* C:N:P data in the Straits of Florida from 1983-1987 and 2010-2018 showed significant increases in the N:P ratio, indicating increasing P-limitation of growth. A 19-year record of observations from MODIS satellite imagery revealed the recent development of a Great Atlantic Sargassum Belt (GASB) that extends across the tropical Atlantic Ocean. The GASB formed in the spring/summer months between 2011 and 2018. In 2015 and 2018, the GASB showed the highest coverage, extending > 8,850 km from west Africa across the tropical Atlantic Ocean and through the Caribbean Sea. The shape of the GASB is consistent with advection by ocean circulation patterns in the tropical Atlantic Ocean. Particle-tracking numerical experiments accounted for the physical transport and growth of *Sargassum*, with weak connections to the North Atlantic and Caribbean Sea. The results suggest that the blooms form locally in the central Atlantic and match other modeling efforts emphasizing the role of the North Equatorial Recirculation Region (NERR). Analyses of satellite-derived change rates in *Sargassum* point to strong upwelling in the eastern Atlantic and Amazon discharges as nutrient sources that contribute to the GASB. This was supported by sampling on cruise # 371 of the RV Thomas G. Thompson in late August 2019, which showed abundant *Sargassum* in reduced salinity (33 psu) surface waters of the Amazon plume.

BIOPHYSICAL DRIVERS FACILITATING A TOXIGENIC CYANOBACTERIAL BLOOM IN A MAJOR GREAT LAKES TRIBUTARY

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The Maumee River is the primary source for nutrients fueling seasonal *Microcystis*-dominated blooms in western Lake Erie's open waters though such blooms in the river are infrequent. The river also serves as source water for multiple public water systems and a large food services facility in northwest Ohio. On 20 September 2017, an unprecedented bloom for this river was reported within the Toledo metropolitan area, which triggered a recreational water advisory by the City of Toledo. A rapid-response survey was conducted to determine the toxicity and taxonomic composition of the bloom. Chemical analysis determined levels of cyanotoxin, specifically microcystins, in excess of recreational contact limits at sites within the lower 20 km of the river while amplicon sequencing found these sites were dominated by *Microcystis*. Historical analysis of ten-years of seasonal river discharge, water level, and local wind data identified two instances in which high retention conditions (e.g., low river discharge, low seiche intensity, and a high proportion of easterly winds) occurred over ≥ 10 days in the Maumee River estuary: in 2016 and during the 2017 bloom. Remote sensing imagery supported the advection of *Microcystis* into the river from the lake during 2017, as well as the lack of a river bloom in 2016 due to a weak *Microcystis* bloom in the lake. These results highlight the need to broaden our understanding of biophysical coupling within the interface between riverine and lacustrine systems, particularly as cyanobacterial blooms are expected to become more prominent in response to changing climate.

MITIGATING HARMFUL CYANOBACTERIAL BLOOMS IN A HOTTER, HYDROLOGICALLY MORE-EXTREME WORLD

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Managing and mitigating the global expansion of toxic cyanobacterial harmful algal blooms (CyanoHABs) along the freshwater-to-marine continuum is a major challenge. Various approaches, including: nutrient load reduction, artificial mixing and flushing, omnivorous fish removal, algaecide applications and sediment dredging, have been used to reduce bloom occurrences. However, managers now face the additional challenge of having to address the effects of climate change on watershed hydrology, temperature, mixing and nutrient loading/cycling dynamics. Warmer temperatures and more frequent extreme weather events, including: tropical cyclones, extratropical storms, floods and droughts, all promote CyanoHABs and affect the efficacy of ecosystem remediation measures. An increasingly common scenario that promotes cyanobacteria bloom events is when periods of excessive rainfall and nutrient runoff are followed by prolonged drought and heat wave conditions. Additionally, earlier spring warming and later fall cooling has resulted in a longer growing season enabling blooms to develop early and persist late into the year. Lastly, an increase in both the frequency and magnitude of precipitation events associated with tropical cyclones and extratropical storm events often stimulates severe cyanobacterial blooms. Since we are unable to alter the climate in the short term, stricter nutrient (including both nitrogen and phosphorus) reduction targets will need to be enacted for bloom control in affected waters. In light of the synergistic effects of climate change with nutrient enrichment, and because the climate continues to change, managers will need to periodically reassess the efficacy of other mechanical, chemical and biological control measures previously used to reduce CyanoHABs.

HABS THEN AND NOW: COMPARING HISTORIC AND MODERN CYANOBACTERIA DYNAMICS OVER THE LAST 5000 YEARS USING THE SEDIMENT RECORD

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The global occurrence of harmful algal blooms is believed to be increasing in response to warming and human impacts. Land use change, hydrological modifications, nutrient dynamics, biological interactions and other drivers have all been identified as triggers for HAB development. Whereas modern HAB dynamics are being identified, placing HABs in a historical context could benefit future models and projections of HAB occurrences. Here, we applied paleolimnological tools to six tropical and subtropical lakes to document the development of dense cyanobacteria communities from the middle Holocene to the present (last ~5000 years). The objectives of the study were 1) to place modern limnological conditions in a historical context against pre-human disturbance periods and, 2) to determine if the drivers of modern cyanobacteria communities differ from historic communities. We utilized paleolimnological techniques to infer past allochthonous inputs (nutrients, organic matter, metals), hydrological modifications, and autochthonous responses (photosynthetic pigments) in Lakes Mattamuskeet (NC), Apopka (FL), Griffin (FL), Ditch (AL), and Amatitlán (Guatemala). Paleolimnological results suggest that historic HABs and cyanobacteria development are linked both with hydrological change and alterations to nutrient inputs. Lakes Mattamuskeet and Apopka showed hydrological modifications as the primary driver of cyanobacteria dominance during historic periods, while Lakes Griffin and Ditch developed hypereutrophic conditions in response to major alterations to the landscape. Lake Amatitlán showed historic hypereutrophic conditions due to ancient human settlements in the watershed, but surface sediments suggest that modern cyanobacteria communities could be different between hydrologically separated basins. These results suggest that HAB occurrences are not unique to the modern era and that historic HAB development is linked to a multitude of drivers.

MONITORING & MANAGEMENT (Sessions I-II, alphabetical by author)

AZADINIUM SPINOSUM AND AZADINIUM POPORUM IN THE INLAND AND COASTAL WATERS OF THE PACIFIC NORTHWEST FROM 2014-2018

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Azaspiracids, toxins produced by the dinoflagellate genera *Azadinium* and *Amphidoma*, can cause azaspiracid shellfish poisoning in humans, with symptoms of nausea, vomiting, severe diarrhea, and stomach cramps. A survey of *Azadinium* species in the Pacific Northwest during the spring and summer of 2014-2018 was conducted after shellfish consumers from Washington State reported these symptoms in the absence of the suspected historical culprits (diarrhetic shellfish toxins or *Vibrio*). Quantitative polymerase chain reaction (qPCR) analyses using probes originally designed to detect species of *Azadinium* from the North Sea measured abundances up to 156 cells L⁻¹ of *A. spinosum* and 10,525 cells L⁻¹ of *A. poporum* at shore-based sites in Washington State. At the site with the highest *A. poporum* abundance, shellfish contained measurable concentrations of azaspiracid-2 and azaspiracid-59, the latter a new azaspiracid first described in Washington State waters in 2014. Abundances of up to 30 cells L⁻¹ of *A. spinosum* and 1,206 cells L⁻¹ of *A. poporum* were measured in offshore coastal waters during research cruises. At sites where neither *A. poporum* nor *A. spinosum* were detected, an Amphidomataceae-specific qPCR assay indicated that other species of *Azadinium* or *Amphidoma* were present. *Azadinium* were detected in conjunction with other harmful species at some outer coast stations, highlighting the need for research into the synergistic effects of marine toxins and the strategic placement of biological sensors like the Environmental Sample Processor (ESP). With appropriate molecular tools, the ESP can provide early warning of multiple harmful algal species simultaneously in Pacific Northwest coastal waters. Detection of *A. spinosum* and *A. poporum* in seawater and azaspiracids in shellfish, coupled with the presence of other species of *Azadinium* or *Amphidoma*, emphasizes the importance of continued monitoring for these organisms and toxins as a proactive approach to human health protection in the region.

OBSERVATIONS ON A MULTI-YEAR DATASET FROM A MID-LATITUDE RESERVOIR EXPERIENCING HAB EVENTS

¹H. Joel Allen, ¹Jingrang Lu, ¹Jorge Santo Domingo, ¹Heath Mash, ¹Toby Sanan, ¹Christopher Nietch, ¹Stefania Guglielmi, ²Kit Daniels, ³Tim Neyer

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HABs related data have been collected on a mid-latitude multiple use reservoir with a history of HAB events over the past five years. The goal of this work is to develop relationships between HAB indicator measures and cyanotoxin occurrence which can provide time-relevant information regarding water quality for DWTP operators and other public health stakeholders. Observations include: in-vivo fluorescence, phytoplankton microscopic enumeration, water quality parameters (nutrients, pH, ORP, turbidity, dissolved oxygen), meteorological measures, genomic markers, and cyanotoxin measurements. Frequency of data collection varies by parameter from ten-minute intervals through daily, weekly sampling events. Year over year analyses indicate that while there are similarities to the course of phytoplankton community succession, there is variation in the timing, and magnitude of cyanotoxin production. A brief summary of the dataset will be presented highlighting key relationships in relevant variables and observed year over year differences. Lessons learned regarding sample collection timing and location will also be shared.

COMBINED LONG- AND SHORT-READ DNA SEQUENCING OF CYANOBACTERIAL LAKE SAMPLES REVEALS GENOME SEQUENCE AND POPULATION STRUCTURE OF EXTANT PACIFIC NW BLOOMS

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Despite the ubiquity of freshwater cyanobacterial blooms and the desire to investigate the precise factors that make them so prevalent, we are at present limited by our knowledge of the genome sequences and population structures of extant blooms. This limits our ability to fully utilize advanced metagenomic and other tools, such as in studies aimed at identifying the drivers of bloom expansion and decline or understanding the relationships among extant freshwater HABs. To address this knowledge gap, we have applied a hybrid sequencing approach using PacBio long-read and Illumina short-read sequencing to analyze standing bloom samples from the Pacific NW, without cultivation. Our studies have emphasized *Anabaena/Dolichospermum*, *Aphanizomenon flos-aquae*, and *Gloeotrichia* blooms, with several goals: 1) identifying the producers and genetic determinants of toxins and taste-and-odor compounds, 2) determining relationships between morphotype and genotype, 3) determining the genetic variation in bloom-forming species within single bloom events, between succeeding years in a single lake, and at regional scales, 4) refining the taxonomy of the *Anabaena/Dolichospermum/Aphanizomenon flos-aquae* (ADA) consortium, and 5) identifying co-occurring bacteria that might constitute commensals, synergists or predators in the phycosphere. Among our results, we have determined the complete genome sequence of the *Anabaena/Dolichospermum* producer of cylindrospermopsin that was the cause of a drinking water crisis in Salem, OR, in June 2018. The same strain was present during 2016 and 2017, with toxin biosynthetic genes appearing to be present on a ~200 kbp extrachromosomal element. In both years, the most abundant co-occurring bacterium was a strain of *Opitutus* (phylum Verrucomicrobia). Our analyses have emphasized that extant ADA CyanoHABs are members of a distinct genome-level clade as first identified by Driscoll et al. (2018) that is a logical group to which the now-commonly used genus name *Dolichospermum* could be applied.

ESTABLISHING A NETWORK OF ADVANCE TECHNOLOGIES: LONG-TERM MONITORING OF CYANOBACTERIA BLOOMS IN THE GREAT LAKES

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Harmful algal blooms (HABs) are influenced by a variety of environmental factors such as temperature, pH, light, and nutrient availability/stoichiometry. In the Great Lakes ecosystem, cyanobacteria HABs (cHABs) have been a recurrent feature since the mid-1990s. The predominant cHAB taxon is *Microcystis aeruginosa*, which produces expansive blooms in western Lake Erie and Lake Huron (Saginaw Bay). Toxin levels associated with these events often exceed the World Health Organization (WHO) recommended limit of 1 $\mu\text{g L}^{-1}$, resulting in a significant threat to ecosystem and human health. Though viewed as a Great Lakes ecosystem, blooms in Lake Erie and Lake Huron are influenced by different physicochemical factors leading to distinct biomass distributions and toxicity. To inform management actions, a comprehensive understanding of the multiple interactive factors that influence *M. aeruginosa* growth and toxicity in the Great Lakes is needed. We will discuss the establishment of a monitoring network within the western basin of Lake Erie and Lake Huron, which includes the use of in situ, real time monitoring sites and remote sensing technologies, such as Environmental Sample Processors (ESP) and airborne hyperspectral imaging. Successes and pitfalls of establishing a Great Lakes ESP network to monitor microcystin levels in western Lake Erie will be presented. Analysis of data from 2016 – 2019 suggests inter- and intra-annual variability in the distribution, extent, and severity of cHABs based on climate factors (wet versus drought years) and timing of precipitation.

PRELIMINARY USE OF SOLID PHASE ADSORPTION TOXIN TRACKING (SPATT) FOR BREVETOXINS IN THE HYPOTHESIZED FORMATIVE REGION OF *KARENIA BREVIS* BLOOMS

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Sampling strategies for harmful algal bloom (HAB) species that manifest in the offshore environment have improved in recent years with applications of new technologies such as autonomous underwater vehicles and buoyed in situ sensors. However, these methods are expensive and are not ubiquitous, and the paradigm of discrete sample collections is even more limited in time and space in the offshore environment. Solid Phase Adsorption Toxin Tracking, or SPATT, has been used in situ to measure and track a suite of dissolved toxins and instead of snapshots in time, offers time-integrated sampling. Here, methods for a novel SPATT application for tracking brevetoxins derived from the impactful harmful alga *Karenia brevis*, which blooms annually in the Gulf of Mexico (GOM), were developed and validated in the laboratory. In situ SPATT deployments were then conducted for one year at a fixed offshore site in the GOM, located in an area hypothesized to be important for *K. brevis* bloom initiation. Monthly deployments of SPATT bags at four depths on a buoyed line showed the presence of brevetoxins throughout the year, in spite of *K. brevis* not being detected in the majority of samples collected nearby. This provides proof of concept that *K. brevis* cells were likely present during the deployments at all depths but were under-quantified and/or all together missed by current sampling strategies. A difference in surface and bottom brevetoxin concentrations occurred during the study period from September to December 2018. Adjacent sites showed a vertically stratified system mirrored by changes in observed maximal brevetoxin concentrations within either layer over time. Further development of this method, including additional deployment locations and concurrent measurements, will strengthen the understanding of bloom dynamics in time and space and could eventually be used as an early warning system for offshore bloom manifestations.

APPLICATION OF A CONVOLUTIONAL NEURAL NETWORK FOR IMAGE CLASSIFICATION TO IMPROVE AUTOMATED EARLY WARNING NOTIFICATIONS OF HABS FROM THE IMAGING FLOWCYTOBOT (IFCB)

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Early warning is one of the best strategies for limiting the negative human health impacts of harmful algal blooms. Since 2012, the Texas Observatory for Algal Succession Time series (TOAST) in Port Aransas, Texas has provided early warning of *Karenia brevis* and *Dinophysis ovum* to state managers through an automated email notification system. This previous IFCB email notification system utilized a random forest image classifier to sort images into 54 different categories based on a set of >200 pre-extracted features and trained with a dataset consisting of images captured between Aug 2007 – Mar 2012. Recently, the use of convolutional neural networks for image classification has become more accessible and is well-suited to classifying phytoplankton images. Utilizing images from over ten years of IFCB field deployments a new classifier was trained to distinguish 112 classes (CNN112). A new classifier using the random forest (RF112) approach and the newer image dataset was also developed for comparison to the original 54-category random forest classifier (RF54). Predicted outputs from CNN112, RF112, and RF54 were compared with a manually classified dataset from prior blooms of *K. brevis*. For early warning purposes, a classifier needs to be accurate at low cell abundances. The previous threshold of 2 cells/ml for notification was again used to determine false positives and false negatives. Both the CNN112 and RF112 classifiers had fewer false positives compared to the RF54 classifier at 2 cells/ml, with the CNN112 classifier having the fewest false positives overall. When looking only at cell abundances <10 cells/ml, the CNN112 classifier had a slightly higher correlation ($r = 0.95$) with the manual classifications when compared to the RF112 classifier ($r = 0.90$). Because the CNN approach uses raw image data, any images that are classified in a HAB category can easily be included into our new email notification.

THE HARMFUL ALGAL BLOOMS OBSERVATION SYSTEM (HABSOS), A HAB DATABASE AND DISTRIBUTION PLATFORM

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The Harmful Algal BloomS Observation System (HABSOS) is a web-based data collection and distribution system for harmful algal bloom (HAB) information in the Gulf of Mexico, operated by NOAA's National Centers for Environmental Information (NCEI). The goal of HABSOS is to provide environmental managers, scientists, and the public a resource for HAB data and information, including the distribution, extent and timing of the blooms. *Karenia brevis* is currently the only HAB species contained in HABSOS, however the potential for inclusion of other species exists. HABSOS operates as a cooperative enterprise with Gulf of Mexico state agency partners that monitor *Karenia brevis* blooms and submit their near real-time data to the HABSOS database. HABSOS uses an ESRI mapping application to display the HAB data housed in its database. The map viewer application displays by default the last 28 days of *K. brevis* data from the current date, however the display period can be set to any time span within the HABSOS database. There are additional data sources incorporated into the map viewer including satellite-derived, oceanographic, and meteorological layers, such as chlorophyll, surface currents forecast, or surface winds. Furthermore, the *K. brevis* cell count data and associated environmental data are archived and publicly available from the NCEI archive, <https://www.nodc.noaa.gov/archivesearch/catalog/search/search.page>. HABSOS is a unique resource utilized by a wide variety of user groups including academia, government agencies, private enterprise, non-profit organizations, and individuals.

ENGAGING COMMERCIAL FISHERMEN TO FILL CRITICAL MONITORING GAPS FOR RED TIDE

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The economies on Florida's west coast are dominated by fishing and tourism. The 2017-2019 red tide bloom had severe impacts on these coastal communities despite red tides being a regular occurrence throughout the documented history of this area. During interviews with commercial fishermen from Pine Island, FL regarding Ecosystem-Based Fisheries Management in August 2018, the impacts of this red tide bloom became apparent to the NOAA Gulf of Mexico Integrated Ecosystem Assessment team (GoM-IEA). A planned NOAA research cruise was enhanced to sample the red tide bloom and associated ecosystem dynamics on the west Florida shelf from the Florida Keys north to Clearwater. A commercial fisherman from Pine Island was included in the science party to help guide our sampling. Data collection on the cruise included observations of a large hypoxic area in the bottom waters offshore of Pine Island where the fishing had decreased substantially. Unfortunately, these data were collected simultaneously with the opening of the stone crab season meaning they could not be used to inform these fishermen's decisions on infrastructure investment and fishing effort. Moreover, this area is not sampled frequently enough for scientists to determine the frequency or intensity of hypoxia in this region, much less its causative mechanism. This lack of data led the commercial fisherman who participated in the cruise to found a non-profit, Florida Commercial Watermen's Conservation, and engage the fishing community around southwest Florida shelf to collect the data to help fill this gap in offshore monitoring. The commercial fishermen sample water quality data along four onshore-offshore transects using an In Situ Aqua Troll 600, a HABScope, and water bottles. The data are transmitted to NOAA, quickly QA/QC'ed and analyzed to inform decisions by the fishermen for upcoming fishery seasons and transmitted to GCOOS for distribution and archiving.

THE GCOOS-RA HAB PRODUCTS AND SERVICES

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As more products and services for HAB monitoring develop, there is an increasing need for a central repository and data coordinating entity. The Gulf of Mexico Coastal Ocean Observing System Regional Association (GCOOS RA) has been hosting HAB data streams for over ten years. GCOOS's first supported HAB product, the data stream from two Imaging Flow Cytobot installations from the Texas Observatory for Algal Succession Time-Series (TOAST), and the information system implemented by Dr. Matt Howard (GCOOS). The next HAB data streams came from Mote Marine Laboratory: the Beach Conditions Reporting System (BCRS) and the Programmable Hyperspectral Seawater System (PHYSS), the follow-on to the Optical Phytoplankton Discriminator (OPD.) The data streams from two emerging HAB citizen science projects, the HABscope and the RT-NASBA are also hosted by GCOOS-RA. These data showcase the additional services that GCOOS can provide. Not only are the data hosted by GCOOS, data processing is embedded in the data stream to improve the utility of the data. Finally, the GCOOS-RA autonomous underwater vehicle piloting and mapping tool (GANDALF) assists glider operators throughout the Gulf in their mission planning and monitoring.

The GCOOS RA Data Management team has product development capabilities, geospatial, data science and network infrastructure to efficiently stream data. All data published in GCOOS data portals follow the open data policies and that are available to all Gulf of Mexico HAB scientists for free. GCOOS also archives data through the National Centers for Environmental Information (NCEI). GCOOS encourages all scientists to take advantage of these facilities: let us do the data management while you do HAB science.

The GCOOS RA envisions a fully built out HAB Integrated Observing System (HABIOS) and will continue to support products and services for that vision.

A FLOW-BASED RISK CHARACTERIZATION MODEL FOR HABS ON THE OHIO RIVER

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In August of 2015 an unprecedented harmful cyanobacteria bloom (CyanoHAB) occurred over 700 miles of the 981-mile Ohio River. The bloom threatened drinking water of five million people and caused five states to set advisories that lasted more than two months. To meet several assistance requests EPA Regions 3 and 5 funded a research effort to better understand the cause and consider what could be done to characterize the risk of Ohio River HABs in the future. First, preliminary analyses revealed unique flow trends during the time periods leading up the 2015 bloom. Next historical river flow data was obtained for 24 sites dating back to the beginning of 1996. A conceptual cause and effects model guided subsequent statistical modelling. Two lag terms were found to be highly explanatory, one indicating the degree the river was experiencing high flows 21 to 55 days prior to the observed bloom in 2015 and the other, the degree the river was under low flow conditions 19 days prior to the bloom. A Bayesian model was developed that uses the ratio of these two lag terms and the number of days the ratio itself was increasing as predictors. By pooling across sites, the model provides predictions and uncertainties in bloom probabilities, where a "bloom" is defined by the 2015 condition. Finally, a risk communication framework was developed to provide river stakeholders a visualization of the model results in real time along with other supporting information. Risk probability plots can be visualized for 20 sites along the river. These qualify the current conditions in terms of those that produced the bloom in 2015. The risk characterization tool for Ohio River HABs is currently being transferred to the Ohio River Valley Sanitation Commission, who will provide password-protected access through their website.

ENVIRONMENTAL AND BIOLOGICAL CONTROLS ON NITROGEN-RICH TOXIN PRODUCTION BY DIAZOTROPHIC AND NON-DIAZOTROPHIC CYANOBACTERIA

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Numerous cyanobacterial toxins such as cylindrospermopsin, microcystin, nodularin, and saxitoxin are N-rich biochemicals that act as hepatotoxins or neurotoxins to mammals. Thus, the occurrence of these toxins is often correlated with the total N concentration or the N:P in surface waters and several experimental studies have shown a link between toxin production and N availability during blooms. Some bloom-forming cyanobacteria (diazotrophs) can also fix atmospheric N₂ into ammonium when dissolved inorganic N is in short supply. Other bloom-forming cyanobacteria (non-diazotrophs) do not have the capacity to fix N₂, but many diazotrophs and non-diazotrophs have the capacity to synthesize toxins. Here I present the results of several modeling, culturing, and field-scale studies to explore the tradeoffs between toxin synthesis and N acquisition through diverse cyanobacterial growth strategies. Briefly, a mathematical model of diazotrophic growth that includes the differentiation of heterocysts and the synthesis of nitrogenase indicates a strong tradeoff between N investment in growth and secondary metabolic processes. This tradeoff is even greater in non-diazotrophic taxa as indicated through culture and field scale experiments. These results collectively point toward N reductions (in addition to P reductions) as a critical management tool for reducing the potential toxicity of cyanobacterial blooms.

WHAT'S LURKING IN YOUR LAKE? AN ASSESSMENT OF STATES' FRESHWATER HARMFUL ALGAL BLOOM PROGRAMS

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Freshwater harmful algal blooms (HABs) are increasing in size and frequency, and freshwater bodies that are used for recreation – such as lakes, ponds, reservoirs, rivers, and streams – are becoming more susceptible to blooms. States recognize the need to safeguard freshwater bodies but are not responding quickly enough to adequately protect public and environmental health. In order to determine to what extent state agencies are addressing the emergent challenge of HABs, we conducted a two-part assessment of states' freshwater HAB programs. The first part scores each state's freshwater HAB program (if it has one) based on the presence and availability of HAB-related information. The second part uses data collected by states to map HAB events from 2008 to 2018. States vary widely in the data they collect, make public, and use in assessing threats to public and environmental health from exposure to HABs. While a few states are making excellent progress with their freshwater HAB programs and developing guidelines to be more proactive in their response to HABs, most states are still reactive to rather than prepared for HABs. We found that states need to better collect and use data and technology as well as develop more comprehensive outreach programs to proactively protect the public from exposure to HABs and curtail environmental and economic repercussions.

HOW TO DEVELOP A CYANOTOXIN RESPONSE PLAN USING A TRIGGER BASED SYSTEM FOR YOUR DRINKING WATER TREATMENT PLANTS

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With the rising temperatures and nutrient loading on lakes across the United States, many water agencies are experiencing an increase in Harmful Algal Blooms (HABs). In 2014, a toxic algae bloom that occurred on Lake Erie resulted in a tap-water ban in Toledo, Ohio. This drinking water ban catalyzed a ten-day EPA Drinking Water Health Advisories (HA) for the cyanobacterial toxins microcystins and cylindrospermopsin. After the HA took effect, California became proactive and encouraged drinking water agencies across the state to develop cyanotoxin action plans. The Santa Clara Valley Water District (SCVWD) is one of many agencies that experienced HABs and was proactive in developing its own Cyanotoxin Response Plan (CRP). The CRP uses a trigger-based system with routine monitoring to better prepare treatment plants for changing their treatment strategies. Since 2017, the SCVWD lab has been collecting samples from three treatment plants' raw water sources, in accordance with the CRP, for analysis of

fluorescence, phycocyanin, algae speciation, and Enzyme-Linked Immunosorbent Assay (ELISA). These findings show that when a phycocyanin and fluorescence trigger is hit on a source water reservoir that is prone to cyanotoxins, then most likely cyanotoxins will be present in that raw water source influent. Thus, using a trigger-based system for conducting ELISA and algae speciation is an acceptable first line of defense to prepare treatment plants for changing treatment strategies.

MITIGATION & CONTROL (Sessions I-II, alphabetical by author)

EVALUATING DIFFERENT APPROACHES FOR CONTROLLING TOXIC ALGAL BLOOMS

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Harmful algal blooms (HABs) often occur in water bodies experiencing excess nutrient inputs. In freshwater systems, HABs are often dominated by cyanobacteria, such as *Anabaena* and *Microcystis*, that can negatively impact aquaculture ponds and recreational and drinking water reservoirs through the production of toxic secondary metabolites. Consequently, controlling HABs is imperative. Cyanobacteria are known to be vulnerable to a number of chemical compounds (e.g., hydrogen peroxide, copper sulfate) that can improve water clarity if dosed correctly. In this study, we evaluated the effectiveness of seven different chemicals at reducing cyanobacterial abundance at two scales, including a 14-day microcosm laboratory trial and a 28-day field mesocosm trial. Results from the laboratory experiment showed dramatic decreases in phytoplankton, including cyanobacteria, for most treatments, including hydrogen peroxide and copper sulfate-based products. In contrast to our laboratory findings, the decline in cyanobacteria was not as rapid or as sustained for several of the treatments. The disconnect between results produced from complementary laboratory and field experiments shows a greater need for further field experiments aimed at elucidating the mechanisms mediating the control of HABs with algicides.

EFFECTS OF DINOFLAGELLATE-SPECIFIC ALGICIDE (IRI-160AA) ON MICROBIAL COMMUNITIES: A NEXT GENERATION SEQUENCING APPROACH

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The use of naturally occurring bacterial algicides is a promising approach to prevent or mitigate HABs, but risks to non-target organisms must be evaluated before application. The marine bacterium *Shewanella* sp. IRI-160, secretes algicidal compound(s) that induce cell death in dinoflagellates, yet have no significant impact on other eukaryotic species tested, including laboratory cultures of non-target phytoplankton species, copepods, oyster or crab larvae, or juvenile finfish. Here, the effects of the algicide, designated IRI-160AA, on microbial communities in the Delaware's inland bays were evaluated using a suite of molecular methods including next generation sequencing. A natural community experiment was conducted to evaluate impacts of repeated dosing at concentrations of the algicide necessary to achieve 5%, 50% or 95% reduction in dinoflagellate abundance. Changes in dinoflagellate abundance were determined by quantitative real-time PCR (qPCR), while changes in the entire eukaryotic microbial community were evaluated using both molecular fingerprinting methodology (T-RFLP) and next generation sequencing of the 18S rRNA gene. Results demonstrate a dose-dependent decrease in dinoflagellate abundance, yet overall increases in photosynthetic biomass. Shifts in the eukaryotic community were also dose-dependent based on both sequencing data and community fingerprinting. Sequencing revealed increases in the relative abundance of diatoms, cryptophytes, and ciliates, and decreases in the relative abundance of raphidophytes and chlorophytes, along with expected decreases in dinoflagellates. When the remaining dinoflagellate community was evaluated separately, sequencing revealed an increase in dinoflagellate diversity, along with an increase in the relative abundance of thecate dinoflagellates compared to athecate species. Results of this investigation suggest that application of IRI-160AA may be an effective means to prevent or mitigate dinoflagellate blooms while restructuring the microbial community to promote the growth of non-harmful phytoplankton species and heterotrophic protists.

EFFECTS OF HARMFUL ALGAL BLOOMS ON REGULATED DISINFECTION BYPRODUCTS: FINDINGS FROM UTILITY CASE STUDIES

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During a harmful algal bloom (HAB) event, public water systems face the challenge of treating the bloom and associated toxins as well as controlling disinfection byproduct (DBP) formation. While laboratory studies have shown the link between HABs and DBP formation, there have been few full-scale studies that monitor for the impacts of bloom events on DBP formation. Results from a pilot monitoring study conducted during the 2018 bloom season illustrate the impact of source water quality and treatment and management of HABs on DBP formation with five case studies. The results can provide insights to other utilities faced with managing the complex issues related to HABs and DBPs in their systems.

A REVIEW OF LOCAL, STATE AND FEDERAL *KARENIA BREVIS* PREVENTION, CONTROL, AND MITIGATION RESEARCH FROM 2009 TO PRESENT

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Blooms of the toxic dinoflagellate *Karenia brevis* occur annually in the eastern Gulf of Mexico, significantly impacted human and environmental health as well as local economies. Public and scientific attention has recently focused on mitigation (i.e. the management categories of prevention, control and mitigation) of these blooms. Prevention efforts focus on stopping blooms from occurring or minimizing and limiting their extent. Control efforts focus on the organisms themselves, either killing them or removing cells and/or toxins from the water. Mitigation efforts focus on relieving the impacts of blooms without dealing with the cells and toxins directly. Current (i.e. post 2009) local and state mitigation efforts are focused largely on direct control of blooms and their associated toxins. It is therefore timely to review *K. brevis* focused prevention, control and mitigation efforts that have occurred within the framework of a variety of local, state and federal programs since the last review in 2009. Available literature on the subject was compiled and synthesized from peer reviewed and grey literature, grant reports, and patents. Prevention efforts have focused on local governing bodies reducing watershed nutrient delivery to blooms by banning fertilizer usage and dead fish cleanups on beaches and in bays. Control efforts have included in situ clay flocculation and ozone field experiments as well as laboratory studies of algicidal components of bacteria and allelopathic impacts of various macroalgae. Mitigation efforts such as improved monitoring, commercial shellfish bans, dead fish removal from beaches. Bloom forecasting and prediction, and bloom model development have increased in sensitivity and become available to the public. Additionally, education and outreach programs specifically targeted for the public have increased as have citizen science initiatives. The limitations of practical control and some mitigation efforts is extensive however, and needs better representation in public outreach and education.

IS THERE AN ECO-FRIENDLY TREATMENT TO CONTROL CYANOBACTERIAL HARMFUL ALGAL BLOOMS?

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In February 2017, a *Microcystis aeruginosa* bloom (maximal biomass of 55 $\mu\text{g}\cdot\text{L}^{-1}$ chl *a* and microcystin-LR equivalent concentration of 40 $\mu\text{g}\cdot\text{L}^{-1}$) occurred in a freshwater pond in Padre Island National Seashore, south Texas. This bloom caused mortality of migratory redhead ducks and local animals. To treat the pond without further damage to the ecosystem, we proposed to use H_2O_2 . A nomograph of H_2O_2 required for oxidizing different initial cyanobacterial biomass levels to achieve desired final microcystin and chl *a* concentration was developed, then an outdoor mesocosm experiment with four treatment levels was tested. With chl *a* concentration $>50 \mu\text{g}\cdot\text{L}^{-1}$, the

nomograph predicted >97% removal efficiency when 50 mg·L⁻¹ peroxide was used. In the mesocosm experiment, discrete samples assessed cell morphology, pigment, and toxin content (n = 3) at six different time intervals during the 48-hr treatment period. Cell morphology was affected after 4 hrs exposure at 50 mg·L⁻¹ H₂O₂ treatment, with a significant decrease in filter-retained toxin and pigment concentration, as well as a significant increase in dissolved toxin (p-value < 0.05). Overall, >75% of microcystin and >80% of chl *a* was destroyed after 48-hr exposure to the 50 mg·L⁻¹, whereas at 10 mg·L⁻¹, 34% of chl *a* and 19% of microcystin were destroyed. The pond was treated with 10 mg·L⁻¹ H₂O₂, which reduced chl *a* by 60% and microcystin by 35% after two days. Control of the cyanobacterial bloom using H₂O₂ was effective in lab and mesocosm experiments, but the cyanobacterial bloom recovered at PINS two weeks after the treatment. The recovery resulted from insufficient H₂O₂ addition at the concentration of H₂O₂ suggested by the regulatory protocols. Overall, H₂O₂ residual was depleted by biological and physical oxidation resulting in no long-term damage to the environment, however there is a need to re-evaluate current protocols for bloom management.

A TWO-DIMENSIONAL MOLECULARLY THIN SKIN TO FLOCCULATE AND SINK HARMFUL ALGAE

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We report an effective method for the mitigation of algal blooms using environmentally benign materials to flocculate and sink algal cells in clusters. Such flocculation is accomplished through the use of a two-dimensional metal organic framework known as a metal phenolic network (MPNs). MPNs are composed of natural polyphenols such as tannic acid (TA) coordinated to metal ions such as Fe(III) to form the two-dimensional network. Hydrogen bonding interactions lead to polyphenol attachment to algal cells, and the adhesion of such materials mimics the underwater adhesive properties of mollusks through catechol-based proteins. When used to flocculate *Chlorella vulgaris* as a model algal species, we observe clusters of up to 100 cells in a floc. With *Karenia brevis*, clusters of 10 or more cells are easily captured in the MPN based flocs. The system is extremely efficient, and the flocculation is independent of electrostatic effects implying use in saline and in fresh waters. The sinking of these flocs is accelerated through the use of small amounts of clays with the focus of our work being the use of naturally occurring clay nanotubes known as halloysite. Such tubular nanoclays are of interest since the lumen can be used to sequester algaecides which become targeted directly to algal cells in the flocs. High resolution electron microscopy reveals that the MPNs can be used to anchor the nanotubes to the flocculated cells thus allowing algaecide to be used with maximum efficiency and minimal environmental impact.

OZONE FOR ON-SITE RESPONSE, REMEDIATION, AND MITIGATION OF RED TIDES: FEASIBILITY STUDY

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Recently, the state of Florida has tasked numerous researchers to find products that may be feasible for mitigation of Florida red tide (*Karenia brevis*). The studies presented here were conducted with a Mote patented ozonator design, to assess the feasibility of using ozone as a means to remove *K. brevis* cells and toxins and restore natural conditions to red tide impacted areas. The ozonator brings water into an enclosed chamber for ozone treatment, returning the treated water back to the source. Two large scale (25,000 gal) mesocosm studies were conducted using seawater collected from New Pass in Sarasota, FL. The first study used seawater with no *K. brevis*, as the control for non-bloom conditions. The second study used seawater with a natural bloom of *K. brevis* (~50,000 cells/L). Results of the mesocosm studies demonstrated that ozone was effective in destroying *K. brevis* cells and toxins, and reoxygenating the water without adversely affecting water quality. A larger ozonator system was used to treat a small, dead end canal impacted with a *K. brevis* bloom (~20,000 cells/L) exhibiting hypoxic conditions and dead fish, to determine the effectiveness during a natural bloom. The ozonation process was found to be effective in reducing cell counts and toxins, as well as improving several water quality parameters in the ozone-treated seawater. However, anoxic conditions in the canal rapidly scavenged oxygen from the ozone-treated seawater, demonstrating that much higher capacity ozone generating equipment would be needed to overcome the drain on the oxidation-reduction potential. These studies showed proof of concept for the use of ozone as a means to mitigate the adverse effects of *K. brevis*

cells and toxins in a red tide-impacted canal and, with sufficient ozonating capacity, to restore the canal system back to water quality conditions for marine life to return.

OVERVIEW OF MULTIPLE CONTROL STRATEGIES FOR BLOOMS OF *KARENIA BREVIS* ON THE FLORIDA GULF COAST

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The 2017-2019 *Karenia brevis* bloom along the SW Florida coast emphasized the need for viable control strategies that reduce effects of these harmful algal blooms (HABs) through removal of *K. brevis* cells and toxins. While numerous strategies for combating HABs in coastal marine waters have been proposed, most show potential long term adverse impacts to the environment. With a focus on developing control and mitigation strategies for blooms of *K. brevis*, Mote's Phytoplankton Ecology Program has explored a number of biological, chemical and physical options to potentially combat these bloom events. Experimental results with whole macroalgae and macroalgal extracts demonstrate the ability of multiple species to kill or inhibit growth of *K. brevis*. Polyunsaturated fatty acids and other compounds extracted from several macroalgae species have been demonstrated to reduce *K. brevis* by 100% in 24 hours or less in laboratory assays. Other studies have focused on the ability of algicidal bacteria to selectively kill or inhibit *K. brevis* and reduce brevetoxins. Further research suggests that *K. brevis* and other phytoplankton produce signaling compounds that can induce algicidal activity in some bacterial strains. A collaborative effort, led by Woods Hole Oceanographic Institute (WHOI), quantified the impacts of modified clay application on *K. brevis* and other dominant phytoplankton taxa. In field studies without *K. brevis* present, application of clay preferentially removed larger phytoplankton (>10 um) while smaller size classes were less affected. Laboratory studies testing clay flocculation with a constructed phytoplankton community using laboratory grown cultures of *K. brevis* and a variety of other phytoplankton representing multiple taxa and size classes show similar results. This presentation will overview and summarize results from these projects, consider the efficacy and viability of these approaches as bloom control strategies, and discuss the feasibility and challenges of applying such strategies to a natural bloom.

IMPLICATIONS FOR HATCHERY MANAGEMENT OF HARMFUL ALGAL BLOOMS: INTERACTIONS BETWEEN *KARLODINIUM VENEFICUM*, HATCHERY FEED ALGAE, AND EASTERN OYSTER LARVAE (*CRASSOSTREA VIRGINICA*)

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Karlodinium veneficum is a harmful algal bloom (HAB) species known to cause mortality of fish and other aquatic organisms, including Eastern oyster larvae (*Crassostrea virginica*). This mixotrophic HAB species has been observed in low densities in oyster hatchery water and feed algae, raising concern about impacts on larval oysters and their food source. 96-hour toxicity bioassays in 1-mL microbeakers were used to assess acute effects of different initial *K. veneficum* cell densities (0, 1, 5, 10, 50 K cells/mL) on larval oysters and the hatchery feed alga *Pavlova pinguis* (25 K cells/mL). Algae counts and larval mortality were assessed daily. With initial cell densities >0 cells/mL, *K. veneficum* "swarmed" the larvae, leading to mortality, which was influenced by HAB cell density, the inclusion/exclusion of *P. pinguis*, and initial larval health. Larvae impacted population growth of both *K. veneficum* and *P. pinguis*, however, there was no evidence of allelopathy between algal species. In a separate 96-hour toxicity bioassay designed to better reflect hatchery conditions, oyster larvae were incubated in 1-L jars with 25 K cells/mL of *P. pinguis* along with *K. veneficum* at 0, 0.5, 1.25, 2.5, or 5 K cells/mL, with daily water changes. In contrast to the small-volume bioassay, larval mortality was low in the 1-L jar bioassay. These toxicity bioassays suggest that prolonged physical contact between *K. veneficum* and oyster larvae results in oyster mortality, especially later in the spawning season when spawns are less robust. Hatchery management for *K. veneficum* break-through should focus on monitoring and maintaining water flow to reduce the risk of prolonged contact between larvae and this HAB species. These results suggest that experimental design plays an important role in the outcome of toxicity bioassays and must be carefully considered when extrapolating to real-world applications to avoid erroneous conclusions.

IMMOBILIZATION OF ALGICIDAL BACTERIUM *SHEWANELLA SP.* IRI-160 AND ITS APPLICATION TO CONTROL HARMFUL DINOFLAGELLATES

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Shewanella sp. IRI-160 is an algicidal bacterium isolated from Delaware Inland Bays that can secrete water-soluble algicidal compounds to control harmful dinoflagellates. In this research, *Shewanella* sp. IRI-160 was immobilized to different porous matrices, including agarose, alginate hydrogel, cellulosic sponge, and polyurethane foam; the retention of *Shewanella* sp. IRI-160 within these matrices were examined at 4 and 25 °C for 12 days. Results indicated that 84 to 99.93% of *Shewanella* cells were retained in each matrix after 12 days, and temperature did not have a significant effect on the density of immobilized *Shewanella* sp. IRI-160 in each matrix except polyester foam. *Shewanella* sp. IRI-160 immobilized to alginate beads at different bacterial concentrations were used to test their algicidal effects on harmful dinoflagellates *Karlodinium veneficum* and *Prorocentrum minimum*, compared to the non-harmful cryptophyte *Rhodomonas* sp. and to effects of free-living bacteria. Results indicated that both immobilized *Shewanella* sp. IRI-160 in alginate beads and the free-living bacteria were able to control the growth of *K. veneficum* and *P. minimum* effectively after 24 hours of treatment, while no negative impacts of immobilized *Shewanella* sp. IRI-160 on the non-harmful control species *Rhodomonas* sp. were observed. Overall, results of this investigation demonstrated that immobilized *Shewanella* sp. IRI-160 may be used as an environmentally neutral approach to mitigate or prevent harmful dinoflagellate blooms.

VARIABILITY IN THE EFFECTS OF ALGICIDAL BACTERIA ON *KARENIA BREVIS*

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Karenia brevis occurs naturally in the Gulf of Mexico along the coast of southwest Florida, forming near-annual blooms that lead to massive animal mortality events, negative health effects in humans and economic loss. Potential management strategies include prevention techniques, control strategies and mitigation approaches. More natural approaches seem to be better accepted by the public. Bacteria occur naturally in sea water and some strains produce algicidal compounds affecting HAB-forming phytoplankton species and some have specificity in their target. Here we present an approach focused on the effects of algicidal bacteria on the growth, survival and proliferation of *K. brevis*. Three algicidal bacteria strains provided by NOAA and the University of Delaware were used for the studies presented here. Additionally, sea water was screened for naturally algicidal bacteria and potential algicidal bacteria were isolated. Cell counts were performed daily and algicidal rates (AR) were estimated. Results indicate that media type can impact algicidal activity and not all *K. brevis* strains are equally perceptible to algicidal attack. Our results confirm those of previous research that found algicidal activity towards *K. brevis* of both the *Shewanella*-IRI 160 strain and the S03 strain, with AR up to 100. Adding *Shewanella* filtrate to *K. brevis* culture (8 mil. cells^{-L}) led to an AR = 100 ($p > 0,005$) within 4 h for *K. brevis* strain Wilson, while other strains were less affected. Bacterial strain S03 lead to deformation of *K. brevis* cells and chloroplasts, when directly added to *K. brevis* culture, after 48 h ($p > 0,005$). We did not observe algicidal activity towards *K. brevis* for bacterial strain 41-DBG2. Results for bacterial community assays in different media will be presented. Ongoing work includes the attempt to identify algicidal compounds. This research could lead to a novel, natural, and highly targeted strategy for HAB control.

CAN INTAKE DEPTH BE USED AS A TOOL FOR MANAGING TOXIC CYANOBACTERIAL BLOOMS IN SURFACE DRINKING WATER RESERVOIRS?

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Safe drinking water is a basic necessity of all life on Earth, and many contaminants threaten this important resource. In 2015, the US EPA created a drinking water advisory for two toxic secondary metabolites of cyanobacteria, namely microcystin and cylindrospermopsin. In 2018 through the Fourth Unregulated Contaminant Rule, the USEPA required all surface water utilities to regularly monitor for these, and other, cyanobacterial toxins. To understand the threat that cyanobacteria and their toxins pose to citizens of Alabama, my lab and our colleagues at the Alabama Department of Environmental Management worked with all of the State's surface water utilities to collect basic water quality data, including nutrients, phytoplankton abundance, taste and odor compounds, and cyanobacterial toxins for two years. During this project that included 420 samples, cyanobacteria and their toxins were low to non-existent across all of the surface drinking water sources. Also, two common taste and odor compounds, geosmin and 2-methylisoborneol, were present and tended to be associated with sites that had low nitrogen-to-phosphorus ratios (15 by weight). Intake depth appears to be mediating the patterns we are observing. In summary, Alabama's surface drinking water sources appear to have high water quality, including no-to-low concentrations of cyanobacteria or their toxins.

FOOD WEB DYNAMICS & IMPACTS

WHAT IS SAFE FOR SEA TURTLES TO EAT DURING AN EXTREME SOUTHWEST FLORIDA RED TIDE?

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During an extensive red tide event between November 2017 and October 2018, 247 sea turtles (including loggerheads, Kemp's ridleys, and green turtles) stranded on or near Sanibel and Captiva Sanibel, on the southwest Florida coast. Tissue and gut content samples were taken from dead sea turtles that stranded between May and September 2018. Gut contents were removed from 60 of the turtles. Samples of tissues (liver, kidney, muscle) and stomach and intestinal contents were tested for brevetoxins (PbTx-3) using ELISA. Forty samples were analyzed by LC/MS for other brevetoxin congeners. The remainder of the gut contents was dried, and diet items were identified. PbTx was detected in high concentrations (from 10 to >1600 ng g⁻¹) in most of the stranded turtles, and most had sufficient levels of brevetoxins in their gut contents to have caused their deaths. The dietary items found were mainly seagrass blades in the green turtles. Loggerheads and Kemp's ridleys consumed four main prey categories: crustaceans, mollusks, chelicerates, and fish. Crabs and mollusks (whelks, conchs, moon snails and clams) were the dominant prey consumed by both loggerheads and Kemp's ridleys, occurring in 95% of gut samples. Less frequently observed prey items were fish and spider crabs in Kemp's ridleys. After a large dead zone formed in the Gulf of Mexico off of Sanibel in August 2018, Kemp's ridley strandings increased and their diet shifted to horseshoe crabs and fish. Understanding sea turtle dietary habits and brevetoxin uptake will be useful for predicting the consequences of increasingly severe harmful algal bloom events.

SPATIOTEMPORAL DISTRIBUTION AND TROPHIC LAG OF CARIBBEAN CIGUATOXINS IN FISH SECONDARY CONSUMERS FROM THE US VIRGIN ISLANDS

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Toxicogenic species of *Gambierdiscus*, have been shown to exhibit spatiotemporal differences in site prevalence and CTX production. However, fish toxicity within and across regions, and through time is considered to be patchy due to several key knowledge gaps in fate and transport of CTXs. For instance, CTX bioaccumulation in intermediate trophic level fish contributes to the limited ability of preemptive detection and modeling of CP risk. In this study we aimed to evaluate spatiotemporal trends of CTX distribution in trophic level two and three reef fishes from a CP endemic region in the USVI. Further, through laboratory oral CTX exposures we investigated bioaccumulation and depuration rates to estimate toxin trophic lag. To identify risk patterns in seasonality and spatial distribution of CTX, lower trophic level fish (damsel fish, Pomacentridae) (n=55) and mesopredatory grouper, *Cephalopholis cruentata* (graysby) (n=50), were selected from our quarterly sampling (2015-2016) from four reefs on the southern side of St. Thomas. To investigate timing of CTX uptake and depuration from tissues, we performed 40-day (20 bioaccumulation; 20 depuration) oral-exposure studies with *Lagodon rhomboides* (pinfish) fed Caribbean CTX (C-CTX-1) at an environmentally relevant dose (0.03 ng g⁻¹ fish weight). Fish tissue was chemically extracted using standardized methods and CTXs were quantified using the mouse neuroblastoma assay (N2a). Damsel fish (*Stegastes leucostictus*) exhibited consistently higher (up to 10 fold) toxicity than simultaneously collected *C. cruentata*. In the laboratory study, bioaccumulation continued throughout the 20-day exposure period. When placed in recovery with non-toxic food *L. rhomboides* depurated approximately 25% in five days compared to the bioaccumulation peak, but then plateaued, with no significant differences observed over the remaining time course to 40 days. These data will aid in the refinement of predictive models for CTX risk in reef systems and furthers our understanding of toxin pulses in intermediate reef fishes.

PREVALENCE OF PARALYTIC SHELLFISH TOXINS IN THE MARINE FOOD WEB OF SOUTHCENTRAL AND SOUTHWEST ALASKA

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Saxitoxins (STXs) produced by *Alexandrium catenella* are potent neurotoxins that accumulate in Alaska shellfish and cause paralytic shellfish poisoning (PSP). Recent data indicate STXs reach Alaskan marine mammals and seabirds via the food web, although the relevant trophic transfer pathways are not well understood. Here we present preliminary data from two ongoing projects examining the occurrence of PSP toxins in the marine food webs of southwest and southcentral Alaska. Study sites include Prince William Sound, Kachemak Bay/Lower Cook Inlet, and the Kodiak, Aleutian and Pribilof Islands—locations with low, moderate and high incidences of shellfish toxicity, respectively. The projects focus on toxin levels in phytoplankton, zooplankton, bivalves, forage fishes, commercially important predatory fishes, and other biota. STX levels were quantified via enzyme-linked immuno-sorbent assay/high performance liquid chromatography. Data indicate toxicity was generally low (<100 ng STX Eq. g⁻¹) in forage fish species during summer/autumn 2018 commensurate with low *Alexandrium* abundances and shellfish toxicities observed in much of south-central and southeast Alaska during this period. However, there were sporadic incidences of moderate toxicity (100-500 ng STX Eq. g⁻¹) in some forage fish samples. STXs were also detected in predatory fish during the same period, with the highest concentrations in digestive organs (≤305 ng STX Eq. g⁻¹) and lower levels in kidney (170 ng g⁻¹), liver (133 ng g⁻¹), muscle (108 ng g⁻¹), and roe (~0 ng g⁻¹). Early results from 2019 indicated more intense *Alexandrium* blooms in the Aleutians with higher toxin concentrations in bivalves (to 10,000 ng g⁻¹), forage fishes (>2,000 ng g⁻¹), predatory fishes (to 800 ng g⁻¹) and invertebrates (to 1,220 ng g⁻¹). Prevalence of STXs in predatory fish viscera rather than muscle tissue indicates low human health risks, but heightened risk of trophic transfer to marine mammals, seabirds and other piscivorous predators.

ALGAL TOXINS IN ALASKAN ARCTIC FOOD WEBS: SEAWATER, ZOOPLANKTON, BIVALVES, FISH, ICE SEALS, WALRUSES AND WHALES!

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Changing ocean conditions threaten to increase harmful algal bloom (HAB) frequency, severity and geographic extent in northern seas raising concerns regarding the trophic transfer of algal toxins in marine food webs and potential exposure risks to marine wildlife and humans. Coastal Alaskan communities in Arctic and subarctic regions rely

heavily on non-commercial harvest of marine wildlife for nutritional, economic, and cultural well-being. Thus, the health of marine wildlife is not solely a wildlife conservation issue, but includes public health and food security issues. Here we present data on the prevalence of the algal toxins domoic acid (DA) and saxitoxins (STXs) in multiple levels of Alaskan Arctic food webs. DA and STX are neurotoxic and are responsible for the shellfish poisoning syndromes known as Amnesic Shellfish Poisoning (ASP) and Paralytic Shellfish Poisoning (PSP), respectively. We will present algal toxin prevalence results from long-term data sets (up to 15 years) of subsistence-harvested marine mammals including bowhead whales, walrus, and four species of ice seals (bearded, ringed, spotted and ribbon) representing well over 1,000 animals. We will also present preliminary results on the presence of algal toxins quantified in seawater, zooplankton, bivalve, and fish samples collected during 2019 research cruises in the Beaufort Sea, Chukchi Sea, Bering Strait Region, Bering Sea, and Gulf of Alaska. Both DA and STX were detected in all regions examined and the potential for increased algal toxin prevalence and food web transfer in Arctic waters will be discussed.

MARINE SNOW CONSUMPTION FACILITATES A NOVEL ENTRY PATHWAY FOR DOMOIC ACID INTO THE MARINE FOOD WEB

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Domoic acid (DA) is a known neurotoxin, produced by diatoms from the genera *Pseudo-nitzschia* and *Nitzschia*, responsible for amnesic shellfish poisoning; its transfer through marine food webs may pose a threat to human health. Various pelagic and benthic organisms act as vectors for DA and the toxin accumulates in the food web despite loss variables favoring quick degradation in the water column and depuration from animal tissue. The observation that DA can be detected in higher trophic level organisms when toxin-producer abundance and toxicity are low, suggests there may be an alternative entry pathway for DA into the food web. Laboratory experiments demonstrate that organic polymers can aggregate (e.g. marine snow, transparent exopolymers or TEP) and scavenge dissolved DA (dDA) (2.57 ± 0.56 nmol DA: $\mu\text{mol C}$). We demonstrate assimilation of this TEP-bound DA by the copepod *Acartia tonsa* in both laboratory and field conditions — a pathway which does not require direct ingestion of the toxin-producing diatom. In the laboratory, copepods suspended in filtered seawater spiked with DA accumulated 24.8 ± 4.7 pg DA copepod⁻¹ in triplicate measurements. These laboratory results were validated in one field experiment. Copepods were suspended in particle-free seawater with the ambient dDA and accumulated 14.4 ± 3.8 pg DA copepod⁻¹ via this indirect pathway, which represented ~34% of the total assimilated DA. Among the five field experiments, this successful experiment had the highest *Pseudo-nitzschia* spp. abundance (~225,000 cells L⁻¹) and cellular toxicity (maximum, 0.88 pg DA cell⁻¹). Our results demonstrate the potential for DA to enter the marine food web through a novel pathway and have considerable implications for understanding the transfer of DA through marine food webs, how we monitor DA, and whether other phycotoxins can enter the food web in this manner.

PERSISTENT DOMOIC ACID IN THE SEDIMENTS AND BENTHIC INFAUNA IN CALIFORNIA

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Domoic acid (DA), produced by the diatom genus *Pseudo-nitzschia*, has been documented in coastal California waters for decades. However, less is known about the extent and magnitude of DA in the benthic environment or the impact of DA on the benthic food web. Recent reports from around the United States indicate that the benthos may act as a reservoir for DA and increase the risk of food web contamination long after blooms end. Notably, the Dungeness crab fishery was closed for nearly a year after the historic 2015 West Coast *Pseudo-nitzschia* bloom ended, resulting in

severe socioeconomic impacts. Here we will present data from central and southern California that illustrates the spatial extent and temporal persistence of benthic DA contamination in sediments and benthic infauna. Work in central California revealed DA concentrations as high as 336 ng/g sediment, as well as DA in the tissues of a variety of infauna species. A recent toxigenic *Pseudo-nitzschia* bloom in southern California associated with marine animal strandings led to the sampling of sediments and infauna in the region. All of the sediment samples collected contained DA except one, with concentrations reaching as high as 168 ng/g and all benthic infauna samples contained measurable DA. In response, the Southern California Bight Regional Monitoring Program prioritized monitoring benthic DA and conducted a regional assessment of DA in sediments and infauna in southern California. Additionally, a spatially limited 12-month time-series of sediment and infauna was also collected.

ENGAGING CITIZENS & STAKEHOLDERS

COLLABORATIVE RESEARCH BETWEEN STATE, UNIVERSITY AND CITIZEN SCIENTISTS AIMED AT UNDERSTANDING HABS IN THE GREAT STATE OF NEW JERSEY AND BEYOND

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Harmful algal blooms (HABs) are a globally pervasive and growing problem impacting the commercial, recreational, and aesthetic value of aquatic ecosystems. New Jersey has a history of HABs and is arguably a high-risk area for HAB occurrence and expansion stemming from the combination of eutrophic conditions and climate change impacts on the state's lakes and coastal waters, a well-established summer tourism economy, and a growing aquaculture economy. To that point, summer of 2019 saw unprecedented HABs in New Jersey's Lake Hopatcong that resulted in closures and economic losses; HABs and related hypoxia are common in estuarine waters of the Shrewsbury / Navesink system; *Pseudo-nitzschia* spp. (PN), documented annually in NJ waters in the last decade, remain largely uncharacterized for species composition and toxicity. A collaboration between undergraduate research students at Monmouth University, the NJ Department of Environmental Protection (NJDEP), and citizen scientists was started in 2017 with the goal of better understanding the conditions leading to HABs in NJ waters. In collaboration with the NJDEP HAB monitoring program, student researchers conduct hypothesis-driven research focused on specific HABs in the state. A participatory citizen science network (Coastal Lakes Observing Network, CLONet) was started to better link communities with state and university efforts to understanding the causes of HABs in these environments. Key findings about NJ HABs are emerging, including the roles of temperature, DIP and DIN in coastal lake cyanoHABs, and the species composition and limiting nutrient of estuarine dinoflagellate (*Akashiwo sanguinea*) blooms related with recurring hypoxia and fish kills. The collaboration between state, university and community members to document and characterize HABs will facilitate mutual understanding among members of HAB ecology, current and potential future impacts, as well as opportunities for mitigation.

CONNECTING STAKEHOLDERS TO ECOSYSTEM CHANGE WITH HARMFUL ALGAL BLOOM AND HYPOXIA FORECAST MODELS IN THE CALIFORNIA CURRENT SYSTEM

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The Pacific Warm Anomaly (2013-2016) was characterized by a spatially coherent oceanic structure and sustained by a "ridiculously resilient ridge" of high air pressure, eventually leading to ocean warming along the entire west coast of North America and the largest, most extensive toxic *Pseudo-nitzschia* event ever observed. Extensive food web impacts were recorded from Santa Barbara to Alaska, and the Dungeness crab fishery closures from domoic acid contamination led to losses on the order of \$100 million for the West Coast. Recent reports of warm water anomalies in the Eastern Pacific are raising concern of a return to a "marine heat wave," a class of phenomena that is increasing globally. In support of the U.S. Integrated Ocean Observing System (IOOS), three Regional Associations support a

mosaic of ocean modeling activities, run as quasi-operational systems that provide numerous products pertaining to harmful algal blooms and hypoxia. This presentation will illustrate the tight feedback between decision-making and several ecological forecasting efforts: J-SCOPE (JISAO Seasonal Ocean Coastal Prediction of the Ecosystem), LiveOcean, and C-HARM (California Harmful Algae Risk Mapping system). The HAB forecast and prognostic bulletins that are disseminated to end users are close collaborations state and tribal as well as NANOOS, CeNCOOS, and SCCOOS in support of management decisions for fisheries, protected species, and ecosystem/public health. Taken together, these programs and models provide a holistic view of ocean variability that connects the science and data to regional end-users.

ENHANCING STAKEHOLDER ENGAGEMENT TO IMPROVE CIGUATERA RESEARCH AND MANAGEMENT

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Ciguatera fish poisoning (CFP) poses significant health risks as an environmental public health issue. Through research efforts it may someday be possible to predict unsafe fisheries harvest areas and or specific times of year when the risk if harvesting toxic fish is increased. However, to take such measures, increased environmental literacy on CFP is needed among affected communities and stakeholders. Key components of CFP knowledge include research on the ecology of benthic dinoflagellates that form the source of ciguatoxins, food web dynamics, and the fate of toxins in reef ecosystems. But to increase the uptake and impact of this education, bidirectional engagement with the target population is critical. This includes engaging stakeholders through sharing data and working together to plan research priorities so that achieved outcomes are meaningful to communities most affected. We will outline strategies to improve stakeholder engagement for CFP and discuss how education and outreach efforts may guide research (to consider new or emerging areas of CFP risk). Further, we will demonstrate ways in which stakeholder interaction allows for a more thorough assessment of knowledge gaps in CFP health literacy, allowing for development of comprehensive, targeted initiatives to effectively increase education and awareness within key communities.

EVALUATING SCIENCE EDUCATION, AND OUTREACH PROGRAMS

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How do we know that our science education and outreach programs are working as we intended? We need to evaluate them! As scientists, this can seem daunting and outside of our expertise. However, having and executing an assessment and evaluation plan for science education and outreach programs is often a critical piece of reporting to funders and can contribute to receiving future funding. For example, why should we assess attitudes, knowledge, aspirations, skills? How do we do this? What about behavior change? Is qualitative data as valuable as quantitative data? What if we are interested in human interactions, how can those be measured? Any or all of these are important questions to answer depending on your program and its intended and unintended outcomes. Careful planning is necessary to accurately capture the results that allow us to determine which and at what level these outcomes occur. Examples from evaluation and assessment strategies being used for a collaborative NSF-funded project, Partnerships in International Research and Education (PIRE), and NSF-NIH funded Center for Oceans and Human Health (COHH) will show the context, power, and application of assessment.

COMMUNICATING CHAOS: A REVIEW OF SUCCESSES AND DOWNFALLS OF FLORIDA RED TIDE SCIENCE COMMUNICATION AND BREAKING BOUNDARIES THROUGH CITIZEN SCIENCE

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In November 2017, the beginning of a 14-month bloom was detected off the southwest coast of Florida. Intensity of the bloom increased late June 2018, making international news due to mass wildlife fatalities, economic losses due to decreased tourism, inability to use waterways, and adverse impacts to human health. At this time, a cyanobacteria bloom proliferated from Lake Okeechobee to the Caloosahatchee River reaching the Gulf of Mexico. Florida declared a state of emergency. Confusion, fear, anger and misinformation resulted in an emotionally charged public. Citizens felt helpless and sought clear answers to questions scientists did not have. The disconnect between scientists and the public was highlighted. Few of us stepped into these crowds in order to communicate the science behind these blooms. After countless local, national, and international interviews, hundreds of presentations and even TV shows; there were still many people to reach and much misinformation combating our communication efforts. Social media was a challenge with popular private groups sharing misinformation. There are two choices: either ignore incorrect statements or reply in a professional manner to provide facts for those reading the response. Through failures, successes were found. Effective communication strategies include making messages simple and concise, using words easily understood, providing the audience an achievable step toward minimizing their impact, allowing the audience to take ownership by including them in scientific research, and always remain humble and empathetic. The most frequent request from the public was for transparency and the ability to do something. Citizen science has potential to empower the public and bridge the gap between scientists and the public. Now with over one million users of our website and apps, that gap is being closed. The challenges in communication resulted in effective language strategies, creative citizen science programs, and knowledge of how to form community through chaos.

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

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Harmful Algal Blooms (HABs) have occurred in Alaskan waters for thousands of years and remain a present threat to recreational and subsistence shellfish harvesters in the state. Due to their potential to produce toxins, HABs can be responsible for public health risks such as Paralytic Shellfish Poisoning, Amnesic Shellfish Poisoning, and Diarrhetic Shellfish Poisoning. Limited State resources, dispersed and remote populations, and expansive coastlines have precluded the development of a statewide monitoring program or testing facilities for toxins produced by HABs. To reduce the public health risks associated with HABs, the Sitka Tribe of Alaska has organized the Southeast Alaska Tribal Ocean Research network (SEATOR), a community-based HAB monitoring and toxin testing program. SEATOR started in 2013 with six tribes in Southeast Alaska committed to phytoplankton monitoring in their local communities. Currently, the SEATOR network now works with nearly 25 tribes throughout Alaska who monitor HABs in their regions by conducting phytoplankton monitoring and toxin testing for Paralytic Shellfish Poisoning. Over 2400 phytoplankton observations and 1500 shellfish samples have been analyzed by SEATOR partners. The Sitka Tribe and SEATOR network are building a regional database of shellfish toxins and phytoplankton abundances to make shellfish available as a safe, reliable wild-food source. SEATOR will continue mitigating HAB risks in Alaska by expanding their program to test for Amnesic Shellfish Poisoning, and Diarrhetic Shellfish Poisoning. The SEATOR network'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.

THE CyAN MONITORING APP AND OUTREACH TO EDUCATE AND EXCITE PEOPLE ABOUT THE CYANOBACTERIA INDEX

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The Cyanobacteria Assessment Network (CyAN) project is an inter-agency (NASA, EPA, NOAA, USGS) effort to support the environmental management and public use of U.S. lakes by providing an ability to detect and quantify cyanobacteria algal blooms using satellite data. The CyAN project is validating the cyanobacteria index (CI) algorithm for lakes across the United States using the Ocean Land Colour Instrument (OLCI) data on Sentinel-3 satellites and the historic (2003-2011) data provided by Medium Resolution Imaging Spectrometer (MERIS). The CyAN project also includes the goal of information distribution to provide notifications and decision support. The CyAN project achieves its information distribution goals through a variety of approaches including collaborator trainings, end-user interactions, a CyAN app and finally outreach events called Sippin' on Science. The CyAN App is especially exciting because it makes it possible to easily share the data and information with large numbers of people including water managers, state agencies, and concerned citizens. The Sippin' on Science two-week outreach tour, primarily in small town bars and breweries in the upper Midwest lakes region, used hands-on science, an engaging presentation, and trivia to create awareness about water quality and satellite remote sensing. This presentation will cover the CyAN project communication efforts and successes that increase the interest in CI remote sensing product and gets it into the hands of end-users and thereby help to maximize the societal benefits of the science and satellite remote sensing.

SPECIAL SESSION: OCEANS AND HUMAN HEALTH

EARLY CHILDHOOD EXPOSURE AND PRIMING TO ALGAL TOXINS INDUCE MURINE ADULT HEPATIC INJURY FOLLOWING HIGH FAT DIET FEEDING VIA NLRP3 INFLAMMASOMES

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Evidence is strong for early childhood exposure effects individuals with physical, biological, chemical, or psychological stimuli. Results from American Academy of Pediatrics show that infants and children are at risk for early exposure to microcystin. Notably, infants and children are known to consume more water relative to their body weight when compared to adults and thus are at increased risk of water borne exposures to environmental contaminants. The present report tests the hypothesis that early childhood exposure to microcystin (MC), a PP2A inhibitor and a principal component of harmful algal blooms activate Kupffer cells and hepatic stellate cells, which are considered to be crucial mediators in hepatic fibrogenesis in an underlying condition of NAFLD. Mice exposed to subchronic doses of microcystin-LR (MC-LR) for two weeks immediately after the end of weaning and with a parallel high fat diet co-exposure showed classical symptoms of early NAFLD linked inflammation. Cytokines and chemokines such as CD68, IL1 β , MCP-1, and TNF- α , as well as α -SMA, hallmarks of NAFLD-linked inflammation were increased in the groups that were exposed to microcystin with high fat diet compared to the vehicle group. Also, NLRP3 KO mice showed significant decrease in the inflammation suggesting a decisive role of NLRP3 linked inflammasome activation and increased inflammation in these mice. The same trends were observed for liver injury biomarker IL-1 β , indicating a worsening outcome in liver health. Clinically, serum alanine aminotransferase (ALT) levels were significantly higher in the group that was primed to microcystin followed by high fat feeding when compared to controls. The results suggest that toddlers and children might be at a greater risk of microcystin exposure and subsequent liver damage since data generated in mice models are highly translatable to humans. The results also bode well to strong clinical data that suggest increased incidences of NAFLD and NASH cases in children and juveniles in the US and around the developed world.

HAB SCIENCE MADE EASY: TEACHING STUDENTS ABOUT THE ECOLOGY AND TOXICOLOGY OF HARMFUL ALGAL BLOOMS

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Teaching students about chemical ecology and the role of phytoplankton in the marine environment can be challenging because of the limited prior exposure students may have had to these topics. Here, students learn about the link between harmful algal blooms (HABs) and human health, and how scientists collect and use information about the ecology and toxicology of HABs to ensure seafood safety. We will provide two examples of K-12 activities that incorporate chemistry while teaching students about dinoflagellates and HABs. The first example focuses on the Ciguatera Fish Poisoning and how the chemical ecology of toxin-producing dinoflagellates determines how and where ciguatoxin can bioaccumulate in coral reef food webs. In this activity, students construct models of the molecules involved, and gain an appreciation for the complexity and similarity of chemical structures. In addition, students construct food webs with the props provided and then discuss how higher trophic level organisms as well as people can be exposed to the toxins depending on where and what they eat. The second activity focuses on Paralytic Shellfish Poisoning and its impacts on valuable shellfish resources in New England. In this activity, students learn how data on HAB cells and resting cysts are collected and subsequently used to ensure seafood safety and predict bloom magnitude. Each activity includes modifications for visually impaired students, and both conform to Next Generation Science Standards and Ocean Literacy Principles. The ultimate goal of these educational efforts is to encourage students to have a greater

understanding of and appreciation for the marine environment, including the connection between ocean and human health, and to consider STEM careers.

A BRIEF INTRODUCTION TO THE CENTERS FOR OCEANS AND HUMAN HEALTH FUNDED THROUGH COHH3: IMPACTS OF CLIMATE CHANGE ON OCEANS AND GREAT LAKES

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The National Institute of Environmental Health Sciences and the National Science Foundation jointly fund research on marine-related health issues through the Centers for Oceans and Human Health and through individual research projects focusing on oceans and human health as well as the Great Lakes and human health. Grantees are developing techniques for more accurate and earlier detection of harmful algal blooms with the goal of preventing or reducing exposure, and they are studying the health effects of eating seafood that harbors toxins produced by harmful algal blooms. Here, an overview will be provided for the four centers funded through COHH3: Impacts of Climate Change on Oceans and Great Lakes. The Great Lakes Center for Fresh Waters and Human Health, a collaborative effort among Bowling Green State University and eight other institutions, focuses on freshwater, particularly cyanobacterial harmful algal blooms (cHABs). The purpose of the Greater Caribbean Center for Ciguatera Research (a collaboration of five institutions led by Florida Gulf Coast University) is to examine how climate change may affect ciguatera fish poisoning in the Greater Caribbean Region. The Woods Hole Center for Oceans and Human Health has the mission to protect public health through enhanced understanding of how oceanic and environmental processes including climatic variation affect toxin producing organisms using multiple state of the art in situ sampling technologies and modeling approaches. Lastly, the Oceans and Human Health Center on Climate Change Interactions (a collaborative center headed by the University of South Carolina) is assessing climate change effects on ocean health related illness (*Vibrio* bacteria and cyanotoxins) in conjunction with microplastic exposure, nonalcoholic liver disease, and seafood safety. Together, these projects aim to better understand how climate change may influence ocean (and Great Lakes) – human health interactions in order to mitigate merging human health risks.

DEVELOPMENT OF AN INTEGRATED COMMUNITY ENGAGEMENT STRATEGY FOR OCEAN AND HUMAN HEALTH ISSUES ASSOCIATED WITH HABS

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The “One Health” concept recognizes that the health of people, animals, and the environment are interconnected. It is a collaborative, multi-sectoral, and transdisciplinary approach, with the goal of optimizing health outcomes by increasing our understanding of the relationships among people, animals, plants, and their shared environment. Traditionally, scientists have been concerned with anthropogenic impacts on ocean health, but more recently have recognized that “unhealthy” ocean environments created by these impacts adversely affect the health of humans and communities. Thus was born the concept of Oceans and Human Health (OHH), a prime example of “One Health”. Harmful Algal Blooms (HABs) negatively impact the health and well-being of both freshwater and coastal communities, as people, domestic animals, and wildlife may be affected. Climate change and eutrophication have been suggested as factors in the increase of HAB events worldwide. Current research conducted on HABs is dynamic and complex, and requires effective translation of technical findings so that the public, resource managers, and seafood

producers can better understand the risks associated with HAB exposure, and effectively manage affected resources. The NSF and NIEHS have created an OHH Centers program, which emphasizes research on HABs and related risks as well as bidirectional community engagement. Each NSF-NIEHS Center has a Community Engagement Core that works with OHH researchers to communicate research findings on HABs more effectively, and engages community groups, resource managers, other stakeholders, and the public in both the research and the solutions. These OHH Centers met in October 2019 and developed an Integrated Community Engagement Plan for HABs, which includes strategies and tools for facilitating bi-directional communication with the public on a diversity of HAB issues and areas of ongoing research. This presentation will cover the major points of the multi-Center engagement plan.

HIGH THROUGHPUT NEXT GENERATION COMETCHIP PLATFORM FOR ASSESSMENT OF FISH AND HUMAN GENOME STABILITY FOLLOWING EXPOSURE TO HARMFUL ALGAL TOXINS

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Harmful algal blooms (HABs) are a global issue, affecting fisheries, wildlife, and human health in all coastal areas of the world. These phytoplankton blooms and the potent toxins that they produce are increasing in their frequency, toxicity, and geographical range. While large-scale oceanic blooms can often be detected through remote sensing tools, the sub-lethal and combined effects of these toxins are not well understood and may represent a larger long-term problem for the sustainability of local fisheries and indeed human health. Molecular studies of marine toxins have been largely focused on acute toxicity and impact in humans. However, the molecular and pathological effects of these toxins in marine organisms directly and frequently exposed to a plethora of HAB species, is poorly understood. In this study, we developed and evaluated high throughput CometChip technology for the assessment of combined sub-lethal exposure in an effort to link food web and environmental exposure of HAB toxins to human health. This technology has been recently developed for human genotoxicity assessment in cellular models, so this research is the first effort to apply these tools to aquatic organisms. The CometChip platform will allow us to compare cell based models of genotoxicity under a variety of exposure scenarios (short term, long term, combined exposures) and develop the use of this technology in fish exposure models, and in-situ field based exposures to evaluate the impact of HAB toxins in the food web.

USE OF A NOVEL OVARY-ON-A-CHIP TO SCREEN FOR FEMALE REPRODUCTIVE TOXICITY OF MICROCYSTINS

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Harmful algal bloom (HAB) cyanotoxins have been reported to exhibit neurotoxicity, hepatotoxicity, and endocrine disruption. However, effects of HAB toxins on women's reproductive health has been scarcely investigated. The ovary is the primary female reproductive organ and contains various developmental stages of follicles as the functional unit. We have recently developed an ovary-on-a-chip by culturing individual follicles using the alginate hydrogel encapsulation method and microfluidic technology. The ovary-on-a-chip maintains the 3D architecture of follicles and recapitulates follicle development, hormone secretion, oocyte maturation, and ovulation in vivo. Here, we used the ovary-on-a-chip to screen for the ovarian toxicity (ovotoxicity) of six microcystins (MC), including MC-LA, LF, LR, LY, RR, and YR. Secondary follicles were isolated from 16-day old CD-1 female mice and cultured using the ovary-on-a-chip for eight days. Follicles were treated with 0, 0.1, 1, and 10 μ M (equivalent to 0-10⁴ ppb) different MCs from day 0 to 8, and the follicle survival rate, development, hormone secretion, and ovulation were evaluated. Results indicated that MC-LF was the most ovotoxic MC with 100% of follicles dead in the 1 and 10 μ M treatment groups on day 8, and MC-RR was the least ovotoxic MC with 100% of follicles survived in all treatment groups on day 8. The MC-LF at 0.1 μ M and MC-LY at 0.1 and 1 μ M did not change follicle survival and development, but significantly decreased the secretion of 17 β -estradiol, suggesting the endocrine disrupting effect. MC-LR, the most well-characterized MC, did not significantly decrease follicle survival rate in all treatment groups. However, follicles treated with 10 μ M MC-LR had significantly increased androgen production and >50% follicles had abnormal

ovulation on day 8. In summary, our results suggest that MC variants exhibit differential ovotoxicities and exposure to MCs may increase risks of female premature ovarian failure, hormonal imbalance, anovulation, and sub- or infertility.

ANIMAL AND HUMAN HEALTH

COMPARATIVE ASSESSMENT OF CYANOTOXINS OCCURRENCE AND HAZARDS IN INLAND WATERS

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Surface water contamination by cyanotoxins present risks to public health and the environment. Understanding aquatic hazards is critical for cyanobacterial bloom assessment and management, particularly for events increasing in magnitude, frequency and duration. We have been critically examining published data to identify exceedances of guideline values (GVs) globally in various aquatic systems to understand hazards when exposure occurs. We further evaluated available aquatic toxicology information for aquatic life. Probabilistic environmental hazard assessments were employed to identify exceedances of GV and aquatic toxicological thresholds among aquatic system types and geographic regions. We find inconsistent analytical methods employed to robustly quantitate cyanotoxins in water and tissues of aquatic organisms. We further identify a lack of robust aquatic toxicology data, which preempts development of water quality criteria and standards. For example, aquatic effect studies inconsistently analytically verify treatment levels, report purity of cyanotoxins employed for experimental studies and follow standardized experimental methods. Such observations therefore highlight major research needs that we are beginning to engage towards developing an advanced understanding of cyanoHAB risks to water quality.

MICROCYSTIN INTOXICATION OF CANINES: A CASE STUDY AND ADVANCES IN TESTING

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In the summer of 2018, six dogs exposed to a harmful algal bloom (HAB) of *Microcystis* in Martin County Florida (USA) developed clinicopathological signs of microcystin (MC) intoxication (i.e., acute vomiting, diarrhea, severe thrombocytopenia, elevated ALT, hemorrhaging). Successful supportive veterinary care was provided and led to survival of all but one patient. Confirmation of MC intoxication was made through interpretation of clinicopathological abnormalities, pathological examination of tissues, microscopy (vomitus), and analytical MC testing of antemortem/postmortem samples (vomitus, blood, urine, bile, liver, kidney, hair). Gross and microscopic examination of the deceased patient confirmed massive hepatic necrosis, mild multifocal renal tubular necrosis, and hemorrhage within multiple organ systems. Microscopy of a vomitus sample confirmed the presence of *Microcystis*. Three analytical MC testing approaches were used, including the MMPB technique, targeted congener analysis (e.g. LC-MS/MS of MC-LR) and enzyme-linked immunosorbent assay (ELISA). Total ADDA MCs (as MMPB) were confirmed in the liver, bile, kidney, urine, and blood of the deceased dog. Urinalysis (MMPB) of one surviving dog showed a high level of MCs (32,000 ng mL⁻¹) one day post exposure, with MCs detectable >2 months post exposure. Furthermore, hair from a surviving dog was positive for MMPB, illustrating another testable route of MC elimination in canines. The described cases represent the first use of urine as an antemortem, non-invasive specimen to diagnose microcystin toxicosis. Antemortem diagnostic testing to confirm MC intoxication cases, acute or chronic, is crucial for providing optimal supportive care and mitigating MC exposure.

HUMAN ACTIVITY, ENDOTOXINS, AND WATER QUALITY DURING A CYANOBACTERIA BLOOM AT A RECREATIONAL BEACH

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The presence of cyanobacteria blooms (CHABs) may interfere with recreational activities. Endotoxins can occur with CHABs and at high concentrations in air, endotoxins can elicit respiratory illness. We characterized human activity and environmental attributes of a recreational beach impacted by a CHAB and evaluated air and water endotoxin concentrations. We monitored a single site at an inland lake for ten weekend days in California during summer/fall 2017. We monitored water quality three times daily including: endotoxins, cyanotoxins, phytoplankton cell counts, phycology, chlorophyll-*a*, phycocyanin, dissolved oxygen, pH, and temperature. We recorded daily weather and air: temperature, humidity, and endotoxin activity. We recorded human activity hourly by age group in, on, and near the water. We t-tested mean numbers of people recreating by categories of endotoxin exposure. We developed two multivariable linear models, one for air, and one for water endotoxin outcomes. The CHAB was dominated by a *Synechocystis* spp. bloom. Mean phytoplankton counts (SD) were 351,359 (76,090) cells/mL. Microcystin concentrations were all above the LOD of 0.15 ng/mL, mean (SD) = 0.33(0.07) ng/mL. Mean (SD) endotoxin in water and air were 358.11 (140.42) EU/mL, and 4.8 (6.6) EU/m³, respectively. Water endotoxin concentrations were significantly and positively associated with microcystin concentrations, water temperature, controlling for sample time, pH, humidity, chlorophyll-*a*, and phytoplankton cell count. No covariate remained statistically significant in association with air endotoxin concentrations in a multivariable model. We observed significantly more teenagers recreating on the beach during low air endotoxin study days; no other significant differences among human activity categories by endotoxin category were observed. Endotoxins in water were positively associated with microcystin concentrations. This finding deserves further investigation. All air endotoxin concentrations were below irritant thresholds and our highest measured concentrations (18 EU/m³) would not be expected to deter recreational activities. This abstract does not reflect EPA policy.

TOXIC HARMFUL ALGAL BLOOMS' IMPACT ON FEDERAL LANDS AND TRUST SPECIES

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Toxic harmful algal blooms (HABs) are perceived to be increasing in frequency and duration in US surface waters. The US Federal government holds approximately 640 million acres of land, many of which contain surface water, in trust (Federal lands). These lands are managed by federal agencies for flood control, habitat preservation, and recreation. Waterbodies within these holdings are comprised of fresh, marine, and estuary waters and each contains different species of algae and cyanobacteria which may produce toxins that could affect ecosystem, animal, and human health. For this study, peer-reviewed literature, government reports, and government issued warnings/closures were divided into four tiers of increasing likelihood of health impacts: occurrence; occurrence that exceeded a state or local threshold; suspicions of animal deaths or illness; or animal illness or death confirmed to have been caused by HAB toxins. From this review, it was determined that approximately 12% of Federal lands (parks, reserves, reservoirs, etc.) met at least one of the four criteria used for inclusion. Additionally, the US Federal government protects certain species of animals (Trust Species) due to laws and treaties (endangered species, migratory birds, and marine mammals). To date, over 60 trust species have been exposed to toxins produced by algae and cyanobacteria leading to illness and

death. The history of HABs on US Federal lands and trust species, data gaps, and possible future studies will be discussed.

DO AIRBORNE MICROCYSTINS PRESENT A HUMAN HEALTH RISK?

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Extensive *Microcystis* blooms occurred in the tidal Caloosahatchee River and in adjoining Cape Coral canals in southwest Florida in late summer/early fall 2018. Knowing that microcystins were present in the waters affected by the cyanobacteria bloom, people were concerned that they were being exposed to these compounds via inhalation of aerosols or particles (i.e., “algal dust”). To better assess if this path of exposure presented a possible risk to human health, we initiated a pilot study to determine: 1) if microcystins were present in airborne aerosols or particles; 2) if microcystins were associated with particles small enough (<1 µm) to enter the alveoli in people, thereby presenting a viable vector of exposure; and 3) if the airborne microcystin concentrations varied among sites and seasons. Anderson impactor air samplers were deployed in the fall of 2018 at a site where an ongoing *Microcystis* bloom was present (Cape Coral) and at a control site upwind of the bloom (FGCU Vester Field Station). A second deployment occurred in winter 2019 at an inland site at least three miles away from a significant water body to better assess background concentrations of microcystins in the environment. The Anderson filters were extracted and analyzed by ELISA. Results demonstrated that: 1) microcystins were present on airborne particles, including those capable of entering alveoli; 2) microcystins were quantified at comparable levels in the fall 2018 deployments (control and bloom; ~ 1 ng m⁻³); and 3) microcystins were detected at the inland site during the dry season, albeit at concentrations 3x lower than in the fall. These results suggest that airborne microcystins may represent a vector of exposure, although the concentrations measured in this pilot study and the possible ubiquitous nature of its presence prevent an assessment of such a risk of exposure.

INLAND TRANSPORT OF AEROSOLIZED BREVETOXINS FROM A KARENIA BREVIS HARMFUL ALGAL BLOOM

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This study was a collaborative project between Mote and FL Department of Health, to monitor the distance that aerosolized brevetoxins, generated during a *Karenia brevis* HAB, traveled inland posing a potential risk to public health. Eleven Tish Environmental TE-5000 high-volume air samplers were deployed and sampled twice daily in a grid pattern from Gulf beaches to seven miles inland in Sarasota County and ten samplers were deployed in Pinellas County from along the Gulf beaches to 9.6 miles inland. Seawater samples also were collected for cell counts and brevetoxin concentration from representative beaches. Weather data, including wind direction and velocity, temperature, and humidity, were collected at weather stations distributed throughout the monitoring areas. For Sarasota, two air samplers were deployed at Mote Marine Laboratory, one outside and one inside to observed potential indoor impacts relative to outside. Two were deployed at the Lido Beach public pavilion. All additional Sarasota County air samplers and all Pinellas County air samplers were deployed at fire stations for security. PureAir Control Services Inc. assisted with air filter collection and replacement for Pinellas County. The three overriding determinants for the concentration and distance of brevetoxin inland transport were the concentration of brevetoxins in near-shore Gulf water, wind direction and velocity each morning and afternoon. The placement of the air samplers also was a factor for the amount of exposure to aerosolized brevetoxins. Representative results from a Pinellas County inland transect on 26 Oct 2018; westerly winds (15 – 20 mph, on shore), moderate bloom conditions, samplers at 0.3, 4.2 and 9.7 miles inland collected: 5,380; 72; and 51 pg/m³ respectively. On 28 Oct 2018, northerly winds (11 – 12 mph, along shore), the same samplers collected: 503; 7, and 2 pg/m³, respectively.

POSTERS

(Alphabetical by author in sessions)

BLOOM DYNAMICS

DINOFLAGELLATE CYSTS SURVEYS USED IN MANAGEMENT OF HARMFUL ALGAL BLOOMS IN TERRITORIAL WATERS OF ABU DHABI, UNITED ARAB EMIRATES

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Harmful algal blooms (HAB) have increased in the Arabian Gulf Coast with increased coastal activities including offshore oil exploration, coastal dredging, land reclamation, and urbanization. Dinoflagellates are one of the most important groups of phytoplankton, producing harmful algal blooms threatening human health, commerce and well-being. Some species of dinoflagellates produce resting cysts (“seeds”) that accumulate in bottom sediments where they may survive many years until suitable conditions arise to produce harmful algal blooms again. Since cyst analysis is now an established method used in the management of HABs internationally, the Environment Agency-Abu Dhabi initiated a cyst survey as part of the HAB management program. Samples of bottom sediment were collected from 131 sites representing the whole territorial waters of Abu Dhabi Emirate. The study identified unusually diverse assemblages with 72 distinctive types of dinoflagellate cysts including seven species or groups of species known to cause HAB worldwide. Five of these, are known to produce toxins. Other nontoxic species are capable of developing massive blooms that may reduce dissolved oxygen to threatening levels for fish and other marine life. Cysts of harmful algal species were recorded from almost all samples, many of which included toxic species, showing that bottom sediments within the whole region should be considered as probably carrying some potential risk of HAB. Sites of increased abundance of harmful algal cysts (seedbeds) documented and identified areas of significantly increased risk of HAB. The present study discusses the assessment of risk for HAB from future sediment disturbance by coastal development projects and dredging. In addition, sensitive areas were identified showing the need for continuous cyst monitoring in Abu Dhabi coastal waters.

PSEUDO-NITZSCHIA BLOOM DYNAMICS IN THE GULF OF MAINE: 2012-2016

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The toxic diatom genus *Pseudo-nitzschia* is a growing presence in the Gulf of Maine (GOM), where regionally unprecedented levels of domoic acid (DA) in 2016 led to the first Amnesic Shellfish Poisoning closures in the region. Factors driving GOM *Pseudo-nitzschia* dynamics, DA concentrations, and the 2016 event were explored with data from four offshore oceanographic surveys in the GOM (summer 2012, 2014, and 2015, and fall 2016), and surface water samples from a weekly time series in 2013. Samples were processed for particulate DA (pDA), dissolved nutrients (nitrate, ammonium, silicic acid, and phosphate), cellular abundance, and species composition (estimated via Automated Ribosomal Intergenic Spacer Analysis). Temperature and salinity data were obtained from measurements during the surveys and NERACOOS buoys. *Pseudo-nitzschia* biogeography was consistent in the years 2012, 2014, and 2015, with greater *Pseudo-nitzschia* cell abundance and *P. plurisecta* dominance in low-salinity inshore samples, and lower *Pseudo-nitzschia* cell abundance and *P. delicatissima* and *P. seriata* dominance in high-salinity offshore samples. During the 2016 event, pDA concentrations were an order of magnitude higher than in previous years, and inshore-offshore contrasts in biogeography were weak, with *P. australis* present in every sample. Statistical analysis confirmed that pDA increased with the abundance and the cellular DA of *Pseudo-nitzschia* species, but was not correlated with any one environmental factor. The greater pDA in 2016 was caused by *P. australis* – the observation

of which is unprecedented in the region – and may have been exacerbated by low residual silicic acid. The novel presence of *P. australis* may be due to local growth conditions, the introduction of a population with an anomalous water mass, or both factors. A definitive cause of the 2016 bloom remains unknown, and continued DA monitoring in the GOM is warranted.

DETERMINING THE LIMITING NUTRIENT OF HAB BIOMASS IN BRANCHPORT CREEK, NEW JERSEY

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Harmful algal blooms (HABs) are experienced worldwide, exacerbated by nutrient pollution and climate change. These blooms contribute to deoxygenation and can release toxins that lead to fish kills and public health issues. They can also cause damages both environmentally (fish kills) and economically (lake, beach, and fisheries closures). Branchport Creek in the Shrewsbury River, NJ, has been known for its polluted waters and periodic fish kills, but little has been done to characterize the HABs that presumably occur here. The goal of this study is to determine the nutrient causing increased levels of phytoplankton in Branchport Creek. An accompanying study looked at the physical chemical conditions and phytoplankton species composition. Five boat trips were taken in summer of 2019; water quality measurements were obtained from the surface and the bottom water in Branchport Creek, and surface water was collected for bioassays. Chlorophyll *a* in the Branchport Creek bloom area was approximately 10× the levels detected throughout the Shrewsbury / Navesink area. Three sets of nutrient addition bioassays were performed by adding nitrate (N), phosphate (P), and ammonia (Am) in four combinations (N+P, N+Am, Am+P, N+P+Am) to Branchport creek water, including a control. All nutrients were added ‘in excess’ and in Redfield ratio where combined. Chlorophyll *a* was measured before and after a three- to four-day incubation period and compared to the control to determine the limiting nutrient (ANOVA). Addition of N resulted in significantly higher growth ($p < 0.05$) than control, which was indistinguishable from inorganic P additions. This means that inorganic N limits the phytoplankton bloom in Branchport Creek. Future research will target understanding the source of N and P in Branchport Creek, which will be crucial to new management practices in these polluted waters.

PHOTOSYNTHESIS-IRRADIANCE KINETICS OF *MARGALEFIDINIUM POLYKRIKOIDES*, A POTENTIALLY MIXOTROPHIC HAB SPECIES, IN THE LAFAYETTE RIVER, A SUB-TRIBUTARY OF THE CHESAPEAKE BAY

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Irradiance fuels photosynthesis and the level of irradiance can alter the photosynthetic yield of photoautotrophs. The dinoflagellate, *Margalefidinium polykrikoides*, is a harmful algal species that blooms during mid to late summer in the lower Chesapeake Bay. Cell densities can exceed 41,000 cells/mL during blooms. *M. polykrikoides* is known to be mixotrophic but little is known about its photosynthetic physiology or the relative contributions of auto- and heterotrophic metabolisms in the environment as blooms initiate and proliferate in the environment. Here we examine the photosynthetic physiology of *M. polykrikoides*. Because *M. polykrikoides* vertically migrate, their photosynthetic physiology may vary in response to available light. Presented here are photosynthetic-irradiance kinetics for natural phytoplankton populations in the in the Lafayette River, a small, shallow, sub-tributary of the southern Chesapeake Bay, prior to and during a bloom of *M. polykrikoides*. Moored YSI 6600 V2 sondes deployed at near the surface and bottom (~ 5 m) showed elevated chlorophyll *a* concentrations near the surface during daytime and near the bottom at night during *M. polykrikoides* blooms. This pattern was confirmed in 2016 during a diel study wherein sonde casts were conducted and water samples for cell enumeration were collected every hour for 24 hours. Results demonstrate increasing productivity at higher irradiances but photoinhibition when light levels were greater than 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Samples collected near the surface had a higher photosynthetic capacity than samples collected near three meters, the 1% light level. Water samples collected from near the base of the euphotic zone had lower photosynthetic rate maxima and photosynthesis was saturated at lower irradiances.

ALGAL BLOOM SUCCESSION IN LOWER CHESAPEAKE BAY; ENVIRONMENTAL NICHEs AND SEASONAL AND SPATIAL DISTRIBUTIONS

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Dinoflagellate blooms occur throughout the year in the tidal tributaries of Virginia. Of the over 1400 algal species found in the estuary, several taxa are potentially harmful to natural resources including fish and invertebrate communities and/or human health. For these reasons, algal community composition has been monitored by the Virginia Harmful Algal Bloom Taskforce at >50 stations throughout lower Chesapeake Bay monthly since the late 1990s. Presented here is a summary of seasonal algal composition and abundance over a 12-year period (2007-2019). Nine dinoflagellate species were identified as producing annually recurring blooms in Virginia waters. In addition, the potential biotoxin producing *Dinophysis* spp. and *Pseudo-nitzschia* spp. (diatom) were also observed at low densities at different periods throughout several years. *Scrippsiella trochoidea*, *Prorocentrum minimum* and *Gymnodinium* spp. were the most widespread taxa, found in >90% of monitoring stations. Cell densities during most observed blooms generally were greater than 10³ cells ml⁻¹ with maximum concentrations of ca. 3×10⁵ cells ml⁻¹. *Heterocapsa rotundata*, *H. triquetra*, *P. minimum* and *K. veneficum* had peak abundances during winter and spring months, with summer/fall blooms of *Gymnodinium* spp., *S. trochoidea*, *Margalefidinium polykrioides*, and *Alexandrium monilatum*. While dinoflagellates do co-occur, blooms were largely separated temporally and/or spatially. Results support optimal temperature and salinity ranges as driving factors in seasonal succession patterns and maintaining high diversity of dinoflagellates in the landscape. The average size of the cells generally increase throughout the season, suggesting that trophic status and grazing also shape the community composition.

MOLECULAR EVALUATION OF A PERVASIVE MICROCYSTIS SPP. BLOOM IN A NEW JERSEY RECREATIONAL LAKE DURING SUMMER, 2019

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Cyanobacteria are responsible for the largest number of harmful algal blooms (HABs) worldwide, and the genus *Microcystis* is a major contributor. Several species within this genus produce microcystins, a group of hepatotoxins that affect humans, pets, and wildlife. Recurrent *Microcystis* blooms are well-documented in a wide range of freshwater environments, and bloom reports in brackish and estuarine waters are increasing in frequency. Here we describe a wide-spread, toxic *Microcystis* spp. bloom in Lake Hopatcong, the largest lake in the state of New Jersey and a popular recreational area, during the summer of 2019. As a result of the bloom, Lake Hopatcong State Park and neighboring beaches were closed during much of peak season (July-August). This study evaluated water quality, nutrients (primarily nitrogen and phosphorus), microcystin levels, chlorophyll, and phytoplankton from multiple locations surrounding Lake Hopatcong during July and August, 2019. We also characterized *Microcystis* concentrations using sandwich hybridization assay (SHA). 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 a specific rRNA sequence creating a nucleic acid “sandwich”, wherein results are measured by absorbance. Prior work validated a *Microcystis* spp. SHA application in the laboratory using cultured isolates and assessed the combined influences of temperature and irradiance levels on SHA signal. However, this method had not been fully assessed on *Microcystis* populations from environmental samples. Results herein evaluate the capacity for utilizing SHA to detect and quantify *Microcystis* in the field and applying those data to HAB tracking and forecasting efforts.

MICROZOOPLANKTON GRAZING DURING THE PLANKTOTHRIX DOMINATED CYANOBACTERIAL BLOOMS IN SANDUSKY BAY, LAKE ERIE

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Understanding the role of phytoplankton growth change based on internal top-down trophic grazing interactions from zooplankton is necessary to better determine cyanobacterial algal bloom establishment as a parallel to more common investigation into the role of changes in nutrient concentrations, or bottom-up enrichment, on bloom structure and function. Bi-weekly samples were collected from two locations in Sandusky Bay, Lake Erie between mid-March 2019 to mid-September 2019 to capture pre-bloom, early-bloom, and late-bloom conditions. Dilution experiments were conducted to determine microzooplankton grazing rates using previously established methods. After 24 hours, samples were collected for analysis of total chlorophyll *a* pigment concentration using fluorometry and analysis of phytoplankton community structure using a Fluoroprobe that differentiates algal divisions by measuring differences in photosynthetic pigment fluorescence. Results indicated that microzooplankton were actively grazing on the overall phytoplankton community in the majority of experiments conducted in one location, but not the other, based on statistical significances. Grazing rate ranges from 0.13 d⁻¹ – 0.38 d⁻¹. Furthermore, using the Fluoroprobe to estimate community structure, preliminary results indicated that microzooplankton were grazing on all phytoplankton community types with specific results to be provided during the time of the presentation. Overall, this study indicated that microzooplankton can play an important role in the top-down control of *Planktothrix*-dominated blooms in Sandusky Bay, Lake Erie.

TESTING THE ROLE OF LAKE BROWNING IN A GREENING WORLD BY COMPARING ALGAL RESPONSES AMONG THREE LAKES TO A SIMULATED STORM EVENT

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Across the northeast U.S., algal blooms are occurring more often and later in the year than expected. Simultaneously, some lakes have been increasing in dissolved organic matter (DOM), which can accelerate warming of surface waters and cooling and darkening of deep waters. The new temperature gradients may prolong summer stratification and consequently enable the release of nutrients stored in the sediments, such that when a storm event occurs the lake is predisposed to greater algal blooms. The purpose of this study was to test whether bringing nutrient-rich deep water to the surface could be driving rising algal blooms, and whether the response would be the same across lakes of differing trophic status. We tested the hypothesis that higher DOM would predispose lakes to greater algal response. Water from the epilimnion and hypolimnion of three lakes was incubated near the surface of each lake for seven days in June and in August 2018. We tested three treatments, bottom water alone, surface water alone, and a mixture of the two, and measured DOM content, algal content, and total and dissolved nutrients in five samples taken before and after incubation. Algal content increased in both the oligotrophic and dystrophic lakes in June and August, but only in the eutrophic lake in August. Unexpectedly, the greatest algal increase occurred in the bottom-water alone treatment, with over a 30-fold increase in the eutrophic lake. These findings suggest a key role for browning-related changes in light limitation and lake thermal structure in stimulating late-season algal blooms.

PIECING TOGETHER THE PUZZLE OF PYRODINIUM BAHAMENSE BLOOM DYNAMICS

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The nonlinear interactions of environmental drivers and algal bloom dynamics pose a challenge for understanding and predicting effectiveness of management actions aimed at bloom prevention or mitigation. High-biomass blooms of the toxic dinoflagellate *Pyrodinium bahamense* now occur every summer in northern Tampa Bay, Florida, with abundances greater than 100,000 cells/L persisting between one and four months each of the last nine years. The

variability in bloom duration can be a function of the timing of bloom initiation or termination (or both), and our prior work suggests the timing of each depends on multiple, interacting factors (cyst germination/encystment, seasonal warming/cooling of water column, freshwater runoff). Mechanisms underlying interannual variability of bloom magnitude in Tampa Bay are less resolved. This presentation explores a subset of data from a larger effort to characterize and quantify the patterns, rates, and variability of *P. bahamense* growth and biomass. Results from high resolution spatial and temporal sampling over the 2019 bloom season illustrate that *P. bahamense* abundance can vary by an order of magnitude over short distances, both horizontally (within 500 m) and vertically (within a <40-m depth), and similarly over short timescales (< 1 d). Moreover, weekly fine-scale mapping of surface biomass over the study area suggests a fortnightly signal in bloom variability. These patterns could likely be attributed to the interactions of cell division, diel vertical migration behavior, and hydrodynamics of the system. However, higher frequency in situ sampling is needed to accurately quantify fine-scale variability and resolve potential underlying mechanisms, which could lend new insights into the processes that control *P. bahamense* growth and loss over the course of a bloom.

DISTRIBUTION, COMPOSITION, AND TOXIGENICITY OF MICROBIAL PLANKTON ASSEMBLAGES ACROSS A SALINITY GRADIENT FOLLOWING FRESHWATER RELEASE OF THE BONNET CARRÉ SPILLWAY INTO MISSISSIPPI SOUND.

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Coastal salinity gradients can play important roles in the structuring planktonic assemblages, particularly in regions with high levels of freshwater discharge. In 2019, the repeated opening of the Bonnet Carré Spillway (LA) delivered ~83% more freshwater ($3.8 \times 10^{10} \text{ m}^3$) into the Mississippi Sound relative to typical spring conditions. While these openings were necessary to prevent flooding in the city of New Orleans, the resulting freshwater outflow decreased nearshore salinities to some of the lowest observed over a 13-year period. The increased freshwater discharge coincided with an expansive bloom of freshwater toxigenic cyanobacteria (*Microcystis* spp. and *Dolichospermum* spp.), an event never previously reported along the Gulf of Mexico's northern coastline. During a rapid response survey effort from Mississippi Sound to Dauphin Island, AL (~145 km), surface integrated samples were collected from sites along a nearshore transect ~3 km from the coast over several months. Here, we are leveraging genetic tools (qPCR and 16S/18S rRNA amplicon sequencing) to determine the distribution and abundance of cyanobacterial toxin genes and the composition of bacteria and protist assemblages in relation to surface salinity across the survey area through time. This information will improve our understanding of how the freshening of coastal waters may impact microbial plankton assemblages and alter the risk associated with toxigenic cyanobacterial blooms in the Mississippi Sound, which may have large-scale ecological and economic impacts within the region.

KARENIA SPECIES NICHE DIFFERENTIATION DURING THE 2017-2019 *K. BREVIS* BLOOM IN THE EASTERN GULF OF MEXICO

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We examined spatial and temporal trends in the occurrence of *Karenia* species during the severe *K. brevis* bloom that occurred in the eastern Gulf of Mexico from 2017-2019. While *Karenia brevis* was numerically dominant throughout the blooms by more than three orders of magnitude (up to $6.7 \times 10^7 \text{ cells L}^{-1}$), at least seven other *Karenia* species co-occurred with *K. brevis* during the blooms. *Karenia longicanalis*, *K. cf. umbella* and *K. selliformis* generally exhibited coastal distributions throughout Florida waters, while *K. asterichroma*, *K. papilionacea* and *K. mikimotoi* were present both near and offshore. All species were found in the southwest region, panhandle and east coast except for *K. asterichroma*, which was restricted to southwest region, and *K. papilionacea*, which was excluded from the Florida east coast. Temporal distributions support the hypothesis that in 2018 a newly formed bloom reinforced an overwintering 2017 bloom. *Karenia brevis* exhibited the largest temperature range of all the *Karenia* species present (10.4-39.4°C), while the other *Karenia* species were present between 10.4 and 33.3°C. Unlike *K. brevis*, at least three of the other *Karenia* species (*K. longicanalis*, *K. mikimotoi*, and *K. papilionacea*) were able to tolerate salinities as

low as 19.8 while two species (*K. cf. umbella* and *K. papilionaceae*) were present at 17.0. These data suggest that the thermal tolerance of *K. brevis* may have contributed its dominance and the longevity of this bloom.

IMAGE CYTOMETRY FOR CELL CYCLE ANALYSIS OF A MIXOTROPHIC HAB DINOFLAGELLATE IN BLOOMS

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Use of flow cytometry (FCM) for cell cycle analysis in blooms is complicated by a number of factors. Multispecies assemblages of protists occur in blooms, and these cells can overlap the target cells in both size and DNA content. Also, FCM cannot exclude cytoplasmic DNA from nuclear DNA, and this creates a particular problem with mixotrophic bloom-formers such as *Karlodinium* spp. dinoflagellates that eat other protists. Both overlapping non-target cells and vacuolar prey DNA causes error in FCM cell cycle analysis. In the past this has been circumvented by the use of spectrophotometer equipped microscopes that allowed measurement of individual cells of interest. This technique, however, is slow and results in low numbers for cell cycle analysis. Here we describe an image cytometry (ICM) system that allows rapid measurement of nucleus-specific DNA in high numbers of user-selected target cells, avoiding cytoplasmic DNA and non-target taxa. The system also allows permanent raw data archiving as image files. ICM was used to quantify nuclear DNA content and cell cycle progression in the *Karlodinium veneficum* in cultures and in natural bloom samples. ICM fluorescence peak coefficients of variation (CV's) on fluorosphere standards were 2.1%, comparable to FCM. Precision of ICM analysis of DAPI-stained *Karlodinium veneficum* cells was comparable to flow cytometry with CV's as low as 6.2%, and the cell cycle distributions of culture samples analyzed by both ICM and FCM were in agreement. Automated image analysis developed with cultured *K. veneficum* permitted rapid identification and measurement of *K. veneficum*-specific nuclear DNA in field bloom samples, with instantaneous measurement of up to ~200 *K. veneficum* nuclei per image and corresponding CV's as low as 10%. Further performance and application of ICM for cell cycle and other analyses of field blooms will be discussed.

ENVIRONMENTAL TRIGGERS FOR *MARGALEFIDIUM POLYKRIKODIES* ENCYSTMENT IN THE LAFAYETTE RIVER, A SUB-TRIBUTARY OF CHESAPEAKE BAY

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Margalefidinium polykrikoides is a harmful dinoflagellate that blooms worldwide and is responsible for fish kills and food poisoning, which affects fisheries economy. Although we have a general understanding of bloom development and maintenance for this organism, bloom initiation appears to be complex and the combination of environmental factors promoting bloom initiation remains unknown. Resting cysts are thought to play an essential role in bloom initiation. Cysts formed at the end of preceding blooms are thought to provide "seed" populations to the water column in subsequent years. *Margalefidinium polykrikoides* resting cysts have been found in sediment samples from Indonesia, New York, Korea, Russia, and China. However, we still do not understand the combination of factors that induce encystment of *M. polykrikoides*. To better understand the encystment process, cyst traps were deployed daily in the Lafayette River, a sub-tributary of the southern Chesapeake Bay, during summer, 2018, to collect resting cysts during a *M. polykrikoides* bloom. Resting cysts were identified and counted by using a fluorescent in situ hybridization (FISH) assay developed by Hattenrath-Lehmann et al. (2016) to calculate the daily cyst deposition flux during the bloom. The maximum cyst flux observed was 17,930 cysts m⁻² d⁻¹, and cyst flux increased after ammonium concentrations decreased suggesting that ammonium limitation triggers *M. polykrikoides* encystment. In 2019, cyst resuspension and excystment is being examined and compared with bottom water temperature and nutrient concentrations to determine environmental conditions conducive to excystment.

DRIVERS OF CYANOHAB EVENTS: A HISTORICAL WATER QUALITY COMPARISON IN ADJACENT WATERSHEDS

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Harmful algal blooms (HAB) are of growing concern in freshwater ecosystems as they are occurring at a higher frequency, lasting longer, and geographically expanding. Many questions remain regarding the combination of factors that lead to bloom events, though it is believed to be driven, in part by, nutrient loading/cycling and changing climatic conditions. This study compared historical water quality information at two US Army Corps of Engineers-managed reservoirs in similar and adjacent watersheds in Kentucky and Tennessee to identify potential drivers of cyanobacteria HAB (cyanoHAB) events in small inland lakes. Barren River Lake (KY) has experienced routine HAB events while, to date, no reports of blooms have occurred at Dale Hollow Lake (TN). Historically, Barren River Lake was shown to have greater agricultural land-use as well as greater concentrations of total organic carbon, ammonia, total Kjeldahl nitrogen, and chlorophyll *a* when compared to Dale Hollow Lake. Conversely, Barren River Lake was also shown to have significantly lower temperatures, dissolved oxygen concentrations, and specific conductance levels. Data also indicated that some longer-term trends were apparent including a rise in pH and dissolved oxygen, as well as decline in total organic carbon, nitrite + nitrate, and total phosphorus. Additionally, > 90% of the community composition within Barren River Lake was comprised of cyanobacteria. This study suggests that certain water quality parameters including dissolved oxygen, ammonia, specific conductance, pH, and chlorophyll *a*, may be valuable as predictive indicators for assessing HAB susceptibility in this region's small inland lakes. This study also supported the theory that algal bloom events are strongly related to nutrient loading. Reverse source tracking of nutrients through identification of ecosystem imbalances may allow researchers to understand and predict potential bloom conditions in impacted waterways, as well as potentially assist in local and regional management practices to maintain healthy aquatic ecosystems.

CHARACTERIZING DEOXYGENATION AND HARMFUL ALGAL BLOOMS IN BRANCHPORT CREEK, NEW JERSEY

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Harmful algal blooms (HABs) are not only a local crisis but have been expanding globally with negative ecosystem consequences including deoxygenation and fish kills. Branchport Creek, a tributary of the Shrewsbury River in New Jersey, is known to be polluted and experiences frequent fish kills commonly presumed to result from deoxygenation. However, the phytoplankton in these waters have not been characterized. Our objective was to characterize the phytoplankton growing here to determine their role in the deoxygenation and fish kills observed in Branchport Creek. Over the course of twelve weeks, a series of stations along Sandy Hook Bay, the Navesink and Shrewsbury Rivers including Branchport Creek were sampled. In situ sampling of salinity, temperature, oxygen, pH, turbidity, chlorophyll, dissolved inorganic nutrients and water transparency were done to characterize the physical / chemical environment. Phytoplankton were collected with a 35-um net or as whole water samples and analyzed by microscopy and flow cytometry, respectively. Dissolved oxygen in Branchport Creek surface waters was high and associated with a phytoplankton bloom, up to 387 mg m⁻³ Chl *a*, but bottom water oxygen was hypoxic to anoxic and largely devoid of phytoplankton. Microscopy determined that *Akashiwo sanguinea* was the dominant phytoplankton blooming in Branchport Creek, which contrasted with the largely diatom-dominated phytoplankton of the adjacent Sandy Hook Bay region. Fish kills involving Atlantic menhaden (*Brevoortia tyrannus*) were also observed over the time we sampled. Deoxygenation of Branchport Creek likely results from delivery of surface organic matter produced by the phytoplankton bloom to bottom waters. As *A. sanguinea* has been associated with fish kill events, further research is necessary to better understand the relative roles of HABs vs. deoxygenation in fish kills of Branchport Creek.

WATER QUALITY PARAMETERS AND LATE SUMMER HARMFUL ALGAL BLOOMS IN THE LOWER CHESAPEAKE BAY

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Late summer blooms of *Alexandrium monilatum* and *Margalefidinium* (formerly *Cochlodinium*) *polykrikoides* have occurred throughout the lower Chesapeake Bay since 2007. A predictable population progression occurs in the York River with peak cell concentrations of *M. polykrikoides* followed one to two weeks later by peak concentrations of *A. monilatum*. Laboratory bioassays have demonstrated acute dose dependent toxicity of these species to oysters at different stages of development. Local oyster culturists have reported to researchers at the Virginia Institute of Marine Science numerous juvenile oyster mortality events during and immediately following these blooms at grow-out locations in natural estuarine habitats. This has led to increased efforts in tracking bloom activity. Some years, such as 2015 and 2016, blooms of these species have occurred with cell counts greater than 20,000 cells/mL, with some *A. monilatum* cell counts greater than 100,000 cells/mL. Other years, such as 2014 and 2018, there have been no blooms of these species and extremely low cell counts. Annual variations in weather conditions, nutrient levels, and other environmental parameters likely have an effect on the density and distribution of these blooms. For example, increased flushing rates seen in 2018 due to heavy rainfall that summer may have contributed to the lack of blooms. The Virginia Estuarine and Coastal Observing System (VECOS) of the Chesapeake Bay National Estuarine Research Reserve (CBNERR) has maintained Continuous Monitoring Stations in the York River for more than fifteen years. These stations collect water quality data every 15 minutes, giving a fine-scale recording of water quality data, such as temperature, salinity, pH, dissolved oxygen, and chlorophyll *a*. Nutrient level data, specifically nitrogen and phosphorus, has been collected over the years by the Chesapeake Bay Program. We are looking at these data to determine if there are correlations between the various water quality parameters and bloom occurrence.

PHYTOPLANKTON ASSEMBLAGE, BIOMASS, AND NUTRIENT LIMITATION SHIFTS IN RESPONSE TO SEASONAL NITRATE DRAWDOWN

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The rapid response of phytoplankton to changes in abiotic factors makes for an ideal biological indicator for ecosystem assessment (Jindal et al., 2014). Here, we examine the effects of an annually-recurring seasonal drawdown of nitrate on phytoplankton assemblage, biomass, and nutrient limitation status in a mesotrophic Arkansas lake. A 30-year lakes record demonstrates a seasonal pattern of nitrate drawdown during the summer months and an increase in nitrate during late winter into early spring. We conducted monthly sampling events across the temporal drawdown gradient and performed nutrient enrichment (nitrogen; N and phosphorus; P) bioassays. Initial water samples and completed bioassays were analyzed for carbon (C), N, P, and chlorophyll-*a* to assess temporal variability in nutrients as it relates to phytoplankton biomass and assemblage structure. Our initial results suggest P-limitation occurred from April until June, and switched to N & P co-limitation in July and August. Cyanobacterial genera such as *Microcystis* were identified in many samples; however, these genera did not become dominant with nutrient additions in the spring, but shifts were expected for summer samples that will soon be analyzed. We hypothesized that a seasonal shift to N-limited phytoplankton would occur as nitrate became decreasingly available. If this hypothesis is supported, the experiments provide compelling evidence for a long-term pattern of seasonal N-limitation in this lake.

IDENTIFICATION OF THE LAST STEP OF DOMOIC ACID BIOSYNTHESIS

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Pseudo-nitzschia is a cosmopolitan diatom genus that lives in coastal waters worldwide, including off the coast of California. Blooms of *Pseudo-nitzschia* often produce domoic acid (DA), a potent neurotoxin that accumulates in shellfish and can cause serious illnesses in humans and marine mammals. DA biosynthesis was recently decoded by our group (Brunson et al, Science 2018). The group established a cluster of transcriptionally co-regulated genes (dabA,

dabB, dabC, dabD) in several toxic *Pseudo-nitzschia* species that encode enzymes that perform all but one steps of the biosynthesis of DA. This project aims to determine the final missing step of the biosynthesis in which isodomoic acid A is isomerized to domoic acid. We synthesized biosynthetic intermediates to domoic acid labeled with ¹⁵N and deuterium and fed these compounds to live diatom cultures of a high domoic acid producer *Pseudo-nitzschia multiseriis*, a low domoic acid producer *Pseudo-nitzschia pungens*, and a non-domoic acid producer *Pseudo-nitzschia delicatissima* to evaluate the production of labeled domoic acid and intermediates. Results that [¹⁵N,D]-L-NGG could be converted into [¹⁵N,D]-domoic acid were confirmed using LC-MS, achieving higher yields of the labeled domoic acid than reported in previous work (Maeno et al., Sci Rep 2018). Similar feeding experiments are currently underway with four other labeled intermediates: 7'-hydroxy-L-NGG, carboxy-L-NGG, dainic acid A, and isodomoic acid A.

IMPROVEMENTS TO THE ORGANIZATION AND CURATORIAL PRACTICES OF THE ALGAL RESOURCES COLLECTION

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The Algal Resources Collection (ARC) was formally established as a public service collection in September 2016 after transitioning from an established private research collection that was started in 1987. The collection supplies toxic algal strains for researchers around the world, with many collaborations, loans, and exchanges. Recently, the ARC has focused on improving its organization and curatorial practices, with gains in the efficiency of its daily operations. We successfully updated and reorganized our database, synchronizing it with a barcode-labeling system that provides rapid access to the metadata of any strain by scanning a barcode on their labels. We also redesigned our website (www.algalresourcescollection.com), which has significantly enhanced its organization and aesthetic, allowing potential users to access desired information more intuitively. Finally, we are performing the characterization of the 424 strains currently housed in the collection, based on light photomicrographs, DNA sequencing, and toxicologic bioassays. The obtained information is periodically updated on our website, which will allow ARC users to obtain accurate information on the distributed strains. These significant achievements are attributable to an award from the National Science Foundation (NSF 1756414).

NUTRIENT POLLUTION AND HARMFUL ALGAL BLOOMS IN COASTAL LAKES OF MONMOUTH COUNTY, NJ

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Harmful Algal Blooms (HABs) in freshwater environments have been increasing nationwide in recent years, exacerbated by nutrient pollution and climate change. Coastal Lakes in New Jersey sometimes experience HABs, while others in adjacent watersheds remain HAB free. The relationship between HABs and nutrients was investigated through (1) intensive examination of the largest coastal lake in NJ (Deal Lake) starting in 2017, and by (2) comparing time series of nutrients and HAB across ten different coastal lakes in 2019. HAB abundance was monitored through phycocyanin and chlorophyll fluorescence measurements and microscope cell counts. Water quality measurements were made in the field and nutrients were measured by NJ DEP labs. The Deal Lake analysis indicated seasonal shifts in nutrient ratios, with elevated DIN:DIP in winter transitioning to low DIN:DIP in summer when HABs tend to occur. Chl *a* was strongly correlated to DIP in Deal Lake, but nutrient addition bioassays showed P-limitation of winter-spring non-HAB assemblages and N-limitation of summertime HABs. In the inter-lake comparison (2019), summer HABs were consistently detected in some lakes but not others. Average summertime HAB abundance was strongly correlated with DIP and pH, but HAB biomass responded to storm water runoff that was enriched in ammonium but not DIP, indicating N-limitation of summertime HAB assemblages again. The combination of observational and experimental results suggests that HABs cause elevated water column DIP and pH, whereas DIN inputs play an important role in fueling and maintaining HABs in these lakes.

INNOVATION & ADVANCES IN HAB DETECTION

THE CHALLENGING ROAD TO OPERATIONS: TRANSITIONING A LAKE ERIE HARMFUL ALGAL BLOOM FORECAST

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Developed by the NOAA Office of Oceanic and Atmospheric Research (OAR) Great Lakes Environmental Research Laboratory (GLERL), the Lake Erie 3D Harmful Algal Bloom (HAB) Tracker predicts bloom position of cyanobacteria, accounting for vertical mixing and buoyancy of cyanobacterial colonies. This capability improves upon the traditional 2D model in which conditions underneath the surface, which are critical for water intake managers, are ignored. In 2018, the research product was transitioned to the NOAA National Ocean Service (NOS) for operational implementation. To ensure a successful transition, the research tool was re-engineered to fit the technical requirements of an operational product. The HAB Tracker must be reliable and, when needed, fail gracefully with clear messages for quick resolution. Moreover, the operational tool must run efficiently to avoid reaching hardware limitations at any time. To evaluate forecast quality, studies were performed using GLERL test data in 2011, and both offices are running HAB forecasts in parallel in 2019. NOS is also comparing the forecast from the 3D HAB Tracker and the traditional 2D model. If comparisons demonstrate performance alignment and stakeholder feedback indicates support, the 3D model will fully replace the legacy 2D model in 2020. To improve user-friendliness and consistency, the software has been automated to reduce repetitive, manual inputs and to deliver daily forecasts incorporating the most recent satellite imagery and updated hydrodynamic forecast. For public users, an interactive web interface was designed to easily visualize forecast data. In 2019, NOS is engaging with stakeholders on input for this improved product and will incorporate stakeholder feedback into the product to ensure that it suits their needs for the 2020 implementation. NOS is ensuring a smooth transition of the software to an operational product to ensure reliable, robust, and sustainable HAB forecasts in Lake Erie are disseminated to stakeholders.

CRYPTIC BLOOMS: INVESTIGATING THE PRESENCE, ABUNDANCE AND ENVIRONMENTAL DRIVERS OF BENTHIC CYANOBACTERIAL BLOOMS IN OHIO

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In recent years, there has been an increased reports of benthic cyanobacterial blooms worldwide. With new species and changing phytoplankton communities comes possible production of harmful cyanotoxins, including the neurotoxins saxitoxins and anatoxins. To further understand the potential threat these toxin producing cyanobacteria have, routine sampling and experiments were conducted throughout Ohio, primarily focusing on the Maumee River and Alum Creek. Chemical and biological water column sampling occurred at consistent sites from midsummer to early fall of 2018 and 2019. Toxin concentrations were measured through ELISA and LC-MS/MS and gene copies for cylindrospermopsin, saxitoxin and microcystin-producing cyanobacteria were quantified with qPCR. Based on previous benthic surveys, toxin analyses and qPCR results, one site in western Lake Erie (Put-In-Bay), three sites in the Maumee River, and two in Alum Creek were chosen to conduct nutrient amendment experiments. The in-situ experiments focused on nutrient diffusing substrata for benthic algal growth and toxin production. The setup array included ten replicates for each of the six treatments (Control, Ammonia, Nitrate, Phosphorus, Ammonia + Phosphorus, and Nitrate + Phosphorus). Each cup was filled with a 2% agar and nutrient solution and topped with a porous crucible cover on which the phytoplankton could grow. The cups were attached to trays and deployed into wadeable (~0.25-0.5m) portions of the river and left for two weeks. For collection, every crucible cover was measured on a fluoroprobe and for each treatment, three covers were analyzed for chlorophyll, three for DNA (qPCR), three for

toxins, and one for phytoplankton identification and enumeration. These results will provide critical insight needed to better understand benthic cyanobacterial growth and toxin production in Ohio waterways.

SYSTEMATICS OF THE (SOMETIMES) HARMFUL *CRYPTHOCODINIUM*-LIKE DINOFLAGELLATES

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Crypthecodinium spp. heterotrophic dinoflagellates can be harmful to industrial production of the red algae *Porphyridium*, a valuable source of fatty acids, pigments, and sulphonated polysaccharides with a broad range of pharmaceutical applications. Contaminating *Crypthecodinium* feeds on *Porphyridium*, causing collapse of the aquaculture. Although important in this respect, *Crypthecodinium* is poorly understood systematically, and is thought to represent a species complex containing members that are only superficially similar. Although many geographic strains are in culture, most have been identified only based on their lack of pigmentation and ability to grow on solid media. Some strains show gross morphology inconsistent with the original description of the genus, as well as differing physiologies such as ability to graze on microalgae. In order to better understand diversity within this group, we conducted 18S phylogenetic analysis of multiple *Crypthecodinium* strains, including one that has been taxonomically shown to have the correct plate tabulation for the original description of the type species, *C. cohnii*. Resulting phylogenetic trees showed that the examined strains formed a monophyletic group, but as an early branching lineage within dinoflagellates with long evolutionary distance and species-level differences in sequence divergence. In order to understand the cryptic diversity we build on previous 18S phylogenies and compare compensatory base changes in the folded structure of ITS2 rDNA sequences as indicators of species level divergence. Future research will involve more closely examining divergent strains for taxonomically important morphological and, physiological features. As well as, mapping phylogenetic data to a model mutant complementation system that can be used for testing biological species boundaries in *Crypthecodinium*. The overall goal is to revise the systematics of this group, and to use *Crypthecodinium* as a model system to test species concepts in protists.

QUANTIFICATION OF MICROCYSTIN DEGRADATION RATES BY LAKE ERIE *MICROCYSTIS* BLOOMS USING EXPERIMENTAL METHODS

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Determining the spatial and temporal drivers of microcystin degradation rates are needed to enhance current forecasting of cyanobacterial blooms for the western basin of Lake Erie. Throughout the 2019 summer, eight microcosm experiments were conducted with *Microcystis* dominated lake water collected from a near and offshore location. In clear polycarbonate bottles, 2-L of whole and 0.2- μ m filtered lake water were spiked with 1 μ g/L of N15 labeled dissolved microcystin LR to measure degradation rates of this congener due to biotic and abiotic factors under ambient light and temperature incubation. Subsamples were collected every three hours for 24 hours, then at hour 36 and 48 for measurement of microcystins (LC-MS/MS). Degradation rates were notably due to biotic factors. Development of a forecast of cyanobacterial bloom toxicity accounting for degradation of microcystin would aid water treatment and resource managers in establishing action protocols for toxic water.

ECOTOXICITY OF CYANOTOXINS IN FRESHWATER HABITATS - A SYSTEMIC REVIEW OF THE LITERATURE

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In recent decades, cyanobacterial harmful algal blooms have increased in frequency and intensity in freshwater ecosystems. These blooms can impact water quality by lowering dissolved oxygen concentrations and producing potent toxins. Across the U.S., a variety of cyanotoxin classes have been detected in lakes, rivers, and drinking water reservoirs. Acute exposure to these toxins has caused severe illness, and in extreme cases death, in humans, pets, and livestock. Thus, U.S. Environmental Protection Agency (U.S. EPA) developed drinking water and recreational advisory guidelines for two of the most prevalent toxins, microcystins, and cylindrospermopsin. Additionally, individual states have issued guidelines for up to four cyanotoxin classes. However, it is unclear if any of these numeric guidelines are protective of aquatic life exposed to sublethal levels of cyanotoxins for prolonged periods of time. In this project, over 170 peer-reviewed articles were critically evaluated to better understand the impact of waterborne or dietary exposure of four cyanotoxins classes: microcystins, saxitoxin, anatoxin-a and cylindrospermopsin, in aquatic organisms. For this study, aquatic organisms were defined as fish, aquatic invertebrates, amphibians and birds living or exclusively feeding in freshwater habitats. This presentation will summarize 1) the number of published studies for each toxin, 2) the most common acute and chronic endpoints evaluated, and 3) the number of studies per organismal class. Studies that met our acceptability criteria were further examined to identify the no-observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC) for each toxin. These datasets will be critically discussed and compared to existing monitoring guidelines to determine whether these criteria are also protective of non-targeted aquatic species.

CELL & MOLECULAR ADVANCES

BIOMARKERS OF BREVETOXIN EXPOSURE IN *MERCENARIA CAMPECHIENSIS*

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Brevetoxins, the causative agent of neurotoxic shellfish poisoning (NSP) produced by the marine dinoflagellate *Karenia brevis*, are a significant concern in seafood safety due to the increasing number and severity of red tide events in the coastal southeastern United States. The commercial shellfish industry is particularly vulnerable to these events as a result of the sessile, filter-feeding life cycle of clams and oysters. 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. Until recently, the NSP MBA has been the only National Shellfish Sanitation Program (NSSP) approved method for regulatory NSP testing. An ELISA that determines the composite B-type brevetoxins has been approved recently as a limited use method for NSP testing in Eastern oyster (*Crassostrea virginica*), sunray venus clams (*Macrocallista nimbosa*), and hard clams (*Mercenaria mercenaria*). Brevetoxins are extensively metabolized in hard clams and Eastern oysters. The most persistent and abundant metabolites contributing to overall toxicity are identified as cysteine and taurine conjugates of B-type brevetoxin. These metabolites, BTX-B1, BTX-B2, and S-desoxy BTX-B2, have been identified as biomarkers of brevetoxin exposure in oyster and hard clam and correlate well with the composite toxin measurements by ELISA. In this study, *K. brevis* bloom-exposed *M. mercenaria* and southern hard clams (*Mercenaria campechiensis*), a species of growing commercial interest, were examined by N2a cytotoxicity assay, ELISA, and LC-MS. Results suggest that *M. campechiensis* and *M. mercenaria* metabolize brevetoxins similarly and BTX-B1, BTX-B2, and S-desoxy BTX-B2 could serve as biomarkers of brevetoxin exposure in *M. campechiensis* for confirmation by LC-MS.

BLOOM-FORMING *MICROCYSTIS* HARBOR UNIQUE BACTERIA IN RESPONSE TO HIGH LIGHT AND FLUCTUATING OXYGEN LEVELS

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Cyanobacterial bloom-forming *Microcystis* species produce toxins, creating environmental issues for human and aquatic species. During the summer bloom season, *Microcystis* form coenobial aggregates in which strong diel variations of light, dissolved oxygen, and pH occur. The aggregates also contain abundant dissolved organic matter which can be mineralized by heterotrophic bacteria. However, little is known definitively about the function of bacteria living inside *Microcystis* aggregates. In this study, we applied both culture-dependent and culture-independent methods as well as genomic sequencing of isolates to understand the diversity, physiology, and potential interactions of *Microcystis* aggregate-associated bacteria (hereafter, associated bacteria). Our results show that associated bacteria are unique bacterial consortia different from planktonic communities in the surrounding water. Network analysis suggests that intense and close interactions might be formed between associated bacteria and *Microcystis*. A total of 225 bacterial strains were isolated from *Microcystis* aggregates during the peak of bloom and ten representative associated bacteria were selected for whole genome sequencing. In contrast to the free-living bacteria, the genomes of associated bacteria have a higher frequency of photosynthesis gene clusters, suggesting that phototrophic bacteria could be abundant in the aggregates. The dominance of anoxygenic phototrophs in the community of associated bacteria was also confirmed by exploring the *pufM* gene using PCR. Three associated bacteria encoded denitrifying gene clusters, and their denitrification activities were confirmed, suggesting that denitrification could be important to the associated bacteria. Comparative genomics revealed that associated bacteria contained rich and unique chemosensory systems which were not found in the free-living bacteria. We hypothesize that within *Microcystis* aggregates, phototrophic bacteria are active during the day, while low oxygen conditions that occur at night create a unique niche for denitrifying bacteria.

APPLICATION OF HIGH-RESOLUTION METABOLOMICS APPROACHES TO MARINE PHYTOPLANKTON

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The application of metabolomics to marine phytoplankton affords an unprecedented view into the suite of organic molecules produced by primary producers in the oceans. We know that phytoplankton create a vast pool of organic matter that includes products of central metabolism as well as toxins, compatible solutes, and signaling molecules. These compounds are then consumed, transformed, and/or accumulated within the food web. Yet, the chemical makeup of these tens of thousands of metabolites is inherently complex, a product of the diversity of the hundreds of thousands of different planktonic organisms that make up seawater communities. Defining the subset of phytoplankton-derived metabolites that have ecological and environmental impact is critical to track, predict, and model phytoplankton bloom dynamics. Here, we use combined targeted and untargeted liquid chromatography-mass spectrometry-based metabolomics approaches to explore the intracellular metabolites in >20 axenic cyanobacterial and eukaryotic phytoplankton taxa. We find that patterns in the presence and absence of metabolites is predictive of taxonomy. Further, some of the most abundant and taxonomically-constrained molecules in eukaryotic phytoplankton lineages (i.e., diatoms, dinoflagellates, haptophytes, and prasinophytes) are charged, low-molecular-weight organic sulfur- and nitrogen-containing molecules that likely act as nutrient and energy sources for bacteria in the microbial food web. We also explore the use of metabolomics for detection of signaling molecules and toxins and their co-occurrence with other cellular metabolites. We anticipate that these high-resolution metabolomics approaches will be useful for identifying novel phytoplankton metabolites, putative biosynthetic pathways, and bio-active molecules in seawater communities.

DEVELOPMENT OF MOLECULAR MARKERS AND QPCR ASSAYS FOR *ALEXANDRIUM CATENELLA* AND *A. OSTENFELDII* (DINOPHYCEAE) IDENTIFIED FROM PATAGONIAN FJORDS

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Alexandrium catenella and *Alexandrium ostenfeldii* are globally distributed dinoflagellates species capable of producing Paralytic shellfish poisoning (PSP). In the case of Chile during the last years, *Alexandrium* blooms have increased their frequency and amplitude in the coasts, from Magallanes to the Los Lagos region (Guzman et al. 2002, Mardones et al. 2015). This increase has generated big impacts on the salmon industry and the environment, becoming an important challenge for those responsible for managing coastal resources and protecting public health. This study describes the development of a quantitative real-time polymerase chain reaction (PCR) assay for identification of *A. catenella* and *A. ostenfeldii* from Patagonian fjords samples. DNA sequence information for the large ribosomal sub-unit (LSU) was obtained, following single cell PCR of *A. catenella* and *A. ostenfeldii* cells isolated from Chile coastal locations. PCR primers specific for the detection of both species were designed for real-time PCR on the LightCycler™. The LightCycler™ software melt curve analysis programme determined that *A. catenella* was identified by a melt-peak at 81.01°C, while *A. ostenfeldii* cells produced a melt peak at 81.42°C. Also the primers were tested in water samples preserved with Lugol's. The real-time PCR assay identified *Alexandrium* species in all of the samples of the months in the year 2018 where no blooms occurred. The results were compared with analysis by light microscopy and had differences between relative abundance and fold change calculations for CT values. These differences could be due to many facts that affect this method, or even unknown species in sample waters. However, the qPCR assay can identify and discriminate *A. catenella* and *A. ostenfeldii* at low numbers in fjord samples and has the potential to rapidly allow countermeasures against red tides, to prevent the mass mortality of cultured fish.

ASSESSMENT OF A TOXIC CYANOBACTERIAL EVENT IN NEW BRUNSWICK, CANADA, USING A COMBINED TAXONOMIC, CHEMICAL, AND GENETIC APPROACH

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A potent biotoxin produced by cyanobacteria, anatoxin-a (ATX-a), has been implicated in poisonings and deaths of dogs, livestock and waterfowl that have been exposed to water containing blooms of cyanobacteria. Such an event occurred in July 2018, when three dogs died after visiting the water's edge at two locations on the St. John River near Fredericton, New Brunswick, Canada. All showed signs of toxicosis following exposure, and necropsies revealed non-specific pulmonary edema and multiple microscopic brain hemorrhages. Samples of vomitus, stomach contents, water and biota from the mortality sites were analysed to determine the cause. Light microscopy identified cyanobacteria belonging to the poorly-resolved *Oscillatoria-Phormidium-Microcoleus* species complex. Targeted and untargeted analysis by liquid chromatography high-resolution mass spectrometry (LC-HRMS) verified the presence of anatoxins in all samples. The highest levels were measured in a sample of a dried algal mat that two of the dogs were eating before falling ill, with concentrations of up to 390 and 980 mg/kg for ATX-a and dihydroanatoxin-a (H2-ATX), respectively. Species of cyanobacteria were identified from the original samples using 16S rRNA gene sequencing, including a known ATX producer, *Microcoleus autumnalis*. Concurrently, 80 filaments of cyanobacteria were isolated from the original samples generating 30 clonal cultures. A representative set of clones covering all morphotypes were subjected to 16S rRNA sequencing, which identified 12 of the 15 isolates as *Microcoleus autumnalis*. Of the 15 cultures, nine tested positive for the presence of the anaC gene involved in anatoxin-a biosynthesis, eight of which were confirmed to produce ATX-a, 11-carboxy-ATX, and H2-ATX by LC-HRMS. The other six isolates were negative for the anaC gene, although anatoxins were detected in two of these. Chemical analyses revealed a highly variable toxin profile and concentration among clones and over time, and in some cases complete cessation of toxin production was observed. This study demonstrates how a combined approach including microscopy, genetic methods, and chemical analytical techniques are necessary to elucidate the cause of cyanobacterial toxicity events.

EUGLENOID TOXICITY: A REVIEW SINCE ITS DISCOVERY IN 2004

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In 2004, a fish kill event was reported from New Bern, NC with evidence for toxin production by *Euglena sanguinea*. Additional fish kills were identified in several states and additional toxic strains were isolated. In 2010, after several years of work, the toxin was identified conclusively as a new to science compound that was named euglenophycin. Toxic isolates were obtained from four continents and toxic blooms in 22 US states since this initial work. Toxicity was confirmed in six of the seven strains and a phylogenetic survey was published in 2017 which identified toxin formation in a total of seven species of euglenoids. Anticancer activity was noted in this toxin, and several studies have demonstrated efficacy of euglenophycin as a prophylactic precancer treatment and as a post-cancer mechanism to reduce blood vessel development or mTOR activity. Occurrence of euglenophycin has been documented in drinking water sources, in finished drinking water, recreational lakes and rivers, farm ponds and wetlands. Mortality of cattle, fish, and laboratory mice has been demonstrated. Toxic euglenoids can survive in hypersaline lakes and streams (up to 70 ppt recorded) and blooms often appear when conditions are rapidly altered (e.g., stagnant water due to low wind mixing, fall overturn, after four to five days of cloudy weather). Laboratory doubling rates exceed 2.4 doublings/day. Stress results in nearly complete temporary cyst formation-these cysts sink to the sediment surface and can become motile within minutes of positive cues. If conditions are stressful or extended periods of a palmelloid stage is formed. Climate change will result in many areas having lessened precipitation. Euglenoid blooms are likely to increase given decreased water levels, extreme weather events, and increased salinization.

METHOD VALIDATION & REFERENCE MATERIALS

DETECTION OF ALGAL TOXIN IN CLINICAL SAMPLES: LABORATORY SUPPORT FOR PUBLIC HEALTH

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The Emergency Response Laboratory (ERB) at the Centers for Disease Control and Prevention (CDC) is developing laboratory capabilities to diagnose human exposure to algal toxins. Recently, algal blooms have become more prevalent and persistent in freshwater and marine environments worldwide. Diagnosis of human exposure to algal bloom toxins requires sensitive analytical methods to identify classes of toxins, as well as various toxin analogues. ERB is approaching this challenge using multiple strategies. Sensitive screening methods, such as activity assays, immunoassays, and receptor assays, identify the presence of toxins groups according to their biological functions. Targeted detection typically relies on mass spectrometry and uses available certified reference materials (CRMs) to identify and confirm the most prevalent and potent forms of toxins. Additionally, high-resolution mass spectrometry may be used to confirm the presence of toxin congeners not addressed with the targeted methods. ERB is exploring the use of natural materials to establish standard parameters and library spectra, since CRMs for these compounds are often unavailable. Data gathered from acute exposures and epidemiological studies using these techniques guides method improvement to provide beneficial laboratory support for public health response.

ECOPHYSIOLOGY

KARENIA BREVIS EFFECTS ON SOIL MICROBIAL ACTIVITY IN A COASTAL WETLAND ECOSYSTEM

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Karenia brevis is the dinoflagellate responsible for red tides recently observed along Florida's coast. *K. brevis* releases neurotoxins (brevetoxins) when it lyses, which have numerous negative recorded effects. Previous studies indicate brevetoxins can persist in marine ecosystems for 1-2 months after a red tide event occurs, but the effects of these toxins on coastal wetland soil respiration have not been studied. This study aimed to understand the effects of *K. brevis* on soil microbial respiration and enzyme activity in a coastal wetland soil. To do this, soil samples from a mangrove swamp on Weldon Island in St. Petersburg were mixed with three different concentrations of *K. brevis* and a control of 0 cells/L in laboratory incubation. Methane and carbon dioxide production rates were measured every 48 hours over five weeks using a gas chromatograph. Extracellular enzyme activity (β -glucosidase (BG), β -N-acetylhexosaminidase (NAG), and alkaline phosphatase (AP)) and microbial biomass carbon were measured before and after the four-week incubation to evaluate the potential effect of *K. brevis* on the microbial community. Chlorophyll-a concentrations were measured daily to track the abundance of *K. brevis*. On day seven, chlorophyll concentrations dropped rapidly to control levels, indicating cell death. Treatments did not significantly differ in methane production, carbon dioxide production, BG, or NAG activity. In contrast, AP activity was significantly reduced in the high *K. brevis* treatment. This drop in AP activity could indicate significant impacts to phosphorous availability and shifts in coastal soil microbiomes in the event of a red tide.

DIETARY TRACERS FOR DETERMINING THE ROLE OF ECOLOGICAL PARTITIONING ON CIGUATOXIN BIOACCUMULATION IN MARINE HERBIVOROUS FISH

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The frequency and geographical extent of ciguatera poisoning (CP) throughout the Greater Caribbean Region and northern Gulf of Mexico has increased in recent decades. In order to improve the prediction and prevention of human exposures to ciguatoxins (CTX), it is important to identify ecological processes affecting trophic transfer of these toxins. The accumulation of toxic algal metabolites by herbivorous fish represents a primary entry point into food webs and a key link in CTX fate. Despite occupying the same trophic level, herbivorous fish can exhibit markedly different feeding strategies, which may affect their exposure to and subsequent uptake of CTX through dietary pathways. To investigate whether differences in feeding strategy influence CTX accumulation in herbivorous fish, trophic niches of ocean surgeonfish (*A. bahianus*, n=56), redband parrotfish (*S. aurofrenatum*, n=43), and stoplight parrotfish (*S. viride*, n=22) were estimated using ¹³C, ¹⁵N, and ³⁴S stable isotope analysis (SIA). Trophic overlap was determined using maximum likelihood and permutation procedures conducted within a Bayesian framework, which revealed substantial niche overlap of surgeonfish and redband parrotfish (appx. 57.3 and 90.3%, reciprocally; $\alpha=0.95$). A subset of samples from these species (n=20) were further compared using profiling and ¹³C compound-specific SIA of fatty acid methyl esters (FAMES) and examined using a series of multivariate approaches (e.g., Bray-Curtis dissimilarity, principal components analysis). Based on these data, we found clear differentiation of comprehensive fatty acid profiles between species and that a large driver of variation among samples is the dinoflagellate indicator FAME c22:6 ω 3, for which targeted analyses showed substantial patterns of variation between species and site groupings (ANOVA, p=0.062; post-hoc multiple comparisons, p>0.80). Interestingly, qualitative patterns of toxicity differed among surgeonfish and redband parrotfish, which suggests a strong link between toxicity and dietary differences in herbivorous fish and highlights the utility of using chemical dietary tracers in HAB ecotoxicology.

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The toxigenic marine algae *Prymnesium parvum* is a mixotrophic species that forms harmful algal blooms (HABs). When exposed to sub-optimal conditions, such as phosphate limitation, *P. parvum* increases in toxicity and heterotrophy. It has been speculated that when engaging in heterotrophy, *P. parvum* attaches itself to its prey using the haptonema - a specialized flagellum-like structure. Previous studies have shown that as salinity increases, so does toxicity. Increasing salinity also increases available calcium. Previous studies have used calcium as a co-factor to increase toxicity. It has been shown that calcium limitation can hamper prey collection in related haptophytes such as *Chrysochromulina* by altering haptonema coiling. If the mechanism in *P. parvum* is similar, even at high salinities and toxicities, calcium limitation may impede or reduce *P. parvum*'s ability to capture prey. We are testing this hypothesis using a *Rhodomonas salina*-flow cytometry based bioassay. Preliminary results indicate that calcium is indeed important to *P. parvum* growth, regardless of P availability. Results of predation trials investigating the roles of calcium and phosphate limitation on heterotrophy by *P. parvum* are underway and will be presented.

CLIMATE

THE INTERACTION OF NITROGEN ENRICHMENT AND PREVIOUS, CURRENT, AND FUTURE CLIMATE SCENARIOS ON THE GROWTH OF *MICROCYSTIS AERUGINOSA* (UTEX 2385)

Ashley Allen, Seth Buscho, Grace Phillip, Berkeley Sheppard, Lauren Ferguson, Anu Kannan, Ashley Ricciardelli, Christopher Sharon, Jonathan Deleon, Emily Quach, Sandi Win Nuang, Jacquelyn Duke, J. Thad Scott

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Many cyanobacterial toxins are nitrogen-rich biochemicals that act as hepatotoxins or neurotoxins to mammals. The concentrations of these toxins are often correlated with the total N concentration or the N:P in surface waters. In addition to warming the planet, climate change is also increasing the availability of carbon dioxide dissolved in water when it is in equilibrium with the atmosphere. Because temperature, CO₂, and N availability often interact to control phytoplankton growth, cyanobacterial blooms may become more prominent in future climate scenarios, especially if it coincides with N enrichment. *Microcystis aeruginosa* (UTEX 2385) produces microcystin-LR, a N-rich polypeptide with a carbon:N ratio of less than 5 that previous research has shown to be strongly related to N supply and the biological stoichiometry (C:N ratio and N:P ratio) of *M. aeruginosa*. We conducted a hierarchical experiment to test the effect of CO₂, temperature, and N availability on the growth of *M. aeruginosa* in experimental bloom conditions. We will present the preliminary results of this ongoing experiment aimed at testing both the immediate environmental controls on *M. aeruginosa* growth, and the potential adaptation of this species to prolonged exposure to these conditions.

ECOPHYSIOLOGICAL RESPONSES OF *PSEUDO-NITZSCHIA* TOXIC SPECIES TO ENVIRONMENTAL VARIATIONS RELATED TO CLIMATE CHANGE

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The diatom *Pseudo-nitzschia* includes certain toxic species capable of producing domoic acid (DA), an amnesic neurotoxin. The proliferation of these toxic species presents many risks ranging from serious ecological and economic damage to threats to public health. Several species and strains of *Pseudo-nitzschia* have been studied to better understand and predict their physiological responses to future climate change. In particular, the experiments carried out in this study allowed to explore the impact of salinity variations (stress and acclimation) and ocean acidification (decrease of pH) on the growth, photosynthesis and DA content by several *Pseudo-nitzschia* species and strains isolated from several geographical regions. The results showed that *Pseudo-nitzschia* species are flexible, able to adapt, proliferate and produce DA under varying conditions of salinity and pH, despite the large diversity of responses depending on the species or strains studied. In addition, an *in situ* monitoring of the presence of *Pseudo-nitzschia* spp. and the toxin domoic acid was carried out during the spring-summer period for two years (2016-2017), in two stations strongly influenced by the contributions of the rivers “Loire and the Vilaine” in France. Bi-monthly water column sampling of these two stations was conducted to monitor the change in physico-chemical parameters (turbidity, salinity, temperature, and nutrients availability) and cell density of *Pseudo-nitzschia* spp. The data collected during these two years allowed to determine the presence of *Pseudo-nitzschia* species, and evaluate the concentrations of toxins produced during each bloom, as well as the physico-chemical conditions of the environment in these two stations in order to treat in a preliminary manner, some of the questions concerning the conditions of appearance, intensity and duration of these blooms and the load of free or retained toxin in the cells.

A FIRST REPORT OF POTENTIALLY TOXIC ALGAL BLOOMS AT URAL RIVER AT KAZAKHSTAN

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Cyanobacterial algal blooms occur in freshwater rivers, lakes and reservoirs and brackish water bodies throughout the world. Cases of fish-kills are regularly occurred along the river Ural and have been reported in 2018-2019 (December–Atyrau, April–Uralsk, June–Orenburg). During December–March 2018-2019, mortality about a hundred tons of farmed sturgeon and fish in the river were reported. A preliminary analysis of algal samples from Caspian sea delta of Ural river up to 100 km was carried out. The collected phytoplankton samples were analyzed for potentially toxic species by light microscopy and by flow imaging cytometry with FlowCam instrument (10x and 20x objectives). As dominant phytoplankton genera, we identified cyanobacteria *Cuspidothrix issatschenkoi*, *Dolichospermum flos-aquae*, *Dolichospermum affine*, *Pseudanabaena limnetica* and diatomea *Fragilaria acus*. High abundances of potentially toxic algae were found in the areas with low circulation rates and increased water temperature such as a mouth of river duct Peretaska. Spectral signatures of phytoplankton communities from these areas confirmed a significant presence of phycocyanin-expressing cyanobacteria. The concentrations of nitrates at these locations reached 4-4.5 mg/liter. The study documented the occurrence of potentially toxigenic phytoplankton species in the water samples associated with places of large fish-kills this year. The finding suggests a potential risk of cyanoHABs in the Ural river near Atyrau.

MORE THAN MICROCYSTINS! INVESTIGATING THE EFFECTS OF TEMPERATURE ON THE GROWTH AND TOXIN PRODUCTION OF SAXITOXIN, ANATOXIN AND CYLINDROSPERMOPSIN-PRODUCING CYANOBACTERIA

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Blooms of cyanobacteria are a growing global issue that influence the health and socioeconomic status of those dependent on impacted water bodies. Variation in water surface temperatures driven by changing global climate has allowed for increases in the growth of toxigenic cyanobacteria. It has been documented that a range of some species have shifted with increasing temperature, causing some strains to be able to invade new aquatic environments. While previous studies have investigated the effects of temperature on microcystin-producing cyanobacteria, in the current study here, we test the effects of temperature by culturing cyanobacterial strains capable of producing either saxitoxins, cylindrospermopsins or anatoxins across a thermal gradient of 17 to 30 °C under common garden light and replete nutrient conditions. The goal is to determine the strain specific optimal temperatures and limits for growth and toxin production in these toxigenic cyanobacteria. We are in the process of using this framework to test several different genera including, *Aphanizomenon*, *Dolichospermum*, *Cylindrospermopsis*, *Anabaena*, and *Scytonema*. By understanding the influence temperature has on cyanobacterial growth and toxin production, we will be able to better forecast peak bloom season and composition, as well as toxicity.

CLIMATE CHANGE INFLUENCES ON CONNECTIONS BETWEEN CIGUATERA FISH POISONING AND CORAL REEFS

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Impacts to coral reefs such as bleaching events and disease outbreaks have been shown to provide new substrate for macroalgae to colonize. It has also been hypothesized that these events could be associated with outbreaks in seafood poisoning epidemics, primarily ciguatera fish poisoning (CFP). Toxigenic species of the epiphytic benthic dinoflagellate genus, *Gambierdiscus*, are considered to play a major role in CFP due to their ability to produce potent neurotoxic compounds called ciguatoxins. Because *Gambierdiscus* cells reside on macroalgae, it becomes imperative to investigate the dynamics of benthic macroalgae and the driving forces impacting coral reef ecosystems. Implications associated with ecological disturbances, such as coral bleaching events, could exacerbate CFP by providing an increase

in suitable habitat for *Gambierdiscus*. A substantial coral bleaching event occurred August-October 2014 in the Florida Keys which coincided with the highest abundances of turf algae and *Gambierdiscus* cell densities recorded over a five year period. This event could be indicative of the long proposed hypothesis that CFP outbreaks are associated with coral reef disturbances. With impending impacts of climate change, potential connections between coral reef bleaching and CFP merit further consideration for future management strategies.

FLOW-THROUGH EXPERIMENTAL APPROACH FOR INVESTIGATING THE EFFECTS OF OCEAN ACIDIFICATION AND WARMING ON *KARENIA BREVIS*

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Florida red tides (*Karenia brevis*) occur nearly annually along the Florida gulf coast and cause numerous adverse effects on the environment, the economy and human health. Prediction of Florida red tides is difficult at present, and in light of a changing climate and environmental conditions, may become more challenging in the future. Increasing atmospheric CO₂ concentrations are causing the oceans to acidify (ocean acidification) and to warm (climate change) which may cause alterations in plankton growth and physiology, and ultimately shifts in phytoplankton composition. Previous studies have shown *K. brevis* sensitivity to changes in pCO₂, but have been limited by short term studies or batch experimental design. Here we attempted to understand how growth rates, rates of photosynthesis, and rates of respiration of a cultured strain of *K. brevis* would be affected by elevated pCO₂ (~1400 µatm) and elevated temperature (29°C) in a multifactorial 10-day study in a flow-thru chemostat system. Chemostats are homogenous, open systems that can allow the influx of treated seawater (high pCO₂) to match the outflow. Ideally, under ambient conditions (27°C, pCO₂~450) the cultures in the chemostat will exhibit a constant growth rate which can be compared to simultaneous experimental conditions, such as elevated pCO₂ and elevated temperature on growth rate and other physiological processes. Preliminary results showed growth rates of the cultured *K. brevis* were reduced in elevated seawater temperature with no effect from pCO₂. Rates of photosynthesis were elevated in the combined high pCO₂ and elevated temperature treatment tanks (end of century predictions) while rates of respiration were reduced in the high temperature treatment tanks. This experimental approach differs from batch or semi-continuous culture studies enabling us to supply a constant feed of elevated pCO₂ and temperature seawater to the cultures which are more representative of real world conditions.

LAKE SEDIMENT CORE ANALYSIS DATES THE EXPANSION OF ANATOXIN-PRODUCING *ANABAENA/DOLICHOSPERMUM* IN ANDERSON LAKE, WA

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Anderson Lake is a 25 ha lake with mean depth of 3.7 m situated in a semi-rural setting. Land adjacent to the lake was developed for agricultural use in the early 1900s until it became a state park in ~ 1970. The lake has suffered cyanobacterial blooms since monitoring began in 2006. Anatoxin-a levels over 100 µg/L have been recorded many times and result in closure of the lake every summer; microcystins are sometimes also detected. *Anabaena/Dolichospermum* sp. WA102 has been identified as the anatoxin-a producer (Brown et al., 2016). In order to determine when cyanobacteria and anatoxin-a production became established in Anderson Lake, we analyzed a 73-cm long sediment core sample taken from the deepest point of the lake, spanning the period 2018 to the early 1700's. Pigment analysis by HPLC indicated that cyanobacteria have been present since the 1700's, but the highest flux occurred during the farming years. On the other hand, droplet-digital QPCR (ddPCR) analysis of DNA extracted from core slices showed a steep increase in cyanobacterial 16S rDNA after about 1970. Genes for anatoxin-a (anaF) and microcystin (mcyE) synthesis were detected by ddPCR, from the 1980 core slice in the case of anaF and at a lower level and since 1987 for mcyE. Based on the ratio of anaF to cyanobacterial 16S gene copies, the representation of anatoxin-a biosynthetic capacity increased since 1995, intensifying since 2000. This increase was also detected by 16S rDNA amplicon deep sequencing detection of *Anabaena/Dolichospermum* WA102, the extant anatoxin-a producer. Metagenome analysis of the 1995 core slice detected multiple reads corresponding to this genome, showing that *Anabaena/Dolichospermum* WA102 has been present in Anderson Lake since 1995.

THE INTERACTION OF NITROGEN ENRICHMENT AND PREVIOUS, CURRENT, AND FUTURE CLIMATE SCENARIOS ON THE BIOLOGICAL STOICHIOMETRY OF *MICROCYSTIS AERUGINOSA* (UTEX 2385)

Lauren Ferguson, Anu Kannan, Ashley Ricciardelli, Christopher Sharon, Jonathan Deleon, Emily Quach, Sandi W. Nuang, Ashley Allen, Seth Buscho, Grace Phillip, Berkeley Sheppard, Jacquelyn Duke, J. T Scott

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Many cyanobacterial toxins are nitrogen-rich biochemicals that act as hepatotoxins or neurotoxins to mammals. The concentrations of these toxins are often correlated with the total N concentration or the N:P in surface waters. In addition to warming the planet, climate change is also increasing the availability of carbon dioxide dissolved in water when it is in equilibrium with the atmosphere. Because temperature, CO₂, and N availability often interact to control phytoplankton growth, cyanobacterial blooms may become more prominent in future climate scenarios, especially if it coincides with N enrichment. *Microcystis aeruginosa* (UTEX 2385) produces microcystin-LR, a N-rich polypeptide with a carbon:N ratio of less than 5 that previous research has shown to be strongly related to N supply and the biological stoichiometry (C:N ratio and N:P ratio) of *M. aeruginosa*. We conducted a hierarchical experiment to test the effect of CO₂, temperature, and N availability on the biological stoichiometry of *M. aeruginosa* in experimental blooms conditions. We will present the preliminary results of this ongoing experiment aimed at testing both the immediate environmental controls on *M. aeruginosa* stoichiometry, and the potential adaptation of this species to prolonged exposure to these conditions.

FRESHWATER HARMFUL ALGAL BLOOMS IN VIRGINIA: EXPANDING MONITORING AND MANAGEMENT EFFORTS TO MEET EMERGING NEEDS

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The Virginia Harmful Algal Bloom Task Force has been coordinating harmful algal bloom responses in The Commonwealth of Virginia for 20 years. While marine and estuarine blooms continue to occur, the largest increase in reported algal blooms in the last five years are freshwater cyanobacteria events. Recreational advisories related to *Microcystis* and microcystin concentrations in Virginia date back to 2004, with more recent advisories based on a growing number of toxigenic filamentous species and toxins. Significant recent multispecies blooms of *Cuspidothrix*, *Dolichospermum*, *Planktothrix*, *Raphidiopsis* and others represent growing technical and management difficulties. Analyses have included taxonomic enumerations using microscopy, microcystins and cylindrospermopsin assays by ELISA, and microcystins and cylindrospermopsin assays by fluorescence planar waveguide biosensor. These efforts have resulted in a growing dataset of regional species compositions and toxin concentrations. In addition to laboratory methods, expansions to management have reflected recent federal advisory thresholds and increased outreach and educational components including an online bloom reporting tool and an interactive map of ongoing blooms and advisories in the state.

CHARACTERIZING HARMFUL ALGAL BLOOMS (HABS) USING UNMANNED SYSTEMS IN THREE FRESHWATER LAKES IN THE US

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New technologies with quick turn-around times are needed to predict, track, and mitigate harmful algal blooms (HABs). Cyanobacteria in aquatic environments may produce cyanotoxins that can affect the nervous and respiratory systems of domestic animals and humans. Little is known about the transport of HABs in the air and water. Water samples were collected at Grand Lake Saint Mary's (OH), Lake Erie (OH), and Lake Anna (VA) during August and September, 2019. We collected 180 samples using a DrOne Water Sampling SystEm (DOWSE). The DOWSE consisted of 3D-printed sampling device tethered to a drone, and was used to collect surface water samples at different distances from the shore (10 to 100 m). Aerosol samples were collected with a new DROne Aerosol Monitoring System (DROAMS) to characterize aerosols over two HABs. The DROAMS consisted of an impinging device and an optical particle counter mounted above a large commercial quadcopter. A remotely operated vehicle (ROV) was equipped with a fluorometer and an underwater GPS system to quantify the extent of the HAB underwater (concentration, time, latitude, longitude, and depth). Coordinated unmanned systems may be used to detect, track, and assist in mitigating HABs in aquatic systems.

THE INTERACTION OF NITROGEN ENRICHMENT AND PREVIOUS, CURRENT, AND FUTURE CLIMATE SCENARIOS ON MICROCYSTIN-LR PRODUCTION BY *MICROCYSTIS AERUGINOSA* (UTEX 2385)

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Many cyanobacterial toxins are nitrogen-rich biochemicals that act as hepatotoxins or neurotoxins to mammals. The concentrations of these toxins are often correlated with the total N concentration or the N:P in surface waters. In addition to warming the planet, climate change is also increasing the availability of carbon dioxide dissolved in water when it is in equilibrium with the atmosphere. Because temperature, CO₂, and N availability often interact to control phytoplankton growth, cyanobacterial blooms may become more prominent in future climate scenarios, especially if it coincides with N enrichment. *Microcystis aeruginosa* (UTEX 2385) produces microcystin-LR, a N-rich polypeptide with a carbon:N ratio of less than 5 that previous research has shown to be strongly related to N supply and the biological stoichiometry (C:N ratio and N:P ratio) of *M. aeruginosa*. We conducted a hierarchical experiment to test the effect of CO₂, temperature, and N availability on the microcystin-LR production of *M. aeruginosa* in experimental blooms conditions. We will present the preliminary results of this ongoing experiments aimed at testing both the immediate environmental controls on *M. aeruginosa* microcystin-LR production, and the potential adaptation of this species to prolonged exposure to these conditions.

CIGUATERA AND HERBIVORY: GRAZING DYNAMICS OF THE PINFISH (*LAGODON RHOMBOIDES*) ON *THALASSIA TESTUDINUM* AND *HALIMEDA INCRASSATA* IN THE FLORIDA KEYS

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Ciguatera Fish Poisoning is a form of food poisoning in humans who consume tropical reef fish that have accumulated high levels of ciguatoxins. Ciguatoxins are naturally produced lipid-soluble toxins produced by the benthic dinoflagellate, *Gambierdiscus*, which enter reef food webs through the consumption of these epiphytic cells by herbivorous fishes. The objective of this experiment was to understand how ciguatoxins are introduced to the marine food web through grazing. Heine Grass Bed in the Florida Keys was chosen for this experiment because it is an

ongoing study site from current ciguatera research and is known to harbor toxic *Gambierdiscus* spp. Additionally, seagrass beds are a nursery ground for many juvenile commercially important reef fish species making them highly susceptible to ciguatoxin accumulation. This experiment was designed to examine the grazing dynamics of the pinfish (*Lagodon rhomboides*) on *Thalassia testudinum* and *Halimeda incrassata* under multiple temperature treatments. Results of the study will be used to: 1) quantify grazing rates for input into a ciguatoxin flux model; 2) determine if pinfish prefer grazing upon one substrate versus the other; and 3) examine the influence of temperature on grazing rates and preference.

MONITORING & MANAGEMENT

IMPACTS OF ENVIRONMENTAL CHANGES ON PHYTOPLANKTON BIODIVERSITY IN DESTIN, FL DURING SUMMER 2019

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Harmful algal blooms (HABs) are becoming increasingly more common as temperatures rise around the globe and fertilizer runoff fuels their growth. As a result, learning to forecast these blooms is essential to protect our coastlines and water sources. Additionally, determining the cause of HABs may give researchers an insight into controlling the spread of these dangerous phytoplankton. We investigated the relationship between Gulf Coast phytoplankton biodiversity, HAB abundance, and changes in environmental factors such as salinity, air temperature, water temperature, air pressure, humidity, light intensity, and weather. Environmental factors in Destin, FL were monitored between June 1st, 2019 and August 24th, 2019. Water samples were taken biweekly from the Destin Harbor, preserved with Lugol's, and observed under a microscope in the lab. A *Pseudo-nitzschia* bloom was recorded in late June along with high concentrations of *Chaetoceros*. High concentrations of *Ditylum* and *Coscinodiscus* were also recorded later in the study. We are still analyzing our data, but preliminary results indicate that *Chaetoceros* and *Pseudo-nitzschia* abundance are positively correlated to salinity. The data is currently being analyzed with various statistical software programs and biodiversity calculators such as the AI Young Biodiversity Calculator and The R Project.

THE SUMMER OF 2019: OVERVIEW OF HARMFUL ALGAL BLOOM THAT AFFECTED MISSISSIPPI'S BEACHES

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The Mississippi Department of Environmental Quality (MDEQ) through its Beach Monitoring Program documented an algal bloom with the potential to become harmful on June 21, 2019. Salinities in the western Mississippi Sound had dropped to low levels---< 3 ppt beginning in March 2019--- due to recent heavy rains, and the opening of the Bonnet Carre' Spillway for an unprecedented second time in 2019. A *Dolichospermum* bloom was discovered offshore in mid-June by the Mississippi Department of Marine Resources (MDMR) which notified MDEQ as this bloom moved nearshore, and it was confirmed microscopically in the nearshore environment where it would come into contact with bathers on June 21, 2019 in Hancock County, in western MS. This resulted in a "Water Contact Warning" being issued the next day for Hancock County. By June 24, 2019 both *Microcystis* and *Dolichospermum* were detected in Beach Monitoring Program samples. Within 2-3 days, the bloom consisted only of *Microcystis*. The MDEQ staff, with much assistance from the HAB Group at the Mississippi Department of Marine Resources (MDMR) began intensively sampling and identifying samples collected from all Beach Monitoring Program sites on a daily basis. By July 7, a water contact warning had been issued for all MS beaches. After Tropical Storm Barry came ashore on July 13, 2019, dumping large amounts of freshwater over the area. By July 18 another *Dolichospermum* bloom was noted in Hancock County. Again, the *Dolichospermum* bloom persisted for only 2-3 days after which the bloom was exclusively *Microcystis*. The sampling continues daily across the Mississippi Gulf Coastal beaches. The *Microcystis* bloom that began on June 21, 2019 has persisted throughout the summer and continues to this date all along the MS Gulf Coast, and has been noted in salinities ranging from 0-29 ppt.

WASHINGTON DEPARTMENT OF FISH AND WILDLIFE: MONITORING, RESPONSE, AND MANAGEMENT TO APPARENT INCREASE IN DOMOIC ACID ALONG THE WASHINGTON COAST

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The Washington Department of Fish and Wildlife monitors harmful algal blooms (HAB) along the Washington Coast in collaboration with the Olympic Region Harmful Algal Bloom (ORHAB) partnership. ORHAB is composed of researchers, oceanographers, phytoplankton experts, state and tribal fishery managers, and human health experts. ORHAB is focused on building cost effective early warning capabilities for HABs along the Washington Coast. To do so, a series of “action levels” for cell counts of *Pseudo-nitzschia* spp. were established to trigger event response when partners began monitoring nearshore waters in 2000. Event response includes analyzing seawater for particulate DA (pDA) concentration using the Enzyme-linked Immunosorbent Assay (ELISA) method. If an elevated level of pDA is detected additional seawater and tissue samples are collected and tested for DA using ELISA. Results from the additional tests are communicated to the partners. Management actions including additional tissue toxin testing by the Department of Health may also be taken. One of the most recent changes to the ORHAB monitoring program was to decrease the *Pseudo-nitzschia* spp. large cell type action level from 50,000 cells/L to 30,000 cells/L. This change was in response to the 2017 bloom that produced enough DA at less than 30,000 cells/L to close the razor clam fishery within two weeks of its first detection. Since then, *Pseudo-nitzschia* spp. have occurred as early as February and as late as December often resulting in an increase of DA in razor clams along the coast. Particularly when the *Pseudo-nitzschia australis* subtype is present, even at low abundance pDA levels can be elevated enough to result in a quick uptick of DA in razor clam tissue. Adjusting this action level will allow for earlier detection of DA in razor clams and for a safer and more efficient management of the fishery.

DETERMINING THE TRENDS AND DRIVERS OF CYANOHABS IN CLEAR LAKE

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Harmful cyanobacteria blooms (cyanoHABs) have gained international attention over the past decade due to the increased frequency and severity of blooms, including in California, where many water bodies have experienced unprecedented issues from cyanoHABs. Clear Lake, the largest freshwater lake in California and a famous bass fishing location, is a cyanobacterial hotspot. Clear Lake has a decades long history of observational reports of blooms and large surface-scum forming cyanoHABs, which have occurred every summer at least since 2009. These events have impaired key beneficial uses within the lake including impacts on tribal traditions and cultural uses, recreational activities, and drinking water. The lake has been listed on the 303d list of impaired waterbodies since 1986 and has a decades long database of water quality measurements. However, cyanoHAB monitoring has only been occurring formally since 2014 when Big Valley Band of Pomo Indians and Elem Indian Colony initiated a collaborative, formal program. Since 2014, maximal yearly microcystin levels detected in the lake have varied across five orders of magnitude, with maximal toxin concentrations exceeding California recreational trigger levels for microcystins in five out of the six years of cyanoHAB monitoring. Here we will discuss the trends and potential drivers of cyanoHAB events in Clear Lake. Additionally, we will assess a suite of parameters that might be informative for developing stress response models that could be used to develop simple risk assessments relationships for toxin concentrations based on basic water quality parameters.

PROTECTING PUBLIC HEALTH THROUGH CITIZEN SCIENCE: VALIDATION OF NEAR/REAL TIME QUALITATIVE REPORTING OF HAB EFFECTS

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Neurotoxins from *Karenia brevis* blooms threaten aquatic and human health, recreation, tourism, and economy. Informing the public of beach conditions is an effective strategy to protect beachgoers by encouraging visits to beaches not experiencing effects. Mote's Beach Conditions Reporting System (BCRS) is a citizen science website and smartphone application which has attracted over 1 million users and over 4 million page views since it's redevelopment in 2015 (due to various efforts which will be discussed in this presentation). Reports are made by trained beach sentinels twice daily and include parameters of respiratory irritation, dead fish, water discoloration, winds, surf and other sought information when choosing a Florida beach. This study analyzed nine years of historical BCRS reports (supported by citizen reports) for correlation with environmental data to determine the reliability of citizen science qualitative reporting. Sarasota Healthy Beaches Program water sample data (water chemistry, cell concentration and brevetoxins) as well as wind speed and direction, were statistically modeled to determine parameters (or combination of parameters) most likely to be associated with respiratory irritation. To support these data, results from brevetoxin aerosol studies in Sarasota and Pinellas counties were also included in analysis. Aerosol transport algorithms were developed using particle size distribution, fluid dynamic modeling, and ArcGIS to support BCRS correlation statistics. The strongest correlation was determined to be that of PBTx-3 and respiratory irritation (0.82). PBTx-3 is the brevetoxin most likely to aerosolize and impact respiratory health. This information gives insight to the validity of citizen science, and also may give insight to stakeholders when determining budget allocations for monitoring technologies focused on protecting public health. To overcome brevetoxin sensor obstacles of sparse data collection and cost, an expansion of the BCRS proposes to develop sensors for deployment on citizen science vessels.

USING LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LCMS) TO DETECT AND QUANTIFY MICROCYSTINS IN THE HERBIVOROUS ZOOPLANKTER *DAPHNIA PULEX*

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Microcystins are hepatotoxins commonly produced by the cyanobacterium *Microcystis*, one of the most common genera of bloom-forming harmful algae in fresh waters. As cyanoHABs increase in frequency and intensity around the world, it becomes more important to effectively monitor and quantify their toxins, especially in food webs. Yet measuring the microcystin in an organism is problematic for two reasons. First, there are over 250 congeners of microcystin, and even the best commercial detection kits can only measure about five. Second, these toxins covalently bind to tissue, rendering commercial detection kits ineffective. Using LCMS circumvents these problems. The fragment of the microcystin molecule shared by most congeners can be identified and quantified, even after covalent binding occurs. Methods have been developed to examine the microcystin in fish and plant tissue, but not zooplankton. Here, I will present initial findings on the viability of using LCMS to detect and quantify microcystins in zooplankton, and on whether microcystins accumulate in *D. pulex* tissue.

A TIERED APPROACH TO ROUTINE CYANOBACTERIA MONITORING: FROM CELLS TO TOXINS

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Harmful algal blooms (HABs) have increased in both frequency and intensity in recent years and directly impact US Army Corps of Engineers projects including: locks, dams, lakes, reservoirs, and navigation waterways. Protecting these resources requires novel approaches for HAB control and mitigation, which first starts with advancing our ability to rapidly and accurately detect low-levels of these problematic organisms. Of the conventional monitoring methods, microscopic identification is the classic approach to identifying the bloom species but is costly and time intensive. Pigment analysis provides a rapid estimate of bulk biomass but neglects to provide information on the overall genera

present. Furthermore, enzyme linked immunosorbent assays (ELISAs) for cyanobacterial toxins have a limited dynamic range and are also time and cost intensive. Our research group aims to provide monitoring strategies for management, providing rapid turn-around to give resource managers time to implement an appropriate management plan. We therefore use molecular approaches to streamline water quality monitoring to save time and money and detect low copy numbers of cyanobacteria and their associated toxin genes. Our pipeline is as follows: upon sample receipt deoxyribonucleic acids (DNA) is extracted and broad cyanobacteria 16S rRNA-targeted polymerase chain reaction (PCR) is conducted for presence/absence. If positive, the following steps are implemented: 1) quantitative real-time PCRs (qPCRs) for microcystins/nodularins, cylindrospermopsin, and saxitoxins using the PhytoXigene CyanoDTec kit along with PCR for anatoxin-a and 2) 16S rRNA genus-level PCR screening for *Microcystis*, *Dolichospermum*, *Oscillatoria*, *Nodularia*, and *Aphanizomenon*. If samples are positive for cyanotoxin genes, targeted ELISAs are conducted. This tiered monitoring approach can provide lower detection limits and faster turn-around times at a lower cost than conventional broad-scale monitoring strategies. Additionally, rapid genus-level information is often necessary to identify appropriate management strategies, as it is unlikely that one strategy will work for all cyanobacteria.

EFFECT OF PREVENTATIVE DOSING OF ALGICIDE IRI-160AA ON NON-TARGET MICROBIAL COMMUNITIES

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Bloom-associated bacteria have the potential to function as biological controls for harmful dinoflagellate species present in blooms. IRI-160AA is an algicide produced by *Shewanella* sp. IRI-160 and is being investigated as a means to prevent or mitigate harmful dinoflagellate blooms. Prior work showed that application of the algicide during dinoflagellate blooms resulted in a significant restructuring of the eukaryotic community, including a decrease in bloom-forming dinoflagellate abundance. For this project, two experiments were conducted to determine the effects of algicide IRI-160AA application as a preventative on non-target microbial communities when dinoflagellates are not the dominant species. In Experiment 1, changes in both eukaryotic and prokaryotic community structure were evaluated after application of the algicide using molecular fingerprinting methods, T-RFLP and ARISA. Results of Experiment 1 demonstrated that algicide dosing caused a shift in both eukaryotic and prokaryotic microbial communities over time compared to controls. To determine if effects on the prokaryotic community were due to the algicide application alone or were the result of a shift in the eukaryotic community, a second experiment was performed in which eukaryotes were filtered out before adding the algicide. Results of Experiment 2 showed that the algicide alone was responsible, at least in part, for the shift in prokaryotic community structure. This project demonstrates that application of the algicide IRI-160AA as a preventative will affect non-target eukaryotic and prokaryotic members of the microbial community, and that impacts on bacterioplankton may be independent of impacts on the eukaryotic population.

STAKEHOLDER ENGAGEMENT SHAPES HAB PRODUCT DEVELOPMENT

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Harmful algal bloom (HAB) operational forecast products from the National Oceanic and Atmospheric Administration (NOAA) are decision support tools driven by stakeholder needs. Understanding the decisions our partners make to mitigate economic and environmental impacts from HABs are crucial to creating useful products. The key to enhancing this product relies on gathering input and feedback from essential decision-makers and stakeholders. Using an agile approach to development promotes regular communication with stakeholders to ensure their requirements are implemented into robust operational products. This process reinforces the R2O2R feedback loop (i.e. Research to Operations (or Applications), then Research again) to bring established HAB research directly to end-users. Continuously integrating scientifically accurate products creates a resilient network of evidence-based, oceanographic information -- specifically designed for those who need it. Engaging our partners during the development and transition phases results in steady improvements to product look and delivery, and helps prioritize the most important data sets needed for better forecasts. For example, the request for higher resolution satellite imagery was met through integrating Sentinel-3 imagery as soon as operationally available.

NOAA launched operational Lake Erie HAB forecasts in 2017, which provides satellite imagery, bloom analysis, and transport forecasts to more than 4,000 subscribers. The recent integration of the HAB Tracker 3D hydrodynamic model into the Lake Erie operational product suite demonstrates the value of agile development and the R2O2R feedback loop. Product usefulness has increased by modeling projected cyanobacterial density at the surface and at depth. Winds mix toxic surface algae into the water column, alerting communities of the threat to municipal water supplies. A dashboard has been created to highlight the HAB Tracker's model projections, lake conditions, true color and chlorophyll index satellite imagery, and vertical mixing profiles for all sample locations.

COMMUNITY BASED PSP TESTING FOR SUBSISTENCE AND RECREATIONAL SHELLFISH HARVESTING IN SOUTHWESTERN ALASKA

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Despite high risks of paralytic shellfish poisoning (PSP) to subsistence harvesters in southwest Alaska, the State currently has no capacity for routine testing of non-commercially harvested shellfish. This project aims to provide tools to inform local shellfish harvesting decisions through community-based toxin monitoring and development of new PSP testing methods. Five communities in Kodiak (Old Harbor, Ouzinkie, Kodiak) and the Aleutians (Sand Point, King Cove) participated with monthly butter clam sampling. Toxin testing was performed by high performance liquid chromatography at the NOAA Beaufort Laboratory, with comparative testing by the Alaska Dept. of Environmental Conservation. Results through Year 2 of the study showed clam toxicity in Old Harbor reached high levels in the spring/summer, which coincided with seasonal water temperatures and mirrored patterns at the Aleutian sites. In contrast, eastern Kodiak sites exhibited lower toxicities with a more irregular seasonal pattern and more frequent toxicity during winter months. An improved enzyme-linked immunoassay (ELISA) for PSP was developed with greater sensitivity toward gonyautoxins (GTXs), congeners largely undetected by other ELISAs currently available, but that sometimes accounted for 70-80% of shellfish toxicity. To address community interest about butter clam preparation methods, we also examined the distribution of toxins in butter clam tissues and how removal of these tissues affected PSP risk. Results indicated congeners saxitoxin (STX) and neosaxitoxin (neoSTX) were often concentrated in the clam siphons during the winter, accounting for >50% of the toxicity in individual clams. During spring *Alexandrium* blooms, however, GTXs in clam digestive tissues could account for most of the toxicity. Removal of these tissues sometimes yielded a substantial decrease in overall toxicity, but variability was too high to provide a reliable margin of safety for consumers. Non-commercial butter clam harvesters are cautioned not to rely upon traditional preparation methods alone to reduce PSP risk.

THE ALASKA HARMFUL ALGAL BLOOM NETWORK - AN INTRODUCTION

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Harmful algal blooms (HABs) are not new to Alaska and include both toxic and non-toxic phytoplankton. The most well-known and destructive HABs in Alaska include the toxic dinoflagellate *Alexandrium*, that produces paralytic shellfish toxins, and the toxic diatom *Pseudo-nitzschia*, that can produce amnesic shellfish toxins (or domoic acid) under certain environmental conditions. Paralytic shellfish toxins have been well documented in shellfish and have killed or sickened people in Alaska through consumption of untested contaminated shellfish. While long suspected to impact Alaska marine mammals and seabirds, both of these toxins have only recently been confirmed present in these upper trophic level species. Although the causes of harmful algal blooms are not well understood, climate change is likely to increase the threat of HABs in Alaska, resulting in increases in HAB duration, intensity and regional expansion. The Alaska Harmful Algal Bloom Network (AHAB) was formed in 2017 to provide a statewide approach to HAB awareness, research, monitoring and response in Alaska. AHAB coordinates a diverse group of coastal stakeholders to address human and wildlife health risks from toxic algal blooms. Objectives of the group include reducing health risks due to HABs and facilitating safe supply of seafood, improving effectiveness of HAB response

and HAB education and outreach, expanding HAB monitoring and developing forecasting capabilities, and identifying needs and data gaps related to HABs. The network is a partnership of regional stakeholders dedicated to this mission. The group is co-sponsored by Alaska Sea Grant and the Alaska Ocean Observing System. The website <http://www.aos.org/alaska-hab-network> was formally launched in January 2018 to share information statewide, describe current monitoring and research, provide real time results and provide access to statewide experts and resources. This poster shares the objectives of the network and introduces the content of the AHAB website.

UNTARGETED HIGH RESOLUTION MASS SPECTROMETRY APPROACHES FOR IDENTIFYING NOVEL MICROCYSTINS

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Microcystins (MCs) are a group of hepatotoxic heptapeptides that inhibit protein phosphatases, and are produced by many species of cyanobacteria. Most MCs contain the unusual β -amino acid “Adda5”, γ -linked D-Glu6, and N-methyldehydroalanine (Mdha7) at positions 5–7. There are over 250 unique microcystin congeners reported in the literature, and new ones are regularly being discovered. In addition to methods capable of measuring total MCs or targeting known congeners, improved methods are needed for identifying new candidate MCs, to ensure the safety of recreational and drinking waters. Untargeted high resolution mass spectrometry (HRMS) methods allow for simultaneous analysis of a tens of thousands of known and unknown chemicals in complex biological or environmental samples. Typically, identification of novel MCs by HRMS involves only analysis in positive ionization mode with detection of a characteristic fragment from Adda5 at m/z 135.0804. However, some MCs contain structural modifications that change their fragmentation and are not readily detected in this manner. We have recently shown that thiol-derivatization of the Mdha7/Dha7 group in MCs is an effective tool for identifying even trace amounts of novel MCs in complex samples by LC–MS. Here, we present a new approach using metabolomics software for semi-automated detection of novel MCs based on mercaptoethanol derivatization and accurate mass detection of precursor and characteristic product ions in negative and positive ionization modes. Taken together, this approach targets any molecules containing Adda5, D-Glu6 or Mdha7/Dha7, one or more of which is present in every microcystin reported to date. Furthermore, it is these three residues that are in closest contact with the catalytic centre of protein phosphatases, and which appear to be most important for toxicity. We demonstrate the power of the combination of these novel approaches with the identification of numerous novel MCs in laboratory culture of a *Microcystis* sp.

MAKING SENSE OF SENSORS: HOW NJDEP IS USING VARIOUS TOOLS TO MONITOR AND TRACK BLOOMS IN IMPACTED AND AT-RISK WATER BODIES

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The use of continuous real time, handheld, and multi- or hyper-spectral data sensors to monitor impacted water is still an evolving tool that is quickly becoming adopted into “response kits” for blooms. While these sensors can provide a wealth of data they are not without drawbacks. New Jersey experienced multiple large high-profile bloom events which impacted the use of several water bodies for extended periods of time during the 2019 season. The use of various in field, continuous real time, and over flight data collectors to monitor and assess these events were employed as part of our response. Many of these sensors measure the cyanobacteria specific pigment phycocyanin, or more broadly chlorophyll-a. Their reporting units can be either relative fluorescence units (RFU) or a standard concentration to volume – but making sense of those values and how they possibly track back to a cell concentration is important for those agencies which have cell count response thresholds. The NJDEP conducted work to draw a correlation between cell count values and extractable phycocyanin to establish a calibration curve for field and flight meters. Handheld unit sensors appear to be weakly correlated ($\sim r^2=0.5$) to cell counts; whereas real-time data buoys appeared to have a higher correlation ($\sim r^2=0.9$). Here we present the various sensor data that was collected and the interpretations we’ve made from the data. We also present the use of fixed wing hyperspectral sensor data to broadly monitor bloom status in a number of inland lakes. We ultimately believe the use of these various sensors were valuable during a bloom response, not only for determining severity; but for delineating bloom extent and for optimizing resources and sampling collections. The continued use of these sensors and their potential interpretation for sampling triggering will be continued to refined in the upcoming bloom season.

EXPANDING THE HORIZON! ANALYSIS OF CYANOBACTERIAL TOXINS BY UPLC/MS/MS DETECTION USING A UNISPRAY ION SOURCE

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Analysis of toxins, including microcystins and other compounds, using Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) has been shown to be a powerful tool in monitoring. What is especially attractive about LC/MS/MS is its sensitivity and selectivity of the technique. Typically, electrospray ionization (ESI) is the mode of ionization used for these compounds. In this poster, we investigate the use of a new and novel ion source (called UniSpray) which allows for enhanced sensitivity of these compounds (as well as a variety of other compounds). This source allows for greater ionization efficiency as well as providing a rugged interface. Comparison of microcystins, anatoxin-a, and cylindrospermopsin in various matrices to traditional ESI and Unispray ionization will be shown. Data from various toxin blooms in the United States will be shown and discussed using both techniques.

SOCIAL AND POLITICAL ASPECTS OF HARMFUL ALGAL BLOOMS

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Significant research exists on the formation, spread, and mitigation of harmful algal blooms (HABs) but not as much research is available on the social and political relations to all the aforementioned processes of HABs. Some of the social research on HABs considers the impacts of HABs on tourism or human health but there is not as much research on the policy arena governing HABs and how this policy environment also interacts with societal perceptions of HABs. Through a review of research related to the policies governing HABs, we map out the HAB policy landscape in the state of Florida and illustrate how this landscape can unwittingly contribute to further increasing HABs within the state. We conclude with some recommendations for organizational restructuring which might help ameliorate the HAB phenomenon in the state of Florida, and possibly the rest of the United States.

COASTAL LAKES OBSERVING NETWORK (CLONET): A PARTICIPATORY CITIZEN SCIENCE NETWORK TO UNDERSTAND LAKE WATER QUALITY AND HARMFUL ALGAL BLOOMS

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The coastal lakes of Monmouth County, NJ historically provided a variety of recreational opportunities and served as important fish and wildlife habitats. However, decades of intensive development and human activity in the watersheds of these lakes have impacted their ecology and resulted in impaired recreational uses and aesthetics, including degraded water quality and harmful algal blooms. Additionally, HABs result in regulatory actions by state and county officials, limiting lake use and resulting in some economic disruption. Although work to manage and restore coastal lakes has proceeded over several decades, these efforts lacked leadership that would connect efforts across the lakes. The Coastal Lakes Observing Network (CLONet) was created to bring together community members to participate in lake monitoring and research, and to improve communication among lake groups and university / state researchers. In March 2019, community leaders associated with various coastal lake advocacy groups, as well as state and county environmental officials, attended a Coastal Lake Summit hosted by Monmouth University where CLONet was introduced. Over the following summer Monmouth University faculty and students trained groups from seven lakes (Lake Takanassee, Lake Como, Deal Lake, Spring Lake, Sunset Lake, and Wesley Lake) in basic water quality sampling, including Secchi depth, conductivity, water temperature, pH, dissolved oxygen, ammonia, phosphate, and nitrate. Groups sampled weekly and data were uploaded to an online database developed for this project. Accompanying weekly measurements of HAB abundance and water quality were made in the same (and three additional) lakes by Monmouth University and NJ DEP. Community water quality data showed distinct patterns

among different lakes sampled, and this corresponded with Monmouth and DEP measurements. HAB abundance varied widely among lakes, although two lakes experienced significant blooms. CLONet participants will reconvene to refine the program, interpret findings and plan for future sampling over upcoming seasons.

IMPACTS OF *MARGALEFIDINIUM POLYKRIKOIDES* AND *ALEXANDRIUM MONILATUM* ON OYSTERS CULTURED IN LOWER CHESAPEAKE BAY

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Harmful algal blooms (HABs) and their impacts are expanding globally. In the lower Chesapeake Bay, two dinoflagellate species, *Margalefidinium polykrioides* and *Alexandrium monilatum*, form extensive, sequential blooms most summers. Previous laboratory studies have demonstrated mortality and tissue damage in oysters exposed to both species. However, impacts of sequential exposure to the two species on oysters in the natural environment are less well understood, particularly in the context of oyster aquaculture. To address this question, an oyster monitoring study was conducted from summer to early fall in 2017 and 2018 in the York River-Mobjack Bay area within the lower Chesapeake Bay. In 2017, oysters were deployed inter-tidally and sub-tidally on-bottom at a high-energy HAB-endemic site, a low-energy HAB-endemic site, and an intermediate-energy reference site where the two HAB species do not typically occur. In 2018, an additional site was added, an intermediate-energy HAB-endemic site, where oysters were also grown in floating cages. Throughout summers 2017 and 2018, water quality was measured continuously. Cell concentrations of *M. polykrioides* and *A. monilatum*, and multiple metrics of oyster health and survival, were also monitored regularly. Modest blooms of the two species were detected in 2017, and blooms of neither species were detected at any of the study sites in 2018. In 2018, salinity was lower than the long-term annual average. In both summers, oyster health and survival were more related to planting location and water quality factors not directly related to HABs. Higher mortality was associated with intertidal planting versus subtidal planting. In a more HAB-intensive year, it is possible intertidal planting might compound with stress associated with HAB exposure. However, additional years of data are likely necessary to fully investigate the impacts of sequential exposure to *M. polykrioides* and *A. monilatum* on oysters cultured in the lower Chesapeake Bay.

USING HANDHELD QPCR TECHNOLOGY FOR ON-SITE DETECTION OF HAB SPECIES

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Advancements in mobile platforms continue to improve our ability to rapidly and specifically identify HAB species in varying environments in support of stakeholder needs. One such device, the Liberty16 (Ubiquitome; New Zealand), is based on qPCR technology and supports both SyBr-based and more specific probe assays (e.g. Taqman). Features such as a 16-well sample capacity, 6 hour battery charge, and simplistic Bluetooth supported iPhone app allow for flexibility in sampling strategies and locations. During 2019, we performed preliminary deployments in support of ongoing routine monitoring efforts of HABs at the Monterey Municipal Wharf (Monterey Bay, CA) and the Monterey Bay Aquarium, which relies on raw seawater for some exhibits. These field efforts were used to develop an initial pipeline for DNA extraction, towards a 'quick and dirty' approach for determining qualitative presence of target species (e.g., *Pseudo-nitzschia*, *Cochlodinium*, *Dinophysis*). The process allowed for rapid reporting of results to the Aquarium in monitoring for the threat of HAB events, and assessing their current filtration methodologies to mitigate potential impacts.

CYANOBACTERIA BLOOMS IN RECREATIONAL WATERS OF VIRGINIA: ANALYTICAL CHALLENGES AND MANAGEMENT

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Cyanobacteria blooms have occurred in 2018 and 2019 on Lake Anna, located in central Virginia, resulting in swimming advisories in excess of 4 months per season. In addition, a growing number of blooms in other public and private water bodies across the state have been reported, requiring an increasing amount of response, management, and guidance. The Virginia Harmful Algal Bloom Task force utilizes a hybrid approach for implementing HAB advisories; including microcystin and cylindrospermopsin concentrations when in exceedance of the May 2019 EPA criteria (8 and 15 ppb respectively), and when cell densities of *Microcystis* species exceed 40,000 cells/mL or, the total of potentially toxigenic cyanobacteria densities are greater than 100,000 cells/mL. While densities of cyanobacteria in Lake Anna, including *Aphanizomenon*, *Cuspidothrix*, *Dolichospermum*, *Microcystis*, *Raphidiopsis* and others, have varied by 4 orders of magnitude, with highest densities >106 cells/mL, toxin concentrations for microcystin and cylindrospermopsin have generally remained at either just at or below detectable levels (0.15 and 0.05ppb). In addition, prior to the start of the 2019 HAB season, the VA Task Force revised the advisory approach to simplify advisories and public messaging by eliminating a mid-level advisory which was issued when cyanobacteria densities were 20,000-100,000 cells/mL. A comparative analysis of the resulting advisory duration of the two management approaches is included along with a comparison of environmental parameters that varied over the 2-year period. An ongoing challenge for monitoring and management decisions associated with a cell-density approach is building consensus on the genera that should be included as potentially toxigenic. Development of health advisory criteria for neurotoxins (anatoxin-a and saxitoxin) are also needed to inform recreational contact advisories where the species capable of producing them are found to be present.

OVERVIEW OF THE KANSAS HARMFUL ALGAL BLOOM RESPONSE PROGRAM

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Since 2010, Kansas Department of Health and Environment has operated a response-based monitoring program for Harmful Algal Blooms. As an agency that serves both environmental and human health priorities under one roof, we deal with many facets of HABs: health concerns, water supply protection, evolving science and regulation, unpredictability of blooms, bloom prevention and mitigation, watershed management, and public education, as well as inter-agency coordination. In 2010, Kansas had nine lakes on advisory; from 2011 to 2018, the average was just over 22 per year, with a range of 16 to 33. As of Sep 1, 2019, there are 35 on advisory. The first advisory thresholds were founded on World Health Organization's recommendations for cell count and microcystin levels in recreational waters. Over time, these have been adjusted based on regional science as well as national and international guidelines. Each year, we synthesize data, incorporate new research findings and regulatory guidance, and incorporate stakeholder concerns to update our approach. The product of this work is a dynamic document, KDHE's "Harmful Algal Bloom Response Plan." As blooms have increased in frequency and duration, we have rebalanced available resources to meet the challenges. In 2019, KDHE also established a voluntary pilot program for Public Water Supplies to monitor cyanotoxins in raw and finished water, an initiative that so far has attracted about 1/3 of all surface water producers in the state. We maintain close coordination with human and animal health colleagues in our agency and others. This year, too, we have placed renewed emphasis on public education and outreach. USEPA's new toxin guidance, along with a changing landscape and climate, require continued adaptation of our Response Plan. This represents an overview of how the KDHE HAB response program was built, what we have learned, and what the future may bring.

THE NATIONAL WATER QUALITY MONITORING COUNCIL

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The National Water Quality Monitoring Council (NWQMC, or Council) provides a national forum for coordination of comparable and scientifically defensible methods and strategies to improve water quality monitoring, assessment, and reporting. The Council promotes partnerships to foster collaboration, advance the science, and improve management within all elements of the water quality monitoring community. Vital to this role, the Council provides a voice for monitoring practitioners across the Nation and fosters increased understanding and stewardship of our water resources. The Council, which is administered through the Advisory Committee on Water Information (ACWI), was created in 1997 as a vehicle for bringing together diverse expertise needed to develop collaborative, comparable, and cost-effective approaches for monitoring and assessing our Nation's water quality. These approaches are fundamental to the successful management and sustainability of our waters. Water issues continue to become more complex, and the demand for high-quality water continues to grow in order to support a complex web of human activities and aquatic ecosystem needs. Members of the Council include representatives from USEPA, USGS, USDA, USACE, NOAA, USFWS, National Park Service, and Bureau of Land Management, along with representatives from Tribes, States, interstate monitoring consortia, municipalities, industry, the volunteer monitoring community, nongovernmental organizations, and a variety of academic and professional groups. The mission of the Council is achieved through various activities and products of its workgroups. The Council hosts the National Monitoring Conference, which is held every two to three years and serves as an important clearinghouse for water monitoring professionals. The Council also supports the Water Quality Portal, a user friendly web-based interface to access water monitoring data from USGS, USEPA, state agencies, and many other sources. In addition, the Council produces a newsletter, factsheets, webinars, and other resources designed to foster continued education and collaboration across organizations.

MULTI-YEAR EXECUTION OF AN EMERGENCY HARMFUL ALGAL BLOOM (HAB) SAMPLING AND ANALYSIS PROGRAM IN EPA REGION 8, AND PARALLEL ANALYSIS WITH ELISA AND LC/MS/MS METHODOLOGIES

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In 2014, the EPA Region 8 Laboratory initiated algal toxin analyses for recreational and drinking water source water bodies in response to a need identified by regional water quality managers throughout Colorado, Montana, North Dakota, South Dakota, Utah, Wyoming, and Region 8 Indian Country. A program was devised to provide technical assistance to stakeholders in identifying blooms, distribute emergency sampling kits, and offer a 48-hour turn-around window to issue results to requesters. Routine sampling projects were also designed at specified water bodies. Samples were analyzed in parallel with both enzyme-linked immunosorbent assay (ELISA) and liquid chromatography with tandem mass spectrometry (LC/MS/MS) methods. Presented herein is a comparison of six years of monitoring data for ELISA and LC/MS/MS analyses. This poster also describes identified best practices for establishing a rapid-response HAB program.

APPLYING THE RED-BAND DIFFERENCE CHLOROPHYLL-A FLUORESCENCE ALGORITHM FOR SATELLITE DETECTION OF HIGH BIOMASS HABs OTHER THAN *KARENIA BREVIS*

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Chlorophyll-a fluorescence has been a useful indicator of *Karenia brevis* blooms in Florida. The Red-Band Difference (RBD) fluorescence algorithm, developed for the Moderate Resolution Imaging Spectroradiometer (MODIS), was deemed the most useful in detecting and monitoring *Karenia brevis* blooms in Florida. The algorithm was particularly useful in monitoring the 2018 *K. brevis* bloom, which will be demonstrated. RBD separates high fluorescing biomass blooms from background chlorophyll and is less susceptible to interference from sediment and shallow bottoms when compared to other fluorescence-based algorithms. When applied to the higher resolution Sentinel 3a and b Ocean Land Colour Imager (OLCI) image products, 300 m RBD imagery can be used as a useful monitoring tool for high biomass fluorescing blooms in more turbid estuaries, as well as the open ocean. While not species specific, the RBD has been used to determine the timing and possible source population for a bloom of *Karenia mikimotoi* in Kachemak Bay, Alaska in 2013 and detected another *K. mikimotoi* bloom off the coast of Ireland in 2012. In addition to *K. brevis*, *Pyrodinium* blooms in Tampa Bay have been observed. The RBD has been a useful tool for state managers in Maryland and Virginia, monitoring HABs in the tributaries and mainstem of Chesapeake Bay. The RBD has provided early warning and further monitoring of *Margalefidinium polykrikoides* and *Alexandrium monilatum* in Virginia, while monitoring *Prorocentrum minimum* and several species of *Heterocapsa* in Maryland, among other dinoflagellates.

MITIGATION & CONTROL

MICROCYSTIN-LR BINDING AND SEDIMENTATION USING PHOSLOCK

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Microcystins [and its congener microcystin-LR (MC-LR)] are considered the most common cyanotoxin produced by cyanobacterial harmful algal blooms. The intracellular MC-LR is released into the water column upon cell lysis during treatment or senescence, where it can remain stable for weeks and lead to adverse health effects. The ideal treatment method for MC-LR-producing strains is one that can remove or degrade both cells and toxins from the water column. We tested the hypothesis that MC-LR can bind to Phoslock®, removing it from the water column by sedimentation. In order to test this, MC-LR was dissolved in deionized water and diluted to 5, 20, 50, 100, and 500 ppb. A stock solution of Phoslock® (1 gL⁻¹) was used and kept homogenized on a magnetic stirrer and applied into solutions containing MC-LR at concentrations of 50, 100, and 150 ppm (in triplicate) including a control without Phoslock®. The tubes were incubated at room temperature for 24-48hr, and then processed for MC concentration using ELISA. Results indicate that MC-LR is removed from the water column when higher concentrations of both Phoslock® (100 and 150 ppm) and MC-LR (100 and 500 ppb) are present. Future work aims at deciphering the *in situ* effects of Phoslock®, in combination with other algaecides and flocculants to improve the efficacy of toxin removal.

IDENTIFICATION AND QUANTIFICATION OF CHEMICAL SIGNALS THAT INDUCE BACTERIAL ALGICIDAL ACTIVITY ON *KARENIA BREVIS*

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Blooms of the toxic dinoflagellate, *Karenia brevis* (*K. brevis*), occur frequently in the Gulf of Mexico and cause human respiratory irritation, fish kills, marine mammal mortalities, and significant economic loss. Mitigation strategies to combat HABs are categorized as physical, chemical, or biological controls. Naturally occurring biological compounds, particularly produced by bacteria, have been suggested to play a role in the regulation of blooms. Interactions between HABs and surrounding bacterial communities has not been fully explored, and previous studies have shown the ability of select bacteria strains with algicidal properties to negatively affect HABs. Few bacterial strains have been shown to be algicidal against *K. brevis* either through the release of algicidal compounds or direct contact. However, specific biological mechanisms and conditions that trigger algicidal activity have not yet been thoroughly defined. Literature suggests that specific compounds, including phenolic acids, generated by *K. brevis* cells may act as signaling compounds prompting the production and release of algicidal compounds from surrounding bacterial communities, negatively affecting *K. brevis* cells. Here we present preliminary findings on the identification and quantification of four phenolic acids that may serve as signaling compounds released by *K. brevis*. Additionally, we determine the growth stages these compounds are being released. Results from this study have the potential to develop new red tide mitigation strategies through the utilization of natural compounds to promote algicidal activity in bacteria.

REDUCING EFFECTS OF FLORIDA RED TIDE VIA BIOFILTRATION

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Florida red tide blooms are caused by the microscopic dinoflagellate *Karenia brevis*, which produces a neurotoxin called brevetoxin. Elevated concentrations of *K. brevis* cause fish kills and other wildlife fatalities, as well as neurotoxic shellfish poisoning and respiratory irritation in humans. Research shows that filter feeding organisms, such as clams, mussels, and whelks accumulate brevetoxins, removing them from the water column (Pierce et. al., 2004). Artificial reef structures promote the colonization of bivalves and other sessile, filter-feeding organisms. Laboratory tests were conducted to determine the filtration and clearance rates of (artificial reef colonized) tunicates from City Island, Florida. Tunicates were exposed to Wilson LT and New Pass strains of *K. brevis* from Mote Marine Laboratory culture. Results showed a notable steady decline in Wilson LT *K. brevis* cells and a staggered decline of New Pass *K. brevis* cells when tunicates were introduced. By deploying artificial reef structures in residential canalways, additional substratum is provided for invertebrates and other filter feeding organisms, possibly providing alleviation of bloom effects to residents. Volunteers owning homes with personal boat docks along two canals in Boca Grande were recruited for a citizen science initiative to study the impact of artificial reef structures in the natural environment. During the course of the project, volunteers carried out weekly assessments of the artificial reef structures as well as water sampling. Data extrapolated from these assessments will be analyzed to determine the potential impact of these structures for mitigation of red tide bloom effects. In the future these data may be used to isolate and promote certain species for colonization in order to increase filtering capacity.

ULTRASONIC TECHNOLOGY FOR CYANOBACTERIA CONTROL: A PILOT STUDY ON TWO NEW YORK CITY RESERVOIRS

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The increased occurrence of Harmful Algal Blooms (HABs) regionally has prompted the New York City Bureau of Water Supply to increase monitoring and control strategies. In 2018, a pilot project using ultrasonic platforms was initiated to determine the effectiveness of ultrasonic treatment in preventing and reducing algal blooms. Two areas of the East of Hudson watershed that historically have experienced high concentrations of blue-green algae during the summer months were selected for the study. A sonic platform was activated in June in Croton Falls Reservoir in an attempt to prevent an algae bloom. A second sonic platform was activated in August in New Croton Reservoir in an attempt to diminish an algal bloom that occurred. Each unit has two sonic heads, which emit sound in four directions using two bandwidths for control of different algal groups. The units are designed to interrupt the gas vesicles within the cells of algae, which should then cause the algae to lose buoyancy and sink in the water column out of the photic zone. The system is designed to be effective on green algae and diatoms to a range of 150 meters radially from the platform, and blue-green algae to 400 meters. According to the manufacturer, the sonic signal will not disrupt the cells or have any impact on higher organisms. Weekly monitoring of phytoplankton, chlorophyll a, total phosphorus, total dissolved phosphorus and dissolved oxygen profiling was conducted. Biweekly monitoring of zooplankton was performed using both fine and coarse mesh tow nets for vertical tows at each site. The study results did not show differences in water quality at the control or treatment sites in terms of chemical or biological parameters. DEP plans on redeploying one of the sonic platforms at site 5 on Croton Falls in 2019 to provide additional data for comparison.

THE EFFECTS OF ALGAECIDES AND HERBICIDES ON A *MICROCYSTIS* WINTER BLOOM IN LAKE OKEECHOBEE, FLORIDA (USA)

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Microcystis-dominated cyanobacterial harmful blooms (cyanoHABs) are a recurring problem in Lake Okeechobee (LO). As public awareness on their risks increases, there is a need for studies on both short- and long-term management. To provide science-based best management practices or treatment options, we tested various concentrations and combinations of algaecides/herbicides. Bloom waters, dominated by *Microcystis wesenbergii*, with some *M. aeruginosa* and *Dolichospermum circinale*, were collected from LO in November 2018. The material was exposed to 15 different algaecides, herbicides, or combinations, at four concentrations each. Cell abundance and morphology, chlorophyll a, phycocyanin and microscopic analyses were undertaken at collection and 24 and 72 hours post-treatment. Microcystin (MC) concentrations were measured from the crude bloom, but determined too low for further analyses. The most efficacious chemicals for treating this bloom were sodium carbonate peroxyhydrate; copper (Cu) sulfate pentahydrate; Cu ethanolamine complex; and combinations of diquat dibromide with endothall, Cu gluconate/citrate and Cu ethanolamine. Other promising methods include combinations of flumioxazin with Cu gluconate/citrate and endothall with liquid H₂O₂. Some chemicals, including liquid H₂O₂ and endothall alone, were unable to deplete cyanobacterial abundance, thus considered an ineffective treatment option for *M. wesenbergii*-dominated blooms. Future work aims at treating toxic blooms and monitoring toxin degradation.

INFLUENCE OF DISSOLVED HUMIC COMPOUNDS ON *K. BREVIS* CELL VIABILITY, INTRACELLULAR BREVETOXIN CONCENTRATIONS AND BREVETOXIN AEROSOLIZATION

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Karenia brevis, the dinoflagellate responsible for Florida red tides, produces a suite of potent neurotoxins called brevetoxins (PbTx) which cause massive fish kills, marine mammal and seabird mortalities, and commercial shellfish bed closures. Brevetoxins can be aerosolized and carried by winds onshore, causing human eye irritation and respiratory symptoms. Dissolved humic substances (DHS), a form of dissolved organic carbon that dominate estuaries during periods of heavy rainfall and runoff, have been shown to stimulate growth of some dinoflagellate species and accelerate brevetoxin breakdown. We examined the impact of two different DHS, commercially-sourced DHS derived from leonardite, and natural DHS extracted from the Peace River, on *K. brevis* cell viability, intracellular brevetoxin composition, and brevetoxin aerosolization. A benchtop mitigation trial with *K. brevis* cultures in 1 L beakers exposed to 0, 5, 15 and 30 mg L⁻¹ DHS was conducted over a 48-hour period, and aerosolization was simulated with the Mote Aerosol Generator for a 30-minute period at t = 48 hr. Commercial and natural DHS both significantly (p<0.05) inhibited *K. brevis* survival and viability, with up to 39% cell removal efficiency, but had no significant impact on intracellular brevetoxin concentration or composition. The addition of DHS, however, significantly (p<0.05) reduced brevetoxin aerosolization: Peace River DHS additions of 30 mg L⁻¹ were 80% effective at preventing brevetoxin concentrations in aerosols. These data demonstrate that natural DHS are a potentially effective treatment for reducing brevetoxin aerosol during coastal *K. brevis* blooms.

EFFECTS OF MULTIPLE POTENTIAL MITIGATION PRODUCTS ON *KARENIA BREVIS*, TOXINS, AND NUTRIENT PRODUCTION

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The west coast of Florida has experienced large expanses of red tide blooms of the toxic algae *Karenia brevis*. As a result of some of these long-term blooms, new studies are underway to determine if blooms can be stopped or controlled. A number of commercial chemical products have been presented as possible mitigation chemicals against *Karenia brevis*. To assess these products prior to field deployment, a tiered approach of lab-scale to mesocosms-scale tests were developed to assess the efficacy of products to destroy *K. brevis* cells and toxins with minimal adverse impacts on non-target organisms and water quality. The first tier consisted of, range-finder toxicity tests to determine the effective concentration range of each product. Definitive toxicity tests were then conducted to identify the lowest effective concentration. Out of 14 preliminary chemical products, three compounds were deemed appropriate to continue testing in the bioassay experiment phase. Bioassay tests were conducted in 20-Liter carboys to assess impacts on the local phytoplankton community and if there were any water quality impacts. In addition, representative marine organisms (marine snails, bivalves, and tunicates) local to the area were exposed to the three products to determine if there were any adverse impacts. To date, 1 of the 3 products has been deemed appropriate for further mesocosm testing. Additional products will continue to be evaluated at Tier-1 and Tier-2. Future Tier-3 assessment will include field testing of chosen products once a Florida Red Tide bloom is present.

TRANSCRIPTOMIC RESPONSE OF HARMFUL DINOFLAGELLATE *KARLODINIUM VENEFICUM* TO ALGICIDE IRI-160AA

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Previous research identified an algicide IRI-160AA produced by bacterium *Shewanella* IRI-160 that was able to control the growth of dinoflagellate species, but with no negative impacts on other algal taxa tested. Further study indicated that this algicide negatively impacted chloroplasts, PSII and photosynthetic transport chain, as well as nuclear and chromosome structures in dinoflagellates. Research shows that the death of dinoflagellates was accompanied by DNA degradation, reactive oxygen species production, and cell cycle arrest. No study has been focused on the cellular response of dinoflagellates to algicide IRI-160AA at a transcriptomic level. Recent research demonstrated that ammonium is one of the active compounds in algicide IRI-160AA, although its contribution to the algicidal activity is unclear. Here, to assess the response of dinoflagellates to algicide IRI-160AA at a cellular level, RNA-seq was conducted on *Karlodinium veneficum* treated with algicide IRI-160AA. Results were compared to the transcriptome of *K. veneficum* treated with ammonium at the same concentration as in the algicide to better understand the contribution of ammonium to the algicidal effects of IRI-160AA. Fragments from RNA-seq were de novo assembled and over 7000 genes were found to be highly differentially expressed across treatments and controls. Genes were annotated and subjected to Gene Ontology term enrichment analysis. Results of this research will illustrate the cellular mechanisms behind the algicidal effect of IRI-160AA as well as the contribution of ammonium to the activity of this algicide.

EVALUATION OF THE SAXITOXIN RECEPTOR BINDING ASSAY FOR MEASURING HUMAN EXPOSURE TO PSP

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The saxitoxin receptor binding assay (STX RBA) is used to quantify the integrated toxicity of paralytic shellfish toxins (PSTs) from shellfish samples. An interagency collaborative study was conducted on human urine and serum samples to assess the STX RBA as a diagnostic tool to investigate human poisonings by PSTs. The application of the STX RBA in human PST poisonings investigations would be advantageous because all saxitoxin-like activity in a sample would be measured relative to its molecular toxicity and independent of the specific PST congeners present. Human urine or serum was spiked with various voltage gated sodium channel site one ligands, blinded, and analyzed. Specifically, saxitoxin, gonyautoxins 1-4, neosaxitoxin, and μ -conotoxin CnIIIC were spiked individually and in combination into serum or urine at various concentrations, some representative of past human poisoning events. Percent accuracies of individual urines spiked with STX ranged from 94-142% and from 116-232% for individual sera spiked with STX. Relative standard deviations of undiluted STX spiked samples ranged from 7.4 – 28 for serum and from 9.8 - 12 for urine. The calculated method LOD for undiluted urine is 11.4 ng/mL and is 3.34 ng/mL for undiluted serum. Future experiments incorporating sample dilution or clean-up may improve quantitation of human serum and urine by the STX RBA.

FOOD WEB DYNAMICS & IMPACTS

DETERMINATION OF THE PRESENCE AND BIOMAGNIFICATION OF CARIBBEAN CIGUATOXINS AND BENTHIC ALGAL TOXINS IN FISHES FROM THE FLORIDA KEYS NATIONAL MARINE SANCTUARY

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Ciguatera fish poisoning (CFP) is a common syndrome affecting coastal communities in the Atlantic and Indo-Pacific regions, including the Greater Caribbean. This severe illness is caused by the ingestion of reef fish contaminated with ciguatoxins (CTXs). The source of CTXs has been associated with epiphytic dinoflagellates of the genus *Gambierdiscus* (among others) which reside on macroalgae that are grazed by herbivores. The entry of algal CTX precursors (often referred to as gambiertoxins) into the food web initiates a cascade of trophic transfer events and biotransformations that have not yet been fully elucidated in the Greater Caribbean region. A critical knowledge gap addressed in the present study was to gain better predictive capability of CTX presence in fishes across trophic levels in a sub-tropical hotspot for CFP (i.e. the Florida Keys). A variety of fish including herbivores (e.g., representatives of Acanthuridae and Pomacanthidae) up to apex predators (e.g., *Sphyrena barracuda* and *Mycteroperca bonaci*) were collected from a long-term monitoring site in the Florida Keys. Extracts of fish were assessed for neurotoxicity and CTX activity using the sensitive in vitro neuroblastoma (N2a) assay. Twenty five of the 66 fish (38%) examined in this study expressed sodium channel-dependent toxicity in both sensitized and non-sensitized cell treatments, highlighting the likely presence of multiple toxin classes in these reef fish. Monospecific sodium channel activity was observed in 3% of fish represented by one species: *S. barracuda*. Stable isotope analysis was used to determine fish trophic level and in the calculation of a food web magnification factor (FWMF) of 1.114 for CTX and CTX-like compounds, indicative of the biomagnification of these toxins. The presence of toxic fish indicates a need for risk assessment and management consideration in the Florida Keys.

EFFECTS OF SAXITOXIN INGESTION IN A MODEL AVIAN SPECIES

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Since 2014, widespread annual mortality events involving multiple species of seabirds have been documented in the Gulf of Alaska, and the Bering and Chukchi seas. In 2017, nearly 1600 birds were found dead along the western coast of Alaska, and emaciation was determined to be the principle cause of death among individuals submitted for diagnostic evaluation at US Geological Survey's National Wildlife Health Center. However, due to concerns about harmful algal blooms in this region, a subset of these seabirds was also tested for the algal toxins saxitoxin (STX) and domoic acid (DA). No DA was observed but STX was detected in stomach contents, cloacal contents, or liver tissue in 60% (15/25) of individuals tested. Since few data are currently available that would help determine the contribution of STX exposure to this large-scale mortality event, we conducted experimental trials to describe the effects of STX in birds. Specifically, mallards (*Anas platyrhynchos*) were used as the model species to determine levels of STX that result in morbidity and mortality in birds. The LD50 (dose at which 50% of birds die) of STX in mallards was determined to be 167 ug/kg. STX was detected in fecal samples, collected from mallards that survived initial dosing, for up to 48 hrs. Among tissues, we detected STX in the livers of 2/7 birds that died acutely (<2 hr) and in 1/3 sub-acute dosed birds euthanized 24 hrs after receiving the last of 7 daily doses of STX, and more infrequently in kidney, lung, heart, and breast muscle tissues. No gross or microscopic lesions were observed that could be attributable to STX exposure. Given its acute toxicity to birds, the challenges associated with detection, and its frequent occurrence in the Alaska marine environment, additional research on STX in seabirds is warranted.

EXPOSURE TO MULTIPLE ALGAL TOXINS AMONG RESIDENT BULL SHARKS, *CARCHARHINUS LEUCAS*, IN FLORIDA'S INDIAN RIVER LAGOON

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Extensive population growth and human development over the past century have subjected Florida's Indian River Lagoon (IRL), an estuary of national significance, to heightened nutrient loading that has increased the frequency of harmful algal blooms (HABs) in this region. This includes two large scale cyanobacterial blooms during the summers of 2016 and 2018. A thorough understanding of the effects of HAB toxins on the IRL ecosystem is critical to mitigate risks to associated coastal communities; however, managers face challenges from a lack of information on ecosystem wide impacts. While studies on red tide toxins (brevetoxins) have been conducted in nearby systems, information on the effects of other HAB toxins (i.e. microcystins, saxitoxins, etc.) that are more prevalent on Florida's east coast is lacking. This study aims to evaluate exposure and potential effects of HABs on this ecosystem using the bull shark (*Carcharhinus leucas*) as a sentinel species. Liquid chromatography-mass spectrometry was used to measure a suite of HAB toxins in bull shark tissues including stomach contents, blood plasma and liver. Baseline concentrations were assessed from samples (n=47) collected in Fall 2018 and Spring 2019 using a standardized fishery-independent survey and will be compared to those from animals collected during HAB events. In addition, visual inspection of stomach contents combined with stable isotope analysis of muscle tissue will be used to assess dietary sources of toxins. Analysis of samples has thus far demonstrated the presence of multiple toxins (microcystins, brevetoxins, domoic acid, and okadaic acid) in the tissues of bull sharks during non-bloom periods across the lagoon. Additional collection and analyses are ongoing, but we expect our results will provide valuable data on exposure and potential impacts of previously unmeasured toxins in this iconic estuary.

THE EXAMINATION OF GRAZING PREFERENCES EXHIBITED BY HERBIVOROUS INVERTEBRATES ON THE COMMON MACROALGAL HOSTS OF *GAMBIERDISCUS* SPP.

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Understanding the basis of the marine food web is essential in determining the uptake and possible control of the toxins that cause ciguatera fish poisoning (CFP), predominantly ciguatoxins. Ciguatoxins are produced by the marine dinoflagellate *Gambierdiscus*, and when transferred through the food web, have serious environmental and health implications. Ciguatera outbreaks are common in coral reef environments where macroalgae are present. As ocean conditions change and more coral reef ecosystems shift to macroalgal domination, the risk of ciguatera outbreaks may increase. Since the first discovery of causal agents for CFP, the general consensus has been that the ciguatoxin pathway goes through herbivorous fish; however, some previous studies have suggested that herbivorous invertebrates may be another vector. Because hogfish have been found to be one of the high-risk fish species for CFP, and they are also known to consume invertebrates, more studies are necessary in order to accurately evaluate the invertebrate role as such vector. The purpose of this study, therefore, was to determine the grazing preferences of various herbivorous invertebrates on the common macroalgal hosts of *Gambierdiscus*, in order to examine the uptake of ciguatoxin into the food web. Four marine macroalgae were tested (*Laurencia gemmifera*, *Halimeda incrassata*, *Dictyota menstrualis*, and turf algae) against three different taxa of herbivorous invertebrates. Consumption rates were determined from changes in algal biomass. Preliminary results indicated that there was a significant food preference for turf algae among all three species tested. There was also a significant food preference for *Dictyota menstrualis* for two of the species tested and *Laurencia gemmifera* for the other species tested. These results will expand our knowledge on herbivore feeding preferences and toxin uptake, leading to a better understanding of this crucial step in the introduction of ciguatoxin into the reef food web.

SOUTHERN CALIFORNIA WILDFIRE, HARMFUL ALGAL BLOOM, AND FISH KILL IN LAKE ELSINORE, CA

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The Holy Fire wildfire in Southern California began on August 6, 2018 and reached full containment on September 13, 2018 with a total burned area of 35.9 square miles. Lake Elsinore lies at the bottom of several large canyons draining the burned area. Since the fire containment, the area received numerous storms of varying strength from <0.25 to 5.5 inches, totaling approximately 25 inches of rain from October 2018 through March 2019. Debris flows from the burn area deposited a large sediment delta in the lake. Shortly after the first two major storms in late November 2018 hit the area, a large fish die-off was observed in Lake Elsinore which continued through January 2019. Based on multiple lines of evidence this die-off was attributed to the Golden Algae, *Prymnesium parvum*, a species not previously observed at high densities in the lake. The City of Lake Elsinore embarked on a study to determine if runoff from the Holy Fire had triggered the Golden Algae bloom and subsequent fish kill. Chemistry and toxicity analyses were performed on both water and sediment samples in and around the fire sediment delta. Samples for phytoplankton taxonomy were also collected in and around the fire sediment delta, as well as during monthly routine mid-lake sampling. A comprehensive analysis of results will be presented showing the magnitude of effect on lake water and sediment quality and potential for long-term impacts.

SPECIAL SESSION: OCEANS AND HUMAN HEALTH

OCEANS AND HUMAN HEALTH: THE GREAT LAKES CENTER FOR FRESH WATERS AND HUMAN HEALTH

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The Great Lakes Center for Fresh Waters and Human Health at Bowling Green State University was funded by the NIEHS/NSF program COHH3: Impacts of Climate Change on Oceans and Great Lakes program. Owing to the need to protect our recreational freshwaters and drinking water from the expansion of cyanobacterial HABs, the Center supports three research projects, a Facilities Core providing water chemistry and cyanotoxin analyses, and a Community Engagement Core (CEC) aimed at engaging participant scientists and stakeholders to communicate research findings and establish future priorities. The CEC also provides a collaborative connection to the CECs from the other OHH Centers. In total, senior personnel from eight participating institutions participate in Center activities, allowing an expansion of existing research collaborations established in the wake of the 2014 Toledo Water Crisis. Specifically, the Center research projects address the following issues: first, the environmental drivers that yield different types of blooms (e.g. *Microcystis* vs. *Planktothrix* vs. *Dolichospermum*); second, characterization of novel cyanobacterial secondary metabolites; and third, determination of best methods for bloom and toxin detection. The research project focus on 'omics approaches allow a detailed examination of cHAB formation, persistence and decline.

DETECTION OF BREVETOXIN IN HUMAN PLASMA TO DIAGNOSE POTENTIAL ENVIRONMENTAL EXPOSURES

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In recent years, Florida Red Tides, formed by the dinoflagellate *Karenia brevis*, have become more prevalent and persistent in the Gulf of Mexico. During bloom events, brevetoxin (PbTX) can be produced in high concentrations, released into the water, and subsequently aerosolized via sea spray. PbTXs are a public health concern since the consumption or inhalation of PbTX may cause neurotoxic shellfish poisoning (NSP). NSP commonly causes symptoms such as respiratory irritation and, in rare cases, can also produce more severe symptoms including bradycardia and convulsions. Prior studies have detected PbTX in the blood and urine of marine organisms, however, there are no validated ELISA methods diagnosing environmental exposures of PbTX in human blood. Therefore, to address this gap and to prepare for potential exposures, our lab evaluated the effectiveness of an available ELISA kit to detect PbTX in human plasma. Brevetoxin-3 was spiked into pooled plasma at varying concentrations to generate standard curves and quality control samples. To validate the method, 20 calibration curves and quality controls were evaluated to determine method performance, 50 individual unexposed matrix samples were measured to determine LOD and ensure no false positives, and analyte stability in matrix was assessed. The establishment of this method expands our lab's capabilities to provide diagnostic support for public health exposures to PbTX.

A BRIEF INTRODUCTION TO THE CENTERS FOR OCEANS AND HUMAN HEALTH FUNDED THROUGH COHH3: IMPACTS OF CLIMATE CHANGE ON OCEANS AND GREAT LAKES

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The National Institute of Environmental Health Sciences and the National Science Foundation jointly fund research on marine-related health issues through the Centers for Oceans and Human Health and through individual research projects focusing on oceans and human health as well as the Great Lakes and human health. Grantees are developing techniques for more accurate and earlier detection of harmful algal blooms with the goal of preventing or reducing exposure, and they are studying the health effects of eating seafood that harbors toxins produced by harmful algal blooms. Here, an overview will be provided for the four centers funded through COHH3: Impacts of Climate Change on Oceans and Great Lakes. The Great Lakes Center for Fresh Waters and Human Health, a collaborative effort among Bowling Green State University and eight other institutions, focuses on freshwater, particularly cyanobacterial harmful algal blooms (cHABs). The purpose of the Greater Caribbean Center for Ciguatera Research (a collaboration of five institutions led by Florida Gulf Coast University) is to examine how climate change may affect ciguatera fish poisoning in the Greater Caribbean Region. The Woods Hole Center for Oceans and Human Health has the mission to protect public health through enhanced understanding of how oceanic and environmental processes including climatic variation affect toxin producing organisms using multiple state of the art in situ sampling technologies and modeling approaches. Lastly, the Oceans and Human Health Center on Climate Change Interactions (a collaborative center headed by the University of South Carolina) is assessing climate change effects on ocean health related illness (*Vibrio* bacteria and cyanotoxins) in conjunction with microplastic exposure, nonalcoholic liver disease, and seafood safety. Together, these projects aim to better understand how climate change may influence ocean (and Great Lakes) – human health interactions in order to mitigate merging human health risks.

THE GREATER CARIBBEAN CENTER FOR CIGUATERA RESEARCH: CLIMATE CHANGE AND HUMAN HEALTH

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The Greater Caribbean Center for Ciguatera Research (GCCCR) is one of the centers funded by the joint NSF/NIEHS Centers for Oceans and Human Health program (COHH3). The primary purpose of the GCCCR is to examine how climate change may affect ciguatera fish poisoning in the Greater Caribbean Region. Ciguatera fish poisoning (CFP) is the most common form of phycotoxin-borne seafood illness across the globe, affecting tens of thousands of people annually. CFP is caused by the consumption of seafood (primarily reef fish) contaminated with ciguatoxins. Gambiertoxins, precursors of ciguatoxins produced by the (sub)tropical benthic dinoflagellate genus *Gambierdiscus*, enter reef food webs when herbivores and detritivores consume *Gambierdiscus* directly or indirectly by grazing on macroalgae. These precursor molecules are transferred to higher trophic levels by bioaccumulation, bioconversion and biomagnification until they reach predatory finfish species that are targeted in many commercial and recreational fisheries. When people subsequently consume the contaminated fish, they are exposed to the toxins, thereby experiencing CFP. Historically, CFP outbreaks have been linked with warm water temperatures and coral reef impacts, both of which are expected to increase in the setting of climate change. Consequently, CFP is predicted to increase on geographic and temporal scales. The goals of this center are to: 1) examine the role climate change may play in the geographic and temporal expansion of CFP into more temperate latitudes; 2) obtain a better understanding of the toxic metabolites produced by certain *Gambierdiscus* strains, and the subsequent transfer and biotransformation of these compounds into coastal/reef food webs; 3) study the genotoxicity and impacts on cellular metabolism caused by these

toxins upon exposure in order to 4) better protect and treat people from this malady in an era of climate change processes. This presentation provides a brief overview of the activities of the three Research Projects and Community Engagement Core addressing these goals.

SPEED TALKS + POSTERS

(Alphabetical by author)

THE USE OF BREWER'S SPENT GRAIN TO INHIBIT *MICROCYSTIS AERUGINOSA* BLOOMS AND LC-MS/MS ANALYSIS OF THE INHIBITORY COMPOUNDS

[Mitigation and Control]

Taylor Armstrong, Ernest Williams, Allen Place

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In Lake Williston, Eastern Maryland, toxic *Microcystis* became a major threat and caused closures from 2009-2011. Every closure caused an economic burden on the Girl Scout camp who use the lake for recreational activities. For the past few years, two strategies have been effective in suppressing the toxic blue-green algae: opening the dam to freeze the over-wintering algae needed for inoculation of a summer bloom, and putting out barley straw which release active algaecides when rotting that inhibit the growth of some *Microcystis* species. Phenolic acids and flavonoids, two likely classes of algaecides, were confirmed to increase post barley bale deployment in the lake. However, barley straw dispersal can be time consuming, labor intensive, and its effectiveness is dependent on deploying bales months prior to the bloom formation. Brewer's Spent Grain (BSG) is the insoluble part of barley grain that constitutes as much as 85% of breweries total by-products. The bound phenolic acids and flavonoids is an estimated five-fold concentration higher to that of barley straw and is easily degraded by microbial communities. Here, we investigate the use of BSG as a more effective method for inhibiting toxic *M. aeruginosa* LE3. Barley and BSG were placed in a 30C, 60% humidity, dark room. Ten percent water rinsed through BSG at day 0, 2 weeks, and 1 month immediately inhibited *M. aeruginosa* LE3 within 3 days by 70%, 94%, 96% (n=6). This continued research will help scientists and managers find more effective solutions to inhibit toxic algal blooms.

IS *SARGASSUM* A VECTOR FOR ENVIRONMENTAL TOXICANTS?

[Food Web Dynamics & Impacts]

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As a free-floating holopelagic genus of brown algae, *Sargassum*, is widely distributed throughout the Gulf of Mexico, Caribbean and Western Atlantic. And, in effect, the floating mats in this basin collectively represent the largest "algal bloom" in the world. Floating mats in pelagic and neritic waters represent vital habitats for a wide range of marine life. However, *Sargassum* is able to propagate continuously, as it travels with ocean currents, and large and often overwhelming quantities are, therefore, found accumulating along an expanding range of coastal beaches, representing an emerging concerns for coastal communities in these regions. Although the genus is not associated with any known biotoxins, *Sargassum* is known to have considerable biosorption properties with respect to metals, including several toxic metals, and perhaps other environmental pollutants (e.g., persistent organic pollutants [POP]). As such, *Sargassum* may represent a vector for accumulation and transport of pollutants to beaches and nearshore waters, and thus, a potential environmental health concern. It is also possible that accumulation of toxic pollutants may impact the diverse fish and invertebrate communities associated with floating mats. To investigate these possibilities, we are applying analytical techniques to identify and quantify a range of environmental pollutants including toxic metals and POP associated with *Sargassum* collected from beaches of South Florida, and other sites in the region. In addition, we are investigating the possible desorption and release of these pollutants with senescence as occurs, in particular, when biomass reaches and accumulates in beach and nearshore waters. Results of these initial and ongoing studies will be presented.

A *KARENIA BREVIS* MITIGATION STRATEGY USING NATURAL COMPOUNDS DERIVED FROM MACROALGAE

[Mitigation and Control]

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The brevetoxin-producing dinoflagellate, *Karenia brevis*, is known to cause vast mortality of many marine organisms in the Gulf of Mexico when cell abundances increase to bloom conditions. As harmful algal blooms pose significant risks to marine ecosystems, human health, and local economies, finding an effective, sustainable mitigation strategy that minimally impacts ecosystems is vital. Research shows macroalgal terpenoids and polyunsaturated fatty acids (PUFAs) have algicidal effects against some HAB species, including *Heterosigma akashiwo* and *Karenia mikimotoi*. Previous work at Mote has also shown that some macroalgae demonstrate algicidal and growth-inhibitory effects against *Karenia brevis*. Here, we investigate the effects of compounds released naturally and extracted from macroalgae on the growth and survival of *K. brevis*. We exposed *K. brevis* to live macroalgae and macroalgal extracts using two polar protic solvents (water and methanol) and a polar aprotic solvent (acetone) to determine the efficacy of these different extract fractions in reducing or eliminating *K. brevis* cells. In less than 24-hours of exposure to intact “drift-algae” species and *Padina*, a brown macroalgae, 100% *K. brevis* lysis occurred. Aqueous extracts from *Caulerpa*, a green macroalgae, resulted in 100% reduction of *K. brevis* after a 2-hour exposure, while *Padina* and drift species caused 100% loss after a 15-hour exposure. Methanol extracts of *Caulerpa* and *Padina* caused 100% loss of *K. brevis* within 7-hours of exposure, and *Gracilaria*, a red alga, caused 100% loss of *K. brevis* in 15 hours. *Caulerpa* acetone extractions caused 100% cell lysis in less than 4-hours, and *Padina* extracts in less than 7-hours. Acetone and methanol extracts showed the highest statistical significance (ANOVA, $p < 0.05$) in all tests, with no significant difference between solvent and media controls. These results suggest that extracts of macroalgae could provide a viable mitigation strategy for red tide and other HABs.

REDUCING THE OCCURRENCE OF HABS THROUGH LOW IMPACT DEVELOPMENT

[Monitoring & Management]

Sarah Caywood, Tracy Fanara

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Stormwater runoff is the largest nonpoint source of pollution. Urbanization and traditional flood prevention systems have changed the natural water cycle resulting in high runoff volumes, high peak flows, and low water quality. This causes erosion, flooding, deterioration of wildlife, reduced groundwater recharge and algal blooms. Low impact development (LID) is a stormwater management technique that strives to mimic the natural water cycle by encouraging stormwater infiltration and treating water at the source. LID designs aim to strategically implement small-scale structural and nonstructural hydrologic controls for the management and protection of stormwater quality. Modeling is an effective way of evaluating LID implementation and the nutrient load reduction. BAM (biosorption activated media), a commercially available filter media, is often used in LID for its high removal efficiency of nitrogen and phosphorus from stormwater. A column study was conducted to determine BAM’s ability to remove glyphosate: the most world widely used herbicide. Biochar Now, an activated carbon media, was also column tested for removal efficiency. This study aims to evaluate nutrient loading and effective treatment design strategies a Sarasota site prone to eutrophication and cyanobacteria blooms selected for commercial development. LID designs were modeled using the EPA’s SWMM5 and BMP Trains programs with support of lab scale column tests to determine removal efficiencies of possible media for LID implementation. By implementing the max LID (including pervious pavers, green roof, infiltration trenches and rain gardens, phosphorus load was reduced over 90%, runoff discharge was reduced 79.9% and infiltration was increased 77.2%. Field samples at this site show that typical land use assumed nutrient loads are underestimated. For the column study, Biochar Now had the highest glyphosate removal efficiency of 23.62%, then BAM at 20.02% and quartz sand at 10.18%.

THE EFFECT OF RED TIDE (*KARENIA BREVIS*) ON THE FLORIDA STONE CRABS (*MENIPPE MERCENARIA*)

[Food Web Dynamics & Impacts]

Cody E. Cole, Philip Gravinese, Stephanie Kronstadt, Talib Clemente, Emma Saso, Patricia Blum, Michael Henry, Richard Pierce, Vincent Lovko

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The stone crab fishery is economically important, generating nearly \$30 million per year in Florida. The majority of the fishery is located on Florida's Gulf coast, which is prone to blooms of the red tide dinoflagellate, *Karenia brevis*. The impacts of *K. brevis* blooms on the stone crab fishery are unknown. We determined the lethal and sublethal effects of *K. brevis* by exposing sublegal crabs for nine days to three treatments: a control with no *K. brevis*, a low-toxin *K. brevis* strain (Wilson-LT), and a toxic strain (New Pass). During the exposure period we monitored food consumption, reflex impairment, and survivorship. The toxic treatment resulted in a 67% decrease in food consumption and significant reflex impairment, both of which resulted in a 42% decrease in survivorship. Some blooms can be widespread (e.g., 2017-2019) and coincide with the stone crab reproductive season. After hatching, stone crab larvae are transported by currents offshore where *K. brevis* blooms originate which may increase the chances of larvae encountering a bloom. We therefore also monitored larval stone crab tolerances to high concentrations of *K. brevis* in a separate study. Newly hatched larvae were exposed to "high" (10^6 cells·L⁻¹) and "medium" (10^5 cells·L⁻¹) concentrations of *K. brevis* and a control treatment with no algae present for 96-hours. Larval survivorship, geotaxis (vertical swimming behavior), and oxygen consumption were monitored. There was 100% and 30% mortality in the "high" treatment and "medium" treatments respectively after 96-hours. Larvae also reversed their swimming behavior in the toxic treatments; however oxygen consumption did not differ. Combined, these results suggest that stone crabs are susceptible to red tide blooms throughout their life-history eliciting both lethal and sublethal effects, which may reduce the resiliency of subpopulations in regions that have experienced repeated blooms.

EFFECT OF MICROCYSTIN ON THE PHYCOBILISOME ANTENNA COMPLEX IN *MICROCYSTIS* SPP.

[Cell and Molecular Advances]

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Cyanobacteria use phycobilin pigments, along with chlorophyll, to collect a broader range of photosynthetically active radiation (PAR) for photosynthesis. Unlike the well-studied photosystems of plants and algae, cyanobacteria use a phycobilisome antenna system (PBS). Multiple proteins make up the PBS, including phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE). PC and PE join to extend the antenna-complex and it is attached to the APC core. Under low-light conditions, more PE and PC subunits should organize to make the antenna longer. Under high light conditions, PC and PE should dissociate to make the antenna smaller. The mechanism involved with this increase or decrease in antenna size is unknown. *Microcystis* cultures were grown under four different light intensities. Optimal growth was obtained at 30 $\mu\text{mol m}^{-2} \text{sec}^{-1}$. Chlorophyll production decreased with higher light intensities (140 and 310 $\mu\text{mol m}^{-2} \text{sec}^{-1}$) as did the intrinsic cross sectional area of the antenna complex. Bulk PC measurements were not significantly different between light levels when expressed on a per cell basis. This suggests either the fraction of total PC associated with the antenna increased with decreasing light intensity or the fraction of PBS associated with the PSII reaction centers increased under decreasing light. In higher plants, protein phosphorylation plays an important role in the association between the antenna complex and the photosystem reaction centers. Microcystins are potent protein phosphatase inhibitors, so we examined the effects of changing light intensity in a microcystin biosynthetic pathway mutant. No change in microcystin per cell was observed PCC7806 under changing light levels. Results on the effect of changing light intensity on both the bound and free PC pool in both PCC7806 and its -mcyB mutant, as well as its impact on the size of their antenna cross sectional areas, will be presented.

DIVERSITY AND DYNAMICS OF MACROALGAL EPIPHYTE COMMUNITIES IN THE FLORIDA KEYS AND US VIRGIN ISLANDS, 2014-2015

[Ecophysiology]

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Ciguatera fish poisoning is the most common phycotoxin-related seafood illness globally. It is caused by toxins produced by dinoflagellates in the genus *Gambierdiscus*, which are commonly found as epiphytes on macroalgae. Multiple species of *Gambierdiscus* can occur within a single site or macroalgal host, and are difficult or impossible to distinguish using light microscopy. The aim of this study was to understand the diversity of *Gambierdiscus* communities and how it relates to the composition of the wider epiphyte microbial community as well as environmental conditions. We examined monthly epiphyte samples during 2014 and 2015 from four sites each in the Florida Keys and St. Thomas, USVI. High throughput sequencing of both large and small subunit (LSU & SSU) ribosomal RNA gene regions was used to determine the relative abundance of *Gambierdiscus*, as well as other microbial taxa, within these ciguatera-endemic regions. Details of spatial and temporal variation in community composition, how community structure relates to environmental conditions, and potential biotic interactions within the communities will be discussed. In addition, we will compare the utility of the LSU and SSU regions for sequencing efforts, and how sequence results compare to those obtained by other methods.

DRONE IMAGERY FOR ALGAL BLOOM MONITORING

[Monitoring & Management]

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Early detection is imperative for managing the potential ecological, economic, and public health risks associated with harmful algal blooms (HABs). While traditional methods, such as water quality sampling and satellite remote sensing, are effective tools for measuring algal and cyanobacterial abundance, they can be costly and require training that many stakeholders, such as aquaculture farmers and drinking water utility managers, lack. Unmanned aerial vehicles (UAVs), or drones, are a cost-effective tool that allow users to apply satellite remote sensing principles for HAB monitoring without the limitations of cloud coverage and image turnover rate associated with satellite remote sensing. In this study, UAV imagery from red, green, blue, and near-infrared wavelengths were collected from aquaculture ponds of various sizes, depths, and productivity levels and used to calculate 12 spectral indices (band ratio algorithms). Index results were then compared to in situ chlorophyll-a (all phytoplankton) and phycocyanin (cyanobacteria) concentrations to generate predictive models that differentiate between low-productivity systems, phytoplankton-dominated blooms, and potentially toxic cyanobacterial blooms. Models generated from this study will assist managers, farmers, and researchers in using UAVs for monitoring HABs at greater spatial and temporal scales, thus leading to better-informed decisions for understanding and managing blooms in their systems.

DMSP PRODUCTION IN *GAMBIERDISCUS* SPP. EFFECT OF SALINITY AND VARIATION WITHIN THE GENUS

[Ecophysiology]

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Dimethylsulfoniopropionate (DMSP) is produced by many species of marine phytoplankton and has been reported to provide a variety of beneficial functions including osmoregulation. Dinoflagellates are recognized as major DMSP producers; however, accumulation has been shown to be highly variable in this group. We explored the effect of hyposaline transfer in *Gambierdiscus belizeanus* between ecologically relevant salinities (36 and 31) on DMSP accumulation, Chl a, cell growth, and cell volume, over 12 days. Our results showed that *G. belizeanus* maintained an intracellular DMSP content of 16.3 pmol cell⁻¹ and concentration of 139 mM in both salinities. Although this intracellular concentration was near the median reported for other dinoflagellates, the cellular content achieved by *G. belizeanus* was the highest reported of any dinoflagellate thus far, owing mainly to its large size. DMSP levels were not significantly affected by salinity treatment but did change over time during the experiment. Salinity, however, did have a significant effect on the ratio of DMSP:Chl a, suggesting that salinity transfer of *G. belizeanus* induced a physiological response other than DMSP adjustment. A survey of DMSP content in a variety of *Gambierdiscus* species and strains revealed relatively high DMSP concentrations (1.0 to 16.4 pmol cell⁻¹) as well as high intrageneric and intraspecific variation. We conclude that, although DMSP may not be involved in long-term (3 to 12 day) osmoregulation in this species, *G. belizeanus* and other *Gambierdiscus* species may be important contributors to DMSP production in tropical benthic microalgal communities due to their large size and high cellular content.

EFFECTS OF AGE ON THE SUSCEPTIBILITY TO NEUROBEHAVIORAL TOXICITY FOLLOWING ACUTE DOMOIC ACID EXPOSURE IN A MOUSE MODEL

[Special Session: Oceans and Human Health]

¹**Alicia M. Hendrix**, ²**Kathi A. Lefebvre**, ¹**David J. Marcinek**

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The harmful algal bloom (HAB) toxin domoic acid (DA) contaminates marine food webs in the western United States, and acts as a potent glutamate receptor agonist following ingestion. With the duration, severity, and toxicity of HAB events expected to increase in coming years, spatial and temporal windows of concern for DA exposure are widening. It is critical that we characterize risk for members of the human communities that depend on safe seafood resources. Reduced resilience in response to physiological stress is a hallmark of aging. This reduced resilience is associated with higher systemic levels of oxidative stress and inflammation, both of which are also associated with DA toxicity. In light of knowledge that the elderly are disproportionately represented among those that have suffered from acute DA toxicosis, and new research suggesting they are most at risk for chronic DA exposures, it is important to identify possible increased susceptibility of the elderly to the toxic effects of DA exposure. To this end, we are conducting a dose-response study assessing convulsive and subconvulsive responses to acute administration of DA (1-5 mg/kg) to young (7-9 mo) and aged (22-24 mo) mice. By assessing both subconvulsive and convulsive responses we will assess sensitivity to DA across a range of exposures. Tissue concentrations of DA at the time of observed behaviors will be measured in an effort to identify possible differences in renal clearance. Additional markers of oxidative stress, endoplasmic reticulum stress, neuronal apoptosis, and ultrastructural damage will be assessed to identify biochemical and histological correlates of the interaction between age and DA toxicity in the mammalian brain. A comparison of dose-response curves will allow for discussion of the extent to which age may play a role in DA susceptibility.

DOMOIC ACID EXPOSURE INDUCED TRANSCRIPTIONAL CHANGES IN THE BRAIN – POTENTIAL EFFECTS ON MICROGLIA

[Special Session: Oceans and Human Health]

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Domoic acid (DA) is a neurotoxin synthesized by the diatom *Pseudo-nitzschia*. DA accumulates in filter-feeders and can result in neurotoxicity when consumed. Despite significant public health concern, there is little understanding of the effects of low-dose DA exposure on the brain. The objective of this study is to characterize the gene expression changes associated with DA exposure. Adult zebrafish were exposed to two concentrations of DA (symptomatic dose = 1.10 mg/g wet weight; asymptomatic dose = 0.31 mg/g) and brain tissues were sampled at 1, 2 and 7 days post-exposure. Microarray and pathway analyses were performed to characterize the transcriptional responses to exposure. Our analysis revealed that transcriptional responses to DA are dose-dependent, with 319 genes differentially expressed under the asymptomatic condition compared to the 485 in the symptomatic. Pathway analysis suggests enrichment of IGF1, EIF2, corticotropin-releasing hormone, and NRF2, signalling pathways. In response to both doses, downregulated genes represent neuregulin, PI3K/AKT, P2Y, and calcium signaling pathways. These down-regulated pathways play an important role in both neuronal and non-neuronal cells in the brain. In particular, they have been implicated in the activation and proliferation of microglia, the resident immune cells in the CNS. There is very little understanding on the impact of DA on non-neuronal cell targets including microglia. In order to understand the effects of DA exposure on this cell type, we are using transgenic zebrafish, tg(mpeg1:GFP), which labels microglia. We are currently testing the hypothesis that DA exposure affects distribution and morphology of microglial cells in the brain. The results from this study will demonstrate the potential effects of exposure to HAB toxins on microglial cells, important players in responses to CNS injury and neurodegenerative disease. This work is supported by a NSF Graduate Research Fellowship, NIEHS P30 ES00703, NIH P01ES028938 and NSF OCE 1840381, and an internal NOAA grant.

THE CONTRIBUTION OF EPIPHYTIC DINOFLAGELLATES TO THE CHEMOECOLOGY OF THE MACROPHYTE HOLOBIONT

[Ecophysiology]

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Tropical benthic macrophytes play host to complex communities of microorganisms (e.g. bacteria, fungi, protists) that together with the macrophyte can be considered as a single functional unit or holobiont. The community structure of these holobionts are determined by highly specialized symbiotic and competitive interactions which can be facilitated through the production and release of secondary metabolites by many taxa involved. Dinoflagellates belonging to the genera *Gambierdiscus*, *Ostreopsis*, *Coolia*, and *Prorocentrum* have been implicated as producers of marine biotoxins involved in ciguatera poisoning (CP) and are common members of the holobiont of tropical macrophytes. However, the contribution of these metabolites to the chemical diversity and ecology of the macroalgal holobiont remains unclear. Traditionally, many of the known metabolites from these dinoflagellates have been extracted directly from cells. The integration of SPATT technology to assess the proliferation of HAB toxins in nature has revealed that many of the known toxins are present in adjacent waters and culture media suggesting an extracellular target and ecological mechanism. The goal of this study was to investigate the presence and bioactivities of known and novel metabolites in the media of cultured dinoflagellates to better understand their potential to influence the chemoecology of the macrophyte holobiont. In this study, Diaion™ HP-20 resin was used to extract metabolites from the media of *Gambierdiscus* sp., *Ostreopsis* sp., and *Coolia* species. Multiple bioassays including the mouse neuroblastoma (N2a) assay, the Abraxis PP2a OA assay, and Biolog® Ecoplate™ were integrated with an untargeted assay guided fractionation workflow supported by LC MS/MS to isolate and identify known and novel dinoflagellate metabolites from culture media. This study identifies multiple novel dinoflagellate-metabolite associations. Additionally, the

dynamics of CP outbreaks may be further complicated if the co-occurrence of multiple toxins influences CP symptoms.

WHAT HAPPENS WHEN THE OCEAN AND TOXIC HABs MIX? AN EXPERIMENTAL ASSESSMENT
[Bloom Dynamics]

Felicia Osburn, Nicole D. Wagner, Raegyn B. Taylor, C. Kevin Chambliss, Bryan W. Brooks, J. Thad Scott

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One management approach to mitigating the effects of harmful algal blooms (HABs) is to flush freshwater environments, where they are formed, to downstream estuaries. However, the effect of increasing salinity on the growth and toxin production/leakage has not been widely studied. Coastal regions are also beginning to experience how encroaching salt water conditions, due to rising sea levels caused by climate change, are affecting freshwater phytoplankton, with HABs being a key concern due to their ability to produce cyanotoxins. The production of nitrogen (N)-rich cyanotoxins such as microcystin has been shown to increase with N availability to blooms, but N is often the limiting nutrient in coastal marine ecosystems. To test how an increase in salinity will affect microcystin-LR production both over time and under differing N availabilities, we grew *Microcystis* in batch cultures for approximately a month under low (4) and high (50) N:P ratio environments. We then simulated flushing events to ocean water (OW) environments by spiking cultures with differing amounts of artificial OW (0-30%) after 21 days of growth. After, we monitored growth, carbon (C) and N stoichiometry, and toxin dynamics for 10 days. Similar to other studies, we found that N availability affected microcystin-LR production. In the high N:P treatments, salinity greater than 16‰ OW had a negative effect on toxin production and growth over time, but this was not seen for the low N:P treatments. Microcystin-LR concentrations were greatest when *Microcystis* was exposed to high N and low salinity. Combined, our results suggest that the N:P of growth conditions determines how both time and salinity concentration will influence microcystin-LR production/leakage.

INTERACTION OF GRAZER EXPOSURE AND NUTRIENT REGIME ON TOXIN PRODUCTION AND GROWTH IN *ALEXANDRIUM CATENELLA*
[Ecophysiology]

Gihong Park, Hans G. Dam

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The goal of this study is to simultaneously address the roles of grazer exposure, nutrient regime (varying limitations of nitrogen and phosphorus) and their interaction on toxin production and cell growth of *Alexandrium catenella* (strain BF-5). We designed an assay to test the interaction of 'previous' cell exposure to grazers and nutrient regimes on cell toxin content (CTC) and cell growth rate (CGR) throughout a growth stage (exponential, stationary, and declining phases). The results demonstrate significant effects of both grazer and nutrient on CTC and CGR. First, the effect of the nutrient regime on CTC and CGR is most evident in the exponential and stationary growth phases, with CTC increasing in N-replete media, but dependent on treatment (previously direct grazer-exposure > previously indirect grazer-exposure > no grazer-exposure). Second, the effect of elevated N:P ratios on CTC is only significant in the absence of grazer cues. Third, cells previously directly exposed to grazers show the lowest CGR, which is consistent with a reverse pattern (a defense-growth trade-off) of CTC that persists for generations after the grazers are removed. Lastly, there is a significant positive correlation between induced toxin production and estimated direct cost. Although the cost of PST production is constrained by both grazer-exposure and the nutrient regime, the effect of the former is more pronounced.

SIMULTANEOUS EXTRACTION AND SEPARATION OF CHLOROPHYLL AND PHYCOCYANINE FROM CYANOBACTERIA WITH OCTANOL-WATER: STABILITY AND HIGH RESOLUTION FLUORESCENCE STUDIES

[Cell & Molecular Advances]

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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 marine and freshwater ecosystems worldwide. Cyanobacteria are characterized by the presence of phycocyanin (PC), a blue, proteinaceous, water soluble pigment, in addition to chlorophyll a (chl_a), a green, hydrophobic pigment. The amounts of PC and chl_a in water bodies are useful parameters 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. Chl_a extraction procedures employ organic solvents which may denature PC, and chl_a is insoluble in the aqueous-based extraction procedures for PC. We have found that the representative species of cyanobacteria grown in culture, *Microcystis aeruginosa* and *Limnithrix* 144, can be gently extracted with 1:1 mixtures of octanol:water, resulting in chl_a concentrated in an upper octanol phase, and PC concentrated in the lower aqueous phase. The separation of PC and chl_a into their respective hydrophilic and hydrophobic phases is complete, facilitating their respective measurements, as their absorbance and fluorescence spectra overlap. Measurements of the absorbance spectra over time suggests that the chl_a peaks are quite stable. However, the chl_a and its degradation product pheophytin a share similar spectra with both absorption and fluorescence. Work in progress is focused on distinguishing chl_a and pheophytin a in octanol, and optimizing the biphasic extraction for environmental samples. Funding is provided by East Georgia State College.

DOES *DINOPHYSIS ACUMINATA* PREY ON THE RAPIDOPHYTE, *HETEROSIGMA AKASHIWO*?

[Bloom Dynamics]

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Dinophysis acuminata is a toxic dinoflagellate that is found worldwide in coastal regions, including the Delaware Inland Bays (DIBs). It produces the toxin, okadaic acid, and has the potential to cause diarrhetic shellfish poisoning. Previous studies demonstrated that lab cultures of *D. acuminata* acquires plastids from its prey, the ciliate, *Mesodinium rubrum*, which, in turn, acquired plastids from the cryptophyte, *Teleaulax amphioxeia*. Reports of *D. acuminata* from field samples, however, suggest a broader range of prey as well as plastid retention from other sources. This project is seeking to better understand prey selection by *D. acuminata* and determine if *D. acuminata* will prey on an alternate food source, *Heterosigma akashiwo*, a species of harmful algae that co-occurs during *D. acuminata* blooms in the DIBs. Feeding on *H. akashiwo* by *D. acuminata* and/or *M. rubrum* was observed using microscopic observations and fluorescent dyes. Interestingly, all *M. rubrum* died when fed *H. akashiwo* at a predator to prey ratio greater than 1:5, suggesting that this toxic alga has deleterious effects on *M. rubrum*. Future work using fast repetition rate fluorometry will investigate the potential for *D. acuminata* to retain functional plastids of *H. akashiwo*. This project is ongoing, but results will add to our understanding of *D. acuminata* bloom dynamics and potential factors stimulating blooms of this species in the DIBs.